

## Report

# European contribution to the science, prevention and management of HIV infection

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*The objectives of the European Commission Biomed AIDS Programme are to enable Europe to pool its intellectual and financial resources in the control, treatment and prevention of HIV infection and AIDS. In order to facilitate this aim the Commission has allocated 40 to 50 million ECU over the past 6 years for concerted action of the Biomed projects on AIDS by the countries of the European Union. This is only a small proportion of the real cost spent by the member countries on this epidemic. © 1997 Elsevier Science Ltd.*

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At the International Conference on AIDS in Vancouver the leaders of the European Concerted Action on AIDS were commissioned to report on the *State of the Art and Future Priorities in Research on HIV infection and AIDS*. The report is addressed to the scientific community at large, to the members of the Institutions of the European Union, and for consideration at the forthcoming preparation of the 5th Framework Programme of the EC. In general, there is a realisation that the epidemic is too complex a problem for any one country to solve, and the virus does not recognise national boundaries. Furthermore, the severity of the epidemic in developing countries has been of great concern and an additional reason in making AIDS a high priority for research among the EC scientists. Basic and medical scientists have pooled their resources to work as single multi-national teams. A brief outline of the report of the eight working groups, covering the entire field of AIDS is presented below.

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### Epidemiological monitoring of HIV and AIDS

Monitoring has involved investigations of genetic variations of HIV in Europe and indeed the world and established the classification of HIV-1 into genetic subtypes in different parts of the world. Paradoxically, with the increasingly detailed knowledge of nucleotide sequences of virus strains, the subtype strains became less well defined. Recombinant viruses have been identified, further contributing to HIV variation, and leading to potentially dangerous changes in the pattern of the epidemic. Functional or biological phenotyping was not concordant with the genetic subtypes; for instance markers of virulence were found across the genetic subtypes. An innovative approach has been the development of mathematical models studying the epidemiology, economy and social impact of HIV and AIDS on the European Society.

Future priorities in Epidemiology will be to monitor the AIDS epidemic and this includes surveillance of the HIV strains by molecular analysis. The establishment of a European network of identification and early warning of emergence of new strains of HIV has been advocated. This may be especially pertinent with the increasing therapeutic use of a variety of antiviral drugs. The inclusion of Eastern European countries within the EC may create further variation in HIV strains. It is desirable to integrate molecular epidemiology and virology to create a common database and to utilise these in mathematical modelling. Whereas reliable conventional epidemiological data continue to be important, molecular epidemiology offers a novel approach, which should include social and behavioural modelling.

### Pathogenesis of HIV infection and the subsequent development of AIDS

This has been subjected to immunological, virological and histological investigations. Resistance to HIV infec-

tion in subjects exposed to the virus (e.g. commercial sex-workers and partners of sero-positive subjects) has been associated with CD8 suppressor factors and  $\beta$  chemokines, generating protective immunity. The missing co-receptor to CD4 for HIV-gp120 to bind and fuse with the CD4<sup>+</sup> T cell has been recently identified as the CCR-5 receptor for  $\beta$  chemokines (RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$ ) which preferentially bind to macrophage tropic and fusion receptor which binds T cell tropic HIV strains. IL-16 in addition to the  $\beta$  chemokines is another factor identified within the culture supernatant generated by stimulating CD8<sup>+</sup> T cells that are capable of suppressing HIV/SIV replication. These T cell factors and their corresponding receptors have opened a new approach to investigations of transmission and prevention of HIV. There is evidence in favour of a modulating influence of TH-1 cytokines (IL-2 and IFN $\gamma$ ) and the converse effect of TH-2 cytokines (IL-4 and IL-10), on the development of AIDS and generating CD8-suppressor factors against HIV.

The development and breakdown of humoral and cell-mediated host immunity is fundamental to our understanding of the pathogenesis of HIV infection and AIDS. This includes not only systemic immunity, but also secretory IgA and cellular immune responses at the mucosal surfaces which are the portal of entry of HIV in both homosexual (anal) and heterosexual (vaginal) transmission of HIV. The virus, its antigens and immune complexes are carried to the follicular dendritic cells (FDC) in the germinal centres (B cell zone), or to the interdigitating dendritic cells (IDC) in the parafollicular T cell zone. The permissiveness of dendritic and Langerhans cells has now been established. The role of these cells and FDC and IDC in transmission of HIV to CD4<sup>+</sup> T cells, as well as to the development of humoral and cell-mediated immune responses and memory are central to the pathogenesis of HIV infection. In addition virucidal mechanism and programmed cells death (apoptosis) play an important part in the loss of T cells, apparently during the early phase of HIV infection, when the rate of cell death equals the rate of cell renewal. This kinetic equilibrium, however, breaks down after a variable time, measured usually in years, with progression to AIDS. An important fact has been established that a steady state of relatively high viral replication prevails during the clinical latent state (from the onset of HIV infection to the development of AIDS). However, with the onset of immunodeficiency a breakdown of protective mechanisms takes place, that allows opportunistic infections to develop (e.g. *Pneumocystis carinii*, mycobacteria, herpes viruses, candida) and tumours [predominantly Kaposi's sarcoma (HHV8) and malignant lymphoma (EBV)].

Future priorities in studying pathogenesis need to deal with the role of CD8 suppressor factors and chemokines in relation to their specific receptors in prevention of HIV infection in various tissues (lymphoid, blood and CNS), as well as the development of AIDS. Modulation of receptors to these factors will need to be studied not only in T cells, macrophages and dendritic cells in the circulation and lymph nodes but also in Langerhans cells found in mucous membranes. *In vivo* experiments will have to be carried out in macaques to establish the function of these CD8 cell generated factors. As specific protective immune correlates have so far been difficult to demonstrate, renewed efforts will have to be made to

find out if these CD8 generated factors in association with antibodies and/or other cell-mediated functions prevent infection and AIDS. Decreasing the viral load during infection has now become a prime objective, as recent evidence suggests that the lower the viral load in infected circulating cells, the longer is the latent clinical period before the clinical manifestations of AIDS develop. The rapid advances in this area suggest that a dual approach, with anti-retroviral drugs and agents restoring the immune system should be pursued. The mechanism of destruction of CD4<sup>+</sup> T cells has to be re-examined with reference to the newly discovered receptors, as well as mechanism of apoptosis. The pathogenesis of opportunistic infections and tumours is related to the breakdown of cell-mediated and humoral immunity, due to pathological changes in lymphoid and antigen presenting cells.

### Molecular virology

Molecular virology has progressed rapidly since the discovery of HIV-1 and HIV-2 as the aetiological agents in the development of AIDS. The related SIV (simian immunodeficiency virus) is capable of inducing AIDS-like manifestations in macaques. The target for HIV or SIV is the CD4 glycoprotein, and the binding, conformational changes and determinants between CD4 and the envelope gp120 have been well defined. The discovery of co-receptors (CCR-5 and CXCR-4) to enable fusion between HIV and the CD4<sup>+</sup> cells has been only very recently established. The structure and function of the envelope gp120 and core p24 have been extensively investigated, and recently the structure of the matrix protein has been reported. The regulatory proteins have also been studied, and particularly the structure and function of tat and to a lesser extent nef have been clarified. Our understanding of the regulation of HIV by transcription factors, notably NF $\kappa$ B and the nature of transduction signals have been significantly enhanced.

Future priorities in molecular virology should be concerned with the application of basic science to anti-retroviral intervention. HIV entry into CD4<sup>+</sup> cells should be controlled by blocking the interaction between chemokines and their receptors. HIV tropism is also linked to the expression of different chemokine receptors. Rational drug design should be especially concerned with targeting tat and rev proteins, and control of HIV transcription by interfering with transduction signals and transcription factors. Administration or induction of appropriate chemokines according to the tropism of the HIV strains isolated, or downregulating the corresponding receptors, is a novel type of antiviral intervention to be investigated in experimental models and in patients.

### Prevention of HIV infection by vaccination

Vaccination against HIV has been a principal objective of the AIDS programme. The major criteria for a successful vaccine have been to elicit total immunity or to decrease significantly the viral load, by intravenous and/or mucosal challenges, with a vaccine devoid of serious side effects, in which the mode of administration is acceptable, the mechanism of immunity is understood and the vaccine is cost-effective. These are stringent criteria, but the requirement for sterilising immunity has

now been relaxed, as this has not been essential for other viral vaccines and there is convincing recent evidence that a decrease in viral load can delay the onset of AIDS. It is noteworthy that at least three experimental vaccines have been developed in preventing SIV infection in non-human primates:

- (1) (a) *Xenogeneic immunization* of macaques with inactivated whole cells of SIV grown in human T cells has consistently elicited almost total protection from SIV infection by the IV route. This vaccine however, is dependent on HLA class I and class II stimulation and its applicability to human immunization is unlikely.
- (2) (b) *Gene deleted or live attenuated SIV or HIV vaccines* are highly protective in macaques but not chimpanzees and the lack of safety of such vaccines precludes presently their application to humans.
- (3) (c) *Subunit envelope antigens* have been consistently protective in chimpanzees and in some instances in macaques.

Rectal mucosal protection has been achieved with recombinant envelope SIV gp120 and core p27 antigens in alum but immunization had to be targeted to the iliac lymph nodes draining the mucosa. Thus, protective mucosal immunity can be elicited by targeting the draining lymph nodes with a safe subunit vaccine. The correlate of protection in chimpanzees appears to be neutralising antibodies to HIV, but no consistent correlation has been demonstrated with SIV neutralising antibodies and protection in macaques. Another correlate of protection in humans and some immunized macaques is HLA class I restricted CD8 cell cytotoxicity. Very recently, CD8 virus suppressor factors and  $\beta$  chemokines were correlated with protection against mucosal SIV challenge. Whilst no single mechanism appears to account for protective immunity, a working hypothesis for mucosal infection has evolved which suggests that total protection from HIV/SIV infection or decrease in viral load takes place by several local immune factors that function at three barriers: the mucosa, regional draining lymph nodes and circulating blood.

Future priorities need to deal with the correlates of protective immunity, induction of long-lasting effective immunity, and development of protective mucosal vaccine strategies. The role of  $\beta$  chemokines and their putative control by TH1 and TH2 cytokines has to be studied in vivo, in order to determine if they prevent infection or decrease viral load and prolong survival of infected macaques. Recombinant subunit SIV envelope (gp120), core (p27) and regulatory gene products tat, nef and rev will need to be tested in combination. The SHIV reagents have enabled HIV envelope vaccines to be tested in macaques and this approach should be actively pursued, using different clades. Indeed, there is a need for high quality standardised HIV and SIV reagents. Experiments with the B clade found predominantly in Europe, needs to be extended to other clades which affect developing countries. As most HIV infections take place via the mucosal surface, strategies for vaccination and challenges have to be addressed towards achieving effective protective mucosal immunity. Indeed, local immune mechanisms in the rectal and cervico-vaginal mucosa, and the draining iliac lymph nodes, in relation

to the central systemic immunity have to be studied. The evolving pre-clinical vaccine strategies in non-human primates can now be applied in clinical phase 1 and 2 trials in carefully defined cohorts of subjects.

### Clinical manifestations of AIDS

Manifestations vary and there are some 25 conditions which may affect patients with AIDS. Opportunistic infections (e.g. *Pneumocystis carinii*, tuberculosis, herpes viruses and fungi) and malignant neoplasms (especially Kaposi's sarcoma and non-Hodgkin's lymphoma) are most commonly found. Depending on the severity of immunosuppression and individual susceptibility, most patients will develop only 2–3 disease manifestations. There are, however, significant variations among the European countries in clinical manifestations, as well as prophylactic and therapeutic regimens. The existing EC promoted concerted actions have enabled cohort studies, therapeutic trials, investigation of oncogenesis and specific opportunistic infections (such as *pneumocystis carinii*, toxoplasmosis and tuberculosis).

Future priorities need to be addressed at a European level in order to assess the efficacy, compliance, toxicity and cost-effectiveness of prophylactic treatment in different parts of Europe. The effect of anti-retroviral therapy on preventing AIDS has to be studied with special reference to the new multiple drug therapies. The development of drug resistance and the possibility of emergence of resistant HIV strains, has to be closely monitored. Furthermore, the emergence of atypical mycobacteria, and drug resistant mycobacteria, fungi and bacteria have to be recorded. Special attention has to be paid to the management of the distressing manifestations of HIV infection of the central nervous system. Rare manifestations of AIDS are best managed on a multinational basis in Europe.

### Prevention and treatment of HIV infections

This section deals with prevention of HIV infection by vaccination (dealt with separately above) and treatment of established HIV infection in proven sero-positive subjects. Multi-centre European trials have made major contributions in assessing the therapeutic value of a number of anti-retroviral drugs, in delaying the development of AIDS and prolonging life in cohorts of sero-positive subjects. However, the development of drug resistant strains is likely to be a major obstacle to the successful treatment of HIV infection.

Future priorities are to pursue the multi-centre trials with the new generation of anti-retroviral drugs, and using combinations of three or more drugs, so as to diminish the chances of development of drug resistance. Molecular virus studies will be necessary to determine the changes in the virus strains, including the development of drug resistance. Long-term strategies need to be developed to monitor drug toxicity, especially in view of the accelerated approval of some of these drugs. In order to derive maximum benefit from these drug trials, a multi disciplinary strategy is likely to prove of greatest benefit. In parallel to the antiviral treatment, measures to restore the immune system deserves high priority. The virus load should be monitored by the quantitative plasma RNA assay. Serum and mucosal IgG and IgA antibodies, as well as T cell mediated responses are

valuable in assessing the effect of treatment and probably in predicting the prognosis.

### Paediatric aspects of HIV and AIDS

Paediatric aspects are of great importance, as vertical transmission from infected mothers to their infants occurs in 15–20% of cases. Maternal HIV disease and breast feeding are associated with increased risk of infection. Although about 25% of infected infants progress rapidly to AIDS and die during the first year, most children survive with relatively mild clinical manifestations, but persisting as a chronic disease. Furthermore, in a small proportion of children (<2%) who become sero-negative, HIV has been detected on one or more occasions. The immune mechanism in these long term survivors and sero-negative but HIV-positive infants has not been clarified and needs to be studied. Preventive treatment of the mother with Zidovudine may significantly reduce vertical transmission of HIV.

Future priorities should include studies of specific clinical manifestations and pathogenesis in HIV infected children, as these are ill-defined and require further investigation. There are important public health implications in families with children and their mothers being infected with HIV. Combination trials with anti-retroviral drugs, both to prevent vertical transmission and to delay progression of the disease in infected children should be initiated on a multi-centre European basis.

### Health care

Health care deals with epidemiological, social, health and economic problems of HIV infection and AIDS. Quantitative methods have been applied by mathematical modelling, that involves risk factors, testing for HIV sero-positivity, control of sexually transmitted diseases, social and behaviour factors, education and economic considerations. Health care has strong relations to epidemiology (see above).

Future priorities should consider standardization of data collection, with European integration and monitoring of the information. Special attention should be paid to the analysis of the relation between health status, health care utilisation and cost of HIV infection and AIDS. The effects of prevention and treatment with the anti-retroviral drugs need to be evaluated. Furthermore, the epidemiological, economic and social impact of AIDS on society, on other diseases, and the competing claims for resource allocation has important medical and political implications.

### General comments

A general perception that European scientists and medical specialists are learning to integrate in a single, multi-national team has been a striking feature of the 8 working groups and needs to be encouraged. The interdisciplinary meeting of minds was also appreciated, as exchange of ideas and concepts between different specialities will benefit the research objectives improve management of patients and lead to prevention and cure of HIV infection. There was also a strongly felt view that a

comprehensive investigative programme of HIV infection will have a beneficial spin-off for other infections and oncological diseases and programmes. This applies especially to sexually transmitted diseases with mucosal infections, vaccination in general and the advances in prophylaxis against opportunistic infections is of significance in the management of other immunodeficiency diseases.

The eight working groups share the need for standardisation in evaluation of data, and proposals for designated centres or networks as indicated below:

- (1) (a) Epidemiology of the natural history of the disease in adults (as has been established by the EC for children infected by vertical transmission of HIV), to assess the effect of preventive and therapeutic interventions on the epidemic and to address behavioural and economic issues.
- (2) (b) For molecular analysis of HIV strains within the European countries, and monitoring any changes due to therapeutic intervention.
- (3) (c) For pre-clinical vaccine studies, using limited and expensive non-human primates.
- (4) (d) To investigate the safety and efficacy of preventive vaccines in humans.
- (5) (e) To utilise the expertise in mucosal immunology in the development of effective vaccines.
- (6) (f) To pursue multi-disciplinary therapeutic trials, using standardized protocols, with combinations of anti-retroviral drugs in adults and in infants, the latter also involving infected mothers.

The link between basic and applied sciences must be further strengthened for the ultimate benefit of the patient. The limited duration of 3 years of the EC projects makes it difficult to pursue essentially long-term investigations (e.g. clinical research, epidemiology, anti-retroviral therapy, vaccination and animal studies). There was a good deal of support for pre- and post-doctoral fellowships, to enable training and free flow of young scientists between the European centres of excellence.

In conclusion, there is a consensus that Europe is playing a crucial part in the fight against AIDS. There is guarded optimism that through European actions, such as the Biomed Programmes, there is sufficient expertise in all aspects of medical research among European scientists, to pool their knowledge and expertise, that will maximise the chances of successful prevention and treatment of HIV infection and AIDS.

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The term "Europe" refers in the present context exclusively to members of the European Union.

"Concerted Action" denotes cooperative research involving 2 or more member states of the European Union.