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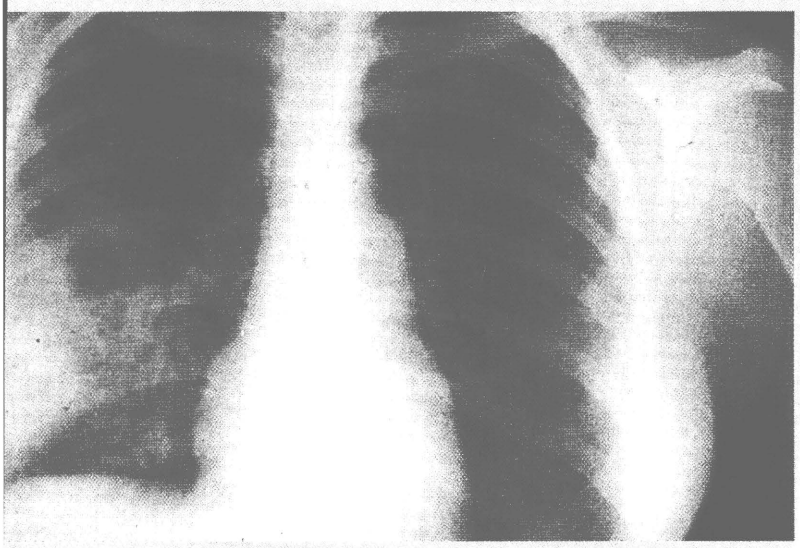

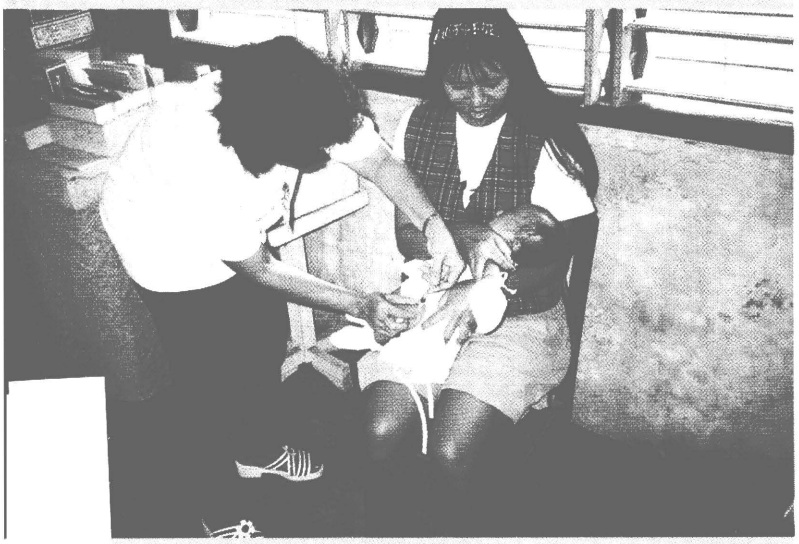

HEALTH RESEARCH WITH DEVELOPING COUNTRIES

Volume 2
VIROLOGY / BACTERIOLOGY NON COMUNICABLE DISEASES RESEARCH

OVERVIEW OF EC SUPPORTED JOINT RESEARCH PROJECTS

RTD INTERNATIONAL COOPERATION

EUROPEAN COMMISSION

Édith CRESSON, Member of the Commission
responsible for research, innovation, education, training and youth

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The front cover photographs have been kindly supplied by M. Pletschette

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HEALTH RESEARCH
WITH DEVELOPING COUNTRIES

Volume 2

VIROLOGY / BACTERIOLOGY
NON COMMUNICABLE DISEASES RESEARCH

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Foreword

Health research with developing countries has been part of the European Commission's research agenda for almost 15 years.

It is currently part of the specific RTD Programme in the field of Cooperation with Third Countries and International Organizations (INCO): activity II of the Framework Programme IV (1994-1998) (see annex).

This document introduces research projects on bacterial and viral diseases together with projects on non-communicable diseases. It combines summaries of completed contracts for STD3 and a catalogue of ongoing and new contracts of STD3 (Science & Technology for Development) and INCO-Developing Countries 1st and 2nd call for proposals. Other volumes of this documentation deal with research on health systems and parasitic diseases.

The research agenda is focused on the frequently occurring and enduring health problems of developing countries. The systematic application of modern biological and clinical research to these problems is in the best tradition of medical research and has resulted in many achievements.

Improving health conditions in developing countries through application of science and technology is recognised as an integral component of development policy.

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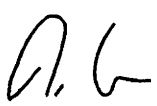


R. Gerold
Director

The INCO-DC Workprogramme

Sector Health

The selection of research areas is based on existing and newly identified health needs in DC's and on the capacity to cope with these needs through research. The tools and the systems developed for the control of health problems in Europe are not always directly applicable in DC's. Specific aspects of health care policy, of health care delivery, and the natural history of diseases require approaches adapted to DC's. Adequate and timely ethical clearance should be obtained whenever appropriate.

Research on the tools for prevention and the fight against the predominant diseases

Vaccines

Vaccines are cost-effective tools for disease control. Research on vaccines in the programme should be concentrated on selection, evaluation and delivery of antigens, with the objective of designing vaccines applicable for DC's. For vaccines against diseases with a significant impact in Europe, emphasis will be on aspects specific to DC's. With regard to the evaluation of antigens, emphasis will be put on immunological mechanisms underlying experimentally induced protection. Research on the non targeted effects of vaccines will also be taken into consideration.

Drugs

Emphasis will be put on targeted drug design and targeted drug delivery, based on the fundamental understanding of biological functions of pathogens and of disease mechanisms. Research on drugs, including bioactive natural compounds is eligible when focused on drugs for predominant diseases in DC's when non-toxic efficient treatments are unavailable.

Diagnostic products

This research should make an initial distinction between 1) the development of diagnostic products as research tools, or 2) the development of diagnostic tools for routine health care. Application of high technology approaches to design robust and simple diagnostic tools for routine health care will be given due attention. The relevance for practical case management of the read-out of routine diagnostic tools should be adequately addressed.

For each project concerning the design and the development of vaccines, drugs and/or diagnostics products, the feasibility of introducing affordable products envisaged should be pre-evaluated.

Research on the biology of the diseases

Biology

The understanding of the processes and biological interactions is a source of new techniques and tools for the control of disease. Research on genome structure and regulation of gene expression is eligible if it supports the investigation of defined biological mechanisms aiming at the control of disease causing pathogens. Metabolic pathways that differ between the human host and the pathogen may be addressed as a source for targeted interventions. Immunological studies should concentrate on the biological function of antigenic molecules and the mechanisms leading to protection against disease. Studies on the genetics of vectors are supported as tools that allow understanding of the physiological processes of the vectors, and vectorial interaction with the pathogenic agent and the host. Studies of intervention based on vectorial biology are eligible provided that monitoring is envisaged on a longer term and on a sustainable basis.

Pre-clinical models

A model may be used for different purposes. Where a model is proposed as a screen in a development process, the relevance of the particular model for human disease has to be established. In vitro and in vivo models for human disease will be the subject of studies aimed at refinement, replacement and reduction of animal experimentation.

Complementary areas bridging science and application

The programme will contribute to improved coordination and to better research methodology in fields of growing interest and for which only a limited number of international links are currently established, linking EU scientists to their colleagues in DC's. The modalities for implementation of this research will be concerted actions for topics not addressed under 1.3.1, 1.3.2 and 1.3.3. Opportunities to combine research with existing initiatives or future development initiatives of the EU and of Member States in these fields will be exploited. These topics are:

Relevance and methodology of health interventions

A large number of problems and of important bottlenecks have been identified for the evaluation of specific tools and of methods for the interventions in health in the field. Apart from technical aspects such as unequivocal contribution of morbidity and mortality of the health problem under study, there are issues of strategy, of ethics and relevance to be covered. Studies and tests of interventions have to be integrated into the existing health systems and the process of this integration can consequently form part of the research. The

research workers' responsibility for the results of their intervention research and for the continuation of the health services depending on these studies should be adequately addressed. In order to be eligible for support under this heading, the health problem for which an intervention is planned should be relevant in epidemiological terms as well as in terms of the needs expressed by the community in which it is studied. Innovative methods on the interventions on health problems will be supported for evaluation in the field. The planning of interventions should take account of ongoing activities in appropriate geographical areas and concentrate preferably on those fields where significant investments have already been made, or are planned, so that optimum use is made of existing capacities.

The European Commission and support for Virology/Bacteriology
Non Communicable Diseases Research

* Geographical distribution

* Context

The portfolio of projects under the research area of microbial and non-communicable diseases is necessarily wide and somewhat dispersed over many topics (figure 1).

However the major health issues are covered with a substantial and coherent effort developed over the active period the STD programme and more and more successfully linked with the INCO-DC exercise.

Such is the case with cholera research where innovative epidemiology is linked to innovative vaccine research but also tuberculosis research where molecular epidemiology is bridging to operational research on control programme management.

Collaboration in other diarrhoeal projects has led to new important findings such as the role of Cryptosporidium in the research of children diarrhoea.

The programme is, with a relatively small number of projects, contributing substantially to the development of tuberculosis subunit vaccines but also to the development of efficient vaccination against the major courses of death in children in developing countries: namely acute respiratory infections.

Based on long-term capitalization with partners in The Philippines, a new vaccine against pneumococcal disease is now under clinical development while other research in Guinea-Bissau, Bangladesh and Sudan is likely to improve vaccination against measles.

STD/INCO HIV research looking at the differences of progression of the disease, the mortality associated with the HIV-2 type and the issues linked to its association with tuberculosis has been among the most productive in the field. Based on numerous new partnerships emerging, a major effort is now with the development of research into the biology of HIV subtypes and variants.

Other pathogens are not forgotten, hemorrhagic fever research has been representing a great part of the European effort in the domain.

There are a few projects on non-communicable diseases showing well also the limits of the definition in the current classification. Scorpion and snake envenomation can represent an important cause of mortality in many developing countries, hence the important research effort on the molecular biology of the diseases.

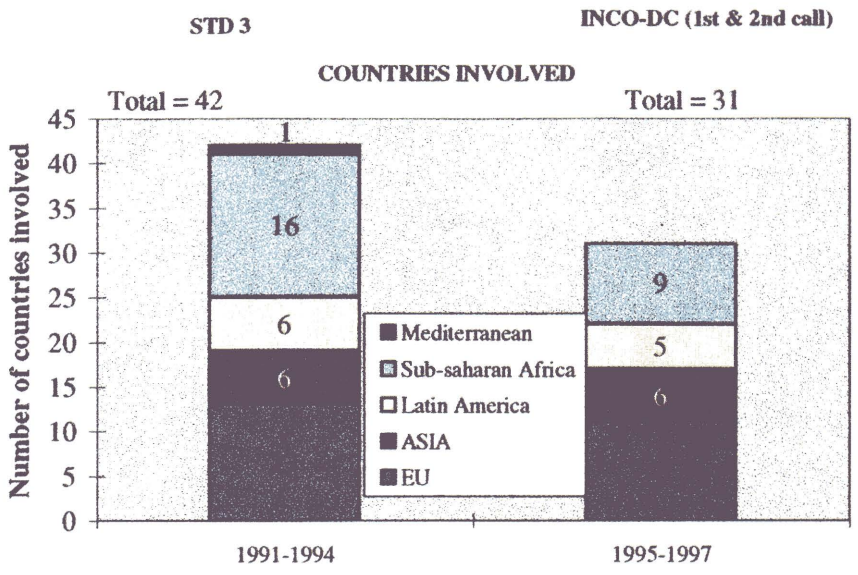
Other problems like metabolic and blood disorders are somewhat underrepresented in relation to the long-standing burden of diseases they represent, but the results are substantial.

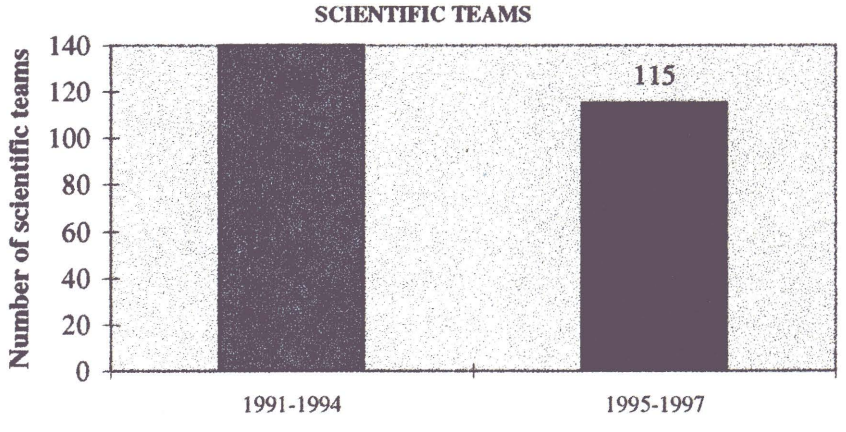
Over the years, the size of projects have increased and the number somewhat reduced but the exercise as such appears well consolidated and remains a strong asset in the field of international health research.

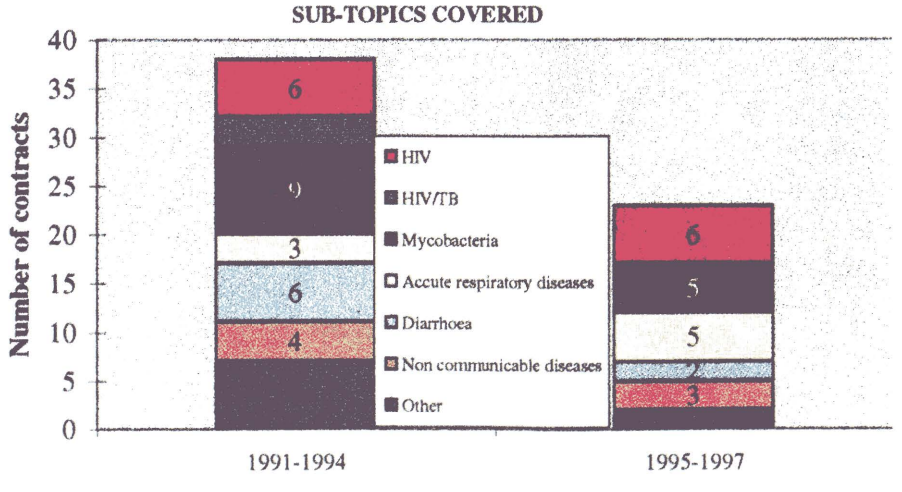
Figure 1.

VIROLOGY/BACTERIOLOGY
NON COMMUNICABLE DISEAS RESEARCH

COUNTRIES, TEAMS, SUB-TOPICS







EC supported joint research projects (1991-1996)

STD3/INCO-DC (1st and 2nd call)

1. HIV/AIDS

HIV & Sexually Transmitted Diseases

Presentation of EC supported joint research projects (1991-1996)
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

1. HIV/AIDS

HIV & Tuberculosis

Contract number TS3*CT910022

STUDIES ON MATERNAL AND NEONATAL MORBIDITY DUE TO SEXUALLY TRANSMITTED DISEASES

Period: January 1, 1992 — December 31, 1993

Co-ordinator: INSTITUUT VOOR TROPISCHE GENEESKUNDE, DEPT OF MICROBIOLOGY, Antwerpen, Belgium (M. LAGA)

Objectives

The overall aim was to assess interventions to reduce complications in mother and child due to sexually transmitted diseases. Specific objectives were:

- To determine risk factors to pre-term delivery (PD) and post-partum endometritis (PPE);
- To validate different diagnostic approaches for the management of gonorrhoea during pregnancy;
- To assess the cost-effectiveness of an intervention programme against gonorrhoea and syphilis to reduce perinatal complications;
- To determine risk factors for syphilis in pregnant women;
- To determine the incidence, microbial aetiology, risk factors, and clinical and histologic features of PPE;
- To assess the influence of gonococcal outer membrane proteins on pregnancy outcome in women with gonococcal infection during pregnancy;
- To develop diagnostic criteria and assess the responses to treatment for PPE.

Activities

* Interventions against gonorrhoea in pregnancy: gonorrhoea detection and Treatment in pregnancy was assessed. The effect of the intervention was estimated by comparing the incidence of adverse pregnancy outcome in both the study and the control group, including prematurity, low birth weight, chorioamnionitis, and postpartum endometritis.
* Studies on syphilis: the impact screening and treatment of syphilis during pregnancy was evaluated by comparing the incidence of prematurity, stillbirth, low birth weight, and congenital syphilis in children from women with and without syphilis during pregnancy.
* Studies on PPE: in a case control design, patients with clinical signs and symptoms of post-partum infection and controls will be studied in order to assess risk factors and the aetiology of post-partum infections, and to develop case management strategies.

Contract number TS3*CT910022

Results

For the intervention study on gonorrhoea in pregnancy a total of 2.383 pregnant women were recruited from two antenatal clinics in Nairobi. Patients in the intervention group (N= 1 060) were screened and treated for STDs, the non-intervention group (N=1 323) received routine antenatal care. Women were followed up at delivery, 2 and 6 weeks postpartum. Of these women 6% had chlamydia infection, 5.1% gonorrhoea, 5.6% syphilis and 16.2% were infected with HIV. For chlamydia infection and gonorrhoea risk factors were age less than 20 years ($p<0.01$ OR 2.5) and not having a fixed sex partner ($p<0.01$ OR 2.1). 3 or more partners during lifetime was a risk factor for contracting chlamydia infection, syphilis or HIV infection but not for gonorrhea.

During pregnancy no clinical signs or symptoms (lower abdominal pain, dysuria, foul smelling discharge, colored cervical swab, ectopia) were correlated with gonorrhoea or chlamydia infection. Two weeks after delivery chlamydia infection was found to be associated with ectopia ($p<0.3$ OR 2.2).

There was no difference in pregnancy outcome (gestational age at delivery, birth weight, PPE and ophthalmia neonatorum between patients of the intervention and non-intervention group. The main reasons were:

⇒ The high incidence of gonorrhoea and chlamydia infection, 14.8% and 17% per year respectively bringing the infection levels after intervention, almost back to pre-intervention levels two weeks after delivery;

⇒ The high loss to follow-up (56.6%) in the intervention group.

Thirteen percent of women developed post-partum endometritis (PPE). A history of lower abdominal pain ($p<0.0001$) and foul smelling lochia ($p<0.0001$) were associated with PPE. Having a Caesarean section ($p<0005$ OR 3.1), gonorrhoea ($p<0.0000$ OR 2.9) and no fixed partner ($p<0.002$ OR 1.8) were risk factors. There was no significant association with chlamydia infection.

10.9% of newborns developed ophthalmia neonatorum (ON). Having a mother with PPE ($p<0.005$ OR 2.3) was a risk factor, as was gonorrhoea ($p<0.0001$ OR 2.6) or chlamydia infection ($p<0.001$ OR 2.6) in the mother. Reintroducing ON prophylaxis with tetracycline in Pumwani Maternity Hospital could prevent 825 cases of ON per year.

For the study on syphilis and pregnancy outcome, 12 296 pregnant women were screened when presenting for delivery at Pumwani Maternity Hospital in Nairobi. More than one in two patients delivering in Pumwani Maternity Hospital were not screened for syphilis during pregnancy (53.5%).

Contract number TS3*CT910022

A total of 1 156 women and their newborns were enrolled in the study. Of these 840 had no syphilis during pregnancy; 140 women were treated; 176 women had serological evidence of recently acquired syphilis but were not treated during pregnancy. Age, belonging to a ethnic group who does not practice circumcision, not having a fixed partner, number of previous pregnancies and abortions were not associated with syphilis. However, 19.4% of women who were seropositive for *T. pallidium* were infected with HIV.

The incidence of perinatal mortality was 1.3% in the control group. Among women treated for syphilis, the incidence was 3.6% and among the untreated women 7.2%. The incidences of low birth weight were 10.0%, 14.4% and 16.8% respectively in controls, treated and untreated women. In the multivariate analysis perinatal death was significantly associated with syphilis (OR=6.7; 95% C.I. 2.2-20.8). Treatment reduced the incidence of perinatal mortality, although treated women still had a higher risk for perinatal mortality (OR=3.8, 95% C.I. 1.0-15.0). Assuming a prevalence of syphilis among pregnant women of 6.5% in Nairobi, it is estimated that 2 cases of perinatal mortality per 1000 births at Pumwani Hospital could be prevented if all antenatal clinical attenders were screened and treated for syphilis during their first visit to the antenatal clinic.

Contract number TS3*CT910022

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Contract number TS3*CT920051

A LONGITUDINAL EPIDEMIOLOGICAL STUDY OF HIV-2 INFECTION IN GUINEA-BISSAU: TRANSMISSION, DISEASE DEVELOPMENT, IMMUNOLOGY AND PREVENTION

Period: April 1, 1992 — June 30, 1996

Co-ordinator: STATENS SERUM INSTITUT, DEPT. OF EPIDEMIOLOGY RESEARCH, Copenhagen, Denmark (P. AABY)

Objectives

Improve the understanding of HIV-2 infection and promote its prevention, through studying:

- The impact of HIV-2 infection on mortality.
- The risk factors for and the probability of disease development in HIV-2 infected individuals, including immunological parameters.
- The risk factors for HIV-2 positivity in people over 50 years of age.
- The rate of transmission of HIV-2 from mother to child/foetus, and its possible risk and co-factors.
- The role of other sexually transmitted diseases (STD) in the transmission of HIV-2.
- The socio-cultural context which influences popular understanding of AIDS and AIDS information.
- The social implications of an increase in the number of AIDS cases.

Activities

* Coordinate the only two community cohorts of HIV-2 infected individuals in urban Bissau and in a rural area.
* Annual surveys to follow mortality in cohorts examined for HIV infection.
* Detailed interviews with people over 50 years of age have been performed in an urban area of Bissau.
* A study of the mortality of children born to HIV-2 infected mothers.
* Analyses of immunological parameters, including T-lymphocyte subsets, neopterin, and beta-2-microglobulin, viral load and co-infections, including HTLV.
* Develop an ELISA for antibodies to *H. ducreyi.*
* Analyze risk factors for concordance for HIV-infection in couples.
* Initiate prospective community study of the interaction between tuberculosis and HIV-2 and HIV-1.

Results

In 1990-93 in the cohort of 671 persons of 50+ years of age a HIV-2 seroprevalence of 14.1% was found while that of HIV-1 was only 0.1%. The main risk factors for infection were younger age group and ethnic group. The more behavioral factors varied between men and women. For men, factors like early sexual experience and having been in the colonial Portuguese army were significantly associated with HIV-2. For women, travelling was a significant risk factor, as was a history of blood transfusion and a history of preparing monkey meat. In a similar analysis of risk factors for HTLV seropositivity in the same population, HTLV was associated with being bitten by a monkey, scarifications, commercial sex, and age of sexual debut. The association with sexual risk factors was much stronger for women than for men and women had a three times higher prevalence than the men.

The overall prevalence of HIV-2 did not increase significantly over the study period from 1987 to 1996. While the incidence was 1 per 100 person-years-at-risk (pyr) during 1987-89, it appears to have declined in recent years being only 0.3 per 100 pyr in the period 1989-93.

More recent data from 1995-96 suggest that the HIV-2 prevalence is in fact declining. However, this is unlikely to be due to the efficacy of preventive activities as HIV-1 has increased strongly during the period 1992-95 from virtually zero to 2.5%. In contrast to the much publicized results from Senegal, we have found no evidence that HIV-2 provides protection against HIV-1 infection.

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Using data from rural Guinea-Bissau, we have conducted an analysis to see whether transmission is related to viral load. The major risk factor for women being seropositive if married to a seropositive man was the age of the woman being over 40. The proviral load did not have a significant effect in this analysis. In both cohort studies in Bissau, we have also found a surprisingly high incidence of HIV-2 infection among women over 40-45 years of age. In urban Bissau, HIV-2 infection was five times higher than for young women even though younger women certainly have more sexual contacts. This suggests that age-related susceptibility may play an important role in the HIV-2 epidemic. An analysis of the prevalence of HTLV among people over 50 years of age in Bandim, Bissau, indicates that the pattern of increased age-related susceptibility for women may also apply for HTLV.

For the oldest cohort in Bissau, we have carried out a survival analysis of the 9-year follow-up of HIV-2 infected and uninfected adults. Mortality was only two times higher among HIV-2 infected individuals, the difference was most marked among younger people under 40-50 years of age. For people over 50, there was a minimal difference in mortality related to HIV-seropositivity. HIV-2 infected individuals who were living with a HIV-2 infected spouse had significantly higher mortality than HIV-2 infected individuals who were living with a spouse who was not infected.

In both cohorts of HIV-2 infected individuals we have found no sign of strain differences between young and old individuals and between AIDS cases and asymptomatic cases. Only strain A appears to be present in Guinea-Bissau. Hence, it is unlikely that strain difference can explain the variable outcome of HIV-2 infection. In a cohort of HIV-2 infected individuals from a rural area of Bissau where pro-viral levels had been determined, we have shown a very strong association between pro-viral level and the mortality during the following 3-4 years. There is no trend to increasing load with age. The interaction with HTLV infection has been examined. There is no sign of amplification of HIV-2 pro-viral load by HTLV.

The immunological samples collected in 1988, 1990 and 1992 from HIV-2 infected individuals and controls have shown very small changes in T-cell subsets over the 4 years of observation. There was no association between T-lymphocyte values and the risk of dying during the follow-up period. T-lymphocyte values were significantly different for individuals who were concordantly infected with their spouse compared to HIV-2 infected individuals who were living with an uninfected spouse. Syphilis antibodies have been measured on samples previously collected in Bissau and the rural area. Data suggest that syphilis may suppress CD4 T-cells and could thus contribute to progression of HIV-2 related infection. It has not been feasible to examine the *H. ducreyi* antibodies on filter paper blood samples.

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Contract number TS3*CT920059

ROLE OF PLACENTA IN TRANSMISSION OF HIV INFECTION FROM MOTHER TO CHILD

Period: November 1, 1992 — October 31, 1994

Co-ordinator: DANISH CANCER SOCIETY, DEPT. OF VIRUS AND CANCER
Aarhus, Denmark (P. EBBESEN)

Objectives

The general purpose is to study the role of the placenta in vertical transmission of HIV.

- Human placental cell types will be characterised with respect to presence of CD4 and HIV. Limited studies will be done with baboon placenta. Immunohistochemical studies will be done.
- Placental thophoblast cells will be isolated and cultured. These will be analysed with FACscan with respect to cell types, presence of CD4 and HIV.

Activities

HIV infected labouring women admitted in a busy maternity hospital in Nairobi will be enrolled in the study. Placenta's will be collected and the neonates will be followed till the age of 3 to 6 months to determine the vertical transmission rate, using PCR techniques (Institute of Tropical Medicine, Antwerp, Belgium).

At the Institute of Primate Research, Nairobi, cryostat placental sections will be characterised with respect to CD4 and HIV/SIV using immunohistochemical studies. Placental cell isolation and FACs analysis will also be done as well as *in situ* hybridisation and PCR studies.

The Department of Virus and Cancer, Danish Cancer Society, Aarhus, Denmark, will study placental cells with monoclonal antibodies developed by the department and *in situ* hybridisation with the probes produced by the department. An ELISA test for trophoblast interferons will be developed and used for determining concentrations in maternal and fetal blood.

Histological studies on fixed placental material will be done at the Department of Human Pathology, University of Nairobi.

Expected outcome

⇒ Improvement of our understanding of the mechanisms of mother-to-child transmission of HIV and the placental cell types involved in transmission.

⇒ Establishing the role of interferon in the vertical transmission of HIV.

⇒ Better understanding of the role of placental inflammation in vertical transmission of HIV.

Contract number TS3*CT920059

Results

A total of 274 placentae and maternal/cord blood samples were collected. Of these 128 samples were ELISA+ and 98 ELISA-. The PCR was done on 57 blood samples from babies collected during follow up and 7 were found to be positive with at least one primer par. This represents an apparent transmission rate of 12%.

A data base on maternal history/risk factors and placental status is set up in two institutes (IPR and Danish Cancer Institute). The FACS-analysis done on 169 maternal samples showed a low CD4/CD8 ratio in 124. An intact syncytiotrophoblast was present in all placentae. Cytotrophoblast cells were rare. Cells staining for CD4 were present in the villus axis within placental mesenchyma and endothelial lining of blood. Presence of HIV proteins was very rare (17%). Maternal/fetal barrier was intact.

All cord sera were tested for alpha interferon and 16% had high levels. It was evident that HIV infection could occur without provoking an enhancement of placental and fetal interferon response. More detailed studies were done in 17 positive and 22 control samples. None of the maternal samples had detectable rheumatoid factor and were negative for HBV core antigen or HCV but malaria was the predominant parasitic infection.

In the 216 placentae studied chorioamnionitis, funistitis and villitis was present in 92, 34 and 16 respectively. Malarial pigments were present in 10 placentae.

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Contract number TS3*CT920122

STUDIES OF THE EPIDEMIOLOGY OF HIV INFECTION AND OTHER SEXUALLY TRANSMITTED DISEASES IN MWANZA REGION, TANZANIA

Period: December 1, 1992 — November 30, 1995

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, EPIDEMIOLOGY AND POPULATION SCIENCES
London, United Kingdom (R. HAYES)

Objectives

- To measure the impact of an intensive control programme for sexually transmitted diseases (STDs) on the incidence and prevalence of STDs over a two year period in a large rural population.
- To identify risk factors for HIV infection and other STDs through case-control studies conducted at baseline and after two years, and to quantify the effects of these risk factors.
- To investigate the aetiologies associated with genital ulcer syndrome (GUS) in this region through studies at an STD clinic; to estimate the percentage of GUS cases at this clinic associated with each aetiology.
- To estimate the incidence of asymptomatic carriage of chancroid in selected urban groups at high risk of HIV and other STDs; to evaluate the field performance of a serological test for *H. ducreyi*.

Activities

This research project complemented the "Mwanza Region HIV/STD Intervention Programme", funded by the AIDS Task Force of the EC (DG VIII) and the UK Overseas Development Administration, which aimed to measure the impact of improved treatment services for STDs on HIV incidence in the general population of rural Mwanza. This project comprised the following activities:

* More accurate documentation of the impact of the intervention on STDs, through syphilis and *H. ducreyi* serology on all 12.000 adults in the main cohort at baseline and after two years.
* Regular follow-up every three months of a sub-cohort of 1.200 men to record the incidence of STDs and related treatment-seeking behavior.
* Annual surveys of STDs among 100 women attending antenatal clinics (ANC) in each of the twelve study communities.
* Case-control studies of risk factors for HIV infection and other STDs. Cases detected from main cohort surveys at baseline and two-year follow-up, with controls sampled from the entire study cohort.
* Etiological studies in GUS patients presenting to the STD clinic in Mwanza town.
* Intensive studies of commercial sex workers and STD patients in a search for asymptomatic carriers of *H. ducreyi*.
* Field evaluation of serological tests for *H. ducreyi*, including serial testing of GUS patients at the STD clinic.

Results

⇒ After two years, the prevalence of active syphilis (RPR +/RPHA+) was 5.0% in the intervention (I) and 7.0% in the comparison (C) communities, a significant reduction of about 30% (P = 0.02). Seroconversion for syphilis (TPHA) was also 30% lower in the I communities, but this difference was not significant. In males, the prevalence of symptomatic urethritis was about 50% lower in the I communities (P=0.056). These effects help to explain the 40% reduction in HIV incidence achieved by the intervention programme.

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⇒ In the male sub-cohort, incidences per 100 person-years were as follows: reported genital discharge syndrome (GDS): 9% (I) and 14% (C); reported GUS: 1.4% (I) and 2.1% (C). About 30% of men reported more than one partner per quarter, and reported condom use was low (<5% of men). Many men did not seek treatment at government health units, but more did so in I communities, where the improved services were in place.

⇒ Data from the ANC surveys showed very high prevalences of reproductive tract infections (RTIs) among pregnant women. At the follow-up survey, prevalences were: *T. vaginalis* 24%, *N. gonorrhoea* 1.7%, *C. trachomatis* 5.2%, active syphilis 10.5% and 42% of women had at least one RTI. There were no significant differences between the I and C communities, presumably because most infections are asymptomatic or poorly symptomatic, and are therefore not covered by syndromic treatment services. Other strategies will be needed to reduce the burden of RTIs in these women. Data from these studies were used to develop a "risk score" to predict cervical infection from simple questions on social and behavioral factors, and this was shown to perform better than clinical approaches to diagnosis.

⇒ Among 154 consecutive patients presenting with GUS at the STD clinic, prevalences of pathogens were: 20% *H. ducreyi* (by PCR: 30% of these were culture-positive); 39% were positive on syphilis serology but few were confirmed by dark-ground microscopy (5%); 13% HSV2 (antigen ELISA); 1% LGV in males (antigen ELISA). There was no identifiable pathogen in 40% of cases.

⇒ A cohort of 40 CSWs were followed monthly for 6 months, but no cases of symptomatic or asymptomatic *H. ducreyi* were observed. STD incidence in this cohort, already enrolled in an HIV/STD intervention programme, was very low. 100 patients presenting to the STD clinic without GUS were also studied, with the detection of *H. ducreyi* by PCR in 1/50 males and 2/50 females, none of whom developed ulceration subsequently. Asymptomatic carriage may play a role in the transmission of this agent in this population.

⇒ The serological test for *H. ducreyi* is being modified and improved, and will be applied to sera from the Mwanza studies in due course.

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Contract number TS3*CT930238

INTERVENTION STUDY OF SEXUALLY TRANSMITTED DISEASES AND HIV INFECTION AMONG FEMALE PROSTITUTES IN ABIDJAN, IVORY COAST

Period: January 1, 1994 — December 31, 1996

Co-ordinator: INSTITUUT VOOR TROPISCHE GENEESKUNDE, DEPT OF MICROBIOLOGY, Antwerpen, Belgium (M. LAGA)

Objectives

- To define effective strategies to prevent HIV infection in high risk populations which are simple, independent of sophisticated laboratory infrastructure and generalizable to large populations.
- To collect data on attitudes and behavior related to STD and HIV infection among prostitute populations in Abidjan, including sexual practices, preventive behavior and health seeking behavior.
- To determine the prevalence of STD and infection with HIV-1 and HIV-2, and associated risk factors in this population.
- To evaluate 3 different interventions aimed at reducing HIV and STD incidence rates among a group of women followed prospectively. The goal is to determine the efficacy for preventing HIV and STD, the feasibility and the cost of the interventions.

Activities

Based on the knowledge that interventions consisting of condom promotion and regular STD screening and treatment can have a considerable impact on HIV incidence, the question remains whether simpler, less sophisticated STD interventions independent of laboratory infrastructure will have the same impact on HIV incidence.

The project will evaluate different levels of STD interventions among a group of female prostitutes in Abidjan. Initially, a cross sectional survey will be performed to collect data about attitudes and behavior related to STD and HIV infection, to determine prevalence of STD and HIV and to validate the diagnostic accuracy of different algorithms. A subsample of HIV negative women will then be enrolled in a prospective study. They will be randomized to one of the three STD interventions. Condom promotion and distribution will be performed for all women similarly.

Women will be followed up monthly for a total duration of minimum 2 years. Every month condom use will be monitored and STD will be treated according to the 2 different algorithms or the gold standard laboratory approach.

The prevalence of STD will be monitored after one and two years intervention in the 3 groups. The incidence of HIV will be monitored on a 6 monthly basis and will be compared in the 3 intervention groups over a period of 2 years.

The cumulative incidence rate of HIV over a 2 year period will be the final outcome measure, and will be compared in the 3 groups after controlling for confounding variables such as sexual exposure.

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Expected outcome

⇒ The definition of more effective strategies to prevent HIV infection in high risk female populations based on condom promotion and STD control. These strategies should not only be effective in controlling HIV and STD, but also be independent of sophisticated laboratory infrastructure, simple and generalizable to larger populations. These strategies will be useful not only for Côte d'Ivoire but also for the rest of the developing world.

⇒ In addition this project should contribute to strengthening the research capability of members of the Ivorian National AIDS Control programme, as well as scientists at the "Institut National de Santé Publique" in Côte d'Ivoire.

Results

A baseline study on HIV infection and sexually transmitted diseases was conducted on 483 commercial sex workers. Of these 23% had a genital ulceration. Gonorrhoea was found in 34.5% of women, chlamydial infection in 10%, trichomonas infection in 24.5% and syphilis in 22%. The overall prevalence of HIV infection was 88%, 50% of women were infected with HIV-1, 2% with HIV-2 and 36% were dually reactive.

Before the start of the intervention consisting of condom promotion, health education and management of STDs the incidence of HIV infection was 23 per 100 women-years. During the intervention this incidence dropped to 3 per 100 women-years.

By the end of 1995, 1201 women were enrolled in the study. Among the HIV infected women (24% HIV-1, 26% dually seroreactive, 5% HIV-2) shedding of the virus was associated with HIV-1 infection, immunosuppression and presence of gonorrhoea, chlamydial infection or genital ulceration.

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Contract number TS3*CT930241

THE WELL-WOMEN CLINIC: STUDIES ON WOMEN'S REPRODUCTIVE HEALTH

Period: January 1, 1994 — December 31, 1995

Co-ordinator: UNIVERSITY OF GENT, DEPT OF OBSTETRICS AND GYNAECOLOGY, Gent, Belgium (M. TEMMERMAN)

Objectives

- To study the prevalence rates of reproductive tract infections (RTI), sexually transmitted diseases (STD) and cervical cancer in women attending a family planning clinic in Nairobi, Kenya.
- To validate diagnostic criteria and risk markers in the management of STD and cervical cancer in family planning clinic attenders.
- To develop training programmes to upgrade the staff in a family planning clinic in the management of STD/RTI.
- To carry out qualitative research into the health seeking and health providing behaviour in an urban family planning clinic.
- To determine clinical and subclinical risk markers for human papilloma virus infection (HPV), in order to identify women at high risk for cervical lesions.
- To compare existing diagnostic techniques for the clearly detection of cervix cancer (Papanicolaou screening) with recently developed new screening techniques (cervicography).
- To examine the feasibility of an integrated programme addressing women's health issues.
- To identify cost-effective means of integrating STD prevention and control programmes into more comprehensive services.

Activities

The site selected for this intervention is a busy planning clinic in Nairobi with 50 to 80 attendants per day. A substantial proportion of these women present with signs and/or other symptoms related to STD or RTI. Many primary health care facilities, including maternal-child health and family planning clinics, do not adequately address RTI in women. STD clinics, on the other hand, are often overloaded and not woman-friendly. Recognition of these issues argues for incorporating STD concerns not a comprehensive program of reproductive health services rather than addressing them in a separate, potentially stigmatizing manner that emphasizes their sexual acquisition. In addition, cancer of the cervix is also very common in Kenya, accounting for high morbidity and mortality rates. National screening programmes for early detection of cervical cancer are hardly implemented due to the lack of adequate cytology facilities, the lack of properly trained staff and quality control.

The findings of these studies allow the development and implementation of simple interventions targeting STD, RTI and cervical lesions in a comprehensive way contributing to the improvement of reproductive health of women in the developing world.

The initial phase of the WWC Supermarket Project was the realisation of an STD/RTI/HIV/cervical dysplasia prevalence study. In order to measure the magnitude of the problem in a population of family planning clinic attenders, over 500 consecutive women attending for family planning purposes were assessed for the presence of STD/RTI and cervical cancer. The STD prevalences were as expected for African urban standards in low risk populations (*C. Trachomatis* 4%, *N. Gonorrhoeae* 2%, syphillis 2%, HIV-1 10%) but striking was the high rate of cervical precancerous lesions. Twelve percent of these women had cervical epithelial dysplasia (CIN) of whom 6% CIN II (moderate dysplasia) or CIN III (severe dysplasia).

During the second phase (1995) more attention was given to:

* Study the interactions between CIN and HIV.

* To expand the training component.

* To establish a referral site for follow-up and management of precancerous lesions of the cervix.

* To the dissemination of the results.

* To assess the syndromic approach for detecting STD/RTI in this group.

* To explore the possibilities of networking with other regional and international organizations in the area of reproductive health.

Based on the results of the first survey, a second study was initiated in 1995. The objectives were to evaluate "risk scores" to predict cervical infections and to develop strategies to reduce the transmissions of STD and HIV including information, education and communication, HIV testing and counselling and the control of sexually transmitted infections (STI). The study was conducted to determine the impact, relevance, access and obstacles to these interventions in women considered to be at low risk.

A new questionnaire has been developed. Again 530 women were enroled and examined at FPAK Ribiero clinic. The results of the second phase indicate that:

* Scoring systems based on historical data and clinical findings may assist in the management of women considered to be at low risk for STI. Sensitivity measures may be improved with the identification of other predictor variables and/or the addition of simple diagnostic testing.

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* The prevalence of cervical dysplasia and aggressive HPV serotype are substantial. Family planning clinics in developing countries provide an important opportunity to implement programmes for the detection and management of cervical malignancies.

* Intervention strategies need to be targeted to women in the general population who remain highly vulnerable to HIV/STD infections despite access to information, condoms and STI treatment. Programmes at family planning clinics may provide an excellent opportunity to increase awareness of vulnerability and implement novel interventions.

Conclusions and recommendations

⇒ The project management structure has been set up well and seems to function satisfactory.

⇒ Overall, the WWC project prevalence study has progressed well. Thanks to the dedication of the participants and the overwhelming clients' response, data collection was much easier than anticipated. The prevalence of CIN was alarmingly high and needs further consideration.

⇒ There is a need for integrated services within existing FP clinics. This was evidenced by the frequency of STI complaints. The especially high prevalence of CIN and HIV also serves to accentuate the need for routine pap smear or other methods of examination of the cervix in this population.

⇒ Strengthening screening facilities without proper treatment and intervention being considered as unethical, a substantial amount of time and effort has been spent to find proper, safe, accessible and affordable treatment facilities for patients identified with CIN lesions. As the public sector in Nairobi was not able to care for these women, arrangements were made with KMWA members working privately to treat these patients. Additional mechanisms to ensure the intervention will be explored. KMWA hopes to avail the colposcopy and CIN treatment facilities to the general public at a small fee. It is known that the Kenyatta National Hospital (KNH) — the only public facility offering colposcopy services — is already overwhelmed with cases. Due to logistic problems and constant breakdown this facility is unavailable most of the time. This leads to long waiting time and possible progression of CIN in many cases. The KMWA facility would serve to provide this very much needed service on top of ofloading some of the KNH clients.

⇒ Additional training and supervision in the field of colopsocopy, cytology and management of STD/RTI and cervical lesions will be required. The remaining budget of this contract (10%) will be used to send one of the senior KMWA members who has been exposed to training to a higher level course in Europe to be better prepared for organizing training courses in Nairobi for Kenya and for East-Africa.

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⇒ To avoid duplication of efforts, networking with other groups working in the area of reproductive health has to be enforced.

⇒ Research experience gained from this project will be useful for KMWA in formulating other research proposals and implementing other research programmes. The KMWA WWC lab is also foreseen to serve as a training lab for updating cyto technicians on cervical cancer screening techniques. The lab would also be a centre for quality control which is lacking in most laboratories offering cervical cancer screening services in Nairobi. KMWA has trained and experienced cyto pathologists able to offer technical assistance in this respect.

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Contract number IC18*CT960050

A STUDY ON THE ROLE OF HIV-1 GENOTYPES ON THE EPIDEMIOLOGY OF HIV IN TANZANIA AND UGANDA

Period: November 1, 1996 — October 31, 1999

Co-ordinator: UNIVERSITY OF MUNICH, DEPARTMENT OF TROPICAL MEDICINE, Munich, Germany (M. HOELSCHER)

Objectives

- Retrospective study (1988-1995) to determine the different subtypes present in the regions, to identify their geographical distribution and possible alterations of the proportions in different population groups over the observation period.
- Consistenc y check, whether the current distribution of subtypes in AIDS patients in a specific region is comparable to the distribution of subtypes in this region seven years ago (at the approximate time of infection.)

Activities

* 1.200 Tanzanian and 900 Ugandan serum samples are tested by V3-serotyping ELISA by the African partners.
* 10% of the samples will be reanalysed as control group by LMU by V3-serotyping ELISA.
* Approx. 20% of the 2100 samples will be untypable by V3-loop ELISA. These will be referred to Europe and will by analysed by HMA (SMH) and sequencing (LMU).
* Consistency check whether the subtype distribution of 100 AIDS patients of the year 1995 is comparable with the distribution of 100 AIDS of the year.

Expected outcome

⇒ The distribution of subtypes in different geographical regions and in different population groups.

⇒ A probable change of predominance of subtypes over the years, which gives an insight into differences in transmission efficacy.

⇒ Detection of new strains from other parts of Africa to the Mbeya Region.

⇒ Detection of new variants.

⇒ Essential information for the preparation of vaccine campaigns in this region.

⇒ Differences between the distribution of subtypes at the time of conversion (5-7 years ago) and the present distribution. This could reveal possible differences in pathogenicity in the different strains.

Contract number IC18*CT960050

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Contract number IC18*CT960110

STUDY OF HIV-1 VARIABILITY IN CAMEROON AND GABON. IMPLICATIONS FOR VACCINE AND ANTIVIRAL INTERVENTION STRATEGIES

Period: April 1, 1998 — September 30, 2000

Co-ordinator: INSTITUTE OF TROPICAL MEDICINE
Antwerp, Belgium (G. VAN DER GROEN)

Overall aim

- To study the biological function of antigenic epitopes inducing broad cross-neutralizing antibodies, and the role they play against infection and/or progression to disease.
- To monitor anti-HIV drug susceptibility and viral load of genetically diverse primary HIV strains circulating in Cameroon and Gabon.

Objectives

- To provide a surveillance system for newly emerging and naturally occurring recombinant HIV-1 strains in Cameroon and Gabon.
- To classify sera from HIV infected individuals according to their capacity to neutralize primary isolates representing different genetic clades.
- To characterize broadly neutralizing antibodies and epitopes in individuals infected with HIV-1 of different genetic subtypes, and evaluate the role they play in disease protection.
- To investigate the susceptibility of HIV-1 strains belonging to different genetic clades, to both existing and experimental anti-HIV compounds and to provide a surveillance system for emerging strains of HIV-1 with naturally occurring mutations conferring antiviral drug resistance.
- To optimize the quantification of HIV RNA in plasma of patients infected with different types, subtypes and recombinant subtypes of HIV.

Activities

* Both heteroduplex mobility assay (HMA) on PCR amplified fragments generated from HIV RNA in serum or plasma of HIV infected individuals and V3-peptide ELISA will be used to monitor the prevalence of different genetic subtypes of HIV-1.
* HMA on both *gag* and *env* fragments of the same isolate will allow to study the prevalence of recombinant viruses.
* Susceptibility of the different subtypes and the natural recombinant viruses towards various HIV inhibitors will be determined.
* Neutralizing antibody patterns in sera of individuals infected with all subtypes (A-H) within group M and representatives of group O to their homologous and heterologous primary isolates will be documented using a peripheral blood mononuclear cell based neutralization assay.
* Neutralization data will be analyzed by a multivariate (spectral mapping) analysis.
* Chimeric viruses consisting of neutralization sensitive and insensitive primary HIV-1 strains will be constructed in order to characterize the epitopes playing a role in the neutralization process.

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Expected outcome

⇒ Making available to developing countries a simple economical serologic test to monitor circulating HIV-1 subtypes.

⇒ Since only a limited number of key isolates are required to document the broad cross-clade neutralization capacity of a particular serum, we hope in future to be able to monitor the efficacy of HIV-1 vaccines inducing high-titer cross-neutralizing antibodies in an economic way.

⇒ To enable some research centres in Cameroon and Gabon to solve some of their specific problems, such as the impact of the extreme high genetic variability of HIV on anti-HIV drug susceptibility as well as on the characterization of conserved neutralization epitopes; discovery of new, not yet identified HIV subtypes and recombinant viruses naturally occurring; development of antiviral drugs efficient against the predominant HIV-1 subtypes circulating in developing countries.

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Contract number IC18*CT960114

MULTICENTER STUDY ON FACTORS DETERMINING DIFFERENTIAL SPREAD OF HIV INFECTION IN AFRICAN TOWNS

Period: December 1, 1996 — January 30, 1998

Co-ordinator: PRINCE LEOPOLD INSTITUTE OF TROPICAL MEDICINE, DIVISION OF MICROBIOLOGY
Antwerp, Belgium (M. LAGA / A. BUVE)

Objectives

- To improve our understanding of the factors that determine the extent of the spread of HIV infection in populations in sub-Saharan Africa where the predominant mode of transmission is heterosexual contact. in view of better targeted interventions to reduce new HIV infections.

Specific objectives

- To compare the distributions of likely population risk factors for the spread of HIV infection, in four large cities in sub-Saharan Africa.
- To establish with policy makers the results of the study and the likey effectiveness of different intervention approaches.

Data analysis and report writing

* In each of the sites data analyses will be carried out immediately following entry nad cleaning of the data. Descriptive analyses at a population level will provide insights in the distribution of suspected risk factors. Multivariate analyses will identify individual risk factors for HIV infection in each of the sites.
* Comparative data analyses will be carried out in Europe. Comparisions will be made of the distributions of sustpeced risk factors (mainly sexual behaviour characteristics, STD prevalence, circumcision) between study sites.
* As well as these standard statistical analyses simulation modelling exercices will enable us to assess whether results from the study are consistent with documented spread of HIV infection in the different sites.

Discussion of study results with policy makers

As soon as the first results of the comparative data analysis are available, a workshop will be organized in each site to which policy makers involved in AIDS control are invited. In this workshop the results of the research will be discussed and recommendations for interventions will be formulated.

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Contract number IC18*CT970216

VIROLOGICAL AND MOLECULAR CHARACTERISTICS OF HIV1 STRAINS IN AFRICA. THE IMPACT OF HIV GENETIC SUBTYPES ON THE PATHOGENESIS AND PROGNOSIS OF HIV INFECTION

Period: September 1997 — September 2000

Co-ordinator: ORSTOM RETROVIRUS
Montpellier, France (E. DELAPORTE)

Objectives

General objective

To analyse the impact of specific genetic subtypes on the natural history of HIV-1 infection.

Specific objective

- To identify the genetic subtype circulating in three existing cohorts of HIV infected patients in Senegal, Cameroon and Guinea-Bissau; to study virological and antigenic characteristics related to the genotype.
- To identify risk factors associated to specific genotypes and to assess clinical, immunological and virological parameters as well as survival over 3 years period in HIV-1 infected patients according to genotypes.

Activities

This project consists on two parts:

First, a transversal study among ongoing cohorts of HIV infected patients in Senegal, Cameroon and Guinea-Bissau will allow to determine the prevalence of the different genotypes circulating in this populations and to characterize their virological properties. Both heteroduplex mobility assay (HMA) and V3 peptide serology will be used to diagnose the different genetic groups and subtypes of HIV-1. An algorithm will be developed to simplify the genetic subtyping of HIV-1. The biological phenotype will be analyzed and correlated with the genetic sequence of the V3 loop. Risk factors associated to genotypes will be analyzed.

Secondly, the possibility to perform a follow-up of HIV-infected patients in Senegal, Guinea-Bissau and Cameroon allows us to initiate a prospective study in order to assess clinical, immunological and virological parameters as well as survivial over a three year period. The biological phenotype will be determined every 6 months and correlated with CD4 counts and clinical parameters. Several clinical, serological, virological and immunological parameters will be studied in order to see if certain genotypes have a different pathogenesis and prognosis for HIV disease development.

Expected outcome

The potential benefits of the proposed projects are multiple. A better clinical care of HIV infected patients is offered through a standardized approach and with the possibility to treat infections due to the immuno-depression.

Contract number IC18*CT970216

The development of a new, simple and rapid strategy to monitor the relative prevalence of the different genetic subtypes of HIV-1 strains. Such data are important for the development of future HIV vaccines. Information will be provided on differences in disease development, prognosis and risk factors accroding to the genetic subtypes. Such data are expected to be important for the management of HIV infected patients.

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Contract number IC18*CT970246

STUDY OF HIV-1 VARIABILITY IN CAMEROON AND GABON. IMPLICATIONS FOR VACCINE AND ANTIVIRAL INTERVENTION STRATEGIES

Period: April 1, 1998 — September 30, 2000

Co-ordinator: INSTITUTE OF TROPICAL MEDICINE
Antwerp, Belgium (G. VAN DER GROEN)

Overall aim

- To study the biological function of antigenic epitopes inducing broad cross-neutralising antibodies, and the role they play against infection and/or progression to disease.
- To monitor anti-HIV drug susceptibility and viral load of genetically diverse primary HIV strains circulating in Cameroon and Gabon.

Objectives

- To provide a surveillance system for newly emerging and naturally occurring recombinant HIV-1 strains in Cameroon and Gabon.
- To classify sera from HIV infected individuals according to their capacity to neutralise primary isolates representing different genetic clades.
- To characterize broadly neutralising antibodies and epitopes in individuals infected with HIV-1 of different genetic subtypes, and evaluate the role they play in disease protection.
- To investigate the susceptibility of HIV-1 strains belonging to different genetic clades, to both existing and experimental anti-HIV compounds and to provide a surveillance system for emerging strains of HIV-1 with naturally occurring mutations conferring antiviral drug resistance.
- To optimize the quantification of HIV RNA in plasma of patients infected with different types, subtypes and recombinant subtypes of HIV.

Activities

The study design will be as follows: both heteroduplex mobility assay (HMA) on PCR amplified fragments generated from HIV RNA in serum or plasma of HIV infected individuals and V3-peptide ELISA will be used to monitor the prevalence of different genetic subtypes of HIV-1. HMA on both *gag* and *env* fragments of the same isolate will allow to study the prevalence of recombinant viruses. Susceptibility of the different subtypes and the natural recombinant viruses towards various HIV inhibitors will be determined. Neutralizing antibody patterns in sera of individuals infected with all subtypes (A-H) within group M and representatives of group O to their homologous and heterologous primary isolates will be documented using a peripheral blood mononuclear cell based neutralisation assay. Neutralisation data will be analyzed by a multivariate (spectral mapping) analysis. Chimeric viruses consisting of neutralisation sensitive and insensitive primary HIV-1 strains will be constructed in order to characterize the epitopes playing a role in the neutralisation process. Human monoclonal antibodies with cross-clade neutralisation capacity will be generated.

Consecutive isolates and sera of long term, slow and rapid progressors will be analyzed in a time paired sequential manner in autologous and heterologous neutralisation experiments, in order to monitor the role of neutralisation antibodies in the progression of the infection.

Expected outcome

Making available to developing countries a simple economical serologic test to monitor circulating HIV-1 subtypes.

Contract number IC18*CT970246

These data are essential for the development of future HIV vaccines, as well as for the development of efficient anti-HIV drugs; multivariate analysis of HIV-1 neutralisation data will allow to monitor the efficacy of HIV-1 vaccines inducing high-titre cross-neutralising antibodies, in an economic way, since only a limited number of key isolates are required to document the broad cross-clade neutralisation capacity of a particular serum. This will also facilitate the selection as well as the design of antigens to be incorporated into a suitable HIV-1 vaccine preparation; to enable some research centres in Cameroon and Gabon to solve some of their specific problems, such as the impact of the extreme high genetic variability of HIV on anti-HIV drug susceptibility as well as on the characterization of conserved neutralisation epitopes; discovery of new, not yet identified HIV subtypes and recombinant viruses naturally occurring development of antiviral drugs efficient against the predominant HIV-1 subtypes circulating in developing countries; strengthening the alliance with research centres in Cameroon and Gabon and leading centres in Europe, with expertise on HIV variability studies in Africa and on studying the anti-HIV drug activity.

The probability of the realisation of the planned objectives is high, since both the overseas laboratories as well as the research centres in Europe, have already an ongoing collaboration and are active in the field of HIV/AIDS since many years.

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Contract number IC18*CT970255

DEVELOPMENT OF A HIV CANDIDATE VACCINE FOR PHASE I AND II CLINICAL TRIALS IN CHINA

Period: November 1, 1997 — October 31, 2000

Co-ordinator: INSTITUTE OF MEDICAL MICROBIOLOGY,
UNIVERSITY OF REGENSBURG,
Regensburg, Germany (R. WAGNER)

Objectives

- To create a basis for testing HIV candidate vaccines in clinical phase 1/2 trials
- To develop a novel HIV-candidate vaccine on the basis of regional virus strains
- To assess the safety, immunogenicity and efficacy of the proposed candidate vaccine in a relevant primate model

Activities

* Careful monitoring of the epidemic situation in a given population is one of the important issues in preparing clinical phase 1/2 trials. Due to the very recent outbreak of the HIV epidemy in the rural population in the Dehong prefecture of Yunnan, the variability of the virus in this area seems to be still restricted to a HIV-1 B^1-Thai subtype. However, the antigenic drift will be carefully monitored by the Chinese Academy of Preventive Medicine (CAPM) and the Health and Epidemic prevention stations (HEPS) assisted by the Institute of Medical Microbiology and Hygiene (RIMMH, Germany).

* In order to optimize the chances of a successful vaccine, recombinant virus-like particles and a corresponding DNA vaccine will be constructed based on a careful molecular characterization of prevalent B^1-(Thai) and C-clade HIV strains of the endemic areas in China (CAPM).

* The long term safety, immunogenicity and toxicity of the different antigen delivery systems will be analyzed in rhesus macaques in collaboration with the Biomedical Primate Research Center (BPRC, The Netherlands) and the Institute of Molecular Biology (IMB, Kunming, China).

* The efficacy of the induced immune responses after heterologous challenge of the immunized monkeys with a pathogenic SHIV chimera will be determined.

* After having demonstrated the safety and efficacy of the antigens in the proposed animal model and in case of a favourable epidemiological situation, clinical phase 1/2 trials will be prepared.

Contract number IC18*CT970255

Expected outcome

It is expected, that this project will lead to a close collaboration between the European and Chinese partners in the area of epidemiology and vaccine development. Furthermore, this study will contribute to the molecular characterization of the prevalent B^1— and C-clade HIV strains from South-East Asia, which will be subsequently utilized for the construction of an innovative VLP and DNA based antigen-delivery system. A systematic evaluation of these candidate vaccines with respect to their safety, immunogenicity and efficacy is considered to be an essential prerequisite in order to proceed to further clinical trial phases.

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Contract number TS3*CT910033

THE APPLICATION OF DNA FINGERPRINTING TO THE PATHOGENESIS OF HIV-RELATED TUBERCULOSIS IN AFRICA

Period: August 1, 1992 — July 31, 1995

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, DEPT. OF CLINICAL SCIENCES, London, United Kingdom (P. GODFREY-FAUSSETT)

Objectives

The study aimed to determine to what extent the development of TB in HIV infected patients in Africa is due to reinfection rather than relapse or reactivation.

Achievements

* Establishment of molecular epidemiology in Zambia and transfer of molecular, computer and epidemiological skills.
* Baseline studies on variability of DNA fingerprint patterns within and between patients in a high prevalence region.
* Prospective study of the molecular epidemiology of TB in a relatively closed community with high prevalence of HIV.
* Contribution to control programme activities of reference laboratory and Lusaka notification register.
* Support to teaching within UNZA medical school.
* Regional interest in possibilities of molecular epidemiology enhanced.
* Extension granted to develop new directions.

Results

⇒ Around 2000 isolates have now been typed and around 1500 stored on a Gelcompar database. The Molecular Biology Unit is funded until 1999 by DFID, the Goldfields Mining Company, and with support from the EU Concerted actions on genetic markers for the epidemiology of tuberculosis and molecular epidemiology and control of tuberculosis (BMH1-CT93-1614 and BMH4-CT97-2102).

⇒ There is enough genetic diversity within a high prevalence community to be useful for epidemiological studies.

⇒ Multiple isolates from most individuals have identical fingerprints. This finding underlies almost all attempts to use fingerprinting as an epidemiological tool in Africa.

⇒ Community based studies will be necessary to answer the question of reactivation versus recent infection. The definition of the community will be vital. The closer one comes to a closed community, the more informative the study will be. Lusaka is therefore a bad choice for such studies; not only is the population much too big to allow complete coverage of the community, but also there is considerable movement of infected patients into and out of the city.

⇒ Studies in the South African Gold Mining industry show that much Tb is due to recent transmission whether subjects are also infected with HIV or not. Whether the same conclusion will be found in a more general population remains open.
Most recurrences are due to persistence of the original strain, but it is difficult to document a cure in many individuals before their recurrence. The original strategy for detecting recurrences was inadequate and the numbers of sets of isolates available for fingerprinting remains small.

⇒ The Department of Pathology and Microbiology of the University Teaching Hospital has now included a Unit of Molecular Biology as part of the institution's structure. Staff have been recruited and space made available. Continuing interest and collaboration should follow.

⇒ Equipment and consumables have been provided to the Chest Disease Laboratory and a collaborative arrangement with the British Public Health Laboratory Services has been established with a two-way exchange of staff and materials.

⇒ Computerisation of the CDL, is continuing to work well and has improved the accuracy of the clerical work and provided rapid feedback on quality assurance to the technical staff and on results to clinicians.

⇒ Other studies that have shared ZAMBART resources include:
1. Paediatric tuberculosis: diagnosis and case definition.
2. Infectivity of HIV positive and negative TB cases, compared to community controls.
3. Epidemiology of bovine TB.

Training

An important goal of the project has been to involve Zambian staff at every level of the work and provide capacity strengthening to promote future collaborative projects.

Molecular Biology

Four Zambian staff and one expatriate, employed on a local contract, have formed the laboratory team. Training in molecular biology, particularly fingerprinting and polymerase chain reaction techniques has been given in both London and Bilthoven. Computer-assisted analysis of fingerprints, which has been being developed with the EU Concerted Action on Genetic Markers (BMH1-CT93-1614), has also been taught both in Bilthoven and at a workshop in Johannesburg. Marian Bruce, a Scottish molecular biologist, spent two years working in the laboratory and was a key to the successful training of the Zambian staff. Dr. Munthali has subsequently moved in to histopathology but hopes to continue to use molecular tools to enhance studies of TB and HIV from autopsy material. One of the graduate technicians, Grace Mbulo (nee Kalenge), took a year out from the lab to complete an MPH degree with the University of Zambia. Her dissertation was developed in collaboration with the project and focuses on transmission of TB in the Lusaka community — a continuing area of interest for the project. She has now returned to the lab and is continuing with fingerprinting and PCR work. Both Dr. Munthali and Mrs. Mbulo spent attachments with Dr. Stuart Wilson at the London School of Hygiene and Tropical Medicine.

The other graduate technician, Catherine Mee joined the lab in late 1994. Following on-site training, she has been responsible for most of the South African fingerprinting. Since September 1996 she has been attending the Masters course in Applied Molecular Biology of Infectious Diseases at the London School. She and Dr. Godfrey-Faussett attended the workshop organised by the RIVM, Bilthoven in Johannesburg to become more familiar with Gelcompar software and the global database of fingerprints now available from RIVM.

Bupe Kambashi was the first technician to join the team and continues as a central part of the team. He has spent six weeks on an attachment to RIVM, working with Dick Van Soolingen and Jan van Embden's groups.

Training has also been provided in Zambia by visits from various European centres. Petra de Haas from Bilthoven, Stuart Wilson from London, Francis Drobniewski from London have all spent time working with the team in the lab. Stewart Cole, from Paris, also visited the lab and lectured on molecular mechanisms of drug resistance.

The result of the training is that the lab is now competent to produce and analyse DNA fingerprints from *M. tuberculosis* without external supervision. If additional techniques are to be introduced further specific training will be needed.

An indicator of the success of the laboratory is that the University Teaching Hospital has agreed to include Molecular Biology as one of the Units within the Department of Pathology and Microbiology. This means that there is a formal commitment to provide staff and career development for them within the administrative framework of the hospital.

Data processing

In addition to the training in computer-assisted handling of fingerprints (see above), we have concentrated on training staff in general aspects of data processing. The project runs its own mini-course on Epi-Info software. The first courses were organised and run by Maria Quigley from the Tropical Health Epidemiology Unit of the London School but more recently in-house courses have been organised for project staff. It is hoped to extend these courses and make them available to medical students embarking on their community medicine projects and to postgraduate students attending the MPH and MMed courses in Lusaka. Epi-info is used for most of the data storage in the project (with the exception of the preventive therapy trial which used dbaseIV for storage and epi-info for data handling). Databases now include Tb isolates stored, fingerprints, paediatric TB patients, Lusaka TB notifications, samples processed at Chest Diseases Laboratory.

Staff seconded to the project by the Ministry of Health and the Teaching Hospital Board of Management have been trained on-site and the project has also funded training in data-processing at the National Institute of Public Administration. This diploma course is run in the evenings but has been very popular with the data-processing staff (Given Kashina, Ovy Moonga, Nasilele Muchola and Sylvia Soko).

Once again, the Ministry of Health and the hospital have shown their commitment to the future of the data processing team by providing staff and office space, which they have made secure for the computers.

Contract number TS3*CT910033

Epidemiology

The project has promoted general training in epidemiology and research methodology and was partly responsible for securing scholarships for Dr. Nanthalile Mugala (who was working for the preventive therapy study), Dr. Lamios Munthali (see above) and Mr. P. Nyanga (one of the senior clinical officers in the TB programme) to attend the Royal Tropical Institute in Antwerp. These scholarships were funded from the Belgian Cooperation and are an example of the catalytic effect of an integrated research project on training in Zambia.

Dr. Godfrey-Faussett attended the Short Course on Advanced epidemiological methods run by the Epidemiology Department of the London School.

Action research

As an addendum to the original contract, we received funds for Dr. Alwyn Mwinga (who runs the preventive therapy study) and Dr. Godfrey-Faussett to have a workshop in Amsterdam with Dr. Marteen van Cleef, Dr. Peter Lever and Dr. Leon Bijlmakers from the Royal tropical Institute. The ZAMBART Urban Tuberculosis Project was drafted, developed further in Lusaka in discussion with the Ministry of Health and has now been funded by INCO-DC.

Cochrane Collaboration

Both Dr. Mwinga and Dr. Godfrey-Faussett attended workshops on methodologies for systematic reviews organised by the Cochrane Collaboration.

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Contract number TS3*CT910034

CHARACTERIZATION OF MYCOBACTERIA FROM HIV ENDEMIC AREA (TANZANIA)

Period: March 1, 1992 — February 28, 1995

Co-ordinator: STATENS SERUMINSTITUT, MYCOBACTERIA DEPARTMENT, Copenhagen, Denmark (A.B. ANDERSEN)

Objectives

To characterize and compare mycobacteria isolated from HIV positive and HIV negative patients. The strains were to be characterized with respect to species and strain distribution and drug susceptibility profiles. The information and experience obtained was expected to contribute to improved understanding of the following topics:

- Are there differences between mycobacteria infecting HIV positive patients compared to HIV negative individuals?
- How may the phenotype (including drug susceptibility) of mycobacteria be correlated to the genotype as assessed by RFLP analyses?
- How do the Tanzanian mycobacteria compare to mycobacteria isolated in other parts of the world?

During the project period the participating laboratories should develop close links to facilitate scientific collaboration and transfer of technology especially within the field of improved laboratory diagnostic methods.

Progress towards objectives

A total of 400 samples were collected at the Central TB laboratory in Dar es Salaam from patients admitted to the Muhimbili Medical Centre through a 7-month period from late 1992 to the beginning of 1993. Clinical recordings are available for almost 80% of these. Most of the samples originate from the Dar es Salaam area and they all represent newly diagnosed cases. History of previous TB — if known — was recorded together with information on HIV status, sex, age, and drug regimen. The information obtained was entered in an anonymized data base at SSI in Copenhagen together with the data regarding the microbiological strain characteristics.

During the same time period a similar data base was established containing data regarding all bacteriologically verified new TB cases in Denmark during 1992.

Contract number TS3*CT910034

Results

The risk of infection with a particular clone of *M. tuberculosis* as assessed by genotype analyses or phenotype analyses appeared not to be influenced by the HIV status of the host.

The genetic diversity of the Tanzanian isolates was high, both in the HIV positive and the HIV negative population.

A fairly high frequency of strains carrying only one (8%) or a few copies of the *IS6110* fragment were observed in the Tanzanian isolates. In our Danish collection we have only observed such strains imported by immigrants. The occurrence of a single or low (up to five) *IS6110* elements per chromosome correlated to the phenotypic appearance of the Asian subtype.

No correlation of RFLP to resistance to any of the traditional anti TB drugs was observed.

In both the studies in Tanzania and in the analyses of strains collected in Greenland there was a clear correlation between geographic origin of the samples and genetically relatedness of the bacterial isolates.

Improved diagnostic methods

Identification of mycobacteria by monoclonal antibodies

At KIT a simple enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (MAbs) for the identification of cultured mycobacteria belonging to the *Mycobacterium tuberculosis* complex, *M. avium* complex, *M. kansasii* and *M. fortuitum* was established. This method may represent a useful and cost-effective alternative to traditional microbiological typing methods. Results of microbiological identification were compared with the ELISA identification.

RFLP on non-cultivable samples or on clinical samples

The RFLP in its present format is dependent on the availability of pure cultures/colonies in order to obtain enough DNA for a Southern blot. In this project and also in other situations it would be preferable if the technique could be adapted to a PCR based method. Thereby it would be possible to analyze old, dead cultures. It would also reduce the time needed for each sample, which in turn would make the method more applicable to clinical use.

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Spoligotyping (fingerprint PCR)

The group at KIT collaborated with Dr. J.D.A. Van Embden and his group on the development of the spoligotyping of *M. tuberculosis* strains (Kamerbeek et al., submitted). Spoligotyping is based on the DNA polymorphism at a unique locus in the *M. tuberculosis* chromosome. The locus contains multiple 36 base pair Direct Repeats (DRs), interspersed by non-repetitive spacer sequences, 34-41 base pairs in length. Since the DRs are extremely well conserved among *M. tuberculosis* strains, each DR copy is a target for PCR. Two DR primers were chosen, which permitted *in vitro* amplification of the whole DR region, including the interspersed spacers. The typing method relies on determining the presence or absence of spacers in the *in vitro* amplified DNA by hybridisation to multiple synthetic spacer oligonucleotides, which are covalently bound to a filter.

Contract number TS3*CT910034

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Contract number TS3*CT930254

DETRIMENTAL EFFECTS OF TB ON NATURAL HISTORY OF HIV INFECTION: VIRAL LOAD, IMMUNE FUNCTION AND APOPTOSIS

Period: January 1, 1994 — December 31, 1996

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE,
DEPT OF CLINICAL SCIENCES
London, United Kingdom (H. DOCKRELL)

Objectives

Using clinical material from patients infected with both *M. tuberculosis* and HIV, we aim to:

Measure HIV viral load with immune function, including CD4 counts, lymphocyte proliferation in response to nitrogen and recall antigens, cytotoxicity mediated by CD4 T cells, cytokine production and T cell susceptibility to apoptosis.

Activities

* A functional immunology laboratory has been established at the Instituto de Higiene e Medicina Tropical, allowing lymphocyte proliferation assays and ELISA assays for cytokines to be performed. Over 140 patients with tuberculosis and with/without HIV infection have now been recruited to the study.

* Two PhD students are being trained through this project in Birmingham and in the Gambia. Over twelve exchange visits to learn new techniques and to discuss progress have been made between the partners to date.

Results

In London, live *M. bovis* BCG has been shown to activate more DC8+T cells than dead BCG, or PPD, and these DC8+T cells can act as cytolytic effector cells.

In Birmingham, a range of techniques for assessing apoptosis within cells in suspension or within tissues, have been established. Lymph node sections from tuberculous granulomas contained a greater number of apoptotic cells than those from reactive lymph nodes, showing that apoptosis is a prominent feature of tuberculosis lesions in immunocompetent subjects.

In Lisbon, lymphocyte proliferation tests showed decreased responses to mycobacterial antigens in patients with tuberculosis compared to controls. Of the antigens tested, the strongest IFN-y cytokine responses were induced by a short term culture filtrate of *M. tuberculosis*.

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In the Gambia, follow up studies on patients with tuberculosis alone have been carried out before and after two months of anti-tuberculosis during therapy. Apoptosis, as measured by the ethidium bromide method, induced by PPD, was greater in patients with dual TB and HIV infections than in those with TB alone, and was primarily occurring within the CD4+ T cell subpopulation. Cytotoxicity assays have shown depressed responses in patients with tuberculosis compared to controls, with no recovery of CTL function at two months.

Techniques for the measurement of HIV-1 and HIV-2 proviral load in peripheral blood mononuclear cells have now been established, and assays for plasma RNA Load will be functional in the near future. Recruitment of patients with dual tuberculosis and HIV-1 or HIV-2 infections is continuing; samples are being collected before anti-tuberculosis drug therapy, and after two months of treatment, and stored for quantitation of viral load.

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Contract number IC18*CT960083

TUBERCULOSIS CONTROL IN AN URBAN AREA WITH HIGH TB/HIV PREVALENCE

Period: October 1, 1996 — September 30, 1999

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, London, United Kingdom (J. PORTER)

Objectives

- To develop capacity for action research in support of urban TB control.
- To develop and evaluate interventions, through action research.
- To assess and document the pros and cons of different methodologies used.

Activities

* To provide training opportunities and technical support to those involved in TB control in the area of action research.
* To quantify the transition coefficients used in the Piot model in Lusaka Urban District.
* To identify and explore service, patient and community factors that determine these coefficients.
* To validate data collected routinely by the national TB control programme.
* To investigate ways to rationalise and assure quality in the use of laboratory services at both reference and peripheral health facilities.
* To document the practical advantages and disadvantages of the different methodologies used in the action research and to disseminate these for use by TB control programmes in other countries.

Expected outcome

⇒ To enhance the understanding of participatory research as applied to urban TB control.

⇒ National or regional conference for dissemination of results.

⇒ Workshops involving TB programme managers, health care providers, representatives of target groups for the reformulation of sustainable TB recommendations.

⇒ Workshops and meetings with policy makers.

⇒ Scientific articles written up and published.

⇒ Manuals written on TB control to identify and prioritise the problem.

Contract number IC18*CT960083

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2. Mycrobacteria

Presentation of EC supported joint research projects (1991-1996)
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

2. Mycobacteria

Contract number TS3*CT910036

A COMPREHENSIVE INVESTIGATION OF ADVANCED METHODS IN MOLECULAR BIOLOGY FOR THE DETECTION OF *M. TUBERCULOSIS* AND *M. LEPRAE*

Period: April 1992 — April 1995

Co-ordinator: KONINKLIJK INSTITUUT VOOR DE TROPEN, DEPT OF BIOMEDICAL RESEARCH
Amsterdam, The Netherlands (A.H.J. KOLK)

Objectives

Comparison of the results of conventional and novel molecular technology for:

- The detection of tuberculosis and leprosy patients.
- The monitoring of mycobacterial viability in tuberculosis and leprosy patients.
- The determination of *M. tuberculosis* infection among household contacts of newly diagnosed tuberculosis patients in a prospective study.

Activities

Tuberculosis patients, household contacts and controls were sampled in Thailand, Portugal and The Netherlands. Microscopy, culture and typing were performed according to standard procedures. Leprosy patients will be sampled from outpatients clinics in Thailand. Clinical, bacteriological and histopathological assessment of the newly diagnosed untreated patients were done according to standard methods. PCR for the detection of *M. tuberculosis* DNA and *M. leprae* DNA were performed as previously described. PCR methodology for the detection of amplified DNA and RNA were optimized in Amsterdam and introduced in Bangkok and Porto.

Results

ad 1 Scientists and technicians from Thailand and Portugal were trained in the PCR technique and subsequently they introduced the PCR technique in their own laboratory. The PCR was further improved with regard to quality control, prevention of contamination and detection of inhibitors. In addition simple methods were developed for detection, identification and quantification of the amplified DNA both for *M. leprae* and *M. tuberculosis*. We investigated the use of different clinical specimens for their applicability in the PCR. We found that (in the order of effectiveness) spontaneous sputum, induced sputum, throat swabs and nose swabs can be used for the detection of *M. tuberculosis* using the PCR technique. *M. tuberculosis* was only detectable in the nose and throat swabs from patients with clearly ZN-positive pulmonary TB and in some contacts of TB patients.

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We have developed a cough filter to simplify sampling of suspected infectious pulmonary TB patients which may be used in mass screening programmes.

We have developed a colorimetric method for the rapid detection and identification of amplified *M. leprae* DNA from skin biopsies and *M. tuberculosis* DNA from sputum samples. We also designed a colorimetric assay based on streptavidin-coated beads or plates to detect biotinylated PCR products with a digoxiginin-labelled detector probe. We have demonstrated that PCR is a useful technique for the rapid diagnosis of pulmonary and extra-pulmonary tuberculosis. In all three laboratories a large number of samples of both pulmonary and extrapulmonary origin were tested in PCR. With pulmonary samples, the sensitivity of the PCR compared with culture varied from 96% - 78%, the specificity from 90% - 76%. For the extrapulmonary samples the sensitivity varied from 100% - 44%, and from 88% - 83% for the specificity.

PCR amplification of the 531-b fragment of the *M. leprae* gene in fresh biopsy and slit skin smear specimens was evaluated for its usefulness in the detection of *M. leprae* in patients in Thailand. Compared with other diagnostic procedures, PCR showed a clear advantage in terms of sensitivity and specificity over both microscopic examination of slit skin smears and serologic detection, especially in paucibacillary patients.

We have further improved and standardized the NASBA for the detection of the *M. tuberculosis* complex in clinical specimens. The NASBA had a similar sensitivity to culture when applied to specimens derived from 20 pulmonary tuberculosis patients. The sensitivity of NASBA was lower than culture when testing extrapulmonary samples.

All 58 control specimens from patients with other diseases were negative in NASBA and culture.

ad 2 We have shown that PCR can be used for the follow-up of patients during anti-tuberculous treatment. Since PCR detects DNA, it can detect both dead and alive bacteria provided the DNA is still intact. This enables one to confirm a clinical diagnosis of tuberculosis when microscopy is negative. All three laboratories found the same results, suggesting that persisting PCR positivity, more than 4 months after the start of treatment, should raise the question of non-compliance or drug-resistance.

We have shown that NASBA for the detection of RNA can be used to assess the viability of mycobacteria *in vitro* as well as *in vivo*. Both PCR and NASBA were evaluated for their potential to help monitor bacterial clearance in leprosy patients during chemotherapeutic treatment. The results on skin samples (smears and biopsies) collected during the course of chemotherapy showed that the number of PCR or NASBA positive cases of both multicabillary and paucibacillary types decreased sequentially.

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The morphology of the mycobacteria in skin biopsies as indicator for viability was compared with the NASBA-signals: the presence of solidly stained mycobateria resulted in strong positive NASBA signals, while fragmented or granular stained *M. leprae* bacteria showed a variable range of NASBA signals, including negative results. No relation between the NASBA results and the bacterial load could be found.

The wide range of NASBA signals observed within each bacterial index (BI) group reflected most likely the number of viable cells present, rather than both dead and viable bacteria. Apparently, the presence of 16S rRNA in skin biopsies of leprosy patients might be indicative for the efficacy of therapy of leprosy patients.

ad 3 Households contacts were studied in Bangkok, Porto and Amsterdam. A positive nose swab could arise from inhaling aerosol droplets containing *M. tuberculosis* from the index case and does not necessarily mean that the individual has an active infection. Laboratory experiments in the KIT have shown that mycobacteria are detectable in the nose of contaminated individuals (laboratory workers that worked with heat killed *M. bovis* BCG) for only a short time (3 - 5 days). In Amsterdam the household contacts were traced by the Municipal Health Centre. The samples from these contacts were collected at least 2 weeks after the index patients were diagnosed and had started treatment. The relatively long interval between start of treatment and sample collection in household contacts may be one of the reasons why all other nose swabs from these contacts were negative in PCR. In Porto and Bangkok contacts and patients were tested at the same time; there was no delay between the diagnosis of the index case and taking the induced sputum from the household contact.

In Porto 52% of the 27 household contacts had PCR-positive induced sputum samples, two proved to be TB patients since both microscopy and culture were positive. One PCR-positive contact was later diagnosed as a TB patient based on additional clinical grounds. The other positive contacts were not further investigated. At the TB Division 15 of 111 contacts were positive in PCR. None of these individuals was treated for TB.

All participants agreed that much was gained from the project both individually and by the local laboratory where the PCR is now done. Given current cost constraints in the health system, it is unlikely that most laboratories will be able to do both nucleic acid amplification and culture routinely all samples. Each diagnostic centre will have to evaluate for its own patient population and economic situation both cost effectiveness and cost benefits of the available diagnostic techniques.

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Contract number TS3*CT920062

MUCOSAL IMMUNITY AND LEPROSY

Period: November 1, 1992 — April 30, 1995

Co-ordinator: UNIVERSITY OF LONDON/INST. OF OPHTHALMOLOGY
London, United Kingdom (I.A. CREE)

Objectives

The primary aim was to develop and use a mucosal challenge test to compare mucosal immunity to *M. leprae* with evidence of infection in an endemic area with a good MDT-based control programme. The aims were further defined in the original 1991 grant application as follows:

- To develop a mucosal challenge test for investigation of mucosal cell-mediated and humoral immune responses to *M. leprae*.
- To determine which mycobacterial antigens are involved in the mucosal immune response and whether they might be associated with protection against leprosy.
- To investigate the suitability of modified antigen and DNA detection methods for the early diagnosis of leprosy.

Activities

To accomplish our aims, we had first to establish a mucosal challenge test and confirm that the mucosal immune response to *M. leprae* was specific and long-lasting. The results of the challenge test development showed that an anamnestic mucosal immune response occurs in individuals exposed to leprosy as long as 10 years post-exposure. No response was observed in those living in a non-endemic country and with no history of travel to an endemic country.

Having shown the feasibility and safety of the mucosal challenge test, we went on to survey groups of leprosy patients, contacts and the general population for both their salivary IgA response to *M. leprae* (MLIgA) and nasal PCR positivity.

Results

A total of 304 subjects were enrolled in the first study: PCR and mucosal challenge tests were performed in 204 of these individuals. MLIgA was present before and/or after challenge in 66% of treated patients, 76% of leprosy workers and 72% of healthy contacts. This is similar to previous studies in Bangladesh and Fiji which predate MDT control programmes (Cree et al., 1988). However, only 33% of local control subjects with no known exposure to leprosy were MLIgA+ in contrast with earlier studies showing 74% positivity among local controls in an area without good leprosy control.

There was evidence of continuing transmission of *M. leprae* in both household contacts (2% PCR+) and local controls (5% PCR+). PCR+ subjects were either MLIgA positive or negative. In a subsequent follow-up study, nasal swabs were taken from 97 of those studied in the first series: of three PCR+ individuals followed up after one year, all became negative, while of the remaining 94 PCR- individuals retested, 2 became positive. A total of 112 subjects were retested with the mucosal challenge test: 22 converted from positive to negative and 12 from negative to positive.

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In addition, saliva samples were tested for the presence of ML-IgM and IgM and IgA anti-LAM, and total IgA. Serum samples were tested for IgG, IgM and IgA anti-whole *M leprae*, anti-LAM and anti-PGL1 (BSA glyconjugate). The results are still being analyzed, but appear to support the PCR and MLIgA data.

Outcome

The implications of these results should provoke some debate. Sub-clinical transmission of *M. leprae* explains the current lack of effect of MDT control programmes on incidence despite the sustained drop in prevalence of active disease. Despite this, there is a general reduction in general population immunity. This suggests that relaxation of MDT control would lead to a recrudescence of disease as the non-immune population became infected. However, partly as a result of this project, we can now detect clusters of infection. Prophylactic antibiotic therapy of all individuals in these clusters would reduce transmission, perhaps to the point where eradication rather than elimination of leprosy became feasible.

Final analyse are now being performed.

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Contract number TS3*CT920099

IMMUNOLOGICAL AND MOLECULAR ANALYSIS OF LYMPHOCYTES AND ANTIGENS INVOLVED IN LEPROSY (NEURITIC) LESIONS

Period: April 1, 1993 — March 31, 1996

Co-ordinator: RIJKSUNIVERSITEIT LEIDEN, DEPT. OF IMMUNOHEMATOLOGY & BLOOD BANK, Leiden, The Netherlands (T. OTTENHOF)

Objectives

The objective of this project is to gain novel and practical insights into the pathogenesis of leprosy lesions, particularly leprosy nerve lesions, in order to be able to:

- design specific and effective measures to prevent and treat nerve damage in leprosy as well as to
- identify antigens that may be useful for the early detection of neuritis or neuritis-susceptible individuals.

Activities

Protective immunity against Mycobacteria is dependent on T lymphocytes. T cells recognize mycobacterial antigens as peptides bound to major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells. T cells can exert many different effector functions, sometimes resulting in immunopathology as seen in granulomatous leprosy lesions with high local cell mediated immune reactions. Leprosy predominantly affects skin and nerves. Irreversible damage to nerves is the most severe clinical complication of the disease and occurs particularly during reversal reactions. This study of immunopathology of leprosy may lead to measures to control and prevent nerve reactions. The local immune response in leprosy skin and nerve lesions will be studied, including the potential involvement of autoantigens.

This project will investigate lymphocytes and *M. leprae* antigens that may be involved in acute leprosy lesions, using a variety of molecular and cellular techniques.

* To isolate and clone lesion infiltrating T cells from (diagnostic) skin and nerve biopsies from acute leprosy lesions and to characterize these T cells with regard to specificity, (antigen/MHC), phenotype (also TCR usage) and function (cytokine release, cytotoxicity).
* To isolate DNA clones from a λgt11 *M. leprae* expression library with sera from leprosy patients with acute (nerve) reactions, and to analyze whether such antigens are associated with neuritis by comparing them with recombinant antigens detected by sera from other leprosy patients' groups. These antigens will also be analyzed for T cell stimulation.
* To study whether these same sera directly recognize neural autoantigens (Western blot of peripheral nerves; a Schwann cell gene library will be constructed) and whether such antigens are also seen by autoreactive cells.

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Expected outcome

⇒ Isolation of T cells from leprosy (neuritis) lesions and defining them with respect to antigen specificity, function and membrane phenotype.

⇒ Several *M. leprae* or auto-antigens will be molecularly and immunologically characterized. From both 1) and 2) it may be possible to identify antigens and/or T cells that are specifically associated with leprosy (neuritis) lesions.

Results

T cells have been isolated from several nerve biopsies from leprosy patients in reversal reaction as well as from skin biopsies. All biopsies were taken strictly for routine diagnostic purposes and only a portion of the material was used for research purposes.

A small piece (3-6 mm) of the radiocutaneous or sural nerve was dissected, peripheral blood lymphocytes removed and freed of surrounding fibrous tissue. Tissue lymphocytes were isolated over a Ficoll gradient. Preliminary analyses show the presence of *Mycobacterium leprae* reactive T cells in nerve biopsies under limiting dilution conditions. Differential expression of a number of adhesion molecules as well as T cell receptor determinants has been observed among nerve infiltrating T lymphocytes as compared to peripheral blood derived lymphocytes.

From some biopsies, Schwann cells could be cultured using a newly designed protocol. These cells are currently under study with regard to (i) their capacity to act as non-professional antigen presenting cells in leprosy reactions, and (ii) their recognition by sera from reactional leprosy cases which have been followed longitudinally.

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Contract number TS3*CT920154

DNA TYPING OF *M. TUBERCULOSIS*: EPIDEMIOLOGY AND EVALUATION OF CHEMOTHERAPY

Period: April 1, 1993 — September 30, 1995

Co-ordinator: NAT. INST. OF PUBLIC HEALTH AND ENV. PROT., UNIT MOLECULAR BIOLOGY
Bilthoven, The Netherlands (J. VAN EMBDEN)

Objectives

The objectives of this study were:

- To develop and implement a highly reproducible DNA fingerprint typing system for *M. tuberculosis* and a system for computer-assisted analysis.
- The establishment of a database of DNA types of hundreds of strains from Africa, Pakistan and the Netherlands for future epidemiological purposes.
- To determine whether unsuccessful chemotherapy of tuberculosis is due to inadequate therapy or by reinfection by different *M. tuberculosis* strains.
- To transfer the DNA technology for molecular diagnosis of tuberculosis and DNA fingerprinting to Antwerp and Karachi.

In Bilthoven a robust and reproducible method has been developed to differentiate isolates of *M. tuberculosis* which allow interlaboratory comparison. For this purpose a robust protocol for IS6110 fingerprinting has been developed and this protocol has been used successfully by the three partners in this project. DNA fingerprints (generated in Bilthoven Antwerp and Karachi) from *M. tuberculosis* isolated in Africa, Asia and Europe showed that transmission within and among countries can be traced using this method, without the need to carry out the experiments in a single laboratory. This now opens the prospect for a global epidemiology of tuberculosis. This may be of particular use as an early warming system to trace the dissemination of multiple drug-resistant *M. tuberculosis*.

By IS6110 fingerprinting many *M. tuberculosis* strains we have been able to define genotypic groups ("clades") of *M. tuberculosis*, which are also related when using other independent other genetic markers. The prevalence of these genotypes differs strongly from country to country and the genotyping may be useful in tracing the global dissemination of *M. tuberculosis*.

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Strain typing of *M. tuberculosis* isolated during chemotherapy disclosed that fingerprinting is a powerful method to monitor the efficacy of drug treatment by being able to distinguish between reinfection and breakdown from a previously acquired infection. In Bilthoven a PCR-based method was developed to simultaneously detect and type *M. tuberculosis* complex bacteria in clinical specimens, which also can be used to detect *M. bovis* BCG from diseased vaccinees.

Much of the technology developed in Bilthoven has been transferred to Antwerp and Karachi, by exchange of researchers and meetings. The DNA typing methods have been described in standard protocols, which have either been published or are available as a protocol from the RIVM.

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Contract number TS3*CT930243

RAPID DETECTION OF MULTIDRUG-RESISTANT MYCOBACTERIA

Period: January 1, 1994 — December 31, 1995

Co-ordinator: INSTITUT PASTEUR PARIS, UNITE DE GENETIQUE MOLECULAIRE BACTERIENNE, Paris, France (S. COLE)

Objectives

Long term & general goals:

- Elucidation of the molecular bases of drug-resistance in *Mycobacterium tuberculosis*.
- Development of rapid methods for detection of drug resistance.

Specific goals:

- Development of a standard protocol for performing polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) analysis of genes associated with resistance to isoniazid, rifampicin and streptomycin. Prospective study of drug resistance in which the results obtained by PCR-SSCP were compared with those obtained by the proportion method.
- Elucidation of the molecular basis of pyrazinamide and ethambutol resistance.
- Implementation of molecular detection strategies in clinical and reference laboratories.

Results

The aim of this project was to study the genes of *Mycobacterium tuberculosis* encoding the targets of front-line anti-tuberculous drugs, to elucidate the molecular basis of drug-resistance, and to use the genotypic information obtained for the development of rapid molecular tests capable of predicting phenotype. Considerable progress was made towards understanding the resistance mechanisms to fluoroquinolones and ethambutol and further insight into the well-established mechanism of resistance to isoniazid was obtained.

Resistance to isoniazid, rifampicin and streptomycin results from alterations to key chromosomal genes and can be readily detected by means of DNA sequencing or PCR-SSCP analysis of selected mutational hotspots. A standardised PCR-SSCP protocol for the detection of isoniazid, rifampicin, and streptomycin resistance was developed and optimised. A blind, prospective, longitudinal study was then conducted in which the results obtained by PCR-SSCP of cultured samples of *M. tuberculosis*, from ±100 specimens obtained from recent tuberculosis cases, were compared with the drug susceptibility patterns established by the generally accepted reference technique, the proportion method.

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In the case of rifampicin, excellent sensitivity and specificity were observed, and the results were concordant for 99% of the samples. Furthermore, identical results were obtained by a second-generation molecular test for drug susceptibility employing solid-phase reverse hybridization. For 87% of the strains, good agreement was seen between the isoniazid resistance profiles predicted by PCR-SSCP and those determined microbiologically. The difference between the two data-sets can probably be explained by the existence of an additional minor resistance mechanism that has not yet been uncovered. Testing for resistance to streptomycin proved to be less reliable by PCR-SSCP since a significant proportion of resistant isolates was not detected. These were probably resistant to low levels of the drug (4 mg/ but 30 mg/ml) and, again the corresponding resistance mechanism has not yet been discovered.

In summary, the molecular methods developed and evaluated during this project proved robust, reliable and, above all, considerably quicker as they allow resistance patterns to be determined in 2 days. This compares very favourably with the two-four weeks required for conventional microbiological procedures.

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Contract number TS3*CT940001

APPROACHES TO THE DIAGNOSIS OF ACTIVE TUBERCULOSIS BASED ON THE DETECTION OF SPECIFIC MYCOBACTERIAL COMPONENTS IN THE URINE

Period: April 1, 1994 — March 31, 1996

Co-ordinator: UNIVERSITY OF BERGEN, CENTRE FOR INTERNATIONAL HEALTH
Bergen, Norway (B. BJORVATN)

Objectives

- To identify specific mycobacterial components in the urine of patients suffering from tuberculosis.
- To develop an efficient, rapid and cheap test for the detection of such components.
- To investigate the practical utility of this test for the diagnosis of tuberculosis under field conditions.

Activities

* To produce the mycobacterial liposaccharide lipoarabinomannen (LAM), and to synthesize glycolipids carrying the terminal trisaccharide 3-0 methylated Rhap and couple those glycolipids to carrier proteins.
* In preparation for an appropriate test, to produce monoclonal and polyclonal antibodies against the above components, and to study their respective specificity and prevalence among representative mycobacterial isolates.
* To optimize methods for urinary concentration, and to establish a highly efficient immunological technique for studies on the (likely) urinary excretion of such components, both in relevant animal models and in different groups of patients with mycobacterial disease, in particular tuberculosis.
* To evaluate the practical utility of the designed assay both in advanced laboratories and in the field setting.
* To provide adequate training of young scientists, both in Europe and in Ethiopia.

Expected outcome

Our research will hopefully result in the development of a reliable, non-invasive, simple and cheap assay for mycobacterial disease, in particular tuberculosis. This would imply a novel and significant contribution to the diagnosis, and thereby control of such diseases.

Summary

Lipoarabinomannan (LAM) is a major cell wall component common to all mycobacterial species and offers highly interesting possibilities for diagnostic approaches. For the last 2 years our network has explored the idea that LAM excretion into the tissues of infected individuals may ultimately become detectable in the urine. If so, efficient detection of LAM in the urine provides a novel approach to the specific diagnosis in patients with active mycobacterial disease. The results so far are highly encouraging.

Our main findings may be summarised as follows:

⇒ LAM is excreted in the urine of patients with mycobacterial disease.
⇒ An indirect ELISA based on polyclonal anti-LAM antibodies has been developed that detects nanogram levels of LAM in the urine.
⇒ Promising preliminary versions of a "dip stick" test for LAM-detection has been developed.
⇒ The specificity of the above tests for the genus Mycobacteria appears to be high.
⇒ A large number of anti-LAP monoclonal antibodies are under production, and await screening for sensitivity and specificity, including species specificity, in the above test systems.

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⇒ Limited clinical studies indicate that LAM regularly (85-90%, or more) is excreted in the urine of patients with active tuberculosis, and that this excretion is significantly reduced following antimycobacterial treatment.
⇒ So far, LAM has only rarely (5%) been found in the urine of randomly selected healthy controls, or in patients hospitalised with other infectious diseases.
⇒ Model systems for TB-infection in mice have been established permitting i.e. studies on urinary excretion of LAM under experimentally standardised conditions.
⇒ Further investigation is needed i.e. concerning optimal pre-assay treatment of the urine, choice of inert plastic tubes for storage and transportation of test material, and selection of an optimal solid phase for the dip-stick test.
⇒ A patent application has been filed, and further commercial development of this test system will be undertaken in collaboration with an appropriate European manufacturer.

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Contract number TS3*CT940299

IMMUNE RECOGNITION OF A NOVEL 45KDA ANTIGEN, SPECIFIC TO *MYCOBACTERIUM LEPRAE*, AND EVALUATION AS A POTENTIAL VACCINE CANDIDATE

Period: January 1, 1995 — December 31, 1996

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, Dept. of Clinical Sciences, London, United Kingdom (H.M. DOCKRELL)

Objectives

- To compare T cell recognition of the 45kDa antigen in leprosy patients, contacts and controls.
- To identify immunodominant epitopes in the 45kDa antigen recognized by T cells.
- To investigate whether immune recognition of the 45kDa antigen identifies leprosy patients at risk of developing reactional complications.

Activities

* To prepare a panel of reagents with which to investigate immune responses to the *M. leprae* 45kDa antigen, including recombinant 45kDa protein and overlapping peptides spanning its sequence.
* To assess lymphocyte transformation and cytokine secretion induced by the 45kDa antigen T cells from leprosy patients, contacts and endemic controls in Mexico.
* To identify immunodominant epitopes in the 45kDa antigen, using synthetic peptides to map the epitopes recognized by peripheral blood mononuclear cells from leprosy patients and contacts, and by human T cell clones.
* To screen leprosy patients with/without a history of erythema nodosum leprosum (ENL) reactions for antibodies to the 45kDa antigen, and monitor a cohort of lepromatous leprosy patients for anti-45kDa antibodies and for development of ENL.

Expected outcome

⇒ These studies will evaluate the specificity of the *M. leprae* 45kDa antigen as a tool for monitoring *M. leprae*-specific immune responses.
⇒ The project will allow transfer of cellular immunology methodology to the National Medical Centre in Mexico City, with training of Mexican scientists.
⇒ Field studies in Mexico will demonstrate whether immune recognition of the *M. leprae* 45kDa antigen can be used as a predictive marker of ENL reactions in leprosy.

Results

Recombinant *M. leprae* 45kDa antigen and a range of control antigens have been prepared by the group in Leiden, as well as a panel of overlapping synthetic peptides for epitope mapping.

A functional cellular immunology laboratory has been established at Centro Medico for work on the cellular immunology of leprosy.

Lymphocyte proliferation assays and cytokine detection by ELISA, as well as ELISA assays for antibodies, can now be performed, and the methodology for cytokine detection using RT-PCR and for epitope mapping of antibody epitopes are being set up.

In London, studies using peripheral blood from healthy BCG-vaccinated donors have shown that these donors can make T cell responses to the *M. leprae* 45kDa antigen, although these responses were stronger than in donors who had not received BCG vaccination. We now aim to clone antigen-specific T cells to allow mapping of the epitopes recognized.

Contract number TS3*CT940299

Normal donors showing T cell proliferation to the *M. leprae* 45kDa antigen also showed parallel secretion of the Th1 cytokine, interferon-gamma. Studies using blood samples from leprosy patients are now being tested in London, comparing mRNA detection using RT-PCR and cytokine product by ELISA.

In Mexico, lymphocyte proliferation assays have been performed with peripheral blood samples from patients with lepromatous leprosy, and their household contacts. Results show that 58.6% of the lepromatous leprosy patients (n=29) responded to the *M. leprae* 45kDa antigen, with higher responses in donors also showing a positive response to a sonicate of *M. leprae*. Additional tuberculoid leprosy patients and endemic controls are being recruited to extend this analysis. The *M. leprae* 45kDa antigen also induced good interferon-gamma from ±65% of both tuberculoid and lepromatous leprosy patients, when tested in a whole blood cytokine assay.

Overall the results obtained to date suggest that *M. leprae* 45kDa antigen induces strong Th1 T cell responses. However it seems that at least some epitopes may be recognized by T cells from donors not exposed to leprosy, suggesting the presence of some cross-reactive epitopes.

Training activities to date include visits of two members of Professor Vega-Lopez' group to the London School of Hygiene & Tropical Medicine for visits of 1 and 4 months to learn methodology for RT-PCR and T cell cloning, and a 1 month visit of a member of Dr. Dockrell's laboratory to Mexico to help set up the T cell work there.

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Contract number TS3*CT940304

PEPTI DES FOR THE EARLY DETECTION AND VACCINATION AGAINST TUBERCULOSIS AND LEPROSY

Period: January 1, 1995 — December 31, 1996

Co-ordinator: MRC CLINICAL SCIENCES CENTER, TUBERCULOSIS & REL. INFECT. UNIT, London, United Kingdom (J. IVANYI)

Objectives

Development of peptide based reagents for the early detection of disease:

- Immunodiagnosis of active tuberculosis (TB);
- Immunodiagnosis of tuberculosis leprosy and type I reactions;
- Formulation of peptides for skin testing using liposomes.

Design of subunit vaccines against tuberculosis involving:

- Identification and characterization of genetically permissive petpides recognized by human CD4 & CE8 T cells;
- Formulatio n of protective forms of antigen delivery, i.e. adjuvants, DNA plasmids;
- Characterisation of the cytokine profiles of protective T cells.

Activities

* Development of robust *in vitro* assays and training of staff from India for testing anti-peptides T cell responses in bulk cultures, T cell lines and T cell clones. Assay of HLA restrictions.
* Testing of a short-list of HLA permissive mycobacterial peptides for their capacity to stimulate the proliferation of blood lymphocytes in sensitized healthy subjects and TB patients from India.
* Identification and characterization of integral membrane protein constituents of mycobacteria.
* Construction of mycobacterial peptides containing liposomes and analysis of their immunogenicity.
* Evaluation of cytokine profiles of peptide reactive T cells from TB patients in the course of chemotherapy.

Results so far

⇒ Analysis of variants with single substitutions in one epitope core showed distinct conformations for HLA-DR1 and BRB5*0101 (Jurcevic et al., International Immunology, in press).

⇒ Analysis of T cell responses to a mixture of mycobacterial peptides with complementary HLA-DR binding profiles showed merely a weak additive effect (Jurcevic et al., Clin Exp. Immunol, in press).

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⇒ Synthetic peptides non-covalently bound to bacterial hsp 70 were shown to have enhanced T cell immunogenicity (Roman & Moreno, Immunology 88:487, 1996).

⇒ Peptides overlapping the sequence of the 35 kDa protein of *M. leprae* were characterized for binding to several HLA-DR molecules. Subsequently, immunodominant peptides were identified on the basis of T cell responses in leprosy patients and endemic and non-endemic healthy subjects.

⇒ Testing of peptides eluted from HLA-DR molecules of a B. lymphoblastoid cell line incubated with or without a mycobacterial extract indicated distinct HPLC profiles.

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Contract number TS3*CT940313

DEVELOPMENT OF A NEW VACCINE AGAINST *MYCOBACTERIUM TUBERCULOSIS*

Period: February 1, 1995 — April 30, 1998

Co-ordinator: STATE VETERINARY SERUMLABORATORY, BACTERIA VACCINE DEPT., Copenhagen, Denmark (P. ANDERSEN)

Objectives

The general objective is to reduce global morbidity and mortality from tuberculosis by vaccination. The immediate goals are:

- To construct an experimental vaccine with a high efficacy in animal models by the identification of adjuvant and antigen components;
- To characterize and conduct detailed immunological investigations of vaccine candidate antigens;
- To identify antigens frequently recognized by TB patients during the first phase of disease.

Activities

Identification of antigen components

Selected proteins purified from a pool of secreted mycobacterial proteins have been identified and characterized. All the purified antigens are tested in the mouse model for the recognition by memory T cells involved in the recall of a protective immune response. A number of purified proteins were able to induce IFN-γ release from memory effector cells tested in this model during the first phase of a protective immune response. Three previously uncharacterized proteins have been identified; CFP-13, CFP-29, and CFP-46. The N-terminal sequence and the amino acid composition of CFP-29 was determined and searches in protein and translated nucleotide sequence databases confirmed that this protein has not been characterized before. Work is in progress to characterize other proteins purified by preparative 2-dimensional electrophoresis.

Identification of the adjuvant components

DDA has proven to be an excellent adjuvant for a vaccine based on antigens from the culture filtrate of *M. tuberculosis*. This Short Term-Culture Filtrate/DDA vaccine is able to stimulate a Th1 type of immune response as judged by the cytokine profile stimulated and it was able to confer protective immunity. Other adjuvants were less efficacious and, notably, Al(OH)3 stimulated a Th2 type of immune response and exacerbated infection. Vaccination with ST-CF and various cytokines or modulators of cytokine expression was examined by antigen specific stimulation of lymphocytes *ex vivo*. IL-12 significantly enhanced IFN-γ production and was able to partially protect mice from challenge with *M. tuberculosis.*

Characterization and detailed immunological investigation of vaccine candidate antigens

In the mouse model ESAT-6 was recognized by genetically different strains. Synthetic overlapping peptides were used to map T cell epitopes. Two T cell epitopes were found and one of these was recognized by an exeedingly high frequency of splenic T cells indicating the involvement of this antigen in the immune response to *M. tuberculosis*.

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The gene encoding ESAT-6 has been demonstrated to be present only in mycobacteria belonging to the *M. tuberculosis* complex and a few other mycobacterial species tested whereas, it is not present in any known *M. bovis* BCG strain.

Identification of protective antigen targets in human

Human T cell screening is currently underway in the South Shoa Region of Ethiopia, 300 km outside Addis Ababa. Hossana houses a 300 bed hospital and serves a population of 3 million people. The project has become an integrated part of the ALERT TB/Leprosy programme in Addis Ababa. So far blood samples from 122 donors have been obtained, transported to Addis Ababa and frozen for later investigation. Clinical evaluation, and X-rays have been used to characterize the groups. Twelve donors with minimal TB, 45 with advanced TB, and 65 healthy contacts have been identified. The donors will be followed for one year to monitor if any of the healthy contacts will develop TB. Laboratory analysis of the *in vitro* immune responses to crude antigen preparations, fractions of culture filtrate and purified antigens are ongoing. Preliminary data show a heteogeneous picture but individual donors show a high response to ST-CF, ESAT-6 and peptides from ESAT-6.

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Contract number IC18*CT960047

MUCOSAL IMMUNITY OF LEPROSY (MILEP2 STUDY) DEFINITION OF LEPROSY TRANSMISSION AND PROTECTION WITHIN AN ENDEMIC LEPROSY POPULATION

Period: December 1,1996 — November 30, 2000

Co-ordinator: UNIVERSITY OF ABERDEEN
Aberdeen, United Kingdom (WCS SMITH)

Objectives

- To establish the relationship between *M.leprae* infection and the development of immunity in a community in which multiple drug therapy (MDT) has been used for more than 10 years.
- To elucidate the pathogenesis of primary nasal infection in leprosy.
- To develop and test a chemotherapy-based intervention strategy for the interruption of leprosy transmission.

Activities

* Whole population survey of 3 villages in India and one village in Africa. to obtain nasal swabs for polymer chain reaction(PCR) for *M.leprae* and specimens of saliva for measuring IgA levels against *M.leprae.*
* Child contact study. Samples will be obtained from 20 children from households with a treated leprosy patient and 20 controls at three monthly intervals over 3 years in 4 age groups.
* Assessment of immunity(IgA) and PCR positivity in newly exposed adults, with 3 monthly follow up over 3 years.
* Assessment of the natural history of infection(PCR status) and immunity (Salivary IgA)in 30 adults identified in the MILEP1 study with 3 monthly follow-up.
* Development of simplified PCR and ELISA protocols (Miraj/AHRI/London/Bergen.
* ENT examination and nasal biopsy to be taken by an ENT surgeon of PCR positive individuals from village surveys which will be formalin fixed and sent to London for diagnosis and immuno-histo-chemistry for lymphocyte subsets in collaboration with Bergen. Blood samples of PCR positive subjects to be obtained to determine cell mediated immunity (CMI).
* Double blind trial of single dose ofloxacin, rifampicin and minocycline in 60 new PCR positive subjects with monthly follow up for 6months and then 3 monthly measurement of PCR and salivary IgA to determine the course of infection.
* Intervention study to interrupt leprosy transmission. 'at risk' groups identified from activities 1-8 will be given a single dose of ofloxacin, rifampicin and minocycline with 3 monthly follow up.

Contract number IC18*CT960047

Expected Outcome

⇒ Assessment of PCR positivity(putative infection) and immunity to leprosy in the endemic community.

⇒ Assessment of immunity and PCR positivity in relation to age of first exposure.

⇒ Establish how newly exposed adults deal with primary infection.

⇒ Determine the course of infection

⇒ Assessment of methods to interrupt transmission of M.leprae and the course of infection through administration of single dose chemotherapy.

⇒ Transfer expertise and knowledge between the partners in a collaborative study.

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Contract number IC18*CT960060

A MOUSE MODEL FOR LATENT TUBERCULOSIS AND PREVENTION OF REACTIVATION OF THE DISEASE

Period: December 1, 1996 — November 31, 1999

Co-ordinator: UNIVERSITY OF BERGEN, CENTRE FOR INTERNATIOAL HEALTH
Bergen, Norway (G. BJUNE)

Objectives

- To identify the sites where dormant bacilli survive, whether their metabolism is different from that of actively growing bacilli and whether they express different antigens.
- To study the nature of the immune response that maintains the latent state and differences between the immune response in men and mice and in latent and progressive tuberculosis.
- To define the immunological and endocrine factors which induce reactivation.

Activities

* Establish a mouse model for asymptomatic lifelong infection with stable bacillary counts to study histopathology and number of bacilli throughout lungs, spleen, liver and bone narrow.
* Study antigen expression in actively dividing bacilli and in dormant bacilli through immune response to specific antigens, purification and characterisation and identification of gene activation.
* Study T-cell subsets and cytokines in various stages of tuberculosis infection in mice and men.
* Follow the antigen specificity of T-cell responses and antibodies throughout infection in men and mice.
* Study the importance of hormones, growth factors and non peptide biological active components from Mycobacterium tuberculosis in latency and reactivation of the disease.

Expected outcome

⇒ A relevant mouse model for latent tuberculosis and reactivation of the disease in man. Knowledge of what antigens and biological mechanisms which induce keep-up and terminate latency and which are involved in reactivation of the disease.

Contract number IC18*CT960060

⇒ To strengthen research capability and training in two DC laboratories in tuberculosis high endemic countries.

⇒ To create a co-operative basis for tuberculosis research and new vaccine development.

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Contract number IC18*CT970236

IDENTIFICATION OF PROTECTIVE IMMUNE RESPONSES TO PATHOGENIC MYCOBACTERIA

Period: November 1, 1997 — April 30, 2001

Co-ordinator: LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE,
Department of Infectious and Tropical Diseases,
London, United Kingdom (H. M. DOCKRELL)

Objectives

To evaluate the role of T cell-associated lytic mechanisms in the killing of intracellular mycobacteria, and the contribution of these effector pathways to immunity against tuberculosis and leprosy.

Activities

The function of various T cell subsets in immunity to tuberculosis will be assessed in patients with tuberculosis, without or with co-infection with HIV, and in normal BCG-vaccinated healthy controls. Further studies will assess expression of the P2Z (P2X7) receptor in patients with tuberculosis or leprosy, and in healthy controls. Specific areas of investigation are as follows:

* To evaluate whether antigen-specific CD4+ and CD8+ T-cell mediated cytotoxicity reduces the survival of intracellular maycobacteria within macrophages, and the relative contribution of various cytolytic effector mechanisms to this T cell-induced macrophage death (LSHTM, London, University of Oxford, and MRC Laboratories, The Gambia)

* To assess the role of the gd T cell subset in cytolysis and cytokine secretion in tuberculosis with/without HIV co-infection (CMDT, Lisbon)

* To investigate the mechanism(s) by which extracellular ATP-induced macrophage death (occurring via the P2Z-receptor mediated pathway) reduces survival of intracellular *M. bovis* BCG, and to extend these studies to other pathogenic mycobacteria (University of Birmingham)

* To investigate the role of genetic heterogeneity of extracellular ATP-induced macrophage death and intracellular mycobacterial killing in conferring resistance to mycobacterial disease, by comparing P2Z (P2X7) receptor expression on monocyte-derived macrophages from patients with tuberculosis (with/without HIV), lepromatous leprosy, and endemic controls (University of Birmingham, CMDT Lisbon, MRC Laboratories, The Gambia and IMSS Mexico City).

Contract number IC18*CT970236

Expected outcome

The study will obtain scientific data allowing the relative importance of these immune responses in protection against mycobacterial disease, and the heterogeneity of the P2Z (P2X7) receptor in susceptibility to mycobacterial disease, to be evaluated.

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Contract number IC18*CT970253

THE PATHOGENESIS OF TUBERCULOSIS; GROWTH RATE REGULATION AND RIBOSOME SYNTHESIS

Period: December 1, 1997 — May 31, 2001

Co-ordinator: MEDICAL RESEARCH COUNCIL, THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH, London, United Kingdom (M.J. COLSTON)

Objectives

To investigate the ways in which pathogenic mycobacteria are able to regulate their growth and survive within an infected host by:

- Analy sing the expression of ribosomal RNA (rRNA) genes when mycobacteria are grown under a variety of conditions, including in host tissue.
- Study ing the role of ribosomal protein S10 in the transcription of mycobacterial rRNA operons.
- Identifying additional transcription factors involved in expressing genes involved in ribosome synthesis.

Activities

The expression of rRNA genes will be investigated by identifying the promoters involved, and studying their relative levels of expression under different conditions. Comparisons will be made between pathogenic (*M. tuberculosis*) and non-pathogenic mycobacteria; between laboratory and clinical isolates of *M. tuberculosis*; and between *M. tuberculosis* grown under different laboratory conditions, including growth in infected host tissues.

The role of ribosomal protein S10 will be investigated by preparing purified recombinant protein and studying its interaction with RNA sequences and with other proteins of *M. tuberculosis*.

Addit ional transcription factors will be identified by establishing *in vitro* transcription assays using purified mycobacterial RNA polymerase. Transcriptional activity will be monitored and factors which affect transcriptional efficiency identified and characterised.

Expected outcome

This study will identify the strategies used by pathogenic mycobacteria to regulate ribosome synthesis, and hence to regulate growth rates. By identifying specific components of the transcriptional machinery of *M. tuberculosis*, we expect to identify potential targets for the development of novel anti tuberculosis drugs.

Contract number IC18*CT970253

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Contract number IC18*CT970254

DEVELOPMENT OF A TUBERCULOSIS VACCINE WITH CONSISTENT EFFICACY IN DIFFERENT REGIONS OF THE WORLD

Period: January 1, 1998 — December 31, 2000

Co-ordinator: STATENS SERUM INSTITUT, Department of TB Immunology, Copenhagen, Denmark (P. ANDERSEN)

General objective

To reduce global mortality and morbidity from tuberculosis by vaccination.

1) Objectives

- To identify, characterize and produce a panel of mycobacterial proteins for vaccine studies.
- To investigate the antigens present in environmental mycobacteria from Karonga district, Malawi.

Activities

Production of an extensive panel of recombinant Culture Filtrate Proteins (CFPs) from cultures of virulent *M. tuberculosis*. Characterization of the antigens expressed by environmental mycobacteria isolated from the Karonga District in Malawi. Investigation of the recognition of these antigens i) *in vitro* by cells from memory immune mice and recently infected mice, ii) *in vitro* by defined groups of TB infected, BCG vaccinated, and unimmunized human donors, and iii) *in vivo* by guinea pigs (skintest). The protective efficacy of the antigens alone or in combination will finally be evaluated in mice and guinea pigs.

Expected outcome

An identification and recombinant production of novel immunodominant antigens. An overview of the immunogenicity and protective efficacy of these. An overview of the mycobacterial antigens that are relevant for protective immunity against tuberculosis in humans.

2) Investigation of experimental vaccines in animal models

Objectives

- To construct and investigate the efficacy and mode of action of experimental vaccines in animal models.
- To study the interactions between environmental mycobacteria and vaccine induced protection in animal models.

Activities

Production of a fusion protein consisting of ESAT-6 and Ag85B. Testing of the immunogenicity of this hybrid molecule. The level of acquired resistance following immunization with the hybrid in the presence or absence of various cytokines and inhibitors will be studied. The influence of pre-vaccination exposure to environmental mycobacteria on immune responses as well as BCG replication will be monitored and the influence on subsequent protection against tuberculosis evaluated.

Expected outcome

Characterization of the mechanisms underlying the vaccine induced protective immunity. Information on the interaction between immunization and sensitization with environmental mycobacteria.

3) Production of ST-CF/DDA vaccine for human use

Objectives

- To produce and test the new ST-CF/DDA vaccine in humans.

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Activities

When ethical clearance has been obtained from the relevant authorities, ST-CF/DDA vaccine will enter phase 1 clinical trial. Following a successful phase 1 trial, the experimental vaccine will be injected in healthy human volunteers from different geographical regions (Denmark and Malawi) and the responses compared with standard BCG vaccination. Cellular parameters such as antigen-specific proliferation, cytokine production, and antigen specific cytotoxicity in response to various recombinant antigens, PPD and ST-CF will be tested.

Expected outcome

The testing of a ST-CF/DDA vaccine in phase 1/2 clinical trial. Detailed information on the type of human immune responses induced following vaccination with ST-CF/DDA.

4) Final construction of new experimental vaccines

Objectives

- To construct an experimental vaccine based on immunologically relevant antigens.

Activities

Production of a new subunit vaccine based on a selection of the best antigens and adjuvants.

Expected outcome

A new subunit vaccine against tuberculosis.

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3. Hemorrhagic fevers

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT920067

DIAGNOSIS AND CONTROL OF CRIMEAN-CONGO HEMORRHAGIC FEVER (CCHF)

Period: January 1, 1993 — December 31, 1994

Co-ordinator: ARISTOTLE UNIVERSITY THESSALONIKI, SCHOOL OF MEDICINE, DEPT OF MICROBIOLOGY, Thessaloniki, Greece (A. ANTONIADIS)

Objectives

- Production of new, non-infectious diagnostic reagents using recombinant DNA technology. These reagents will be applied in immunoassays used in African countries to determine the risk of CCHF zoonotic transmission.
- Development of methods for rapid diagnosis of CCHF virus infections based on oligonucleotide probes and the polymerase chain reaction (PCR).

Activities

* Development of diagnostic reagents.
* Diagnostic reagents will be derived from the S and M RNA nairovirus genomic segments. Recombinant proteins will be derived from the S RNA of CCHF virus, and from the M RNA of DUG virus. Laboratory animals will be immunized with recombinant antigens to produce antibodies directed against the nucleoproteins or glycoproteins for use in immunoassays and antigen detection by antibody capture. In addition, type specific nucleic acid probes and PCR primers will be developed.
* Development of diagnostic techniques.
* Expressed viral proteins will be used as a substrate for viral antibody detection. A variety of standard immunological tests will be employed (ELISA, Western blot analyses, immunoprecipitation and immunofluorescence). In addition, non-radioactive probes (e.g. biotin-labelled nucleotides) will be used so that the techniques can be adapted to African laboratories.
* Validation of immunoassays.
* Preliminary work will aim at establishing a bank of confirmed positive and negative human sera based on immunofluorescent antibody tests and enzyme-linked immunoassays (ELISA) with antigens derived from CCHF 10200 and Greek viral isolates.
* Identification of enzootic foci.
* Surveys will be undertaken in the participating countries. The aim will be to identify endemic areas of CCHF virus and to establish a regime for routine serodiagnosis in African countries.

Expected outcome

⇒ Development and production of recombinant nairovirus proteins and transfer of technology to African partners.
⇒ Production of probes and PCR primers and their application for rapid diagnosis of human infections with CCHF virus.
⇒ Establishm ent of immunoassays in African laboratories using recombinant CCHF virus proteins for rapid diagnosis of human infections as well as for serosurveys in potentially infected animals.
⇒ Data on the distribution and prevalence of nairovirus infections in all countries involved.
⇒ Publications on the development of nairovirus diagnostic reagents and their applications.

Results

The complete nucleotide sequence of the small RNA genomic segment of Crimean-Congo hemorrhagic fever (CCHF) virus, Greek isolate AP92, was determined from cloned polymerase chain reaction (PCR) products.

Contract number TS3*CT920067

The nucleocapsid (N) protein open reading frame was identified by homology to the N protein of a Chinese isolate of CCHF virus, and was expressed to high levels in the baculovirus expression system. Three N protein-derived peptides were expressed in *E. coli* as fusions to glutathione-*S-transferase*, and the antigenicities of these proteins and the Baculovirus expressed protein were tested in an enzyme-linked immunosorbent assay (ELISA). When tested with laboratory animal sera representing all seven serogroups of nairoviruses, the only reactive sera were those raised to CCHF virus (Greek, Nigeria and Chinese isolates) and, more weakly, Hazara virus. When tested with a panel of known positive and negative human sera, the baculovirus-expressed N protein, and the peptide derived from the central region of the N protein, proved to be the best for identifying CCHF virus-specific IgG.

2.440 human sera examined in Bangui for antibody to CCHF and Dugbe viruses. For these viruses variable percentages of seropositives were detected ranging from 10% to 50% in an ELISA test with brain derived antigen. All sera reacting with CCHF virus reacted also with Dugbe virus. In Senegal, 7 CCHF strains were isolated out of 2100 ticks collected from ungulates in several areas. 5 strains of the CCHF virus were isolated form *Hyalomma marginatum rufipes* ticks, 1 from H*yalomma truncatum* and 1 from *Rhipicephalus evertsi* ticks. 1400 human sera (430 from Nigeria and 970 from Greece) examined in Thessaloniki for antibody to CCHF virus by IFA and ELISA tests. Results obtained, revealed 5% of seropositives in Nigeria and 1.8% in Greece. In parallel, 880 ticks were collected in Greece for virus isolation in Vero E-6 cells as well as for virus detection by PCR. Thus far no virus was isolated from ticks. In contrast by PCR in one pool of ticks collected from sheep in CCHF area.

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Contract number TS3*CT940286

EPIDEMIOLOGY OF FILOVIRUS INFECTIONS IN THE CENTRAL AFRICAN REPUBLIC: A RISK FACTORS STUDY ASSOCIATED WITH SUBSISTENCE ACTIVITIES

Period: January 1, 1994 — December 31, 1996

Co-ordinator: ORSTOM, DEPARTEMENT SANTE
Paris, France (J.P. GONZALEZ)

General long term objective

Outside the devastating epidemics of nosocomial origin or the contamination events in laboratories, effects of the Filovirus remain little known and not well documented.

The aims of this study are:

- To define the relative frequency of anti-Filovirus antibodies for different populations.
- To show the risk factors responsible for infection in human populations living in areas where the existence of Filovirus is suspected.
- To define the method(s) of contamination and transmission of Filovirus.
- To evaluate the risks of infection and epidemics in the areas where this virus exists and to propose an information policy for the prevention of epidemics.

Specific objectives to be achieved in this contract are twofold

- Epidemiology and infectiology: to identify the risk factors of contamination by Filoviruses associated with nutritional and cultural habits in populations living in or on the edge of forests in central Africa.
- Anthropology: to define the populations at risk in a well described human and physical environment.

Activities

The proposed study is aimed at the populations living within or on the borders of the central African forests and is dominated by two essential methodological aspects:

Contract number TS3*CT940286

* An epidemiological study centred around the search for a link between contact with wild monkeys and the presence of anti-filovirus antibodies in human populations.

* An ethnological study intended to show the importance of hunting and consuming the meat of wild monkeys in communities of farmers and hunter-gatherers.

The methodology proposed envisages the following activities within the timescale:

* Preparatory phase: Identification of the environment and populations to be targeted from previously obtained data from sero-epidemiological surveys carried out in the Central African Republic. Based on this information, a decision will be made on the choice of area (villages and strength of populations). An objective questionnaire will then be compiled according to populations and to which linguistic group they belong. A practical evaluation will be carried out on the materials necessary to carry out this first field investigation within the timescale and the location (Institut Pasteur de Bangui, IPB, Laboratoire de Langues et Civilisations Traditions Orales, LACITO; ORSTOM).

* Evaluation of the questionnaire will be carried out on a sample of the population selected (IPB; LACITO; ORSTOM).

* This will be followed by the installation of the logistics and the completion of the first sero-epidemiology study, starting in Bangui (IPB; ORSTOM; LACITO).

* Packa ging of the samples for the laboratories will be carried out in part in the field (decanting of serums, preparation of aliquots/splitting the samples, labelling, freezing) and part in the laboratory (distribution, dispatching, IPB).

* Laboratory analyses will be carried out in the virology laboratories (IPB, IV Marburg, ORSTOM/YARU).

* Examination of the questionnaires and the results of the laboratory analyses and data and statistical processing (ORSTOM/LACITO).

* Interpretation of results, with reference to the field, will provide the information required for the preparation of the second visit. This will take place one year after the first visit, and during the same season.

* It will allow follow up of the populations (sero-conversions) and control of the data acquired (sero-prevalence), eventual confirmation of the hypotheses put forward (research on the homologous village groupings), choice of new target specimens (environmental survey in the areas of suspected cases or amongst families with a conflicting prevalence of antibodies) (IPB, ORSTOM, LACITO).

* The second sero-epidemiological survey will be carried out taking into account the results and interpretation of the first (IPB/ORSTOM).

The results of the second study will lead to the final report and then eventually on to a statement of research prospects / perspectives on the epidemiology of Filovirus.

Results

The first analyses carried out on the populations in ten pygmy camps and in two mainly bantu-speaking villages shows:

⇒ There is a more pronounced and higher rate (>15%) of sero-reactivity for Ebola and Marburg virus among pygmy ethnic population.

⇒ The first analysis shows that risk of infection with Ebola virus increases when there is more contact with the forest, particularly when linked to seeking food behaviour.

⇒ The sero-positivity for the Marburg virus is original and demonstrates that this close relative of the Ebola or a similar virus is circulating in this part of central Africa.

⇒ Some green monkeys and one red monkey *(Erythrocebus patas)* were found to be carriers of the antibodies of the Marburg virus but negative for the Ebola antigens, a new finding for this region of Africa.

⇒ Transfer of technology (ELISA, Western Blot, PCR) has been successful, most of the tests having been carried in the Central African Republic. This phase is about to be completed with the installation of the PCR to detect the Ebola and Marburg (or similar) viruses in the specimens collected on the study site in Lobaye in the southern area of the Central African Republic. This technology and reagents have been supplied by the Institut für Virologie in Marburg (Pr Slencka) and Mr Nakoune visited this institution twice.

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⇒ The results obtained so far have been object of several communications and one publication in a highly rated international journal (Journal of Infectious Diseases). The preliminary results have been presented and the first 'Colloquium on research on Ebola virus', held in September 1996 at the Prince Leopold Institute in Antwerp.

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Contract number TS3*CT940290

THE USE OF RECOMBINANT PROTEINS AND SYNTHETIC PEPTIDES FOR DIAGNOSTICS AND ELUCIDATION OF VIRULENCE MARKERS IN DENGUE VIRUS INFECTIONS

Period: October 1, 1994 — September 30, 1997

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE,
DEPT. OF MEDICAL PARASITOLOGY,
London, United Kingdom (A. FALCONAR)

Objectives

To identify dengue viral sequences associated with protection, virulence and identify intra-serotypic strain variations. This will lead to the elucidation of mechanisms involved in haemorrhagic/shock syndromes.

Activities

Selected regions of dengue virus strains showing clearly demarked pathogenic capacities will be sequenced. Intra-serotypic heterogeneity will initially be tested using human sera and neutralizing mouse monoclonal antibodies. Epitopes on the E and prM proteins of these viruses will be identified using computer algorithms, synthetic peptide and recombinant cDNA expression products. These antigens will be assessed for suitability as diagnostic reagents. If particular sequences are associated with virulence, PCR will be employed as a rapid and sensitive method for the identification of virulent strains.

Expected outcome

The data generated in this study will help to design a safe and suitable vaccine against these pathogens and may also have application to other haemorrhagic fever viruses.

Contract number TS3*CT940290

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4. Human Papillomavirus

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT940295

HUMAN PAPILLOMAVIRUS (HPV) INFECTIONS IN THE PATHOGENESIS OF OESOPHAGEAL CANCER IN THE HIGH-RISK AREAS OF CHINA

Period: January 1, 1995 — December 31, 1996

Co-ordinator: UNIVERSITY OF KUOPIO, DEPT. OF PATHOLOGY
Kuopio, Finland, (K. SYRJÄNEN)

Objectives

- To determine the prevalence of HPV infection in the oesophagus by multiple DNA-detection techniques, and evaluate the role of HPV in the pathogenesis of oesophageal precancer lesions and carcinomas in the high-incidence areas of China.
- To study risk factors, HPV genotypes, and interaction of HPV with other infectious agents.
- To analyze the frequency and type of mutations in the tumor suppressor genes p53 and Rb.
- To derive diagnostic, preventive and treatment measures.

Activities

Although a great deal of information on OC (oesophageal cancer) has been obtained, the causative factors of the disease remain to be established. Except for the chemical agents, e.g. nitrosamines, mycotoxins, excessive tobacco use and alcohol drinking, opium use, and physical factors, e.g. coarse and hot food intake, which have been reported as risk factors for OC, evidence is accumulating to suggest that infectious agents may play a role in the etiology of oesophageal cancer. Previous studies in the high-risk area for this disease have suggested that the ingestion of mouldy foodstuffs and pickled vegetables (contaminated with fungi) is closely related to OC. Certain viruses, i.e. human papilloma-virus (HPV), herpes simplex virus (HSV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) have been demonstrated to infect the oesophageal epithelium, and therefore they should be considered as potential etiological agents of OC, although many of the key issues on their mechanisms of action are unclear as yet.

HPV involvement in human oesophageal benign and malignant lesions have been demonstrated by histopathological assessment, immunohistochemical studies and viral DNA-detecting techniques. Our previous studies have shown a high prevalence of HPV infections in oesophageal samples derived from the high-risk areas for OC in China, where this disease has been endemic for generations, with extremely high incidence rates. However, since most of the previous studies have only included small numbers of biopsies, it is necessary to carry out a well-organized and extensive epidemiological study to assess the prevalence of HPV infections in this high-risk population. It is also important to clarify, whether any differences on the prevalence of oesophageal HPV infections exist between the high- and low-risk areas.

Contract number TS3*CT940295

During 1990-1992 our Chinese collaborators in Zhengzhou, China collected a series of more than 2,000 oesophageal specimens derived from 749 patients suffering from a variety of oesophageal squamous cell lesions, with pertinent clinical data available. These specimens have been derived from both the high-incidence area for oesophageal cancer in China and in Finland. Using multiple histopathological and DNA-detecting techniques which have been well established in the laboratories in Kuopio and Turku (Finland), Heidelberg (Germany) and Sienna (Italy), such as light microscopy, electron microscopy, immunohistochemistry, DNA *in situ* hybridization, Southern blot hybridization, dot blot hybridization, polymerase chain reaction, single strand conformation polymorphism, as well as feral isolation, cloning and DNA sequencing techniques, the contractors have been running a systematic analysis of the associations of infectious agents (HPV in particular) with OC.

Results

⇒ The project is progressing on schedule. Technology transfer to the third world is listed among the major goals of the concerted action. A Chinese doctor from the contractors laboratory was trained in Kuopio, Finland. After having returned to China, she has successfully established a number of DNA techniques (e.g. in situ DNA hybridization, PCR, Southern blot hybridization and gel electrophoresis) in her laboratory in Zhengzhou. Additional visits to Sienna and Heidelberg will be realized later.

⇒ Mapping and timing of cancer-related genes in oesophageal carcinogenesis, not practiced by any other laboratory, has been on progress at Henan Medical University. So far, more than 40 surgically resected whole oesophagi have been sampled and processed for detailed histopathological and immunohistochemical analysis for different oncogenes and p53. It was noticed e.g. that a transient p53- immunostaining from dysplastic lesions to the frankly invasive cancer could be clearly mapped in the specimen, suggesting that p53 mutations are involved in the early (precancer) stages of oesophageal carcinogenesis.

⇒ To date, the complete series of 749 carcinomas have been graded by one pathologist (K.S.) and more than 500 cases by all four pathologists. In addition to morphology, the work in Sienna includes immunohistochemistry for cytokeratins and morphometric analysis of the cancer samples. According to our tentative results, the detection of HPV-suggestive lesions among different observers appears to be highly concordant and reproducible. We have noticed the presence of dysplasia in about 20% of the cases, graded as mild (40%), moderate (30%) and severe (30%). Similarly morphological changes suggestive for HPV infection in non-neoplastic adjacent epithelium are present in about 25% of the cases and classified as flat (76%), endophytic (23%) or exophytic (1%) condyloma. The complete analysis of the entire series will take several months.

Contract number TS3*CT940295

⇒ So far, a total of 1,376 oesophageal biopsies derived from 483 patients with invasive squamous cell carcinoma have been analyzed by *in situ* DNA hybridization for the presence of HPV infection. Of the 483 carcinomas examined so far, 113 cases (23.4%) were shown to contain HPV DNA sequences.

⇒ In parallel with the ISH screening, more than 420 biopsies have also been analyzed by the PCR amplification with the L1 consensus primers (MY09/MY11), and followed by Southern blot hybridisation with HPV DNA probes.

⇒ Altogether, 68 cases representing 15.6% of the analyzed biopsies have been shown to contain amplified HPV DNA sequences. Combining the PCR and ISH data, 137/422 (32.5%) seem to show evidence for HPV involvement. This forms the largest series of oesophageal samples thus far analyzed for HPV as a potential etiological agent in the multifactorial pathogenesis of oesophageal squamous cell carcinoma.

⇒ Over 120 DNA samples have been screened for the presence of p53 mutations in Turku using the PCR-SSCP technique. Among them, 70 samples, including 46 oesophageal carcinomas and 24 balloon cytological samples contained enough cellular DNA, from which the p53 gene sequences were successfully amplified by the PCR. P53 mutational spectrum in oesophageal cancer shows most frequently missense mutations and less frequently, gene deletions. These data indicate that inactivation of the p53 gene is a frequent event in oesophageal cancer and such an inactivation might be an important molecular pathway for the development of oesophageal carcinoma. The detection of RB mutations in these samples is underway.

⇒ Detection of other potential tumor viruses, HSV, EBV and CMV in biopsies is completed using immunohistochemistry. Over 100 carcinoma biopsies have been analyzed for both CMV and HSV. All cases remained negative. Recently, more than 100 cancer specimens were also stained with EBV antibodies, all cases remaining negative, however. Part of these samples were further analyzed by PCR with specific EBV primers, but again, no EBV DNA sequences were amplified. These results strongly implicate that these viruses are not likely to be involved in the pathogenesis of oesophageal cancer. These findings are interesting because HSV infection has been epidemiologically linked with oral cancer, and EBV infection has been implicated in the pathogenesis of nasopharyngeal carcinomas, particularly in Chinese patients.

⇒ The ongoing systematic evaluation of infectious agents in pathogenesis of oesophageal cancer using modern DNA- and pathobiological techniques should yield valuable information on the etiology, pathogenesis and prognosis of oesophageal cancer, and therefore could provide reliable data for the prevention and treatment of this disease in the future.

Contract number TS3*CT940295

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Contract number IC18*CT970234

DEVELOPMENT OF HUMAN PAPILLOMAVIRUS (HPV) VACCINES FOR PREVENTION AND TREATMENT OF CERVICAL CANCER

Period: October 1, 1997 — September 30, 2000

Co-ordinator: CANCER INSTITUTE, CHINESE ACADEMY OF MEDICAL SCIENCES
Beijing, China (Y.H. ZHANG)

Objectives

The overall objective is to provide scientific background for the development of efficient and safe prophylactic and therapeutic HPV-specific vaccines.

- To study the prevalence and rate of infection of HPV 16 and HPV58 in high-risk areas for cervical cancer in China
- To develop recombinant vaccines for prevention of HPV16 and 58 infections by eliciting L1-specific neutralizing antibody response
- To develop recombinant therapeutic vaccines based on modified E6 and E7 of HPV16 and 58 capable of effectively inducing cytotoxic T lymphocyte (CTL) response against HPV16 and 58-infected pre-malignant and malignant cells.

Activities

To collect serum samples from approximately 1000 individuals of 15 to 35 years of age in a high-risk area for human cervical cancer to determine antibody titer against HPV 16 and 58. Serum samples from the same 1000 individuals will be taken one year later in order to estimate incidence and prevalence rate. HPV 16 and 58 virus-like particles (VLPs) will be used as the antigens to develop HPV-specific ELISA.

Chimeric virus-like particles (CVLPs) consisting of HPV16 L1 protein fused to mutated E6 or E7 will be made and used as the vaccines. The serological and CTL responses to these CVLPs will be evaluated in mice.

HPV58 L1 gene will be cloned and inserted into expression vector in vaccine strain, replication-deficient vaccinia virus. Serum IgG antibody response upon systemic immunization and IgA antibody response upon mucosal immunization will be measured. Efficacy of vaccination will also be examined by HPV58 epithelial infection.

HPV58 E6,E7 genes will be cloned and modified by site-directed mutagenesis. The loss of their transforming activities will be checked by in vitro transformation assay. The mutated HPV58 E6,E7 genes will be cloned to vaccinia viral vector serving as the therapeutic vaccine. CTL responses will be monitored and their activities against HPV58+ tumour cells grown *in vivo* will be examined.

Expected outcome

The study will also assess the incidence and prevalence of HPV infection and to estimate the statistical power of future vaccination trial with the HPV L1 vaccine in high-risk groups for human cervical cancer.

Contract number IC18*CT970234

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5. Measles

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT910002

THE LONG-TERM IMPACT ON MORBIDITY AND MORTALITY OF MEASLES INFECTION

Period: January 1, 1992 — June 30, 1996

Co-ordinator: STATENS SERUM INSTITUT, DEPT. OF EPIDEMIOLOGY RESEARCH, Copenhagen, Denmark (P. AABY)

Objectives

The original objectives of the project were:

- To determine the magnitude and duration as well as the risk factors for delayed mortality after measles infection through the use of:
  - existing data on the long-term impact of measles infection;
  - organization of further field research on the long-term impact of measles infection.

Due to the unexpected observation of higher mortality among recipients of high-titre measles immunization, we added the further objective:

- To study the long-term impact on survival of high-titre and standard-titre measles mmunization.

Activities

The project has used both re-analyses of existing data and organized new studies on the short and long-term impact of measles infection and measles immunization. The basis of both types of studies have been longitudinal community studies in Bandim, Guinea-Bissau; Bandafassi, Senegal; Niakhar, Senegal and Matlab, Bangladesh, which have registered the incidence of measles infection and measles immunization.

Existing data have been used from the urban area Bandim as well as a sample of rural villages in Guinea-Bissau. Follow-up studies to determine survival and immunological status (allergy, anergy, T-lymphocyte subsets) have been organized in relation to children who had measles in major epidemics in 1979, 1980-83, 1988 and 1991 or received different forms of measles vaccines in immunization trials of medium- and high-titre measles immunization organized in the urban community in the 1980s. Five year follow-up studies were organized on children who received measles immunization prior to 9 months of age in the early 1980s. The impact of measles as well as other vaccines has been assessed in a national longitudinal cluster-cohort study.

In the rural area Niakhar, Senegal, we have followed the impact of measles infection and measles immunization since 1983. Studies have been organized of the acute determinants of the severity of measles, the treatment of measles, the transmission pattern of measles and the long-term survival after measles epidemics in 1983-86, 1989-90 and 1992 as well as the impact of standard measles immunization. High-titre measles vaccines, Edmonston-Zagreb (EZ-Ht) and Schwarz (SW-HT), were used in this area in the period 1987 to 1990 and we have therefore studied the long-term survival impact of these vaccines compared with the standard Schwarz measles vaccine (SW-STD).

Contract number TS3*CT910002

In 1991, an immunological study measured T-lymphocyte subsets, neopterin, albumin, C-reactive protein, retinol, malaria parasitemia, and response to rabies vaccine to assess the possible negative consequences of high-titre measles vaccine. Prospective studies measuring T-lymphocytes, nutritional status and morbidity after measles infection have also been organized in this area.

We re-analyzed data from Matlab in Bangladesh from the 1982-86 period where a measles surveillance was ongoing and measles vaccine had only been introduced in half of the study area to determine the magnitude of post-measles mortality and the impact on survival of measles vaccine.

Due to the interest in the long-term impact of measles infection and its possible relation to immunosuppression, a simple method for determination of T-cell subsets under field conditions was developed.

Results

Measl es infection

Contrary to the initial hypotheses, there was no long-term excess mortality after measles infection and no persistent immunosuppression by measles infection. The tendency is rather the opposite that measles cases have higher CD4 counts after infection. Secondary cases have higher post-measles mortality than index cases of measles. Since the tendency is the same in the acute phase of infection with 3-5 times higher mortality for secondary cases, the indication is that those who have mild acute measles also have less long-term mortality for secondary cases, the indication is that those who have mild acute measles also have fewer long-term complications. Index cases of measles appear to have no increase in mortality relative to community controls without any measles infection and may in fact have significantly lower mortality than controls. Long-term-follow-up in a cohort of teenagers who had measles or measles immunization in 1979 in Guinea-Bissau indicates that measles infection may be protective against allergy as measured by skin prick tests.

Standard titre vaccine

A number of studies including data from all the four study areas involved in the present project have suggested that standard measles vaccine may have non-specific beneficial effects. Standard titre measles vaccine has an effect on survival which is not explained by protection against acute measles or its long-term consequences. The data from Niahkar and Bandafassi, Senegal, have indicated that the non-specific effect of measles vaccine is stronger for girls. In a re-analysis of data from the first measles campaigns in Bissau in 1980-83, children who had been vaccinated at 9-11 months of age (RR=0.61).

High-titre vaccines

Female recipients of high-titre (HT) vaccines had reduced survival compared with female recipients of standard-titre vaccines. The mortality difference is not related to a difference in vaccine efficacy. In both Bissau and Senegal, there was no indication of a significant persistent immunosuppression among the recipients of high-titre measles vaccine. There is no indication that this effect is persisting as we have not found differences in mortality during the last two years of the trials.

Conclusions

This research project ended with the opposite conclusion to the initial hypothesis about a major excess mortality after infection due to persistent immunosuppression. The original hypothesis was inspired by the observation of a major reduction in mortality after measles immunization which could not be explained by protection against acute measles fatality. Hence, it was hypothesized that measles had severe long-term consequences which were also prevented by immunization. However, the present project has shown that the explanation is the opposite. Mild measles infection appears to be better than no infection as it is associated with lower mortality compared with uninfected children. In this respect, the likely explanation of the impact of measles vaccine is that it has a similar non-specific beneficial effect as the natural disease. This perspective became important in relation to the unexpected observation of higher mortality for girls after HT measles immunization.

There is no data to indicate that high-titre vaccine in itself has a deleterious effect. It was associated with higher mortality in areas with low background mortality. It was not associated with higher mortality compared with unvaccinated children. There was no indication that measles is associated with long-term excess mortality which would explain the biological basis for excess mortality after HT vaccines, much less is there a tendency that measles has a long-term negative effect exclusively for girls. There is no data to suggest an important persistent immunosuppression after HT vaccines.

Again st this background, the most likely explanation may be that the mortality difference is a question of the relative benefits of different vaccines. The difference in mortality is only found in comparison with recipients of SW-STD. Since both measles infection and measles immunization are associated with a beneficial effect which is probably due to some form of immunostimulation, the differential mortality between high-titre and standard titre measles vaccines could be due to a difference in the non-specific effects. To a large extent, the reduced survival among recipients of high-titre vaccines is due to the beneficial effect on girls of standard measles vaccine.

While we have no indication of specific biological mechanisms involved in these mortality differences, it seems likely that dose is important. This possibility is also supported by the fact that we have observed long-term differences in morality after natural measles infection depending on whether the person can be assumed to be infected with a small dose (index case) or a large dose of measles virus (secondary cases).

The observations on high-titre vaccines have had the consequence that WHO recommended the withdrawal of high-titre EZ vaccine at 6 months of age in developing countries. The observations on non-specific and sex-specific effects of standard measles vaccine and measles infection warrant futher studies as they may have important implications for future control and immunization strategies.

Contract number TS3*CT910002

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Contract number TS3*CT940302

STUDY OF THE IMMUNE DISRUPTION CAUSED BY MEASLES AND ITS ASSOCIATION WITH CLINICAL PROGRESS IN DHAKA, BANGLADESH

Period: January 1, 1995 — December 31, 1996

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, EPIDEMIOLOGY AND POPULATION SCIENCES
London, United Kingdom (F. CUTTS)

Objectives

- To compare changes in measles-specific antibody profile and cytokine profile after measles or measles vaccination, and determine the duration of abnormalities.
- To trace the expression of measles virus (MV)-specific RNAs in different subpopulations of peripheral blood mononuclear cells (PBMCs) and compare the target cells of MV in natural infection versus vaccination.
- To analyze surface molecule changes (including RCA and LFA-1) on lymphocyte populations as a result of viral infection or vaccination.
- To compare morbidity and the immune response to challenge with hepatitis B vaccines (HBV) among result of viral infection or vaccination.

Activities

A prospective cohort study will be conducted in Dhaka, Bangladesh. The following cohorts of children will be recruited:

Measles cases:

* A community-based cohort of 110 cases of acute measles;
* A hospital cohort of 110 cases of complicated measles.

Controls (age matched to cases):

* A community-based control group of 110 healthy children;
* A hospital-based control group of 110 children attending with diseases other than measles.

Measles vaccines:

* A group of 25 children vaccinated with standard titre measles vaccine at age of 9 months;
* A group of 25 children vaccinated at ages 6 and 9 months.

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The research plan is divided into the following sections:

* Collection of information on morbidity among each cohort, by weekly interviews for six months after recruitment. Measurement of anthropometric status monthly. Children with episodes of illness will be referred to the study physicians for examination and appropriate X-ray and laboratory investigations. Mothers will be invited to bring acutely ill children to ICDDR, Hepatitis B clinic at any time during follow-up.

* Measurement of the serological response to a series of 3 doses of hepatitis B vaccine in each cohort.

* Measuremen t of interferon-gamma (IFN-γ and interleukin-5 IL5 cytokines using whole blood assays in each cohort at recruitment, six weeks and six months after recruitment.

* On a subsample of 25 children in each cohort, *in vitro* analyses of the target cells of measles virus, surface molecule changes, and indices of immune disruption.

Expected outcome

⇒ The project aims to provide information to assist in understanding the basis for the immune disruption after measles, and raise hypotheses for the mechanism of differences in persistence of immunity after measles disease or measles vaccination.

⇒ Bangladesh investigators will be trained in clinical epidemiology. The technique of whole blood assays for cytokines will be transferred to Bangladesh.

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Contract number IC18*CT950011

SPECIFIC AND NON-TARGETED EFFECTS OF A TWO-DOSE STRATEGY WITH STANDARD TITRE MEASLES VACCINE. IMPLICATIONS FOR IMMUNIZATION PROGRAMMES

Period: January 1, 1996 — December 31, 1999

Co-ordinator: STATENS SERUM INSTITUT, DEPT. OF EPIDEMIOLOGY
Copenhagen, Denmark (PETER AABY)

Objectives

The general objectives are:

- To reduce childhood mortality in developing countries through better control of measles infection by developing a better immunization strategy.
- To investigate the hypothesis that standard titre measles immunization is associated with non-targeted beneficial effects on childhood morbidity and mortality in developing countries.

The measurable, specific objectives are:

- To examine whether a two-dose strategy for measles immunization at 6 and 9 months of age can reduce measles incidence by 50% through better coverage or improved seroconversion.
- To examine whether a two-dose strategy for measles immunization at 6 and 9 months of age can reduce childhood mortality by 20% through better coverage, better protection against measles or non targeted beneficial effects.
- To determine the magnitude and duration of non-measles related changes in morbidity patterns after standard titre measles immunization, in particular to test whether measles immunization is associated with a 15% reduction in the risk of diarrhoea.
- To determine non-measles related immunological changes among recipients of measles vaccine in order to establish possible pathways for the non targeted effects of standard titre measles immunization.

Activities

* Conducting the trial of two-dose standard titre measles vaccination in Bissau.
* Conducting the studies of morbidity and anthropometry.
* Performing the immunological studies included in the two-dose trial.
* Performing data-control and data-analysis.

Contract number IC18*CT950011

Expected outcome

⇒ Vaccine efficacy: vaccine efficacy will be assessed both as cumulative incidence from the time of entering the study at six months of age and as protection rate following exposure from a measles case in the same house.

⇒ Measles antibodies: measles antibody responses as well as decay in measles antibodies levels will be analysed for a subgroup of individuals. Since previous studies have shown that antibody titre below 125 miU are not protective, children with such low titres at 18 months of age will be offered reimmunization.

⇒ Mortality: cumulative mortality will be analysed from entry into the study at 6 months of age or later to the censoring date.

⇒ Research training: training abroad of one Guinean physician in research methodology.

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Contract number IC18*CT960054

FACTORS ASSOCIATED WITH THE SEVERITY OF MEASLES AND OF DELAYED COMPLICATIONS OF MEASLES

Period: January 1, 1997 — December 31, 1998

Co-ordinator: LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
London, United Kingdom (F. CUTTS)

Objectives

- To compare levels of IL-5, IL-10, and IFN-(, at recruitment, 6 weeks and 6 months post-recruitment, in children with and without complications within 6 weeks of rash onset, among those who had no complications at enrollment.
- To determine if there is an association between levels of IL-5, IL-10, and IFN-(at 6 weeks and 6 months post-measles, delayed-type-hypersensitivity (DTH) responses at 6 weeks and the development of complications (pneumonia, severe pneumonia, invasive diarrhoea, or hospitalisation for other reasons) 6-24 weeks after measles onset.
- To compare the risk of complications among vaccinated vs unvaccinated cases.
- To determine the association between cytokine profiles at 6 weeks and 6 months post-measles onset and early age at measles infection, sex, and vaccination status.
- To compare cytokine profiles measured by whole blood assays with results of conventional assays on PBMCs on a subsample of children.

Activities

This a nested case-control study within a study evaluating long-term morbidity after measles. Commercial ELISA kits will be used to measure IL-5, IL-10, and IFN-(levels in supernatant from whole blood assays at recruitment, 6 weeks and 6 months at ICDDR,B on approximately 200 measles cases. Cases and controls will be defined among measles cases with and without complications at different times after measles onset. The following factors will be investigated for a potential association with early and late complications from measles: (i) Age; (ii) Sex; (iii) Vaccination status; (iv) Interval between onset of rash and initiating treatment; (v) Primary or secondary case; (vi) Cytokine profile; (vii) DTH response 6 weeks after recruitment. We will have 80% power to detect an odds ratio of 3.5 to 4.5 for different exposures of interest at the 95% significance level.

Expected Outcome

⇒ Information on factors associated with early and delayed complications from measles.

⇒ Confirmation or not of the hypothesis that complicated measles cases have a bias towards a Th2 response.

⇒ Establishment of the capacity to conduct whole blood assays for cytokines at ICDDR,B

⇒ A paper for publication in peer-reviewed journals.

Contract number IC18*CT960054

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Contract number IC18*CT960116

CONTRIBUTION TO THE ELIMINATION OF MEASLES FROM AFRICA

Period: October 1, 1996 — September 30, 1999

Co-ordinator: ERASMUS UNIVERSITY
Rotterdam, The Netherlands (ADME OSTERHAUS)

Objectives

- Implementation of rapid, simple and cheap diagnostic tests for measles that can be used under field conditions.
- Establishment of a diagnostic virological lab, operating according to WHO standards. This lab may participate in WHO eradication efforts for measles in the near future.
- Evaluation of the epidemiology of measles in the Sudan, including vaccine coverage and efficacy.
- Insight in the overall health impact of measles in the Sudan, including estimation of case-fatality ratios.
- Evaluation of naturally acquired and vaccine-induced measles virus-specific antibody and T-cell mediated immunity.
- Knowledge about the mechanisms of measles-induced immunosuppression.

Activities

Field work

* Establishment and follow-up of a cohort for the measurement of maternal antibodies, the estimation of the true incidence of measles and the performance of the new diagnostic field test.
* Establishment of a cohort of acute measles cases for immunological and virological studies.

Lab work

After training in Rotterdam and Lyon, immunological and virological studies will be continued in the Institute of Endemic Diseases in Khartoum, in which a new virology lab will be established.

Training

A MD student (epidemiological work, diagnostic test and a PhD student (immunological and virological studies) from the Sudan will be trained in this project.

Expected outcome

⇒ Technology transfer from WHO reference labs to the Sudan, including the building of an infrastructure and training of personnel, which will be essential in the planned WHO global eradication program for measles.

⇒ Knowledge about epidemiology and health impact of measles in the Sudan.

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⇒ Knowledge about the immune response to measles virus and the mechanisms underlying measles-induced immunosuppression.

⇒ Publication in peer-reviewed international scientific journals.

⇒ An MD and a PhD thesis will be written by the Sudanese students.

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6. Pneumococcal vaccine development

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT920086

POLYVALENT STREPTOCOCCUS POLYSACCHARIDE CAPSULAR VACCINE

Period: April 1, 1993 — March 31, 1995

Co-ordinator: NATIONAL INSTITUT OF PUBLIC HEALTH AND ENVIRONMENT PROTECTION
Bilthoven, The Netherlands (W. WITTKAMP)

Objectives

- To develop and test a multivalent pneumococcal polysaccharide (PS)-protein conjugate vaccine containing the PS of the most prevalent pneumococcal serotypes (at least 1, 5, 6B, 14, 19F, 23F).

- To ensure the implementation of the selected vaccine in the developing world, avoiding developmental cost implementation.

Activities

The research work can be separated into a number of activities:

* Isolation of the capsular PS of the most invasive pneumococcal types (1, 5, 6B, 14, 19F and 23F) was by established methods. The vaccine protein toxoid (TT) produced by standard methods is available for the project.

* Coupling of the PS to the protein carrier will be done by the thioether method. This coupling method gives stable and characterized products. The PS are depolymerized by various methods to shorter molecules of defined length (approx: 20-60 monosaccharide residues). Methods for the depolymerization and control of size are already established.

* All conjugates and the corresponding PS controls will be tested for immunogenicity by immunizing mice and rabbits, and determining IgM and IgG antibodies in their sera to the respective pneumococcal PS after 1 and 2 doses of vaccine.

High antibody response, IgG:IgM ratio, and an increased (booster) response to the second dose of vaccine will be the criteria for T dependency. Animal infection models such as intraperitoneal and intratracheal challenge will be used. Also, sera selected from immunized children will be analyzed.

* In the first phase, at least two alternative methods for conjugation will be tested in order to choose the best one to be adopted for the prototype vaccine.

Contract number TS3*CT920086

Conjugates will be prepared from all selected PS. They will be combined in the prototype vaccine. A large enough batch will be made under GMP to allow extensive field studies for efficacy (at least 100,000 doses).

The prototype vaccine will be subjected to phase I and phase II tests in human volunteers. Sera of immunised volunteers and controls will be tested by EIA (IgM and IgG) to the pneumococcal capsular PS, to C-PS (the C-PS common to all types of pneumococci) and also to the carrier protein.

Expected outcome

The expected outcome is the availability of the technology to produce a multivalent pneumococcal conjugate vaccine.

Result

⇒ Durin g the first years of the project, the laboratory work was concentrated on the preparation of numerous saccharide-tetanus toxoid conjugates of pneumococcal serotypes GB14, 19F and 23F. For this purpose a batch of pneumococcal polysaccharides GB19F (Pn19F), 23F (Pn23F) and 14 (Pn14) and of purified tetanus toxoid (TTd) has been prepared. Polysaccharides were decreased to an approximate molecular weight of either 50kD or 350kD by mechanical sheering. A thioether linkage was used for the coupling of saccharides to protein. After coupling, products were purified using either gel permeation or gel filtration chromatography. Next to the preparation of conjugates with saccharides of 50kD, conjugates with various saccharide: protein ratios have been prepared. The developed conjugate vaccines are being tested for their immunogenicity in animal experiments. Furthermore, assays to control the production process the quality of protein and the final saccharide-protein conjugates were set up. These assays include: estimation of purity of polysaccharides according to rules as established by the American and European Pharmacopoea, the estimation of free protein and free polysaccharide in saccharide-protein conjugates, the estimation of the thiol (SH-incorporated in the polysaccharide) after spacer introduction and furthermore, the determination of the relative amount of cell wall polysaccharide (CPS) in several polysaccharides.

⇒ An animal model for challenge experiments with pneumococci was developed at the Norwegian National Institute of Public Health Oslo. The LD50 for intraperitoneal and intratracheal challenge with *S. pneumoniae* serotypes 1, 5, 6B, 14, 19F and 23F in Balb/c and C57BL/6 has been determined.

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⇒ Serological assays for analysis of anti-polysaccharides antibodies in human sera and study sites for field evaluation were developed at the Finnish National Public Health Institute, Helsinki. Monoclonal antibodies to various serotype specific pneumococcal polysaccharides and to cell wall polysaccharides were developed at the Danish Statens Serum Institute, Copenhagen.

⇒ For purpose of coordination of the development and field trial of a multivalent pneumococcal conjugate vaccine, a coordination group was established within the DNC. The group consists of the project leaders of each of the Scandinavian Public Health Institutes and of RIVM, the Netherlands Public Health Institute. On May 10 (1993) a meeting was held to discuss the financial and technical status of the agreed programme for each of the participants. The group concluded that the joint project was progressing well. Additional meetings for the purpose of coordination of field trial of a multivalent pneumococcal conjugate vaccine were attended by members of the DNC. During those international meetings discussions were held how to establish a network of laboratories in Latin-America which are able to carry out proper diagnostic and epidemiological studies on pneumococcal diseases, future pneumococcal vaccines for use against Acute Respiratory Infectious Diseases in developing countries, and possible sites for trials with pneumococcal conjugates.

⇒ The efforts of the final period of the project (1995 — 1997) are focused on two matters:

1. The preparation of a clinical lot of a tetravalent (GB, 14, 19F, 23F) pneumococcal polysaccharide vaccine conjugated to tetanus toxoid. All procedures have been worked out on a laboratory scale. A clinical lot will be prepared under Good-Manufacturing-Procedures (GMP).

2. The preparation of sites in the Philippines (and Vietnam) which are capable of performing human immunogenicity studies. The study sites and the trained personnel are ready for these tests.

Contract number TS3*CT920086

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Contract number IC18*CT950025

EVALUATION OF RESPIRATORY VACCINES FOR CHILDREN IN SOUTH EAST ASIA: STREPTOCOCCUS PNEUMONIAE (AND Hib) CONJUGATE VACCINES IN PREVENTION OF CHILDHOOD PNEUMONIA IN DEVELOPING COUNTRIES

Period: January 1, 1996 — December, 31 1997

Co-ordinator: NATIONAL PUBLIC HEALTH INSTITUTE
Helsinki, Finland (PH MÄKELÄ)

Objectives

- To consolidate work of the already established vaccine evaluation centre in the Philippines, i.e. the Research Institute for Tropical Medicine (RITM), Manila and to develop a new vaccine evaluation center in Vietnam as collaboration between the Pediatric Hospital No 1 (PHN1) and its counterpart (RH), Copenhagen.
- To obtain baseline epidemiologic information from the two centres on natural acquisition of pneumococcal (Pnc) and *Haemophilus influenzae* type b (Hib) antibodies and of Pnc and Hib in the etiology of ARI in children <5 years of age.
- To monitor the safety and determine the immunogenicity of candidate vaccines (Pnc and Hib conjugate vaccines) in infants in the SEA region.

Activities

* Collect baseline antibody and carriage data of Pnc and Hib in the Philippines and Vietnam.
* Determine the role and types of Pnc and Hib in hospital treated pneumonia in children less than 5 years of age (Philippines, Vietnam).
* Vaccination by public health personnel in local health centers within EPI schedule. Vaccine safety measured by structured parental monitoring and home visits by a nurse.
* A venous blood sample is obtained when the child enters the study at specified time points after vaccination. All antibody analyses will be done at KTL.
* Safety and immunogenicity of the Hib conjugate in infancy in Vietnam will be studied as above. The data will also provide baseline information referred to above.
* Monitoring of the quality of the research, cold chain, laboratory, forms, and data entry will be carried out according to GLP/GCP principles.

Expected outcome

⇒ There will be two experienced vaccine evaluation centers in South-East Asia.

⇒ Safety and immunogenicity of pneumococcal conjugate vaccine established.

⇒ Safety and immunogenicity of Hib conjugate vaccine established in Vietnam.

⇒ Basal data needed for the planning of Pnc and Hib vaccination programs and efficacy trial will be available from two areas in the South-East Asian region.

Contract number IC18*CT950025

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Contract number IC18*CT970219

FIELD STUDIES OF PNEUMOCOCCAL CONJUGATE VACCINES TO PREVENT ARI IN SOUTH EAST ASIA — EFFECTIVENESS OF AN 11-VALENT VACCINE; SAFETY AND IMMUNOGENICITY OF A 4-VALENT VACCINE UNDER DEVELOPMENT

Period: October 1, 1997 — September 30, 1999

Co-ordinator: NATIONAL PUBLIC HEALTH INSTITUTE, Dept. of Vaccines, Helsinki, Finland (P.H. MÄKELÄ)

Objectives

To find an effective primary prevention for a significant fraction of acute respiratory infections (ARI), especially of severe ARI and related severe disease in infants and children in developing countries.

Specifically to:

- prepare, initiate, and carry out the first half of an effectiveness study with a new 11-valent pneumococcal conjugate vaccine (Pnc-c) using meningococcal A,C as control, and administered within the national immunization programme (EPI) on the island of Bohol, the Philippines
- assess safety and immunogenicity in both industrialized and developing country settings (Finland, Vietnam) of the 4-valent Pnc-c developed by the Dutch-Nordic consortium.

Activities

The preparatory activities to pave the way for the effectiveness trial include:

* enrolling infants of the target populations at 6 weeks of age in primary health centers to a randomised, controlled, blinded immunogenicity and safety studies of the Pnc-c
* piloting the crucial EPI activities, randomization and record linkage procedures; upgrading the infrastructure and skills of personnel to meet the standards of Good Clinical Practice (GCP) at the study sites
* setting up vaccinovigilance activities to evaluate the short and long term safety of the study vaccines
* maintaining and setting up surveillance activities and clinical skills to detect cases for the primary endpoint, i.e. pneumonia and severe pneumonia in children 4 to < 23 moths of age both in hospitals and in primary health care settings
* identifying all Pnc pneumonia cases both by standard culture and alternative methods, i.e. antigen detection, and measurement of pneumolysin immune complexes
* within the effectiveness study, follow up a nested cohort of infants for the impact of the Pncc on the upper respiratory tract carriage of Pnc, for establishing serological correlates for protection, and for immunogenicity of the vaccine
* identifying unit costs to measure the cost benefit of the Pnc-c
* establishing monitoring activities to comply with the GCP and ICH guidelines.

Contract number IC18*CT970219

Expected outcome

Demonstrate the immunogenicity and safety of the suggested Pnc-c vaccines. The study, once completed, will provide evidence on the efficacy, effectiveness and cost-benefits and establish serological correlates for protection of the 11-valent Pnc-c. The results will be directly and immediately applicable to countries of the South East Asian region, and more widely to developing countries with a moderate infant mortality rate of 40-60 / 1000. In addition, these studies will strengthen the skills of collaborating researchers in both developing and developed countries to perform small and large scale vaccine trials according to the GCP requirements.

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7. Diarrhoea

Areas of interest:

Contract number TS3*CT920060

INTERVENTION AGAINST DIARRHOEAL DISEASES BY IMPROVING CASE MANAGEMENT

Period: November 1, 1992 — October 31, 1995

Co-ordinator: STATENS SERUMINSTITUT, EPIDEMIOLOGY RESEARCH UNIT, Copenhagen, Denmark (K. MOELBAK)

Objectives

The purpose of the study was to clarify whether the incidence of severe diarrhoea and persistent diarrhoea could be reduced by improving case management and feeding practices, including the promotion of breast-feeding.

Activities

The study was conducted as a randomised controlled trial of a health education intervention. The intervention was undertaken at antenatal-and child-vaccination-visits at a local government health centre. These activities are among the most popular of the health services in most developing countries.

In the first phase of the study, the pre-intervention phase, the optimal form and composition of the health education message was determined. This was achieved by rapid ethnographic techniques: in-depth interviews, group interviews, household observations, and focus group discussions.

The pre-intervention phase included also studies of management of sick children and feeding practices.

The second phase of the study was the intervention phase. All the households in the periurban area of Bandim were clusterwise randomised to an intervention group or a control group. The target population was the mothers with infants born between May 1992 and April 1993. In addition, an external control group, of similar size was followed in order to assess a possible "contamination" of the control group from the intervention — mothers within Bandim. All children were followed by weekly morbidity recall interview in order to measure incidence of diarrhoea and case-management practices.

In the intervention group, a standardized message was passed on to the mother concerning the following aspects:

* Management of diarrhoea, with emphasis on use of ORS (Oral Rehydration Salts) and continued breast feeding.
* Encouraging lactation to the age of two years, and preventing premature termination of breast-feeding.
* The appropriate time of introducing weaning foods (4 to 6 months).
* In addition, information on family planning was provided, as a large part of the early weanings was due to pregnancy of the mother.

The message was delivered to the mother by a social worker on an individual basis. The session lasted about 10 minutes, and included — if appropriate — a discussion of the mother's own experiences with childhood diarrhoea.

Results

A number of findings from the pre-intervention phase have been published. Verbal autopsies were used to investigate care-seeking prior to fatal illness in young children. The vast majority of children with fatal illness were seen on at least one, but often on several occasions, at health centres or hospitals. There was no evidence of mother's delay in care-seeking as an important cause of fatal outcome.

The study suggests that training of health workers and the establishment of appropriate systems for triage and referral may be more important than general public health education for an improved management of sick children.

Contract number TS3*CT920060

A very detailed study of management of acute diarrhoea investigated maternal perception of signs and causes of diarrhoea, the use of ORS, and risk factors for progression to persistent diarrhoea. The study showed that diarrhoea perceived as caused by "teething" is believed to be normal although it is clinically indistinguishable from other types of diarrhoea. This has significant consequences for management of these children. The study also showed that though ORS is commonly used, the quantities are minute. ORS user rates are inadequate measures of the performance of programmes for the control of diarrhoeal diseases.

A case-control study suggested that malaria-parasitaemia was not an important risk factor for diarrhoea.

In an analysis of 945 children, followed from birth until weaning, only 57 (6%) were weaned before 12 months of age. In these cases, termination of breastfeeding was largely associated with illness of the mother or the child, or a new pregnancy. Thus, in Bissau, the children who were among the first to be weaned was a highly selected group, among whom a large proportion had suffered recent illness or were vulnerable in other respects. The mothers' reasons for weaning is an important parameter, which needs to be taken into account in both observational and intervention studies.

The intervention had a significant impact on the introduction on weaning food, which was delayed, and resulted in a significantly increased use of intrauterine devices. The duration of breast-feeding was not affected by the intervention; it remained at the high median of 22 months in both groups, as in earlier studies. There was no increased rate of diarrhoea associated with early introduction of water or supplementary foods. However, to a surprise, we found that late introduction of water or food was associated with an increased mortality rate. The causal mechanism is not clear. However, the finding underscores that, in Africa, emphasis should be given on breast feeding promotion rather than the timing of the introduction of weaning foods. Both the present and earlier studies form Guinea Bissau provide evidence for the beneficial effects of partial breast-feeding.

Assessments of the impact of the intervention on other key outcome variables, including case-management practices, child-morbidity and mortality rates are under way.

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Contract number TS3*CT920064

EPIDEMIOLOGICAL AND MOLECULAR BIOLOGICAL STUDY OF CHOLERA TRANSMISSION DYNAMICS IN THE HORN OF AFRICA: A MULTIDISCIPLINARY APPROACH

Period: July 1, 1993 — July 31, 1997

Co-ordinator: CENTRO INTERUNIVERSITARIO DI RICERCA SUI PAESI IN VIA DI SVILUPPO, Roma, Italy (F. MAIMONE & T. MERTENS)

Objectives

- To describe the epidemiology and ecology of *Vibrio cholera* in the Horn of Africa for planning more rational, specific and realistic prevention and control strategies.
- To develop identification tools of complex modes of transmission and persistence of the infection by combining epidemiological methods with molecular and genetic techniques.

Activities

Epidemiological investigations

Epidemiological surveys of enteric infections associated with diarrhoeal diseases have been conducted in the critical geographic areas of Ethiopia where most cholera foci were active between 1993 and 1995 inclusive. Clinical and environmental specimens have been screened for *Vibrio* spp. and other enteric pathogens.

Transmission dynamics

In order to conduct in-depth studies of transmission dynamics in a representative urban area of the Horn of Africa, a baseline population study of the city of Harar (capital of the Harari People National Regional State of Ethiopia; estimated population: 100,000) has been designed. The first phase has consisted of linking a flexible mapping software package containing the cartographic information on the city to an appropriate data-base programme to enter, process and disseminate demographic, epidemiological and behavioral data.

Genetic and molecular studies

Analysis of *V. cholera* strains isolated in Somalia and Ethiopia has been carried out on the basis of genetic and physical characterization of plasmids, restriction enzyme profiles of the chromosomal DNA, Southern hybridization and polymerase chain reaction (PCR) experiments using DNA probes and oligonucleotide primer pairs for specific sequences, and random amplified polymorphic DNA techniques.

Results

Epidemiological patterns of cholera from 1993 to 1995

In October 1993, *V. cholera* 01 was isolated from patients with acute diarrhoea in Dire Dawa, the second largest city in Ethiopia. In the following months clinical cases and outbreaks occurred in urban and rural areas of numerous zones of different regions. Systematic investigations were mostly conducted in Jijiga town (capital of Somali National Regional State; population: 60,000) and for short periods in the Somali zones of Afder and Liban.

The epidemic in Jijiga town started on December 14, 1993 and ended in mid-June 1994. The final figures were 1039 clinical cases presenting for treatment to Jijiga Karamara Hospital and 19 deaths (case-fatality rate: 1.8%). Attack rates were similar in all town districts (kebeles) and the overall rate was estimated to be 15 per 1000 population in the six months of epidemic.

The sex- and age-specific patterns of the cases displayed no significant differences by sex (males 50%) and a mean age of 20 years (age range 3 months to 80 years); 11% of the cases were infants, 23% were 1 — 4 years of age, 16% were 5 — 14 years of age, 38% were adults 15 — 44 years of age, and 12% were >44 years of age. In Afder and Liban zones the epidemic lasted from March to October 1994.

Contract number TS3*CT920064

The cases were 589 (577 in towns) with 38 deaths (case-fatality rate: 6.5%). The attack rate was estimated to be 2 per 1000 population in the seven months of the epidemic.

During the first half of 1995, cases of suspected cholera were still reported from southern towns and villages in the previously affected regions. Such cases were most concentrated in the areas and communities where the spread of the infection had been lower or silent in 1994. The major series of outbreaks occurred in the Wollayta area in the Southern Administrative Region between April and June 1995. In Ofa District (estimated total population: 135,000) in the April-June period there were over 800 cases (attack rate: 6 per 1000) with a case-fatality rate of 2.2%.

Development of a basic GIS infrastructure for Harar city

A set of 1994 stereoscopic aerial photographs were enlarged at the scale of 1:2000 to form eight photo-mosaics. A detailed map was drawn from this photographic composition by plotting buildings, roads (out-lines and axes), and tree-covered land. The hand-sketched maps were digitized into Geographic Information System (GIS) layers (PC Arcinfo format) according to the following specifications:

⇒ different geographical features belong to different layers;
⇒ each housing unit is coded according to the numbering system adopted;
⇒ the basic projection is UTM (Universal Transverse Mercator);
⇒ dista nces in the UTM projection are in meters and in a reference grid of lines of latitude and longitude.

An *ad hoc* data-base package is being adapted to the specific data collection needs with particular attention to a user-friendly approach and reports print-out capacity.

Molecular epidemiology

The prevalence of *V. cholerae* 01and non-01, the clonal analysis of the strains responsible for the second Somali cholera epidemic, the characterization of the genetic bases for the appearance of drug resistance phenotypes, and the distribution of characters of toxinogenicity and drug resistance in strains of *V. cholerae* non-01, have been defined for most Somali regions of the Horn of Africa during an 8-year period.

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Contract number TS3*CT930255

ORAL VACCINE AGAINST CHOLERA WITH "BUILT-IN" ADJUVANTICITY

Period: October 1, 1993 — September 30, 1995

Co-ordinator: ISTITUTO RICERCHE IMMUNOBIOLOGICHE SIENA
Siena, Italy (S. RAPPUOLI)

Objectives

The aim of this project is the development of new vaccines against diarrhoeal diseases (such as cholera and enterotoxigenic *E. coli*), based on the immunization with live-attenuated strains of *V. cholera* and *Salmonella* expressing non-toxic derivatives of cholera and heat-labile toxins.

To further increase the immunogenicity of the antigens expressed, *in vivo,* by the attenuated strains, we have engineered the *Salmonella typhimurium* strain to express a peptide derived from IL-1β peptide that has been proposed to be a good adjuvant.

Activities

Design, construction, purification and characterization of LT and CT mutants

Heat-labile toxin (LT) is a bacterial protein with ADP-ribosylating activity, produced by enterotoxigenic *E. coli* strains, structurally and functionally related to Cholera toxin (CT). These two toxins share 80% sequence homology and the same 3D structure. LT and CT are organized as AB_5 hexamers, where the homopentameric B subunit binds the receptor on the membrane of eukaryotic cells, while the A subunit is responsible for the ADP-ribosylation of the α subunit of G_s a GTP-binding protein.

Using the known 3D structure of LT and computer modelling analysis, we have identified residues previously not known to be important for enzymatic activity, and provided the rationale to probe their function by changing them by site-directed mutagenesis. We have generated a number of different mutants of LT and CT, expressed them in *E. coli* and *V. cholera* strains, respectively, purified and characterized. Some of them have been found to be completely devoid of enzymatic activity, both, *in vivo* and *in vitro*. We have analyzed the biochemical and immunological properties of the non toxic mutants to define the influence of the mutations in the A subunit on the assembly, stability and immunogenicity of each of the mutant molecules. We have obtained mutants in which the amino-acid substitution had not affected the toxicity; mutants in which aminoacid substitutions had dramatically reduced the toxicity and mutants in which the substitution

had prevented the formation of the A/B holotoxin. By the study of the biochemical and immunological properties of the nontoxic mutants, we have found that, single amino-acid substitions in the A subunit may affect not only the enzymatic activity, but may also have profound effects on the ability to form the AB_5 structure, on the stability during long-term storage, and on the trypsin sensitivity and immunogenicity of the assembled mutant molecules.

Among the non toxic mutants tested, the CT-K63 and the analog LT-K63 mutants, proved to be non toxic, well assembled, stable to trypsin treatment, and able to induce neutralizing antibodies against both the A and B subunit. This property suggests that the A subunit plays an important role in protective immunity and raises the possibility of using these molecules to improve vaccines against LT and cholera.

Expression of CT-K63 in a Vibrio cholera attenuated strain: IEM101

The *V. cholera* IEM 101 strain is an attenuated El Tor strain isolated in China. This strain does not contain the genes encoded by the entire virulence cassette. IEM 101 has been used both in rabbits and humans to study its immunogenicity and toxicity. In rabbits, IEM 101 was able to induce protection against the challenge with a virulent strain after immunization with a single dose. In humans it has been shown to be safe, able to colonize the gut and to induce a strong immune response.

We have studied the ability of IEM 101 to produce, correctly assemble and secrete into the supernatant the wild-type CT as well as the CT-K63 mutant protein, and we have tested the toxicity, *in vivo*, of the recombinant strains. The results showed that IEM 101 was able to produce and secrete into the supernatant, the wild-type toxin, as well as the mutant toxin. The results of the toxicity *in vivo*, in a Rabbit ILeal Loop assay showed that IEM 101 expressing wild-type CT was able to induce fluid accumulation, while IEM 101 expressing CT-K63, did not. The insertion of the mutated gene into the chromosome of IEM 101 is underway.

The interleukin-1β peptide

The nonapeptide sequence VQGEESNDK, corresponding to the aminoacids 163-171 of IL-1β, and the pentapeptide GEESN, has been reported to retain the immunoenhancing properties and to be devoid of proinflammatory activity of the entire IL-1β molecule.

Contract number TS3*CT930255

To increase the ability of *Salmonella* strains to induce immunity against recombinant antigens, we have engineered the strain to express this peptide, derived from IL-1β, using as carrier proteins the flagellin of *Salmonella*, LamB and MalE of *E. coli*.

The level of expression of the recombinant proteins and the immunogenicity induced by the recombinant strains, or by the purified protein, has been evaluated.

The results showed that all the recombinant proteins maintained their functionality and that the amount of each of the native proteins produced were comparable to that of the corresponding chimera proteins. The recombinant strains were used to immunize (i.p.) BALB/c mice. The results showed that the presence of the nonapeptide in the flagellen expressed by *Salmonella* led to an increase in immunogenicity of about 2.5-fold, confirming previous data obtained with the purified protein. In the case of LamB, the immune response induced in mice immunized with *Salmonella* strains carrying the recombinant LamB gene was similar to that induced in mice immunized with the strain carrying the native LamB gene. In the case of malE, the presence of the nonapeptide had a weak immunadjuvant effect, which could only be detected using low doses of antigen, and a low-responder strain of mice. With higher doses or after a booster, and with a high-responder mouse strain, no difference could be seen.

Expression of LT-K63 in attenuated Salmonella typhimurium straius

Four attenuated *S. typhimurium* strains of different serotypes, three of them carrying the virulence plasmid and one which does not contain the virulence plasmid, have been used for the expression of the LT-K63 mutant protein. The gene coding for LT-K63 has been cloned in different plasmids with low, moderate, medium and high copy numbers. The correlation between the copy number of the different plasmids combined with the presence of the virulence plasmid, and the *in vitro* expression level of LT-K63 has been evaluated. The recombinant strains have been used for oral immunization in mice and the immunoresponse induced has been assessed. The results showed that the different *Salmonella* strains were able to produce, assemble and secrete the mutant protein into the periplasm. The *in vitro* expression level of LT-K63 showed a good correlation with the copy number of the different plasmids used, so that the amount of LT-K63 produced was higher when the high copy number plasmid was used.

The results of the immunogenicity experiments showed that after a single oral immunization, the mice immunized with the strain carrying the virulence plasmid and expressing the highest level of LT-K63 mount a high anti-LT IgG response in the sera, as well as an IgA response in the mucosa, starting from the second week after immunization.

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Contract number TS3*CT940292

THE USE OF CONTROLLED RELEASE MICROPARTICLES AS ORAL AND PARENTERAL VACCINES AGAINST *VIBRIO CHOLERA, ESCHERICHIA COLI* AND *BORDETELLA PERTUSSIS*

Period: June 1, 1993 — May 31, 1997

Co-ordinator: UNIVERSITY OF NOTTINGHAM, DEPT. OF PHARMACEUTICAL SCIENCES, Nottingham, United Kingdom (S.S. DAVIS)

Objectives

- To assess the efficacy of microparticles with entrapped antigens as oral vaccines against two important diarrhoeal causing pathogens, *V. cholera* and enterotoxigenic *E. coli.*
- To design and test a single dose parenteral vaccine against *Bordetella pertussis.*

Activities

* The following antigens were provided for the project CTB, CFA 1, PT and FHA. The antigens for the studies were extracted from bacteria, CTB and CFA 1 were supplied by Göteborg and PT and FHA were supplied by the National Institute of Biological Standards, UK.
* Poli (lactide-co-glycolide) (PLG) family of polymers were selected for use in production of vaccine containing microparticles. Two PLG polymers were chosen, primarily for their degradation properties, PLG 75;25 (lactide: glycolide ratio) and PLG 50;50. PLG 75;25 is a slower degrading polymer than PLG 50;50. This would enable us to assess any long term immunological effects of the microparticulate vaccines. The results would be compared to those obtained for PLG 50;50.
* *In vitro* characterisation of the PLG microparticles. Microparticles were produced using an established water in oil in water emulsion solvent evaporation technique, using CTB *B. pertussis* antigens and also tetanus toxoid conjugated to LHRH. The microparticles with entrapped antigens were characterised for protein content and surface morphology. Microparticles with entrapped CTB were investigated for integrity of entrapped as well as surface associated antigen. This was carried out with a GM1 ELISA assay. The results have shown that entrapped CTB retains its GM1 binding activity after release form the microparticles. Surface associated CTB also retains its ability to bind to GM1. Antibodies for the detection and for use in determination of the integrity of *B. pertussis* antigens is still under progress. Once these are available they will be employed to assess integrity of *B. pertussis* antigens and also for quantitation of *B. pertussis* antigens released *in vitro.*
* Immunogenicity *in vivo* of *B. pertussis* antigens PT and FHA entrapped in microparticles was assessed. The routes of immunisation investigated, were intra-peritoneal, subcutaneous, intra-muscular and nasal. The results so far have shown that the nasal delivery of entrapped *B. pertussis* antigens does not induce protective immunity, with low levels of *B. pertussis* specific IgG antibody produced. However, parenteral delivery of *B. pertussis* antigens entrapped in microparticles producing high levels of *B. pertussis* antigens entrapped in microparticles producing high levels of *B. pertussis* specific IgG antibodies.

Contract number TS3*CT940292

* The nasal administration of *B. pertussis* antigens entrapped in microparticles was carried out while the animals were anaesthetised. Previously animals have not been anaesthetised and some degree of protection by soluble *B. pertussis* antigens has been conferred. This was not seen for the studies in this project. This may be a result of the anaesthetic reducing the cilia motility in the nasal mucosa and possibly reducing in uptake of microparticles in the nasal mucosa.
* The ability of microparticles to function as delivery systems for oral vaccines may be enhanced by targeting ligands designed to improve the uptake of particles into Peyer's patches. The targeting ligand which we have investigated in the first instance is CTB. The work so far has concentrated on the use of novel Dextran polymers to stabilise a PLG microparticle, followed by addition of CTB. Conjugation of CTB to the surface of the microparticles was achieved though levels of CTB attached were found to be low. The integrity of the attached CTB was also ascertained by investigation of the ability of CTB to bind to its GM1 receptor. The GM1 binding activity was found to be retained. This work will be extended to include surface attachment of other targetting moieties and also the co-entrapment of CTB with vaccine antigens.

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Contract number TS3*CT940311

PERSISTENT DIARRHOEA IN EARLY CHILDHOOD — A PROSPECTIVE COMMUNITY STUDY

Period: January 1, 1995 — December 31, 1997

Co-ordinator: STATENS SERUMINSTITUT, EPIDEMIOLOGY RESEARCH UNIT, Copenhagen, Denmark (K. MØLBAK)

Objectives

The aim of the study is to improve community — and health-center based management of acute and persistent diarrhoea in early childhood. Furthermore, we wish to suggest vaccine-based or other targeted interventions against acute and persistent diarrhoea, and explore the long term impact of infections with specific enteropathogens on child survival and growth faltering.

Finally, the study will provide a substantial technology transfer to the National Public Health Laboratory in Bissau, and exchange and training of scientists, health workers and technicians.

Activities

The core of the study is an ongoing prospective community based surveillance for diarrhoeal disease, which is carried out in a semi-urban area of Bissau, the capital of Guinea-Bissau. All children below three years of age, residing in 600 randomly selected houses, are followed by weekly visits. At these household interviews, we obtain information on diarrhoeal diseases, other morbidity, and feeding patterns. All children have their height and weight measured at three-monthly intervals. In addition, more detailed anthropometric follow-up and microbiological examination of weekly collected stool samples are carried out in cases of persistent diarrhoea and in the intensive cohort (see below).

Within this frame, the study contains three work packages:

* A community-based, randomized controlled trial (RCT), comparing standard oral rehydration salt (ORS) with reduced osmolarity ORS.
* The development and controlled evaluation of an algorithm for the appropriate management of persistent diarrhoea.
* An intensive cohort study with weekly collection of stool specimens from birth to the age of two years.

The aim with this third package is to determine the microbial etiology of acute and persistent diarrhoea and to characterize in detail persistent, sequential and repeated infections by, as well as disease-to-infection ratios, for major enteropathogens. These data will be used for the investigation of the long term impact of infection with specific enteropathogens.

Results

Data collection for the RCT of reduced osmolarity ORS will be completed by the end of 1996, with a total of 600 episodes of acute diarrhoeal episodes included. If low-osmolarity ORS proves to be more efficacious and/or acceptable than standard ORS, a strong case has been made for changing the official recommendations for the composition of ORS.

Contract number TS3*CT940311

For the management of persistent diarrhoea (PD), an algorithm was adapted during 1995. The algorithm is primarily based on dietary therapy with a frequently offered modified traditional diet, a millet gruel with an energy density of 96 kcal/100 g, and a protein content of 2.3%. In addition, children with PD are examined at a health center, and treated with antibiotics or antimalarials upon scientific indications. Follow-up includes detailed anthropometry, including knee-heel measurements, in order to evaluate catch-up growth. Children with PD from a carefully selected control group receive the same clinical examination, treatment of severe infections, and anthropometric follow-up as the algorithm group.

The intensive cohort, with approximately 150 children, will be assembled during 1996. The sampling scheme, with weekly collection of stool specimens was implemented from early 1996, during the assembly of the cohort. In 1995-1996, the diagnostic setup was established at the National Public Health Laboratory in Bissau. The diagnosis of enteropathogens is undertaken by a combination of conventional and probe-based microbiological techniques. For the detection of pathogenic *E. coli,* bacterial lysates are screened by a panel of DNA probes. For this purpose, a number of different probes identifying the virulence factors of the most important diarrhoeagenic types of *E. coli* as well as *Shigella* spp. have been modified and cloned into the same vector plasmid, PBS, thereby enabling an effective labelling with the non-radioactive marker digoxigenin by the polymerase chain reaction using the same set of primers for all the different probes. Because of the substantial sample load, a system of pooling the individual probes has been developed as well as systems for the handling and transportation of the large number of probe-positive strains.

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Contract number TS3*CT940327

DEVELOPMENT AND TESTING OF AN ORAL B SUBUNIT — WHOLE CELL CHOLERA VACCINE: PROTECTION AGAINST BOTH 01 AND 0139 CHOLERA

Period: January 1, 1995 — December 31, 1996

Co-ordinator: UNIVERSITY OF GÖTEBORG, DEPT. OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY, Göteborg, Sweden (J. HOLMGREN)

Objectives

- To test the safety and intestinal mucosal IgA immunogenicity of a new, bivalent B subunit-01/0139 whole cell (B-01/0139 WC) oral cholera vaccine in comparison with a recently licensed B-01-WC vaccine in volunteers in preparation for later phase 3 field trial efficacy testing of the new vaccine.
- To undertake basic research to further define protective immunological mechanisms and possible additional vaccine candidate antigens in cholera due to *V. cholerae* 0139.

Activities

* To assess in humans the safety and immunogenicity of the proposed new bivalent B-01/0139 WC vaccine and to compare the immune response obtained after vaccination with the responses in convalescents from cholera disease caused by natural infection with *V. cholerae* 0139.
* To develop a methodology for measuring vaccine-specific gut mucosal immune responses by non-invasive techniques in humans.
* To define the possible relevance of capsular antigen as a virulence factor and protective antigen in *V. cholerae* 0139.
* To test the immunogenic properties of a new oral vaccine formulation based on conjugation of LPS and putative capsular antigens to cholera B subunit.

Expected Outcome

The project will significantly contribute to the development of a safe and protective-immunogenic bivalent oral cholera vaccine that could be an effective tool in the prevention and control of cholera disease due to either *V. cholerae* 01 or 0139 organisms/serotypes.

Results

A first phase study of safety and immunogenicity of bivalent B-01/0139 WC vaccine prepared by SBL Vaccine, Sweden compared with the licensed B-01 WC vaccine (same producer) has been completed in Göteborg. As with the B-01 WC Vaccine, the B-01/0139 WC vaccine was found to be safe and immunogenic. Two vaccine doses given 2 weeks apart induced significant mucosal IgA antibody responses in intestinal lavage fluid against cholera toxin in 9 vaccinees and against both 01 and 0139 vibrios in 7 of 9 cases. A third dose of vaccine given after 5-6 weeks did not result in any further increased response.

All of 12 vaccines responded with significant IgA and IgG anti-toxin responses in serum. Such responses are associated with significant vibriocidal antibody titre rises against 012 vibrios in 10 cases (83%) and against 0139 vibrios in 8 vaccinees (67%). The frequencies and magnitudes of the serological responses to the B-subunit and 01 WC components were similar to those induced by the B-01 WC vaccine.

A similar study is in progress in Bangladesh by ICCDR, B. The immune responses after vaccination will also be compared with those in convalescents after clinical infection with *V. cholerae* 01 and 0139, respectively.

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Large phase II studies of consecutive lots of vaccine prepared by SBL Vaccine as compared with a placebo vaccine have been undertaken in 700 individuals in Germany. All the sera collected will be tested "blindly" in Sweden to determine the frequency and magnitudes of serological immune responses to the B and different WC vaccine components.

An extensive basic study in rabbits has been completed in Göteborg defining the immune mechanisms and protective antigens of *Vibrio cholerae* serogroup 0139 as a basis for vaccine development. The results support the critical dependence of 0139 LPS antigen for inducing protection, a lesser role for antitoxic immunity than in 01 cholera but still evidence for clear synergy between anti-0139 antibacterial and antitoxic (anti-B subunit) immunity. A demonstrated cross-reactivity between 0139 LPS and capsule makes the inclusion of specific capsular antigen in the vaccine less a priority than at the time of application and this research line has therefore not been pursued further.

Studies have been undertaken in both Göteborg-Lyon and in Helsinki to define immunological surface markers and especially so-called homing receptors on intestine-derived vaccine-specific circulating B cells after cholera (and for comparisons oral typhoid and injected tetanus) vaccinations. Similar analyses have also been performed on B cells derived in response to clinical cholera infection (collaboration between Helsinki and ICDDR,B). The results clearly indicate that circulating B cells derived from intestine and collected approximately one week after oral vaccination or onset of clinical disease differ from total circulating B cells or specific B cells obtained after parenteral vaccination by expressing the mucosa-homing receptor *alpha4/beta7* integrin (also known as ACT-1). Based on this, we hope to develop a more sensitive assay for detection of specific gut-derived, specific antibody-producing cells that could be used also in young children.

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Contract number IC18*CT960027

EARLY EVENTS IN ROTAVIRUS INFECTION: ROLE OF VIRAL PROTEINS ON PARTICLE INTERNALIZATION AND MEMBRANE PERMEABILITY

Period: October 1, 1996 — September 30, 1998

Co-ordinator: INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE UNITE DE VIROLOGIE ET IMMUNOLOGIE MOLECULAIRES
Jouy-en-Josas, France (J. COHEN)

Objectives

- To identify the domain of VP4 interacting with sialic acids.
- To characterize the mechanism of membrane destabilization and pore formation.
- To determine the viral protein(s) and/or domain(s) responsible for membrane destabilization.
- To study the role of chaperones in folding and oligomerization of ET-associated proteins (VP7, NSP4).
- To elucidate the role of various genes implicated in early events of rotavirus infection.

Activities

* Scientific information exchange between scientists involved in the project in five countries.
* Exchange and dissemination of valuable reagents among the participant laboratories. Exchanges of methodology by short stays of scientists in the lab of another contractant.
* Organizati on of contractants meetings to produce relevant questions and methodology to answer them.
* Common preparation of publication in high standards journals.

Expected outcome

⇒ Insight in the mechanisms that allow penetration into the cell of large nucleoprotein complexes.

⇒ New strategies for prevention of gastro-intestinal diseases.

Contract number IC18*CT960027

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Contract number IC18*CT970231

PATHOGENESIS OF VIBRIO CHOLERAE INFECTION AND PERSISTENCE DYNAMICS IN THE HORN OF AFRICA

Period: December 1, 1997 — May 31, 2001

Co-ordinator: UNIVERSITÀ DI ROMA "LA SAPIENZA", Centro Interuniversitario di Ricerca sui Paesi in via di Sviluppo, Rome, Italy (F. MAIMONE)

Objectives

- To yield new, crucial information on the pathogenesis of *Vibrio cholerae* infection as a necessary basis for creating effective vaccines: role and dual function of the zonula occludens toxin (ZOT) encoded by the *V. cholerae* filamentous phage CTXF.
- To design dynamic models for persistence of primary infection events and secondary transmission of the infection in critical geographic areas for the recurrent spreading of the disease.

Activities

The studies of pathogeneis of *V. cholerae* infection will focus on: 1) identifying the mechanisms of action of ZOT by intracellular signalling and cytoskeleton rearrangement; 2) establishing the effect of ZOT on water and electrolyte transport via the paracellular pathway; 3) analysing the regulatory system and functions of the *zot* gene by TnPhoA fusions and site-directed mutagenesis.

The studies of cholera transmission will concern the screening of aquatic environments as possible *V. cholerae* reservoirs and epidemiological and microbiological investigations based on a Geographic Information System infrastructure (GIS). Collection of demographic baseline data, follow-up studies at the household level, and case-control studies of risk factors will be conducted in Harar (capital of Harari Regional State of Ethiopia) and towns and villages in the Somali Regional State of Ethiopia and in Somalia.

To assess genetic diversity and relationships in the *V. cholerae* population distributed in humans and in the environment, a high number of strains of *V. cholerae* (01 and non-01) from cases with different clinical features and from environmental sources will be analysed by a combination of genotypic methods.

Expected outcome

⇒ The role of ZOT toxin in modulating biochemical modifications of tight junctions up to their disassembly in intestinal epithelium will be characterized;
⇒ ZOT diarrhoegenicity in comparison with cholera toxin will be established;
⇒ Environmental factors and genetic components regulating expression of *zot* gene will be identified;
⇒ The analysis of the microbiological and epidemiological data integrated into a geographic and demographic information system will identify determinants for maintenance of endemicity and for infection propagation. By combining these results with the pattern of genetic relatedness between the prevalent *V. cholerae* strains, dynamic models for persistence and transmission will be proposed as practical contributions for designing realistic cholera control strategies.

Contract number IC18*CT970231

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8. Diphtheria and Tetanus

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number IC18*CT950012

EVALUATION OF DIPHTHERIA- AND TETANUS-TOXIN NEUTRALIZING ANTIBODY ACTIVITY IN HUMAN AND ANIMAL SERA

Period: January 1, 1996 — December 31, 1998

Co-ordinator: NATIONAL INSTITUTE OF PUBLIC HEALTH AND THE ENVIRONMENT (RIVM)
Bilthoven, The Netherlands (C. HENDRIKSEN)

Objectives

- To investigate the nature of the reported absence of correlation between *in vitro* toxin neutralization assays and *in vivo* neutralization tests for diphtheria (and to a lesser extent tetanus) antibodies and to develop a practical solution for this phenomenon.
- To improve research and development capacity in vaccine quality control laboratories of public sector vaccine producers in two developing countries (Indonesia and Vietnam).
- To ensure quality in immunization programmes and local vaccine production by developing and making available scientifically validated test systems that can be used in developing countries.

Activities

* Establisment of anti-diphtheria serumpanels from 4 different species.
* Study of kinetics of neutralization test systems for diphtheria in *in vitro* tests (Vero cell test, ToBI, Double antigen ELISA, DELFIA) and *in vivo* tests (skin test in guinea pigs) using high, intermediate and low doses of toxin.
* Stand ardization of one or two selected *in vitro* toxin neutralization test systems, with established validity for the *in vivo* neutralization mechanism.
* Improve horse hyper-immunization schedules and introduction of plasmaphoresis.
* Provide research training to staff at RIVM (two from Vietnam and two from Indonesia) and implement/improve *in vitro* techniques in Vietnam and in Indonesia.
* Hold two project meetings: one at the onset of the project and one at mid-term, to upgrade research training.

Expected outcome

⇒ Understanding of the factors which have an effect on the mechanism of toxin neutralization.

⇒ Recom mendations to regulatory authorities on the selection and standardization of *in vitro* toxin neutralization tests.

⇒ Research training to 2 Bio Farma and 2 IVAC staff workers for a period of 4 months each.

⇒ The organization of a workshop on the immunological aspects of diphtheria toxin neutralization and antiserum production.

⇒ Improved horse immunization schedules and the introduction of plasmaphoresis for the production of anti-D serum.

Contract number IC18*CT950012

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9. Hepatitis

Areas of interest:

9. Hepatitis

Contract number TS3*CT930259

EPIDEMIOLOGICAL CLINICAL AND SERO-VIROLOGICAL STUDIES OF HEPATITIS C IN GABON AND BRAZIL

Period: February 1, 1994 — January 31, 1997

Co-ordinator: INSTITUT DE MEDECINE ET D'EPIDEMIOLOGIE AFRICAINES/INSERM U13, Paris, France (B. LAROUZÉ)

Objectives

- To evaluate the importance of hepatitis C virus (HCV) in terms of public health in two countries with different levels of endemicity in order to design preventive strategies.
- To collect information on the variability of HCV strains and serological patterns in order to improve diagnostic procedures and contribute to vaccine design.
- To describe the distribution of HCV infection, to identify its risk factors and to study its transmission modes.
- To investigate its relationship to chronic liver diseases and hepatocellular carcinoma.
- To compare the structures of HCV strains circulating in these countries and related serological patterns; to investigate serological cross-reactions with related viruses.

Activities

In order to evaluate the importance of hepatitis C virus (HCV) in terms of public health in two countries with different levels of endemicity (Gabon: high level, Brazil: low level), we will study, in each country, the distribution of hepatitis C infection in different geographical settings, in general and high risk populations. Additional studies will be dedicated to risk factors in a community based study in Gabon where the prevalence of antibody to HCV is much higher (7%) than in Brazil (1-2%). As a complement of this last study, a clinical and virological study of anti-HCV positive subjects will be performed in order to evaluate the clinical impact of HCV infection. In addition, case-control studies in Gabon and Brazil will allow the determination of risk for cirrhosis and HCC attributable to HCV. From the same material, sero-virological studies of HCV will be designed using PCR techniques to compare the structures of HCV strains circulating in these countries and related serological patterns and to investigate serological cross-reactions with related viruses. These studies will provide informations to elaborate prevention strategies (including HCV blood screening in blood banks), to improve serodiagnostic techniques and to contribute to the development of future HCV vaccines.

Expected outcome

By knowing the epidemiology and clinical impact of HCV in Gabon and Brazil, and after the interpretation of the sero-virological studies, much will be gained in terms of introduction of mandatory testing in blood banks and immunoprophylaxis when a vaccine becomes available.

These studies will provide a basis for decision making by health authorities from these countries and from countries with similar HCV patterns. Insight into HCV prevention would complement the effort developed in Africa and in Brazil (programme developed by the Federal Government) to control HCV infection.

The sero-virological studies will allow the design of serological tests adapted to the regional variability of HCV strains and taking eventually into account cross reactivities with

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related viral agents. These tests will be used for epidemiological investigations, clinical diagnosis and, if the health authorities decide to screen blood donors, will be used for this purpose. In the long term, results of the sero-virological studies will contribute to the design of anti HCV vaccines.
The implementation of this protocol will be based on a scientific network which will reinforce links between participants and improve their scientific skills.

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10. Other

Presentation of EC supported joint research projects (1991-1996) continued
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT910035

LEPTOSPIROSIS IN THE CARIBBEAN: A STUDY ON SHORT AND LONG TERM PATHOLOGICAL CONSEQUENCES AND LEPTOSPIROSIS IN BENIN

Period: May 1992 — November 1994

Co-ordinator: KONINKLIJK INSTITUUT VOOR DE TROPEN, DEPT OF BIOMEDICAL RESEARCH Amsterdam, The Netherlands (R.A. HARTSKEERL)

Objectives

The aim of this project was to investigate mortality, pancreatic damage and chronic infection in leptospirosis, and to further develop the polymerase chain reaction (PCR) for rapid diagnosis of leptospirosis.

The objectives are:

- To perform post mortem (PM) studies on humans and animals, comparing the capacities of the methods of culturing, histopathology and PCR for the detection of leptospires.
- To evaluate the PCR on human serum for diagnosis of leptospirosis in the Caribbean region.
- To explore the prevalence of leptospirosis in Benin and its consequences for human welfare and animal productivity.

Activities

* Introduction of culturing techniques and immunofluorescence staining by Veterinary Research Laboratory (VRL) at the Leptospira laboratory (LL) on Barbados and CAREC on Trinidad and subsequent application on post mortem tissues from patients suspected to have died of acute leptospirosis.
* Implementation of PCR on serum and urine specimens at LL on Barbados and CAREC on Trinidad. Development of a PCR assay on post mortem tissues by KIT and implementation of the assay on Barbados and Trinidad.
* Establishment of a leptospirosis research unit in Benin.

Results

A PCR for the diagnosis of leptospirosis using two pairs of primers, i.e. G1/G2 amplifies DNA from the pathogenic species *L. interrogans', L. borgpetersenii, L. weilii, L. noguchi, L. santarosai* and *L. inadai*. Primer set B64I/B64II amplifies DNA from the pathogenic species *L. kirschneri*. The PCR with the two primer pairs was optimized for application on blood and urine samples and successfully implemented in Barbados and Trinidad. To adapt PCR for post mortem tissues, a mechanical disruption of tissue with a mini-bead beater was included in the protocol. Immunofluorescence staining was introduced at MRC and CAREC by VRL, while culturing techniques were introduced by both VRL and KIT. The part of the project entitled "leptospirosis in Benin" started in November 1993 by establishment of a leptospirosis unit (LU) in Cotonou during a working visit of staff of KIT at Cotonou.

Ad. 1 Post mortem tissues On Barbados and Trinidad, PM tissues were collected from acute 16 cases of leptospirosis with a fatal course as well as from 12 cases without leptospirosis that served as control. Leptospiral DNA was demonstrated by PCR in tissue samples from 7 of the 16 patients. Culturing, performed on tissues from 12 patients did result in the isolation of pathogenic leptospires from only one case. IF performed on tissue from 3 cases confirmed the results from PCR in two cases. Histopathological disorders were heart, spleen and kidney.

Contract number TS3*CT910035

Ad. 2 PCR on urine and serum samples On Barbados a total of 82 blood and urine samples from 71 patients with proven leptospirosis (i.e. by seroconversion and/or culture) were tested by culturing and PCR were performed on first samples, consisting of 62 blood and 20 urine samples, mostly before seroconversion occurred. Second samples were used to establish seroconversion. The mortality rate in this group of patients was 16.9% (12 of 71 patients). Samples of 16 patients without leptospirosis were used as controls. Overall, PCR demonstrated the presence of leptospires infections in 44 of the 72 cases (62%), whereas a positive culture was obtained in only 34 cases (48%). Positive PCR results were obtained in 41 blood samples and 33 samples yielded positive cultures. In urine, PCR was positive in 10 samples whereas culturing was positive in 7 samples. These results indicate that PCR, either performed at serum or urine, is a valuable adjuct for early diagnosis of leptospirosis.

Ad. 3 Establishment of a leptospirosis research unit in Benin The microscopic agglutination test using representative serovars from 15 serogroups applied on numerous samples from inhabitants from various cities and districts in Benin suggested a seroprevalence in Benin varying from about 24 to 35%.

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11. Non Communicable Diseases

Presentation of EC supported joint research projects (1991-1996)
STD3
INCO-DC: 1st and 2nd Call

Areas of interest:

Contract number TS3*CT910024

BITES AND STINGS BY VENOMOUS ANIMALS IN BRAZIL: CLINICAL AND LABORATORY INVESTIGATIONS OF ENVENOMING AND THERAPY

Period: April 1, 1992 — March 31, 1995

Co-ordinator: LIVERPOOL SCHOOL OF TROPICAL MEDICINE, VENOM RESEARCH UNIT Liverpool, United Kingdom (R.D.G. THEAKSTON)

Objectives

The aim of the project is to assess the problems caused by venomous bites and stings in Brazil.

Activities

* Complete monitoring of patients with severe envenoming will be carried out. The effect of antivenom in reducing both the extent of swelling and local necrosis will be investigated, as will the problem of possible pituitary and adrenal insufficiency caused by pituitary infarction.
* The use of tourniquets in systemic envenoming will be evaluated in 20 patients and 20 controls.
* The prophylactic use of antimicrobial drugs will be investigated in patients with moderate and severe systemic envenoming, to determine whether or not organisms present in the oral cavity of venom or on the gangs of wild snakes result in wound infection in the bitten patient.
* An investigation of whether routine prophylactic antihistamine prevents both early and late reactions to antivenom will be carried out in both moderately and severely envenomed patients in a randomized double blind placebo-controlled trial.
* Thirt y patients with moderate envenoming will receive half the lowest dose of antivenom given earlier, and 30 will receive the same starting dose as given in the previous project. Recovery from clinical signs, venom clearance, circulating antivenom levels, coagulation and fibrinolytic, hematological and biochemical profiles will be compared.
* Laboratory studies will be carried out to assess the significance of the ELISA method for detection of functional molecules rather than immune complexes.
* Studies on patients envenomed by scorpions and spiders will be carried out in Sao Paulo and Belo Horizonte to investigate the kinetics of envenoming and therapy.
* The detection of specific venom in lesions caused by *Loxosceles* spider bites will be carried out.
* Investigation of the pathogenesis of envenoming by *Crotalus durissus terrificus* in Sao Paulo and Minas Gerais States will be carried out to improve patient treatment.
* To discover the importance of snake bite, to assess the traditional methods of treatment and to try and improve the management of severe cases in indigenous Indian and other associated populations.
* The latest techniques of "molecular genetics" and multivariate analysis of morphological/anatomical features in comparison to venom composition will be applied to provide practical guidelines for antivenom manufacturers to ensure adequate neutralizing efficacy of their products throughout Brazil.
* To clone *Bothrops* myotoxins.

Expected outcome

Knowledge of the pathophysiological effects of venom components and the establishment of appropriate treatment schemes for patients with envenoming.

Contract number TS3*CT910024

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Contract number TS3*CT920081

THALASSEMIA INTERMEDIA SYNDROME: MOLECULAR HEMATOLOGICAL, CLINICAL AND THERAPEUTIC STUDIES

Period: December 1, 1992 — November 30, 1995

Co-ordinator: NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS, DEPT OF PED. THALASSEMIA UNIT, ST. SOPHIÈS HOSP.
Athens, Greece (C. KATTAMIS)

Objectives

Of the two clinical phenotypes of thalassemia (Major and Intermedia), thalassemia (Thal-I) is not well defined and studied.

The main objective of this project is to establish precise criteria for characterization of Thal-I, both at the molecular as well as clinical and hematological levels utilizing existing and new techniques.

Activities

Defin ition of criteria for clinical assessment in a data base format

* Selection of Thal-I patients for study in Athens and Bangkok.
* Chara cterization of common hematologic phenotypes and genotypes.
* Applicatio n of a selection and analysis technique of fetal nucleated cells in maternal circulation.

Completion of characterization of common phenotypes and genes of Thal-I

* Identification of rare — and probably new — mutations.
* Intervention trials with hydroxyurea (HU) (Phases I and II) in Thal-I).
* Application of fetal cell selection in antenatal diagnosis.
* Study of factors enhancing γ-chain production in selected families.

Evaluation of results on common genotypes and phenotypes

* Listing and distribution of rare mutants in the Greek and the Thai population.
* Evaluation of intervention trials with HU.

Expected outcome

⇒ Stand ardized criteria for assessment of clinical severity of Thal-I, and definition of the most quick, accurate and inexpensive methods for diagnosis.
⇒ Transfer and utilization of new technology by the collaborating units; this will result in a precise evaluation of effectiveness and applicability of the new techniques in clinical diagnosis.
⇒ Identification of the genotypes and hematological phenotypes of Thal-I, in which treatment with hydroxyurea may be most effective.

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Results

Further to the primary results summarized in the first progress report, the three centers have proceeded in their respective and collaborative parts of the project according to schedule:

⇒ On the basis of the criteria set out in the first 6 months, the selection of Thal-I patients in Greece and Thailand continued and the number of samples so far collected exceeds 200.
⇒ All patients have been subjected to full clinical and hematological characterization, while the molecular characterization is in progress. Of interest is the characterization of new mutation (codon 29 in the a2 globin gene) amongst the Greek HbH patients.
⇒ Until now amongst the high HbA2-B-thal Thal-I cases, a wide range of previously defined B-gene mutations have been found.
⇒ During this time the DGGE method for a-gene mutations standardized in Leiden has also been set up and applied in Athens.
⇒ Methods for effective harvesting of foetal cells from maternal circulation continues to be investigated in Leiden.
⇒ The initial results from the 6 months clinical trial were positive; the trial will be extended as proposed.
⇒ The three centres are all involved in the organization of a wetlab workshop entitled "Recent advances in the detection of single-base mutations" took place in Bangkok, Thailand 1 - 5 August 1994.

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Contract number TS3*CT920142

NUTRITIONAL INFLUENCES ON THE EMERGENCE OF HIGH BLOOD PRESSURE AND DIABETES IN BLACK AFRO-ORIGIN POPULATIONS IN CAMEROON, JAMAICA, FRANCE AND ENGLAND

Period: April1, 1993 — March 31, 1995

Co-ordinator: UNIVERSITY OF MANCHESTER MEDICAL SCHOOL, CLINICAL EPIDEMIOLOGY UNIT
Manchester, United Kingdom (J.K. CRUICKSHANK)

Objectives overall

To establish the role of nutrition in the emergence of high blood pressure and diabetes in African-origin populations.

Specific:

- To establish random samples of African-origin populations in Cameroon, West Africa, Jamaica and Martinique, West Indies (in both rural and urban samples), in Paris, France and Manchester, UK (urban only)
- To establish in these samples average nutrient intakes by food frequency questionnaire (FFQ) in people aged 25-74 years, weighted towards 40-64 years. Using standardised methods, to relate these intakes to the prevalence of high blood pressure (BP) and glucose intolerance/diabetes as major emerging health problems in these populations.
- To assess how nutrient intake varies with other life style factors including smoking, alcohol and exercise habits, social indices and income.
- To test the hypothesis that decreased potassium (based on 24 hour urine collections) and increasing fat intake are related to increasing BP and deteriorating glucose tolerance (GT) and are associated with disordered insulin secretion and action ("insulin resistance").
- To assess whether a "transition" of the distributions of BP and GT, as well as of insulin secretion, is detectable both within and across the four populations in relation to energy and macro-nutrient intakes.
- To use these data to plan a later incidence and nutritional and lifestyle-based primary prevention programme for high BP and diabetes in the same population samples.

Materials, Methods and Activities

The 3 fieldwork centres were to carry out a closely similar programme with standardised protocols and procedures. Some local variations would be necessary but the general format and order was to be the same in each site, observers being trained by the same techniques.

Contract number TS3*CT920142

1. Population sampling and Glucose Tolerance/Cardiovascular tests
2. Food Frequency Questionnaires (FFQ) built up food diaries/recalls
3. Analysis by between- and within-centre comparisons
4. Planning the intervention trial for primary prevention.

Sampling

A random sample of adults aged 25-79 years, weighted towards 40-64 years olds, was drawn from site specific population registers, originally to reach a total of 1500 per site, with a response rate preferably above 70% of those eligible. Each subject received a letter of personal invitation for a "health check" explaining the study and giving an appointment to attend for their health check, usually at their local health centre. Non-responders were reinvited and then visited at home to establish address or genuine refuser status. After an initial lifestyle questionnaire, BP and anthropometric measurements, including one 24 hour urine, subjects either had, or appointments were made for, the 2 hour 75g GTTs timed precisely over 2 hours and a second 24 hour urine. The FFQ was administered either during this 2 hour period or later at the subject's home, having been piloted on a previous subsample.

Phase 2

The FFQ had a standard format in each site and many basic questions were the same. Local variations were added in the same format, piloted as before. Validation measures were developed for the Manchester sample, including food diaries recorded by a subsample of subjects after their FFQ. Urinary nitrogen was originally to be measured from 24 hour urines, but this was not done, FFQ repeatability measures were carried out in subsamples (Sharma et al, 1996).

Quality Control

During both phases 1 and 2, an important aspect was quality control of all fieldwork, by liaising and exchanging data files, original questionnaires and a random sample of blood/serum samples across field sites.

Phase 3 Analysis

Each centre was responsible for their own data punching, verification and entry, with verification by double entry. Questionnaires and data were exchanged and sent to Paris for merging, analysis and age standardisation. As sample sizes and numbers per age group varied, random samples totalling 400 per site (200 men) for between-site analysis were taken, to average 20 per age/sex band per cell. Within-site analysis used full local data sets. The Paris centre acted as the statistical analysis centre, providing advice and integrating the across/between centre comparisons. Each centre handles its own data as it wishes, collaborating with the others for publication.

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Results→Baseline results for anthropometry, blood pressure and glucose tolerance, as well as indices of social and economic status.→Baseline nutrient intakes in each site, in a subsample of age/sex groups in Manchester from the FFQ with validation/calibration from further food diaries and 24 hr. recalls. The latter have so far only been analysed in Cameroon and Jamaica, although programmes have been written for the FFQ analysis in both sites but staff were available only in Jamaica to do so.

Conclusion

The project has shown that nutritional intakes, anthropometric, BP and glucose tolerance data can be collected uniformly. Here, for the first time, results are now comparable across site, indicating the rapid transition to chronic disease in these 3 African-origin populations. In our view, environmental rather than genetic factors determine this change, as evidenced by the change within Cameroon. Similarly, mainly Jamaican-origin Caribbean migrants to the UK have higher rates than their Jamaican peers, perhaps mainly related to body mass. The dietary data, again unique, show that while quality is generally excellent (initial analyses not shown), clearly quantity is excessive for the degree of physical activity when obesity emerges, except in rural Cameroon where fat (as palm oil) is the major energy source. Obesity is uncommon due to that activity. Despite this, it seems that the maintaining traditional food sources (particularly complex carbohydrates) against the social pressure for high fat "Western" diets may help to avoid epidemics of glucose intolerance/diabetes and hypertension emerging in these developing nations and their migrant populations. Intervention and follow-up trials should indicate what individual and public health interventions may help prevention.

Contract number TS3*CT920142

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Contract number TS3*CT930244

MOLECULAR MECHANISMS OF GENETIC VARIABILITY IN THE EXPRESSION OF MAJOR HEMOGLOBINOPATHIES: PROGNOSTIC VALUE OF GENETIC FACTORS AND THERAPEUTIC PERSPECTIVES

Period: July 1, 1994 — April 31, 1997

Co-ordinator: HOPITAL R. DEBRE, INSERM U120, PHARMACOLOGIE DU DEVELOPPEMENT, Paris, France (R. KRISHNAMOORTHY)

Objectives

- To understand the genetic and molecular bases of the variable phenotypic expression of major hemoglobinopathies and in particular sickle cell disease (SCD).
- To rationalize the therapeutic induction of foetal hemoglobin (HbF) in hemoglobinopathies by pharmacological means.

Activities

* Appreciate: 1) the feasibility of a comprehensive program on SCD based upon neonatal screening, parental education and early medical follow-up in an African setting, and 2) the impact of this program on the related morbidity and mortality (natural history).
* Epidemiology of G6PD deficiency and its interaction with SCD.
* Comparison of severe form of African sicklers with mild ones from India to assess the prognostic value of associated genetic modifiers (genetic polymorphism of the critical regulatory DNA elements of the β-globin gene cluster, α-globin gene status, genetic propensity to express HbF.
* Hydroxyurea treatment in pediatric SCD patients and follow up.
* Recruitment of families for studying genetic modifiers (unlinked to the β-globin gene cluster), involved in the genetic control of HbF expression (F-cell genetics).

Results (to date)

⇒ In the social context of Benin, an affected newborn with SCD is rarely retrieved for regular clinical follow-up. We circumvented this difficulty by focusing our attention on identifying pregnancies at-risk for giving birth to a child with SCD and by providing information and counselling. A total of 2300 pregnancies were followed: 5% of the pregnancies with SCD were managed clinically. 1028 newborns were available for neonatal screening and 12% had SCD with 64% traits. 91 newborns entered the clinical surveillance programme (75% compliance of the parents) which included prophylaxis against infections. Among them 25% had an associated G6PD deficiency (G6PDA-). The outcome of these studies will be assessed only in year/3.

⇒ Contribution to the culture of continuous learning: participation to the first regional specialized course of hematology on SCD held at Cotonou BENIN (12-16 Dec. 1995) with the aim of disseminating the state of the art of biological and clinical aspects of SCD to trainees from 11 different African countries. A "SCD network" among these countries has been created.

⇒ The prevalence of G6PD deficiency by "spot test" in Mauritius is 5.5% (school and blood donor screening n = 1435). Molecular analysis (PCR - RFLP, SSCP, Nucleotide sequencing) confirmed the phenotype data but also revealed the nature of mutant alleles (G6PD Orissa, Kerala Kalyan, Med-Union, Hammersmith, A-) consistent with the ancestral population input. 16 G6PD novel variants await characterization. We could not observe SCD in association with G6PD deficiency in this population. More SCD cases need to be screened. Transfer of knowledge and know how was extremely efficient and a specialized center for hemoglobinopathies was set up and now functions autonomously.

⇒ Extensive comparison of regulatory region polymorphisms of the β-locus in indian and different african sickle cell traits (taking into account the α globin gene status) revealed

that the sickle cell gene from India has intrinsic thalassemic characteristics with resulting reduced expression of HbS and thus might contribute along with elevated HbF expression to the attenuated form of the disease in India as compared to severe form in Africa.

⇒ A large three generation family consisting of 60 members with 16 presenting a β-globin gene cluster dependent HPFH (moderate increase in HbF in the basal state with further increase in response to anemic stress) from Algeria is presently under investigation for defining the molecular basis of such forms of HPFH.

⇒ Clinical trial of hydroxyurea (HU) treatment for the past 2 years of the first generation African SCD children followed at Paris (4 boys and 3 girls, age range 4-16 years) revealed: 1) an excellent compliance; 2) all were "responders" in terms of increase in HbF; 3) SCD with "Senegal" haplotype had higher increment in HbF than others; 4) absence of HU dose — dependence in HbF increase; 5) beneficial affect of HU (in terms of hospitalization for vaso occlusive crisis or acute thoracic syndrome/patient/year is not limited to increment to HbF alone; 6) HU caused reduction in serum iron, supplementation of which, further increased the HbF level all leading to the conclusion that UH treatment appears as an efficient cost-effective alternative for situations where exchange transfusion was the role (excepting strokes) and thus avoiding transfusion-associated risks.

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Contract number IC18*CT950024

DEVELOPMENT OF A VACCINE AGAINST HELICOBACTER PYLORI

Period: January 1, 1996 — December 31, 1998

Co-ordinator: CHIRON SPA, IRIS RESEARCH CENTRE
Siena, Italy (J.L. TELFORD)

Objectives

- To produce a genetically detoxified *H. pylori* cytotoxin protein suitable for evaluation as a vaccine candidate.
- To assess the genetic variability of Chinese isolates of *H. pylori*, and to evaluate the relationship of this variability to the incidence of severe disease.
- To test in the mouse model, vaccine candidates which show promise in protecting against European strains of *H. pylori* for their capacity to induce protection against infection by virulent Chinese strains.

Activities

* A bank of monclonal antibodies raised against purified native toxin wil be screened for their ability to neutralize purified toxin.
* Sequences coding for potentially non-toxic VacA proteins willl be created in *E. coli* by site directed mutagensis.
  Mutated genes constructed in *E. coli* will be transferred into *H. pylori*.
* Mutated toxin proteins will be tested for their ability to induce gastric lesions after oral administration to mice.
* Sera from Chinese patients undergoing routine endoscopy will be analysed for their ability to recognize CagA, VacA and other *H. pylori* antigens to ascertain any correlation with particular conditions such as duodenal ulcer and gastric cancer.
* Strains isolated by endoscopy from Chinese patients will be analysed for the presence of the CagA genotype and by immunoblot for expression of CagA and VacA proteins.
* Selected isolated of different genotype and phenotype will be adapted to mice by sequential passage.
* Non-toxic VacA proteins will be tested for their ability to protect mice from infection with European and Chinese strains of *H. pylori* adapted to colonization of mice.

Expected outcome

⇒ Identification of biologically relevant regions of the VacA protein.
⇒ A genetically detoxified and antigenically intact cytotoxin molecule as a vaccine candidate.
⇒ Serological data on the association of infection with Type I, VacA and CagA expressing strains with gastroduodenal disease in China.
⇒ Genotypic and phenotypic data on the variation of *H. pylori* strains in clinical isolates form Chinese patients and their assocation with disease.
⇒ Pathogenicity *in vivo* and *in vitro* of clinical isolates from China related to their genotype and phenotype.
⇒ Evaluation of the cytotoxin toxoids in conferring protective immunity against infection by European and Chinese strains of *H. pylori*.

Contract number IC18*CT950024

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Contract number IC18*CT960032

SOUTH AMERICAN BITES AND STINGS PROGRAMME

Period: August 1, 1996 — January 31, 2000

Co-ordinator: LIVERPOOL SCHOOL OF TROPICAL MEDICINE, Liverpool, United Kingdom (R.D.G THEAKSTON)

Objectives

- To investigate and improve therapy of bites and stings in different areas of Brazil and Ecuador by clinical testing of antivenoms.
- To investigate the extent of the problem of bites and stings in the above areas.
- To investigate the efficacy of plant extracts as possible alternatives to conventional antivenoms.
- To develop enzymes immunoassay as a tool for epidemiological studies, rapid immunodiagnosis and for studying the kinetics of envenoming and therapy.

Methodology and Activities

* Preclinical experimental assessment of antivenoms before clinical studies (Liverpool).
* Randomized clinical trials of antivenoms using clinical and laboratory methods (Belem, Uberlandia, Sao Paulo-Brazil, Shell Pastaza-Ecuador, Oxford-UK).
* Laboratory tests on the pathological effects of venoms.
* Isolation, purification and study of venom components.
* Taxonomic evaluation of medically-important snake species and associated study of venoms from these (Sao Paulo-Brazil, Bangor-UK).
* Epidemiological survey studies in Amazonian Ecuador and Brazil to establish the true extent of bites and stings in these areas (Sao Paulo and Belem-Brazil, Pastaza, Shell-Ecuador).
* Enzyme-linked immunosorbent assay is being used as a tool for examining the kinetics of envenoming and therapy (Liverpool, Paris).
* Isolates from various plants are being prepared in Hannover and tested for antivenom activity in Liverpool.
* Affinity purification of specific venom antigens is being used to increase the specificity and decrease the time taken for immunodiagnosis (Liverpool, Paris).

Expected outcome

⇒ The optimum antivenoms and antivenom doses will be determined following preclinical tests and possibly clinical trials for use in central and Amazonian Brazil and Amazonian Ecuador.

⇒ Epidemiological studies will result in clarification of the extent of the health problems caused by bites and stings in Brazil and Ecuador.

⇒ The purification and testing of venom components will help in the possible development of novel drugs.

Contract number IC18*CT960032

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Contract number IC18*CT960132

RESEARCH ON THE BEHAVIOUR AND BIOLOGY OF A MAJOR AFRICAN CHILDHOOD MALIGNANCY, BURKITT'S LYMPHOMA, AND ITS ASSOCIATED VIRUS, EBV.

Period: February 1, 1997 — January 31, 2000

Co-ordinator: IMPERIAL COLLEGE SCHOOL OF MEDICINE
London, United Kingdom (B.E. GRIFFIN)

Objectives

- To seek a better prognosis for BL, based on clinical re-evaluation and reassessment of treatment, particularly for patients with non-responsive tumors, correlating patient data with molecular exploration of tumor tissue, and/or sera or whole blood analyses.
- To establish new assays for diagnosis, some of which will be designed for use on site in Malawi, and analyzing viral (and cellular) gene expression, correlating data with clinical information.
- To generate useful questionnaires regarding patient and family histories, as they may relate to onset of the disease, or the different individual clinical patterns observed, looking for possible genetic and/or environmental links to disease.
- To explore viral markers that may have bearing on treatment of disease, aimed at correlation with clinical behavior. To build up a profile of EBV gene expression alongside patients data.
- To train local personnel to help in management of the BL programme, both with regard to data collection, analysis, patient management and laboratory work.

Activities

* To establish an effective communications system among the partners, both with regard to exchange of data and of materials.
* To train local personnel with respect to sample and data collection and interpretation, also with regard to laboratory diagnoses.
* To set up serum, blood and tissue banks on patients, and suitable controls, for assaying with regard to EB viral (and cellular) gene expression.
* To re-evaluate treatment protocols, particularly with regard to patients with tumors that do not respond to conventional chemotherapy, hunting for patterns between EB viral gene expression and tumor response.
* To explore the establishment of BL in an animal model to provide continuing sources of tumor material for analyzing gene expression within a single tumor population; alternatively, using molecular approaches to generate gene expression libraries for evaluation.

Expected outcome

⇒ Data that lead to the re-evaluation of BL, and improved methodologies for correlating gene expression with patient prognosis.
⇒ New assays relevant to diagnosis and treatment of BL, and an overall better understanding of this major childhood tumor in sub-Saharan Africa.
⇒ Trained local personnel to work on BL projects in longer term.
⇒ Distribution of findings to other African countries where BL is a major childhood tumor, and publication of data as a means of distributing information further afield.

Contract number IC18*CT960132

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Annexes:

ANNEX 1:

PARTNERSHIP BETWEEN EUROPE AND DEVELOPING COUNTRIES IN HEALTH RESEARCH[1]

The European Commission has been supporting joint research activities relevant to development, including health related research, since 1983, and has recently issued the second call for scientific proposals within the programme of International Cooperation with Developing Countries (INCO-DC) which has become a broader programme than its predecessor. Health research remains a priority area, together with research on agriculture and the sustainable use of natural resources. The Commission's International Scientific Cooperation Programme is an integral part of the European Union's Fourth Framework Programme on Research. It serves Europe's research policy but it does even more. It is also an instrument to support other European policies, such as development cooperation, external relations or economic cooperation with third countries.

A guiding principle of the European Union is a desire to contribute to worldwide sustainable development. A desire which stems from a sense of co-responsibility for the problems faced by third countries. This responsibility must be shared by every citizen but a special role, undervalued in the past, must be reserved for the scientists who have chosen to address problems of developing countries.

most important commodity for sustainable development is knowledge and knowledge will be the number one production factor in the 21st century. Its importance will far outweigh that of capital and labour in the present century, and if one wishes to safeguard the future and to increase knowledge, more needs to be invested in research now. Research is increasingly perceived as a basis for welfare within the EU. Research must therefore be just as important for the welfare of third countries. This assumption has received too little attention. Knowledge is an invaluable commodity and to acquire knowledge one has to help develop a culture of questioning.

Responsibility and Mandates

The development of a culture of science and research is the responsibility of everyone. Scientists within the individual member states of the European Union are already involved at personal, institutional, regional, or national level in a variety of programmes with a variety of goals related to Health in tropical areas or developing countries. A wealth of research activities are, or can also be, performed or developed on a bilateral basis by each of the European Member States. The Commission strongly encourages all these efforts.

[1] Reprinted with permission from: *Tropical Medicine and International Health* 1996 **5** 553-557.

Moreover, the Commission has a mandate to keep informed of any such initiatives and whenever possible to be in synergy with them, but the Commission's strength lies in its ability to harness expertise at a supra-national level within Europe, in the context of its relations with third countries. Adding a European dimension, complementing the existing bilateral regional and national interactions.

The European Commission will not replace any of the inputs from others. The more support scientists in Europe or Developing Countries receive or acquire from national or other sources, the more European programmes will be meaningful and give room for substantial cost efficient added value. The Commission functions on the basis of subsidiarity, a concept which has been a matter of debate in the EU. At the European level, the Commission does what can be done only at this level, and most importantly, done better at this level. The Commission builds on the valuable contributions to health research of the individual Member States of the European Union. Many agencies like the Institutions of the United Nations, such as WHO, have an alternative agenda focused on different roles and responsibilities, although often in support of common goals.

It is critical that each has their own clearly defined goals and modes of operation. It is also important that all operations are transparent so that what is being done, and where, can be precisely established, thus avoiding wasted efforts and unnecessary duplication. What it boils down to, is the need to establish equitable partnerships which will facilitate the sharing of knowledge. Clearly with the financial and human resources which can be harnessed from within the European Union the opportunity can be provided for partnerships to be established within Europe and with third countries. These resources are not an objective or a solution in themselves. They simply provide the appropriate environment and the means to progress.

For too long it was considered that ideas and practices in health research were "for" Developing Countries, and that they could be exported from Europe to Developing Countries for direct application. While that attitude was not exceptional in times gone by, it is now certainly no longer acceptable. The European Union encourages health research "with" Developing Countries.

In establishing partnerships in health research with Developing Countries, the criteria considered to be most important can be divided in three parts. The first is the scientific aspect. It is essential that the process of science is of a high quality, indeed of international status, and respected on its own merits and rules. There is only one science, whether it is classified as basic, fundamental, strategic, operational or action research. What matters is that science flourishes in a variety of socio-economic and cultural environments.

The second, but equally important consideration is the mechanism to implement the EU policy of scientific cooperation in health research. It would be naive to consider that any scientific interaction can take place in a vacuum, insulated from other aspects of life. There must be consideration of the societal aspects of the science. In other words there must be an expectation that the scientific work will, at one time, bring tangible benefits for society as a whole. Therefore, the work supported by the Commission is aimed at addressing the major health problems faced by developing countries. New practices and technologies have to take account of the context in which they may be applied and the health benefits of their application have to be clearly established. Public health concepts are a common platform for all health research.

The third important point to consider is that health research partnerships cannot be established unless there are partners to establish them with. Partners within Europe are easier to find, but research capacity in Developing Countries is relatively scarce and cannot be created overnight. Although the inputs from national or international research programmes can help, they are not the solution in themselves.

What is needed is a prolonged intensive investment and other support to ensure capacity and capability strengthening. This will only be achieved through the political will and the economic commitment of the countries themselves together with support from other national and international sources. Fortunately the need for this support has been recognised by the European Union through its economic and development cooperation policy which is mainly the responsibility of DGIB and DGVIII within the Commission.

Science should not suffer from any unproductive rivalry, as was the case in the early years of this century, when scientists argued over whether resistance to disease was dependent upon cells or serum. Scientists allied themselves on one side or the other on the basis of their nationality. In the initial stages of the European Union's Research Programmes even the prospect of uniting two European research institutions or laboratories from different member states seemed daunting to less receptive scientists, and brought some opposition. Scientists assumed, falsely, that their efficiency or competitiveness would be diminished by having to participate in what was considered by some to be a cumbersome interaction. But the imposition of this requirement of joint research for eligibility for European Union support has stood the test of time and is helping to change the paradigm for international cooperation to one of equitable partnerships.

The benefits this brings are many. Now, there is an unprecedented level of European cooperation with many examples of the EU programme providing the initial contacts between labs which have blossomed and been extended to address many problems outside the current programme. Competition between laboratories remains a driving force for advances, but this has ensured that scientists tended to become better specialists in their own particular area. There is now an appreciation that the complexity of the problems and challenges being faced, can be solved only through cooperation among scientists with expertise in complementary disciplines.

If establishing links between scientists in Europe needed some persuasion, European scientists found establishing links with developing countries even more difficult to accept. The EC is not interested in providing strictly pre-conceived "European" solutions to problems of development. There is an acute awareness that the complex problems of development cannot be contained within national or regional boundaries, they affect all societies. The aim is to find a common path to achieving improvements in development, bringing together scientists from North and South who will address the problems as an integrated unit, each bringing their own expertise and experience to bear on the problem at hand. If this is done in the right way, the goal of learning to learn will be achieved. A culture of learning, in which scientific methodology becomes an intrinsic part of society will be established. Hypotheses will be tested and development programmes modified in the light of the results, things will not be left to chance. The interactions of the scientists in the Commission's programme leads to the establishment of a culture of learning across the globe.

The Commission continues to encourage interactions among scientists. Links between European laboratories are stronger now than they have ever been. The same can be said for links between European scientists and their colleagues in Developing countries.

But there is an additional benefit from the requirements of the programme, South-South cooperation, and the sharing of knowledge among the third countries anxious to involve their regional neighbours in their quest for knowledge of common problems which are best addressed in partnership. Of course this is not a unique achievement but it is clear that the Programme has done much to facilitate additional steps in this direction.

ANNEX 2:

THE EUROPEAN UNION (EU) FOURTH FRAMEWORK PROGRAMME FOR RESEARCH AND TECHNOLOGICAL DEVELOPMENT (RTD)

Collaborative research with developing countries is carried out against the background of the Commission's Fourth Framework Programme for Research (FP4). The basic aim of FP4 is: To support inter-Member State scientific collaboration, networking and concertation on issues of common concern. To reach this goal FP4 supports multi-centre research projects, concerted actions and accompanying measures which help to improve quality of life and increase European competitiveness, in a global context. More than this, FP4 serves to support other EU policies, such as economic and development cooperation.

The total budget of FP4 (1994-1998) is 12.3[1] billion ECU and is divided over four main activities:

I. Research, Technological Development and Demonstration within the EU Member States
(10686 Million ECU (MECU)),
II. Cooperation with Third Countries and International Organisations (540 MECU),
III. Dissemination and optimization of results (330 MECU) and
IV. Training and Mobility of researchers within the EU (740 MECU).

Activity II, Cooperation with Third Countries and International Organisations has a pivotal role, linking EU policies in science, economic cooperation and development through coherent collaborative research activities with third countries.

Health-related research can take place in activity I and in activity II programmes. The BIOMED and the BIOTECH programmes of Activity I have a budget of 552 MECU and 336 MECU respectively. Many aspects of health research which are trans-disease, can be covered by these programmes and might also be of great importance for collaboration with Developing Countries for example, malaria vaccine development. Within the activity II INCO programme (540 MECU) there is also a health component. In the specific Programme for developing countries a total budget of 63 MECU will be available for health between 1994-1998 and in the component geared towards Eastern and central Europe, health-related research is also covered (INCO-Copernicus).

[1] In 1996, budgets of specific programmes were increased to the new total of 13.1MECU.

The Work Programme

Although they may differ in detail the Commission's research programmes in FP4 have the following modes of implementation:

I Multilateral joint research projects,
II Concerted Actions, covering the actual costs of concertation, such as the search for partners, meetings, common publications
III Accompanying Measures, such as contract holders meetings, networks, studies, targeted research training and mobility and the dissemination of results and
IV Concertation through consultation with the Member states and in the case of the INCO-DC programme consultation with developing countries.

These are set out in the detailed Work Programme together with the main objectives of the programme. For INCO-DC these are, to promote the role of relevant high quality RTD in development in economic cooperation, to encourage scientific collaboration between Europe and DCs, between DCs and within Europe, to help reinforce and maintain RTD capacities, including human capital, in DCs, to contribute to maintaining a competence in Europe in scientific sectors of mutual interest and in those pertinent to problems of DCs, to capitalise on the experience gained during the implementation of previous Commission S&T co-operation activities and to take into consideration the political obligations of the Union and the recommendations of international fora such as the Rio conference concerning research in DCs.

The Work Programme is implemented through Calls for Proposals which are updated from year to year. Details of research themes which are to be supported are provided in these Calls for Proposals.

The Call for Proposals

The Call for Proposals (Anon 1996) contains detailed information on criteria which have to be fulfilled by applicants, for example partnerships, and also on the specific topic areas for which research proposals are invited. The Call for Proposals is updated for each call and permits the Commission to direct the research programme on the basis of the consultation processes with the Member States, developing countries and on the Commission's existing research portfolio. In general the Call for Proposals is issued six months in advance of the deadline for submission of proposals. On arrival at the Commission, proposals undergo a stringent review procedure.

The Evaluation Process

In addition to scientific quality each proposal is evaluated on a variety of aspects, which include:

Is the health problem relevant to developing countries?
Is the problem of national, regional or global relevance?
Is the problem of known, documented magnitude?
Is the problem vulnerable (are there opportunities for cost-effective impact)?
Is there political will to overcome the problem?
Will the research be induce changes in approach or political awareness?
Does the research build on existing and matching capacities?
Does the research partnership have a comparative advantage (including DC)
Is there a demonstration value (spin-off in financial or scientific terms)?
Is there a likelihood of leverage for complementary funding in European Member States and/or DCs?
Is there likelihood of sustainability of the proposed approaches?
What is the research capability strengthening aspect of the proposal?
What is the training and mobility aspect of the proposal?
How is the integration of the DC partner(s) in the national setting?
How does the project fit in the international funding picture?

The evaluation of proposals is effectively done in four tranches:

I. Decision on eligibility by the Commission's Services based on partnership and documentation of the innovation and relevance of the project.
II. Scientific evaluation by selected experts.
III. Evaluation of the highly-rated proposals from the second tranche by a regional panel from developing countries
IV. Prioritisation of the highly-rated proposals based on coherence and complementarity with the Commission's existing research portfolio, partnership value, research capacity strengthening and training value and regional versus national relevance.

Following these procedures the Programme Committee consisting of the representatives of the Member States and associated countries, give an opinion on the proposals. Finally the Commission decides to implement the selected projects and activities.

References

EC (1996) Call for Proposals. *Official Journal of the European Communities* **39,** C75/31.

EC (1997) Call for Proposals. *Official Journal of the European Communities* **34,** C117/27.

ANNEX 3:

LIST OF COLLABORATING INSTITUTES BY REGION/COUNTRIES

AFRICA

GUINEA-BISSAU			Page:
Ministerio de Saude Publica	Bissau	TS3*CT920051	27
Ministerio de Saude Publica	Bissau	TS3*CT920060	153
Lab. Nac. de Saude Publica	Bissau	TS3*CT940311	163
Lab. Nac. de Saude Publica	Bissau	IC18*CT950011	131
IVORY COAST			
Programme National de Lutte contre le SIDA	Abidjan	TS3*CT930238	33
Institut National de Santé Publique	Abidjan	TS3*CT930238	33
KENYA			
University of Nairobi	Nairobi	TS3*CT910022	25
University of Nairobi	Nairobi	IC18*CT960114	45
Ministry of Health	Nairobi	IC18*CT960114	45
National Museums of Kenya	Nairobi	TS3*CT920059	29
Kenyan Medical Women's Association	Nairobi	TS3*CT930241	37
The Population Council	Nairobi	IC18*CT960114	45
MALAWI			
University of Malawi	Blantyre	IC18*CT960132	207
University of Malawi	Blantyre	TS3*CT920059	29
MAURITIUS			
Centre de Recherches Médicales SSR	Moka	TS3*CT930244	201
NIGERIA			
University College	Ibadan	TS3*CT920067	103
SENEGAL			
Institut Pasteur	Dakar	TS3*CT920067	103
ORSTOM	Dakar	TS3*CT910002	127
Université de Dakar	Dakar	IC18*CT970216	47
SOMALIA			
Somalia National University	Mogadishu	TS3*CT920064	155
SUDAN			
University of Khartoum	Khartoum	IC18*CT960116	135
TANZANIA			
National Institute for Medical Research	Dar es Salaam	TS3*CT910034	59
Mbeya Regional AIDS Control Programme	Mbeya	IC18*CT960050	41
African Medical and Research Foundation	Mwanza	TS3*CT920122	31
Tropical Disease Research	Ndola	IC18*CT960114	45
UGANDA			
District Medical Office	Fort Portal	IC18*CT960050	41

ASIA

EUROPE

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Erasmus University 6	Rotterdam	IC18*CT960116	134
Diagnostisch Centrum SSDZ	Delft	TS3*CT930241	37
Rijksuniversiteit	Leiden	TS3*CT920081	195
UNITED KINGDOM			
London School of Hygiene and Tropical Medicine	London	TS3*CT930254	60
Liverpool School of Tropical Medicine	Liverpool	TS3*CT910024	192
Liverpool School of Tropical Medicine	Liverpool	TS3*CT920059	29
Liverpool School of Tropical Medicine	Liverpool	IC18*CT960032	204
London School of Hygiene and Tropical Medicine	London	TS3*CT920122	30
London School of Hygiene and Tropical Medicine	London	TS3*CT930238	33
London School of Hygiene and Tropical Medicine	London	IC18*CT960114	45
Imperial College of Medicine	London	IC18*CT960050	41
London School of Hygiene and Tropical Medicine	London	TS3*CT910033	52
London School of Hygiene and Tropical Medicine	London	IC18*CT960083	62
University of London	London	TS3*CT920062	72
Hammersmith Hospital	London	TS3*CT920099	75
London School of Hygiene and Tropical Medicine	London	TS3*CT940299	82
MRC Clinical Sciences Centre	London	TS3*CT940304	84
University College of London	London	IC18*CT960047	89
London School of Hygiene and Tropical Medicine	London	TS3*CT920060	153
London School of Hygiene and Tropical Medicine	London	IC18*CT970236	92
University College London	London	IC18*CT960060	91
London School of Hygiene & Tropical Medicine	London	IC18*CT970254	97
Medical Research Council	London	IC18*CT970253	94
London School of Hygiene and Tropical Medicine	London	TS3*CT940290	108
London School of Hygiene and Tropical Medicine	London	TS3*CT940302	128
London School of Hygiene and Tropical Medicine	London	IC18*CT960054	132
London School of Hygiene and Tropical Medicine	London	TS3*CT920060	153
Hammersmith Hospital	London	TS3*CT930244	201
Imperial College School of Medicine	London	IC18*CT960132	206
University of Aberdeen	Aberdeen	TS3*CT910024	193
University of Aberdeen	Aberdeen	IC18*CT960047	88
Veterinary Research Laboratories	Belfast	TS3*CT910035	187
University of Birmingham	Birmingham	TS3*CT930254	61
University of Birmingham	Birmingham	IC18*CT970236	93
University of Leeds	Leeds	IC18*CT950024	203
Clinical Epidemiology Unit	Manchester	TS3*CT920142	196
University of Nottingham	Nottingham	TS3*CT940292	160
University of Oxford	Oxford	TS3*CT910022	25
University of Oxford	Oxford	IC18*CT970236	93
Nat. Environment Research Council	Oxford	TS3*CT920067	103
University of Oxford	Oxford	TS3*CT910024	193
John Radcliffe Hospital	Oxford	IC18*CT960032	205
School of Biological Sciences	Gwynedd	IC18*CT960032	205
National Institute of Biological Standards & Cont.		TS3*CT940292	161

LATIN AMERICA

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BARBADOS			
Ministry of Health	St Michael	TS3*CT910035	187
BRAZIL			
Universidad de Sao Paulo	Sao Paulo	TS3*CT930255	159
Escola Paulista de Medicina	Sao Paulo	TS3*CT930259	181
Instituto Butantan	Sao Paulo	TS3*CT910024	193
Hospital Vital Brazil	Sao Paulo	IC18*CT960032	205
Universidade Federal de Uberlandia	Belem	IC18*CT960032	205
Universidade Federal do Para	Belem	IC18*CT960032	205
Universidade Federal de Minas Gerais	Belo Horizonte	TS3*CT910024	193
CHILI			
Universidad of Santiago	Santiago	IC18*CT960027	167
ECUADOR			
Hospital Vozandes del Oriente	Oriente	IC18*CT960032	205
JAMAICA			
Tropical Metabolism Research Unit	Kingston	TS3*CT920142	199
MEXICO			
Instituto Nacional de la Nutricion Salvador Zubiran	Mexico City	IC18*CT960060	91
Instituto Mexicano del Seguro Social	Mexico D.F.	TS3*CT940299	83
Centro Medico Nacional Siglo XXI	Mexico DF	IC18*CT970236	93
Escuela Nacional de Ciencias Biologicas	Mexico DF	IC18*CT970253	95
IBT UNAM	Cuernavaca	IC18*CT960027	167
TRINIDAD			
Pan American health Organization	Port of Spain	TS3*CT910035	187
VENEZUELA			
IVIC-CBB	Caracas	IC18*CT960027	167
IVIC-CMBC	Caracas	IC18*CT960027	167
Instituto Venezolano de Investigaciones Cientif.	Eldo Mirlanda	TS3*CT930243	79

ANNEX 4:

List of scientists

ANNEX 5:

LIST OF PROJECTS BY TOPIC

A. Health Systems Research (Volume 1)

1. Clinical studies for specific health problems

2. Rational organisation of health services

3. Policies for improved practicies

4. Occupational Health

B. Virology/Bacteriology (Volume 2)

1. HIV/AIDS

HIV & Sexually Transmitted Diseases

HIV and Tuberculosis

2. Mycobacteria

3. Hemorrhagic Fevers

4. Human Papillomavirus (HPV)

5. Measles

6. Pneumococcal vaccine development

7. Diarrhoea

8. Diphtheria and Tetanus

9. Hepatitis

10. Other

Non Communicable Diseases

C. Parasitology (Volume 3)

1. Malaria

Vector

Genome

Vaccines and Immunology

Transmission

Drugs

Intervention

2. Schistosomiasis

Development of candidate vaccine antigens

Development of improved immunodiagnostic tools

Epidemiology, Immunology and Morbidity of schistosome infection

3. Trypanosomiasis

Page:

4. Leishmaniasis

5. Filariases

Lymphatic Filariasis

Onchocerciasis

Chemotherapy

6. Other Parasites

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WITH DEVELOPING COUNTRIES
Volume 2 VIROLOGY / BACTERIOLOGY RESEARCH NON COMUNICABLE DISEASES
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