Witness Name: Alice Mackie

Statement No.: WITN2189002

Exhibits: WITN2189003 – WITN2189062

Dated: 30th April 2021

INFECTED BLOOD INQUIRY

EXHIBIT WITN2189012


FD19/153
CLOSED UNTIL 2020

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MEDICAL RESEARCH COUNCIL

MRC: IN CONFIDENCE

Systems: 89/S137 July 1989

PROPOSALS FOR A DEDICATED MAGNETIC RESONANCE IMAGING FACILITY FOR HIV/AIDS RESEARCH

Applications for special project grant support

1. Papers

Annex 1 - Professor M J G Harrison, Dr B E Kendall and Dr S P Newman (University College and Middlesex School of Medicine): "Neurological complications of HIV infection explored by Magnetic Resonance Imaging (MRI)" - application form

Annex 2 - Professor J J K Best, Dr R P Brettle, Dr R E Cull (Edinburgh University and City Hospital, Edinburgh): "Magnetic Resonance Imaging in AIDS research" - application form

Annex 3 - Dr J M Stevens, Dr R I Kitney, Dr K Straughan, Dr D J Thomas and Dr A J Pinching (Imperial College of Science, Technology and Medicine, St Mary's Hospital Medical School Branch): "HIV encephalopathy: natural history and response to therapy: magnetic resonance imaging and neurosciences research" - application form and appendices p1 and P2, N1-3*.

Annex 4 - HIV infection and the CNS: reports from three Working Groups.*

Annex 5 - minutes of the meeting of the Steering Committee on HIV Infection and the Nervous System held on 16 June 1989.

2. Referees

A. Professor R D Cohen (Medical Unit, London Hospital Medical College)

B. Professor G M Teasdale (Neurosurgery, Southern General Hospital, Glasgow)

C. Professor B S Worthington (Diagnostic Radiology, Queen's Medical Centre, Nottingham)

3. Background

1988 July

The Board considered the reports (annex 4) from three Working Groups that had been set up following an MRC Workshop on CNS disease in HIV infection held in June 1987. One of the Working Groups, on "Neuropathology and Imaging", (Chairman: Professor E P G H du Boulay) concluded inter alia that the case for imaging of significantly large numbers of subjects, in carefully controlled groups under properly managed protocols, was extremely strong and the need for MRI units dedicated to HIV research was very pressing. Different and compelling cases could be made for units in Edinburgh and in London; the contribution of each would be greatly enhanced by comparison of the results of both. The Board accepted the need for one dedicated MRI scanning facility but members were not satisfied that the provision of two scanners could be justified. The Board also agreed that the three Working Groups should be amalgamated into one Steering Committee.

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*Copies available from Miss Sarah Wills on request (01-636 5422 ext GRO-C
DBM: Professor J G P Sissons

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Oct The Steering Committee on HIV Infection and the Nervous System was established, with Professor W I McDonald (National Hospital for Nervous Diseases, Queen Square) as Chairman and with members drawn largely from the three Working Groups; there were also representatives from the Systems Board (currently Professor Sissons) and the Neurosciences Board.

Nov The Joint MRC/DH Magnetic Resonance Imaging Monitoring Committee (Chairman: Professor R D Cohen) was asked to advise on the type of MRI facility that would be needed, and where this could best be sited to provide access to the necessary expertise. They agreed that it was important to distinguish between firstly research to evaluate MRI as a method for establishing and monitoring the effects of HIV infection on the nervous system and secondly research into the natural history of the disease and the specific effects of HIV infection on various parts of the nervous system. They endorsed the view that the former, as indicated in the Working Group's report, had already been established; it was the latter where future research effort should be directed. The major consideration when making a decision on the siting of the machine should be the quality of the proposed research programme; this would be more likely to depend on the Centre's expertise in AIDS and the availability of a suitable cohort of HIV-positive individuals, than on its record of MRI research. Members agreed that the type of machine would depend largely on the specific research projects that would be undertaken. In conclusion, the Committee recommended that detailed proposals be solicited from groups interested in working in this area, possibly by placing an advertisement in leading medical journals. (ii)

1989 Feb Following the Steering Committee's endorsement of the MRI Monitoring Committee's recommendation, an advertisement was placed in the British Medical Journal and Lancet inviting outline research proposals.

April Five outline applications were received and were considered by the Steering Committee in the light of comments from referees and from members of the MRI Monitoring Committee. It was agreed that three groups (those from University College and Middlesex School of Medicine (UCMSM), St Mary's Hospital Medical School, and Edinburgh) should be invited to submit detailed proposals. The remaining two (from Charing Cross and Westminster Medical School, and Oxford) were not considered to offer practicable arrangments for establishing a facility for HIV patients.

4. Current Council support

4.1 University College and Middlesex School of Medicine Group

Professor M J G Harrison (with Dr SP Newman and Professor M W Adler) holds a special project grant entitled: 'Neurological and neuropsychological manifestation of HIV infection' (G8709919). Tenure 1.11.87 - 31.10.90. Support was provided for three scientific assistants at a total cost per annum of £28.5k (including NI etc). Expenses have been provided at a level of £61.5k over the tenure of the grant and £3.2k has been authorised to date for equipment. The total support awarded when this grant was approved was £74.6k. The grant has been supplemented with a further £5.8k for a part-time technical assistant, £5.2k for expenses and £3.9k for equipment.

4.2 Edinburgh Group

(i) Dr R P Brettle (with Dr J R Robertson and Professor M W Adler) holds a special project grant entitled 'Risk of heterosexual transmission of human immunodeficiency virus (HIV)' (G8705550). Tenure: 1.9.87 -

31.8.90. Support is provided for one technical asistant at a total cost per annum of £19.5k (including NI etc). Expenses have been provided at a level of £13.8k over the tenure of the grant. The total support awarded when this grant was approved was £26.7k. The grant has been supplemented with a further £22.3k for a nursing sister, £1.6k for expenses and £4k for equipment.

(ii) Dr R P Brettle (with Dr A M Richardson) holds a special project grant entitled: "The natural history of drug misuse related HIV infection with reference to potential cofactors". (G8806652). Tenure 1.5.89 - 30.4.92. Support is provided for two scientific assistants and one technical assistant at a total cost per annum of £39.8k (including NI). Expenses have been provided at the level of £97.6k over the tenure of the grant and £6.7k has been authorised to date for equipment. The total support awarded when this grant was approved was £228.3k.

(iii) Dr R P Brettle (with Dr J R Robertson) holds a special project grant entitled: 'Participation in the MRC/INSERM zidovudine Trial (Concorde 1)' (G8823418). Tenure: one year. Support is provided for one scientific assistant and one technical assistant at a total cost of £28.6k pa (including NI etc). Expenses have been provided at the level of £19.7k over the tenure of the grant and £793 has been authorised to date for equipment. The total support awarded when this grant was approved was £48.6k.

4.3 St Mary's Hospital Medical School Group

(i) Dr D J Thomas holds a special project grant entitled: 'European Carotid Surgery Trial' (G8706396). Tenure: 1.3.87 - 28.2.90. Support is provided for one technical assistant at a total cost of £3.7k pa (including NI etc). The total support awarded when this grant was approved was £3.7k.

(ii) Dr A J Pinching holds a special project grant entitled: 'A study in the biology of HTLV III/LAV infection in Zambia' (G8600697). Tenure: 1.8.86 - 31.7. 89. Support is provided for two scientific assistants and one technical assistant at a total cost of £18.9k pa (including NI etc). Expenses have been provided at the level of £20k over the tenure of the grant and £17.2k has been authorised to date for equipment. The total support awarded when this grant was approved was £64.4k. The grant has been supplemented with a further £1.5k pa for a clerical assistant, £25k for expenses and £10.8k for equipment.

(iii) Dr A J Pinching (with Dr Lesley-Jane Eales and Dr K E Nye) holds a special project grant entitled: 'Studies of membrane signal transduction in normal and HIV infected cells 'G8721658). Tenure: 1.1.88 - 31.12.90. Personal support is provided at a total cost of £14.6k pa (including NI etc). Expenses have been provided at the level of £19.4k over the tenure of the grant and £7.6k has been authorised to date for equipment. The total support awarded when this grant was approved was £77.1k.

5. Present position

5.1 Requests for support

Three detailed applications (annexes 1-3) have been received. Details of the requests are as follows:

(i) University College and Middlesex School of Medicine Group

The applicants are requesting support for two full-time and one part-time scientific assistants, three technical assistants and expenses of £80k pa. (Equipment (the imager) and installation costs have been estimated at £850k and £155k respectively). Total support requested, excluding capital costs, is £977k over a period of five years.

(ii) Edinburgh Group

The applicants are requesting support for three scientific assistants, two technical assistants, and expenses of £85k pa. Total support requested, excluding capital costs, is £948k over a period of five years.

(iii) St Mary's Hospital Medical School Group

The applicants are requesting support for four scientific assistants, five technical assistants, expenses of £85k pa. and £59k for equipment (other than the imager). Total support requested, excluding major capital costs, is £1,325k over a period of five years.

These proposals were considered by the Steering Committee on HIV Infection and the Nervous System at their meeting on 16 June. Members agreed that all were of high scientific merit and that they would wish to recommend that two, those from UCMSM and from Edinburgh, should be supported. However, if they had to recommend only one it would be the proposal from UCMSM. The minute of the Committee's discussion is at annex 5.

None of the proposals has yet received ethical committee approval. No award will be made until written evidence of ethical committee approval has been received.

The referees are those who commented on the outline proposals.

5.2 Funding arrangements

The Council has set aside £1m from the earmarked funds for AIDS research provided as part of the Science Vote in the current financial year for the capital costs associated with the imager, ie the cost of the imager itself and of its installation. Depending on the outcome of other applications for grant support for AIDS research, some additional resources may also be available. The recurrent costs of any award would also be a charge to the earmarked funds for AIDS research provided as part of the Science Vote.

5.3 Acquisition and ownership of the imager(s)

It was originally envisaged that the £1m would be sufficient to provide only one MRI facility. However, in the current state of the market for MR imagers, there are indications that manufacturers may be willing to discount machines by about 50%, if more than one is ordered or if instruments made by two manufacturers are being considered. It may therefore be possible for the Council to provide the capital costs for two facilities within the £1.0m set aside. However, because of the long-term commitment associated with the provision of recurrent expenditure, the Council would have sufficient resources to support the research programme at only one Centre. If the Council were to provide a second instrument and approve the research proposals of a second group, this would assist the group in seeking financial support for recurrent expenditure from other sources. Before making such a commitment, the Council would expect the host institution to meet certain conditions including an undertaking to provide from non-MRC resources the full running costs for the full five years of the research programme. This issue is further complicated

by the intention of the UCMSM to acquire a second imager for the Middlesex Hospital site (outline details are included in annex 1) for local research requirements. Preliminary negotiations are currently in progress, subject to the Board's decisions, concerning the possible purchase of three imagers. An update will be provided at the Board meeting.

It is proposed that the imager or imagers funded by the Council would become the property of the host institution at the outset; however, for the one that would be used for the Council-supported research programme, a firm commitment would be required from the host institution that they would offer the Council the first opportunity to support a research programme occupying up to 100% of machine time for a further five-year period following an initial award, making the period 10 years in all.

5.4 Monitoring of research

The Steering Committee on HIV Infection and the Nervous System has recommended that, because of the Council's substantial investment in this new venture, a condition of any award for a research programme should be that the work would be monitored by a Steering Group set up specifically for the purpose (annex 5). It is proposed that the Steering Group would be chaired by Professor McDonald and its membership would include the Systems Board and Neurosciences Board representatives on the main Steering Committee. The proposed terms of reference are:

(i) to monitor progress on an annual basis;

(ii) to consider and agree changes in the programme;

(iii) to be available for reference and consultation as and when required.

The grant-holders would be required to provide annual reports to the Steering Group which would probably hold its meetings at the Centre.

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6. Action required

(i) Assessment of the merits of the three proposals in the light of such criteria as are relevant.

(ii) Decision on scale and duration of support to be made available for the primary MRI facility, if awarded.

(iii) Consideration of the proposal to provide the capital costs of a second facility, if both can be funded within the budgetary limit determined by Council and the second facility is justified on scientific grounds.

(iv) Endorsement of the Steering Committee's recommendation that a Steering Group should be set up specifically to monitor any research programme supported by the Council.

1. TITLE: MAGNETIC RESONANCE IMAGING IN AIDS RESEARCH : EDINBURGH

2. PURPOSE OF PROPOSED INVESTIGATION

To explore the feasibility of using MRI to detect the effect of the HIV virus on the CNS with the purpose of:

(a) Correlating the MRI observations with clinical, neurophysiological and neuropsychological observations.

(b) Correlating the MRI observations with histology at post mortem.

(c) Correlating the MRI changes with the clinical progress of the disease and monitoring the effects of treatment.

(d) Defining the role of MRI in establishing the diagnosis of opportunistic infections and CNS malignancies in patients with AIDS and ARC.

A long term aim is the development of A.I. methods of quantitative assessment of CNS changes and the evaluation of structured reporting using interactive expert systems.

3. BACKGROUND OF PROJECT

In 1987 the Tayler report estimated the number of HIV infected individuals in South East Scotland measured 1500. This number was made up from 1200 intravenous drug misusers (IVDM), 270 homosexuals and 30 haemophiliacs. The report commented that the highest rate of HIV infection in the United Kingdom amongst a single risk group was found in the drug misusing population in Edinburgh. The figures for AIDS individuals in all categories at risk and deaths from AIDS have been projected to 1994 (Tables I and II). Since 1987, the HIV epidemic in Edinburgh has become more heterosexual in nature for, although only 30% of the IVDM population are women, 60-70% of the drug misusers have non drug-using sexual partners. The date of HIV sero-conversion amongst the Edinburgh drug misusing population can be identified accurately to a period between late 1983 and early 1984.

This background is the reason why the HIV epidemic in Edinburgh has two features that make it different from the pattern familiar to South East England.

(a) The largest group of HIV positive individuals comprise IVDM, the majority of whom are in groups II and III (CDC) of the disease.

(b) The epidemic is heterosexual in nature and at least 56 infants have been born to HIV positive mothers.

3.1 AIDS RESEARCH IN EDINBURGH

AIDS research in Edinburgh is monitored by a Co-ordinating Committee chaired by Professor Ian Bouchier. Recently Dr. R. Covell has been appointed, with SHHD funds, to co-ordinate AIDS research

in Scotland. An HIV immunology laboratory, headed by Dr. G. Bird, has been established and provides facilities for immunological monitoring of HIV infection in South East Scotland.

The research work on the HIV virus and AIDS undertaken in Edinburgh, and supported by central funds, is listed in Appendix A.

The University of Edinburgh NMR Imaging Unit at the Royal Infirmary is currently imaging a cohort of 60 HIV patients (Group II and III), the majority of whom are drug misusers. There have been 22 follow-up scans at an interval of 12 months. The imager is a 0.08 Tesla M & D Technology resistive magnet system. It is capable of quantative T_1 measurements, but the performance is limited by the low field and dated design. It cannot measure T_2 accurately, nor image the spinal cord. The Unit has considerable experience in quantitative CNS imaging.

3.2 HIV AND AIDS PATIENT SERVICES IN EDINBURGH

Hospital services for HIV positive and AIDS patients in Edinburgh are based at the Royal Infirmary for haemophiliac and genito-urinary patients, and at the City Hospital for drug misusers. It is envisaged that the hospital services for HIV positive individuals will remain based on these hospitals. An AIDS Unit, with in-patient and out-patient facilities is being built with central funds at the City Hospital.

Since 1985, the Infectious Diseases Unit at the City Hospital has provided counselling and testing for HIV in adults, together with a concomitant medical surveillance clinic. Whilst this work will continue to be a major concern of the AIDS Unit, the Unit provides in addition, oral substitute drug therapy and a dental care programme provided on site by the Community Dental Service.

Edinburgh is one of the eight centres with children enlisted in the European collaborative study on mother to child transmission of HIV. Currently, 56 children born to HIV sero-positive mothers, have been enlisted in the study as index cases, together with 27 children born to HIV positive fathers, but with negative mothers, who serve as controls. Of the index children, 46 have been followed up since birth, the remaining 10 being referred because of symptoms after the mother was diagnosed HIV positive. A further 10 infants are expected to be delivered during 1989 of HIV positive mothers.

Importantly, because of the developing incidence of vertical transmission of HIV, the Unit provides -

(a) a joint antenatal clinic for HIV positive mothers with close collaboration with Dr. F. Johnstone, Obstetrician at the Simpson Maternity Pavilion.

(b) contraceptive and family planning advice

(c) counselling, testing and medical surveillance of families with "at risk" children.

These aspects of the Unit's work are emphasised because many centres find difficulty in the follow-up care of injection drug misusers. The service provided at the City Hospital has demonstrated the feasibility of follow-up.

Despite the inherent unreliability of the IVDM patient group, the Unit has achieved 70% follow-up because of the close links with other health care agencies in Edinburgh. These links include the prison service, general practitioners and the other hospitals in the Edinburgh area. (Fig.1)

The close working relationships that necessarily exist in services based in a single health authority, together with a stable patient population confer important advantages for longitudinal studies.

4. PLAN OF INVESTIGATION

The proposed plan of investigation reflects -

(1) the importance of studying the Edinburgh IVDM patient group.

(2) the deliberations of the MRC Working Parties in formulating the Steering Committee guidelines.

(3) the advice received following submission of an outline proposal to the Steering Committee.

4.1 HIV POSITIVE AND AIDS PATIENT POPULATION IN THE STUDY

Four groups of patients will be studied.

Group A. Drug users and ex-drug users.

A group of 400 IVDM patients are followed up regularly at the AIDS Unit at the City Hospital. Amongst this group, a cohort of 162 patients have been recruited into an MRC study (A Prospective Clinical Study of Cognitive and Neurophysiological abnormalities in heterosexuals infected with HIV). All have had personal, social, medical and psychiatric histories recorded using the form produced by the MRC Working Party. All these patients have had Auditory-Event-Related Potentials (AEPs) recorded. Since the appointment of a full-time neuropsychologist, Mr. Vincent Egan, with the support of a supplementary project grant, the patients have also been asked to submit to a battery of neuropsychological tests and self-rating mood scales. Eighty have complied fully. It is anticipated that 70% of the full patient cohort of 200 will be tested using a full battery. The rest are willing, in principle, to submit to "high tech" medical investigations (MRI or electrophysiology) but are uncomfortable with the format of neuropsychological testing. It is believed that this is due to significant levels of illiteracy in the group. Despite this difficulty, it is anticipated that core test data will be obtained on most of the patients.

Group B. Patients born to HIV positive mothers

Since January 1986, the paediatric counselling and screening clinic at the AIDS Unit, has followed up all infants at known risk of HIV infection. At present, 56 infants are followed up by Dr.Jacqueline

Mok, 5 are infected, 33 presumed uninfected, and 17 indeterminate. The children are seen on a three-monthly basis. At each visit, the history is taken to elicit the symptoms and signs of HIV disease according to a standard questionnaire. The children are examined and developmental screening performed using the Denver developmental screening test. Any child who fails the screening test is given a Griffith's assessment on mental development (administered by J. Mok). Growth parameters are monitored.

Of the 56 index children, 15 are under the age of eighteen months and therefore classified as indeterminate status in view of the presence of maternal antibody. Five children have so far shown signs and symptoms of HIV disease, and are therefore infected, while of the remaining cohort, 36 children are presumed uninfected having reached the age of 18 months, and tested negative for HIV antibody, HIV antigen as well as virus culture. Investigation of the cohort using the polymerase chain reaction method for HIV DNA detection is now in progress.

Group C. Homosexual HIV Positive Patients

Out of approximately 600 homosexual men regularly attending the Genito-Urinary Out-patient clinic at the Royal Infirmary, Edinburgh, 150 are HIV positive. It is proposed to study a cohort of 50 HIV positive patients with a controlled group of 20 HIV negative patients matched for age and sex. This patient group is the only group not participating in an MRC funded study conforming to the Steering Committee's guidelines. In order that the Steering Committee guidelines are fulfilled, a Research Fellow in neurology will be appointed to undertake the neurological and neurophysiological testing. The Research Fellow will be under the supervision of Dr. R.E. Cull, Royal Infirmary.

Group D. Haemophiliac Patients HIV positive.

At present, 35 haemophiliacs who are HIV positive due to treatment with infected blood products, are followed up at the Royal Infirmary. This group includes 18 patients who were in a group of 32 patients exposed to infection due to Factor 8 in Spring 1984, and the remainder have been exposed to infected blood products at other times. Of this group, three have died from AIDS related complications, and five have presently symptoms of ARC (Dr. C. Ludlam). This group of patients is presently being investigated with neurological and neurophysiological measurements by Dr. R.E. Cull, as part of an MRC funded study (Clinical and Immunological Study of Haemophiliacs treated exclusively with NHS Factor VIII/IX concentrates).

4.2 EXPERIMENTAL DESIGN

The unique patient cohort in Edinburgh allows the question of whether the direct effect of the HIV virus on the central nervous system and the development of HIV encephalopathy may be detected by magnetic resonance imaging. The design of the studies reflects this opportunity and the advice of the MRC Steering Committee. It takes into account the present interest of the University NMR Imaging Unit in the innovative use of MRI as a quantitative tool

and the present research programme at the City Hospital which have already built up a neuropsychological and neurophysiological programme for the detection of cognitive impairment and dementia.

Because of the known time of sero-conversion of the majority of HIV positive patients in the Edinburgh patient groups, the onset of AIDS may be predicted, and the Tayler Report has anticipated a large increase in the number of live AIDS patients in the year 1990 with a subsequent large increase in death due to AIDS two years later (Table II). For this reason, the imaging study is envisaged in being in two phases. Phase I, studies on largely asymptomatic patients able to exist with support from the AIDS Unit as out-patients. Phase II, when the patients in Phase I develop signs and symptoms of AIDS or ARC, may be unwell and require in-patient treatment. It is recognised that there will be considerable overlap between these two phases.

Because of the stable nature of the IVDM population in Edinburgh, together with the successful follow-up programme by the AIDS Unit, it is anticipated that up to 40% of the present cohort being studied will have died from AIDS before the end of the five year programme and the majority of these will have post-mortem studies. It is understood that this is a unique prospect for a research programme into HIV infection in the United Kingdom.

Although the major emphasis of the proposed programme is the study of IVDM HIV positive patients and children born of HIV positive mothers, it is recognised as important to have in the study a cohort of HIV positive homosexual patients. This is to allow comparison of the results of the study in Edinburgh with other studies conducted elsewhere in the United Kingdom, Europe and North America, where imaging studies are presently conducted on populations largely consisting of homosexual patients. It is therefore considered important to contain this limb within the Edinburgh study in order that any differences in the results in the IVDM group can be assessed against the findings in the homosexual group to exclude methodological differences.

4.2i PHASE I

The monitoring of HIV positive patient groups for HIV induced changes in the CNS and correlation with clinical, neurophysiological and neuopsychological examinations.

Details of MRI examination technique in Phase I, see Appendix B.

It is proposed to conduct MRI examinations on patients entered into the Phase I trials at 6-monthly intervals. Patients in the four patient groups are presently being recruited into the AZT asymptomatic patient treatment trial conducted by the MRC, Concord 1. It is proposed to include sufficient numbers into both treated and untreated groups to allow significant differences to be detected.

GROUP A. IVDM.

Dr. R.P. Brettle.

The present cohort of 162 HIV positive patients with a control group of 20 HIV negative IVDM patients (recruitment to continue until the cohort contains 200 HIV positive patients and 50 HIV negative controls). The present study of this asymptomatic patient group is being followed up at 12-monthly intervals in order not to exhaust patient goodwill and to maintain high follow-up rates.

GROUP B - Children

Dr. J. Mok.

The NMR Imaging Unit has experience of imaging neonates and young chilren without a general anaesthetic, under sedation. Imaging techniques will be developed and children in both limbs of the index group studied. The frequency of examination will be determined by experience.

GROUP C. - Homosexuals

Dr. A. McMillan.

A cohort of 50 HIV positive homosexual patients will be recruited together with a control of 20 HIV negative patients. Both groups of patients will be matched by age.

GROUP D. Haemophiliac Patients

Dr. C. Ludlam.

This group of patients is presently involved in MRC funded study which includes CT scanning, neurological examinations and neuro-physiological testing. MRI examinations will be added to the present study. A control group matched by age and severity of disease will be recruited. It is recognised that this is the least compliant group of patients recruited into the MRI study.

4.2ii Phase II.

Demonstration of focal changes in the CNS, differential diagnosis, and correlation with biopsy and post-mortem pathology.

The details of MRI techniques in Appendix B.

Patients in the four patient groups who progress from Phase I of the study into Phase II when they develop AIDS or ARC, will continue in the Phase I of the study until ajudged that they cannot tolerate the MRI examinations as outlined for Phase I. The principle objectives of the Phase II MRI studies are to (a) document CNS changes to allow appropriate sampling at post-mortem, and (b) explore the role of Gadolinium DTPA in characterising the effect of the HIV virus encephalopathy on the blood brain barrier

and on the differential diagnosis of infections and primary CNS malignancy. The registration techniques outlined in Appendix B may allow brain biopsy in selected patients.

4.3. LOGISTICS AND ORGANISATION

An important problem facing researchers in the field of HIV/AIDS is the need not to "over-research" patients. This is of increasing concern to ethics committees and responsible clinicians. It may also be counter-productive as a contributary factor in poor attendance at follow-up clinics. The organisation in Edinburgh allows an optimal balance because of the close working relationship between research and clinical teams (Fig.2). The confidence that the clinical proposal outlined in this application may be successfully completed, is based on the patients acceptance of the present clinical and imaging studies.

4.3i Organisation

The proposed imaging and clinical studies will be integrated into existing protocols and will be monitored by a co-ordinating committee chaired by Professor J.J.K. Best (Sec. Dr. R. Cavell). Professor J.J.K. Best (3 sessions) will oversee the imaging projects, and the day to day management of the MRI Unit at the City Hospital will be by a radiological Senior Research Fellow. The MRI Unit at the City Hospital will be staffed by a Senior Research Fellow, a Physicist and two radiographers, with support from the University Department of Medical Radiology, based at the Royal Infirmary. It is anticipated that the imaging staff will form a focused team with Dr. Goodwin, Ms Ann Chiswick and Mr. Vincent Egan, who are presently involved with MRC supported study with the IVDM patients at the City Hospital. It is planned that the neurology Research Fellow will conduct the neurophysiological testing presently performed by Ms Ann Chiswick. This will allow her to give more time to organising appointments and maintaining patient data files, this forms part of her present job. Mr. Egan has presently responsibility for obtaining and storing all the neuropsychology data and performs all computing operations required for statistical analysis of data on the University main frame computer.

It is proposed to extend the present weekly research meeting to include the imaging component of the protocols.

4.3ii Imaging.

Professor J.J.K. Best, Dr. D.M. Kean and Mr. J. Ridgway, NMR Imaging Unit, Royal Infirmary, Edinburgh, and Senior Research Fellow.

Professor J.J.K. Best will give three sessions to the project and Dr. Kean one session. Mr. J. Ridgway, will be responsible for the physics component of the project and is presently a research physicist involved in the day to day running and maintenance of the present MRI system at the Royal Infirmary. His research interests are the measurement of flow, using MRI techniques. The considerable expertise gained in the past six years by the University NMR Imaging Unit will provide the background expertise

for the MRI Unit at the City Hospital. Other resources from the University Department of Medical Radiology, based at the Royal Infirmary, will also be available to the Unit, including secretarial help. The Radiology Department at the City Hospital will become actively involved in Phase II when sick in-patients are examined.

4.3iii Neurology and Neurophysiology.

Dr. R.E. Cull, Royal Infirmary, Edinburgh and Dr. R. Will, Western General Hospital.

Dr. Will and Dr. Cull will supervise the neurological components of the study and the Research Fellow for whom support is sought in this proposal.

Dr. Cull is presently involved in providing the neurological and neurophysiological component of the MRC funded study involving haemophiliac patients, with Dr. C. Ludlam. He will supervise the Research Fellow in conducting the clinical and neurophysiological component of the study in the homosexual patient group based at the Royal Infirmary (Dr. A. McMillan). It is proposed to measure Evoked Potentials using a BBC-microcomputer based system(MRC funded). This is capable of recording Pattern Reversal Visual Evoked Potentials (VEPs), Somato-Sensory Evoked Potentials (SSEP) Auditory Brain Stem Evoked Potentials (AEP) and P-300 psycho-physiological responses. Additionally, standard measurements of motor and sensory conduction will be performed in at least one arm and one leg of patients. Late responses (F responses and H reflexes) and where clinically indicated concentric electrode EMG studies will be performed.

Dr. R. Will presently provides a consulting neurological service for the Infectious Diseases Unit and the AIDS Unit at the City Hospital, and will continue to do so, supervising the Research Fellow at the City Hospital. The proposed Job Description for the Research Fellow is compatible with a programme of higher professional training in neurology.

4.3iv Psychiatry and Neuropsychology

Dr. G.M. Goodwin, MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Ms. Ann Chiswick and Mr. Vincent Egan, City Hospital.

The original study of neurophysiological and neuropsychological impairment was initiated by psychiatrists, Dr. G.M. Goodwin, and Dr. D. St. Clair. Initially, the need for formal psychiatric screening interviews was seriously entertained, but the point of a major time consuming exercise without specific research questions was regarded as unconvincing. Surveys in the areas of high rates of HIV infection did not support the notion that an AIDS related psychosis occurs.(31) Therefore, while serious mental illness will be diagnosed and reviewed by psychiatrists if and when it occurs, using an appropriate instrument (the schedule for effective disorders in schizophrenia or SADS-Lifetime version) the studied population will continue to be screened by self-rating instruments and clinical interview.

At present, the research supervisor for neurophysiology and neuropsychology is Dr.G.M. Goodwin, Senior Clinical Scientist, MRC Brain Metabolism Unit, who is a named collaborator on the MRC Project Grant investigating cognitive impairment.

4.3v Neuropathology

Dr. J. Bell, Department Neuropathology, University of Edinburgh.

Autopsies will be carried out in the high risk post-mortem room facilities currently available at the Royal Infirmary. The removal of the brain and spinal cord or limited CNS tissue specimens will be supervised by neuropathologists, Dr. A. Gordon and Dr. J. Bell. Autopsy protocols will follow the MRC Working Party guidelines and will include photography, virological studies, electromicroscopy, immunochemistry and studies on SNAP frozen tissues. Further correlation between imaging and pathology will be provided by those cases in which brain biopsy is performed (cases presumed to be due to toxoplasma which do not respond to treatment and CNS lymphoma).

Following discussions with Professor C. Bird, Head of the University Department of Pathology, plans are well advanced for collaboration with Professor P. Lantos at the Maudsley Hospital, as part of the proposal to establish a central brain bank in his department, with a satellite brain bank in Edinburgh. It is envisaged that there will be full exchange of material and data between the banks, greatly adding to the value of the work done in the separate centres.

The potential for neuropathological research in Edinburgh will not be neglected. Dr. J. Bell has a special interest in the development of the central nervous system and proposes to undertake research on the effects of the HIV infection in the immature nervous system, drawing on resources in Edinburgh and the National Brain Bank. A high risk laboratory area is being planned.

Because the increase in post-mortems on AIDS patients does not occur until 1991, the financial implications necessary to set up a study do not have the same immediacy as those for the imaging project. It is proposed that the detailed costings for the neuropathology aspects outlined in the proposal be sought under a separate grant application.

4.4 JUSTIFICATION FOR SUPPORT REQUESTED

4.4i Capital Equipment:

The justification for the MRI imager necessary for the proposal is made in Appendix B. It is understood from the response from the MRC to the outline proposal the Council wishes to go out to tender for the equipment using the Procurement Directorate of the Department of Health. The technical specifications for the equipment requested have been passed to Dr. A.M. Calvert, Department of Health, 14 Russell Square. Preliminary discussions

have taken place with Siemens ltd., on the costings involved in installing a 1 Tesla system in a modular building on a green field site at the City Hospital.

4.4ii Personnel:

Support is requested for five years for -

(a) A radiologist at Senior Research level, to oversee the day to day imaging protocols in the Imaging Unit(this is on the advice contained in the MRC Response to the Outline Proposal.)

(b) A physicist at Senior Physicist/Lecturer grade. The Working Party on Neuro-imaging and Neuropathology in their report regarded a physicist as essential to maintain the imager performance for the quantitative measurements. Additionally, there is a need to develop techniques for quantitative imaging and volume assessment which require considerable computer programming expertise. Support is available from the University Department of Medical Physics and Medical Engineering, but it is regarded that this post shall be full time.

(c) Two Senior Radiographers. The radiographic staff of the MRI Imaging Unit at the City Hospital will be supported by the technical staff of the University Department. Two radiographers, together with technical support are the minimum necessary to provide for holiday cover and sick leave.

(d) Neurology Research Fellow. Support for a neurology research fellow at registrar grade is sought in order that the Steering Committee guidelines for the neurophysiological components of the study are met. Presently, the homosexual group have no neuro-physiological examinations made. Additionally, the registrar will support Ms Ann Chiswick in neurophysiological measurements on the IVDU group at the City Hospital. It is anticipated that this appointment will be for two years in the first instance.

Requests for support for neuropathology and for neuropsychiatry and neurophysiology will be made either as supplementary grant or new grant applications.

4.4iii Running Costs, Consumables and Minor Equipment:

The maintenance and running costs in terms of cryogens for the imager have been assessed after discussion with manufacturers on the basis of a 1 Tesla system.

The costs of consumables are based on the assessment of the present running costs of the NMR Imaging Unit at the Royal Infirmary, and have been kept to the minimum. The annual costs for Gadolinium DTPA are based on the present commercial cost (£55 per bottle). Add an estimate of use which is seen to increase during the course of the project. This may represent a considerable over-estimate.

After discussions with the Unit Management at the City Hospital, no element for services, nursing or portering costs have been included, as these will be incorporated in the AIDS Unit budget.

The equipment requested is for phantoms to callibrate performance of the imager. No other equipment is requested and the resources of the University Department of Medical Radiology and NMR Imaging Unit will be drawn upon as necessary. The Radiology Department at the City Hospital will provide film processing facilities.

DEPARTMENT OF PATHOLOGY

NEUROPATHOLOGY LABORATORY,
WESTERN GENERAL HOSPITAL,
CREWE ROAD,
EDINBURGH, EH4 GRO-C
TELEPHONE: 031-332 2525

10th. May, 1989.

Professor P. L. Lantos,
Department of Neuropathology,
Institute of Psychiatry,
Maudsley Hospital,
De Crespigny Park,
LONDON, SE5 8AF.

Dear Professor Lantos,

Re - Central Brain Bank & Data Base for research on AIDS and the nervous system.

We wish to express support for your proposal to establish at the Maudsley Hospital a central brain bank and data base for research on AIDS and the nervous system. We suggest that it would be desirable, subject to the provision of appropriate resources, to establish a satellite brain bank in Edinburgh.

As you are aware, the problem of HIV infection in S.E. Scotland is large (estimated as 1500 cases in 1987), and rather different from elsewhere since the vast majority are drug abusers. The number of patients developing AIDS is steadily increasing, and the number of deaths is increasing. We have examined the brain in about 10 cases so far. In addition, a significant number of children (>50) have been born to HIV positive mothers, but none has yet died of AIDS. It would be desirable to perform an autopsy on as many cases as possible in order to study nervous system involvement in these two special groups of AIDS patients. If a satellite brain bank were established in Edinburgh, all data and autopsy material would be made available to the central bank.

One of us (JEB) has a special interest in developmental neuropathology and would welcome the opportunity of using the central brain bank to investigate problems of HIV infection in the immature nervous system. The majority of cases in this group may well originate in Edinburgh.

We have shown this proposal to Professor C.C. Bird who is Head of Department and he expresses his approval. It is apparent to us that such collaboration between centres will expedite fuller understanding of the interaction between HIV and the nervous system.

Yours sincerely,

GRO-C

DR. JEANNE E. BELL.

GRO-C

DR. A. GORDON.

c.c. Prof. C.C. Bird

89. HIV Infection and the CNS: report from three Working Groups (88/ST068 file S819/144)

ANNEX 3 to S11/6

The Chairman welcomed Professor McDonald (Chairman of the Neurology and Neurophysiology Working Group) who joined the meeting to introduce this item and respond to members' questions.

Professor McDonald explained that three Working Groups had been set up following the MRC Workshop on CNS disease in HIV infection held in June 1987. The Workshop had drawn attention to the need to define the neurological and psychological effects of HIV infection, to pinpoint the earliest deficits, and to attain a greater understanding of the pathogenesis of the infection. Professor McDonald explained that the scale of the problem of HIV associated abnormalities in the CNS had been indicated by studies in the USA. These had suggested that up to 80% of people dying of AIDS suffered from such abnormalities, and that neurological complications also occurred in asymptomatic HIV sero-positive individuals and in patients with AIDS related complex. However, this data had been derived from cross-sectional studies and there was now a need for prospective longitudinal studies to examine the frequency of HIV associated abnormalities in the UK. There was some suggestion of a lower incidence of the AIDS - dementia complex in the UK; this was not inplausible as different strains of HIV had been identified on the two sides of the Atlantic.

There were a number of factors which favoured the setting up of studies in the UK. Firstly the uniformity of medical training and the links between major centres provided an excellent opportunity for collabortive work in the UK. Secondly the epidemic was at a relatively early stage of development in the UK compared with the US. Thirdly the US data was not in a form to allow comparisons between homosexuals, intravenous drug abusers and haemophiliacs. Such comparisons were required; and concentrations of seropositive members of these groups in different locations in the UK (London: homosexuals, Edinburgh: drug abusers and Oxford: haemophiliacs) made this country particularly suitable for such work. The Workshop had emphasised the importance of data collection being carried out in such a way that data from different centres, could be pooled and compared. The prime objective of the three Working Groups had been therefore to produce guidelines for basic and quantitive assessments to be used by all groups investigating HIV infection on the CNS, and to consider how best the data might be collected.

Professor McDonald then responded to members' questions. In particular Professor McDonald was asked about why it was necessary to have dedicated MRI scanning facilities for AIDS and HIV infected patients. He explained that the proposed serial studies would require considerable MRI time and that existing MRI machines were already heavily committed. It was noteworthy that recent US studies had not included serial studies because of insufficient access to MRI facilities. Two MRC facilities were recommended, one located in London and one in Edinburgh, because these cities had the largest concentrations of HIV positive individuals in the UK and also there were significant differences between the two population groups.

Professor McDonald was thanked by the Chairman for his helpful introduction; he then left the meeting.

The Board agreed that the guidelines for 'core' studies produced by the Working Groups were of considerable value and should now be given appropriate publicity. They had reservations about some of the recommendations in the report from the Neuropathology and Imaging Working Group. The need for one dedicated MRI scanning facility was accepted but members were not satisfied that the provision of two scanners could be justified. They agreed that advice should be sought from the Cell Board's MRI Committee on the type of MRI facility that would be needed; and where this could best be sited to provide access to the necessary expertise.

Members were not convinced by the Working Group's argument for the need to establish four brain banks, and considered that this could involve unjustifiable expense. It was agreed that one central bank with possibly a satellite or satellites would be preferable. The location of the central bank should be selected so that it could be linked to existing Neurosciences Board funded facilties.

The Board agreed that it was appropriate that the three Working Groups should be amalgamated into one Steering Committee. In addition to appropriate representation from the existing three Groups a virologist should be invited to join the Committee so that the question of possible HIV strain differences between the UK and USA, and other relevant virological issues, might be considered in detail. An AIDS physician, a representative from the Neurosciences Board, and observers from the Health Departments should also be invited to join the Committee.

Decisions

(i) Appropriate publicity should be given for the guidelines for 'core' studies and more detailed studies recommended by the Working Groups.

(ii) There is a need for one dedicated MRI facility, though there is insufficient justification for the two facilities recommended by the Neuropathology and Imaging Working Group. Advice should be sought from the Cell Board's MRI Committee on the type and location of the scanner.

(iii) One central brain bank, rather than the proposed four banks, should be established possibly with one or more satellites elsewhere. It should be situated so that it can be linked to existing Neurosciences Board funded facilities.

(iv) It would not yet be appropriate to invite detailed and fully costed proposals at this stage.

(v) The three Working Groups should be amlagamated into one Steering Committee; membership should include representation from the existing three Groups, a virologist, an AIDS physician, a representative from the Neurosciences Board and an observer from the Health Departments.

89/5116

5. Provision of a brain bank

5.1 The Chairman explained that the report from the Neuropathology and Imaging Working Groups had recommended to the Systems Board that one or more brain banks should be set up in conjunction with a central data bank and that the following information should be stored on each AIDS patient who died:

i) General: Serial number, name (initials), referral place and number, age, sex, risk groups;

ii) clinical findings (neurology, psychiatry, neuropsychology), neuroradiology report (CT scan, MRI, SPECT, etc), other investigations (CSF, biochemistry, immunology, virology, bacteriology);

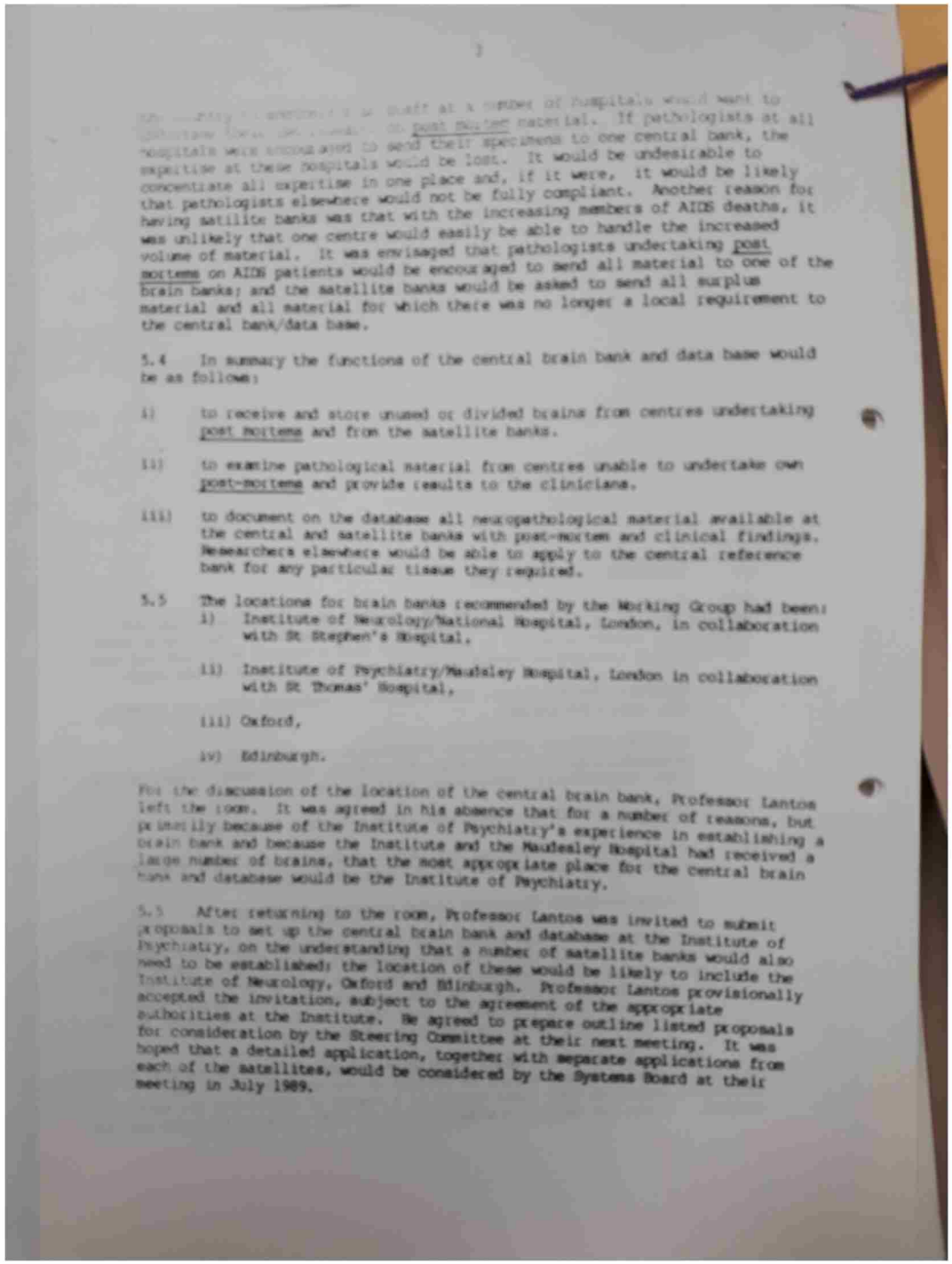
iii) post-mortem findings (diagnoses coded according to SNOMED);

iv) neuropathological material available: brain, spinal cord, peripheral nerves, autonomous nervous system, muscles, CSF, other;

v) Neuropathological diagnosis (coded according to SNOMED).

5.2 The Systems Board had agreed that only one central brain bank should be established, but that it might also be necessary to have a small number of satellite banks at other locations; also that the central bank should be at a location where deaths resulting from AIDS were common and preferably where Council support was being provided already for a brain bank (ie either at the Institute of Psychiatry or at the Institute of Neurology).

5.3 Members accepted the Board's decision and agreed that the central brain bank and central detabase should be located at the same site. It was considered important that there should be a number of satellites for several reasons. It was not easy to transport brain of AIDS patients from one part of



3

[illegible] staff at a number of hospitals would want to [illegible] post mortem material. If pathologists at all hospitals were encouraged to send their specimens to one central bank, the expertise at these hospitals would be lost. It would be undesirable to concentrate all expertise in one place and, if it were, it would be likely that pathologists elsewhere would not be fully compliant. Another reason for having satilite banks was that with the increasing numbers of AIDS deaths, it was unlikely that one centre would easily be able to handle the increased volume of material. It was envisaged that pathologists undertaking post mortems on AIDS patients would be encouraged to send all material to one of the brain banks; and the satellite banks would be asked to send all surplus material and all material for which there was no longer a local requirement to the central bank/data base.

5.4 In summary the functions of the central brain bank and data base would be as follows:

i) to receive and store unused or divided brains from centres undertaking post mortems and from the satellite banks.

ii) to examine pathological material from centres unable to undertake own post-mortems and provide results to the clinicians.

iii) to document on the database all neuropathological material available at the central and satellite banks with post-mortem and clinical findings. Researchers elsewhere would be able to apply to the central reference bank for any particular tissue they required.

5.5 The locations for brain banks recommended by the Working Group had been:

i) Institute of Neurology/National Hospital, London, in collaboration with St Stephen's Hospital,

ii) Institute of Psychiatry/Maudsley Hospital, London in collaboration with St Thomas' Hospital,

iii) Oxford,

iv) Edinburgh.

For the discussion of the location of the central brain bank, Professor Lantos left the room. It was agreed in his absence that for a number of reasons, but primarily because of the Institute of Psychiatry's experience in establishing a brain bank and because the Institute and the Maudesley Hospital had received a large number of brains, that the most appropriate place for the central brain bank and database would be the Institute of Psychiatry.

5.5 After returning to the room, Professor Lantos was invited to submit proposals to set up the central brain bank and database at the Institute of Psychiatry, on the understanding that a number of satellite banks would also need to be established: the location of these would be likely to include the Institute of Neurology, Oxford and Edinburgh. Professor Lantos provisionally accepted the invitation, subject to the agreement of the appropriate authorities at the Institute. He agreed to prepare outline listed proposals for consideration by the Steering Committee at their next meeting. It was hoped that a detailed application, together with separate applications from each of the satellites, would be considered by the Systems Board at their meeting in July 1989.

MEDICAL RESEARCH COUNCIL

MRC: IN CONFIDENCE

Systems: 89/5117 July 1989

DR MARGARET ESIRI and DR P MILLARD (Department of Neuropathology, Radcliffe Infirmary, Oxford).

BRAIN BANK FOR RESEARCH ON HIV INFECTION AND THE NERVOUS SYSTEM: SATELLITE FACILITY AT OXFORD

Application for special project grant support

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1. Paper

Annex - application form

Appendix 1 - proposed research programme

Appendix 2 - details of support requested

Appendix 3 - curricula vitae and lists of publications

2. Referees

A. Professor Ingrid Allen (Pathology, Belfast)

B. Dr Louise Ouzner (Multiple Sclerosis Laboratory, Institute of Neurology London)

3. Background

See previous agenda item.

4. Current Council support

Dr Margaret Esiri holds a special project grant entitled: 'Aspect of the pathology of the nervous system in human immunodeficiency virus (HIV) infection'. (G8801678). Tenure: 1.7.88 - 30.6.91. Support is provided for two scientific assistants at a total cost per annum of £31.3k (including NI etc). Expenses have been provided at a level of £12.9k and £15.2k has been authorised to date for equipment. The total support awarded when this grant was approved was £52.2k.

5. Present proposals

The applicants are requesting support for one part-time technical assistant, expenses of £1.3k over the tenure of the grant and £5.8k for equipment. Total support requested is £48.8k over a period of five years.

The applicants have sought local ethical committee approval; any award will not be made until written evidence of approval has been received.

If awarded this grant will be a charge to the special allocation of funds from the Science Vote for AIDS research.

G89 14151

DBM: Professor J G P Sissons

6. Action required

i) Assessment of the merit of the proposals in the light of such criteria as are relevant.

ii) Decision on the scale and duration of support to be made available, if awarded.

MRC
Medical Research Council

Annex 89/5/17

Application for a project grant

Please type throughout and return this form with nine photocopies

1. Applicant(s)	Applicant 1	Applicant 2	Applicant 3
Surname	ESIRI	MILLARD	
Forename(s)	Margaret	Peter	
Age	47 years	49 years	
Title	Dr	Dr	
Post held	Reader in Neuropathology	Consultant Histopathologist	
No. of hours p.w. on project	4		

2. Institution/Authority (administering grant if approved)
University of Oxford

City or Town Oxford

Department accommodating project (and institution if other than opposite)
Clinical Neurology

3. Title of project **Brain bank for research on HIV infection and the nervous system: satellite facility at Oxford**

4. Type of grant sought
New project

5. Abstract of research

Samples of unfixed central and peripheral nervous system tissues, obtained at autopsy from HIV-positive subjects are to be stored deep frozen for future use. They will be made available as required for research into the pathogenesis of nervous system disease due to HIV either by local investigators (e.g. the applicant, MRC SPG88/01678) or those outside Oxford who get in touch about their requirements. This bank of tissue would act as a satellite to Professor Lantos' proposed brain bank for research on HIV infection and the nervous system.

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6. Proposed starting date 1.8.89

Proposed duration (in months) 60

7. Summary of support requested (See attached sheet for Fourth and Fifth years)		First year £	Second year £	Third year £	Total over period £
(a) Personal support of applicant					
(b) Research staff (medical/scientific)	Number of posts: Whole/part-time				
(c) Technical/other assistance	Number of posts: 1 Whole/part-time	6,173	6,420	6,677	33,160
Addition for superannuation and National Insurance calculated as 26% of salaries in (a), (b), and (c)		1,605	1,669	1,736	8,622
(d) Visiting senior scientist (salary/subsistence, airfare)					
(e),(f),(g) Expenses		250	250	250	1,250
	Total recurrent	8,028	8,339	8,663	43,032
(h) Apparatus		5,816			5,816
	Total support requested	13,844	8,339	8,663	48,848

(PG5)

PROPOSED INVESTIGATION

1. Title of project
2. Purpose of proposed investigation
3. Background of the project
4. Plan of investigation
5. Detailed justification for support requested

Please leave margins blank

Brain bank for research on HIV infection and the nervous system: satellite facility at Oxford

Purpose of proposed investigation:

It is anticipated that during the next five years increasing numbers of post mortem examinations will be performed in Oxford on subjects with HIV infection. Although some of these cases will be likely to have belonged to the high risk groups of homosexuals and drug-abusers, most are expected to have been haemophiliacs infected through the use of factor VIII concentrates. These cases will constitute a prospectively well studied group on which detailed neuropathological autopsy studies may be expected to increase understanding of the effects of HIV on the nervous system. Frozen material will be stored to enable studies to be undertaken that cannot be easily carried out on routinely fixed material. These include nucleic acid hybridisation and polymerase chain reactions and some immunocytochemistry. Some frozen material may be required for the studies being undertaken by the Ist applicant supported by MRC SPG 88/01678, though attempts are initially being made to use fixed material as it is safer. Other investigators in the United Kingdom and elsewhere would also be offered access to the frozen material. It is likely to prove valuable to such groups because nervous system material from clinically well studied cases of HIV infection, particularly from haemophiliacs, is scarce and we have already found that haemophiliacs constitute a group well suited to the study of the early stages of HIV-induced changes in the nervous system (Esiri et al., submitted, copies enclosed). The bank would act as a satellite to the bank to be proposed by Professor Lantos.

Background to the project:

Following on from a workshop convened by the MRC in June 1987 to consider the CNS manifestations of HIV infection, working groups were set up to consider future research on neurological involvement in HIV infection, one of which concerned itself with neuropathological studies. This working group recommended to the Systems Board of the MRC that brain bank facilities should be provided at a few centres where post mortems were performed to enable nervous system material to be frozen and banked for use locally and in other centres where appropriate studies were being undertaken. In January 1989 the Systems Board recommended that a central brain bank should be established. The steering committee on HIV infection and the nervous system invited Professor Lantos to submit proposals for a central brain bank and data bank and agreed that invitations be made to other centres undertaking pathological work to set up satellite brain banks. The proposals made here are in response to such an invitation.

Prospective neuropsychiatric studies of a cohort of 100 HIV-positive haemophiliacs is underway in Oxford with funds supplied by the Wellcome Trust to Dr J. Catalan and Dr C. Rizza. These cases are expected to supply the bulk of the material we propose to store in the Oxford brain bank. Nineteen cases of HIV infection have so far had autopsy examination in Oxford, 13 of them haemophiliacs. The general post mortem examinations have been performed by Dr Millard and the neuropathology by Dr Esiri.

Plan of investigation:

At the time of post mortem examination samples of cerebrum, basal ganglia, brain stem, cerebellum, spinal cord, cerebrospinal fluid, peripheral nerve and muscle will be packaged, labelled and snap frozen in liquid nitrogen and stored at -80°C in a deep freeze reserved for this purpose. Some of the samples will be first exposed to β propiolactone which is known to inactivate the virus (Chaplin et al, 1989, J. Clin Pathol, 42, 318-21). This treatment is known not to interfere with immunocytochemical reactions but it is not yet known if it interferes with nucleic acid studies. If further investigation shows that it does not, all samples for storage will be treated in this way to lessen the risk of infection from handling and cutting sections of this material. When required, frozen sections will be cut on a cryostat reserved for use with infected tissues (already available). Full precautions detailed in the Guidelines published by the Advisory Committee on Dangerous Pathogens for handling tissues and fluids from HIV-infected subjects will be taken. Clinical details, general pathological and neuropathological findings will be carefully documented and transferred to the central data bank proposed to be set up by Professor Lantos. Current post mortem rates are 10-12 per year but are expected to increase. Ethics Committee approval for the procedures involved has been obtained (letter enclosed). Recommendations on removal of tissues made by Health Department will be followed.

Detailed justification for support requested :

A -80°C deep freeze to be used solely for this purpose is requested. Because the material to be banked would constitute a scarce resource the deep freeze should be fitted with an alarm system linked to a constantly manned telephone switchboard, to provide an immediate alert if the system fails.

The services of a half-time fully trained MLSO are requested. Only a highly experienced person (MLSO 2) can be considered suitable for this work in view of the risk involved. Although such a person may not be required to spend all his/her time on this project it would not be feasible to employ someone for less than half time and any extra time available could be spent on other MRC-supported HIV-centred research (SPG 88/01678) for which technical support is badly needed.

A sum of £250 per annum to cover costs of packaging slides, storage boxes and storage of data is included.

The Opus PCV and Wordperfect software is for data storage and will enable data to be transferred by floppy disc to the central data bank.

Oxfordshire Health Authority
Central Oxford Research Ethics Committee
Manor House, Headley Way
Headington, Oxford OX3 9DZ
Tel: Oxford (0865) 817547
Please reply to Anna Truelove

Our ref: GP/APT/jed/1661
Your ref:

22nd April, 1988

Dr M M Esiri
Consultant Neuropathologist
Neuropathology Department
Radcliffe Infirmary
Oxford

Dear Dr Esiri

COREC NO: 1661 - ASPECTS OF THE PATHOLOGY OF THE NERVOUS SYSTEM IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Since your study does not involve clinical subjects, and consent will already have been given for post mortem, I am able to take Chairman's action in approving your study.

Yours sincerely

GRO-C

pp DR G PASVOL
CHAIRMAN
CENTRAL OXFORD RESEARCH ETHICS COMMITTEE

MEDICAL RESEARCH COUNCIL

MRC: IN CONFIDENCE

Systems: 89/S131 July 1989

DR C A LUDLAM (Haemophilia Centre, Edinburgh), DR J F PEUTHERER (Virology, University of Edinburgh) and DR C M STEEL (MRC Human Genetics Unit, Edinburgh)

CLINICAL, IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH PARTICULAR REFERENCE TO HIV INFECTION

Revised application for special project grant support.

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1. Papers

Annex 1 - application form

Appendix 1 - proposed research programme

Appendix 2 - details of support requested

Appendix 3 - curricula vitae and lists of publications

Appendix 4 - letter from Dr Ludlam

Annex 2 - original application considered by the Systems Board in April 1989

Annex 3 - referees' opinions on the original application

Annex 4 - extract from the unapproved minutes of the Systems Board meeting held in April 1989 (to follow)

2. Background

At their meeting held in April 1989, the Board considered an application (annex 2) from Drs Ludlam, Peutherer and Steel to continue studies on haemophiliacs (3ii, below). The Board agreed that the existing work should be continued but had a number of reservations about the proposed new studies, in particular the virology. They agreed that a grant should be awarded but at a reduced level and for one year only and that the applicants should be invited to submit a revised application, taking into account the comments of both the referees (annex 3) and the Board (annex 4).

The grant approved by the Board has not yet been awarded. It will be awarded only if the present application is declined.

3. Current Council support

i) Dr C A Ludlam holds a special project grant entitled: 'Participation in MRC Leukaemia trials' (G8300458). Tenure: 20.6.83 - 19.6.89. Support is provided for a part-time clerical assistant at a cost of £2.2k p.a. (including NI etc). The total support awarded when this grant was approved was £2.2k.

ii) Dr C A Ludlam holds a special project grant entitled: 'Clinical and immunological study of haemophiliacs treated exclusively by NHS factor VIII/IX concentrates'. (G8514756). Tenure: 1.8.86 - 31.7.89. Support is provided for one research assistant and two technical assistants at a total cost of £40.9k p.a. (including NI etc). Expenses have been

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G8902835

provided at the level of £20.2k over the tenure of the grant and £6.5k has been authorised to date for equipment. The total support awarded when this grant was approved was £59.2k.

4. Present proposals

Dr Ludlam and colleagues have submitted a revised application to continue their study on haemophiliacs. It was submitted with a covering letter (annex 1, appendix 4) explaining the changes that have been made in the light of the referees' and Board's comments.

The applicants are requesting support at the level of one scientific assistant and two technical assistants, £58.2k for expenses over the tenure of the grant and £1.4k for equipment. Total support requested is £206k over three years. Referees' opinions have not been sought again.

The proposals have received local ethical committee approval.

If awarded this grant will be a charge to the special allocation of funds from the Science Vote for AIDS research.

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5. Action required

i) Assessment of the merit of the proposal in the light of such criteria as are relevant.

ii) Decision on the scale and duration of support to be made available, if awarded.

ANNEX 1
89/5062

MRC
Medical Research Council

Application for a project grant

Please type throughout and return this form with nine photocopies

1. Applicant(s)	Applicant 1	Applicant 2	Applicant 3
Surname	LUDLAM	PEUTHERER	STEEL
Forename(s)	CHRISTOPHER A.	JOHN F.	C. MICHAEL
Age	42	52	48
Title	Dr.	Dr.	Dr.
Post held	Director, Haemophilia Centre	Senior Lecturer	Assistant Director, Human Genetics Unit MRC
No. of hours p.w. on project	Daily 4-6	Daily 5-10	5

2. Institution/Authority (administering grant if approved)
Univeristy of Edinburgh

City or Town Edinburgh

Department accommodating project (and institution if other than opposite)
Medicine/Haematology

3. Title of project Clinical, immune and virological investigation of haemophiliacs with particular reference to HIV infection

4. Type of grant sought
Special Project Grant

5. Abstract of research This application is for support to continue investigations into HIV virology and immune function in both anti-HIV positive and negative haemophiliacs. The study will include the long term follow up of 32 haemophiliacs, of whom at least 18 became infected by HIV following transfusion of a single batch of NHS factor VIII concentrate in 1984. To date the study has enabled the positive identification of risk factors to infectivity and progression of clinical disease. It is proposed to continue detailed clinical and laboratory follow up including serial determinations of viral, immune and neurological parameters. The project will also include evaluation of anti-HIV negative haemophiliacs who are known to have been exposed to HIV, by the polymerase chain reaction to determine whether they too may be infected. As our previous studies have demonstrated that factor VIII concentrates are also directly immunosuppressive (apart from the effect of HIV), we wish to continue our studies to identify the concentrate components responsible.

For Office use											

6. Proposed starting date 1st August 1989

Proposed duration (in months) 36

7. Summary of support requested		First year £	Second year £	Third year £	Total over period £
(a) Personal support of applicant					
(b) Research staff (medical/scientific)	Number of posts: 1 Whole/part-time	16,750	17,635	18,520	52,905
(c) Technical/other assistance	Number of posts: 2 Whole/part-time	20,284	20,999	22,047	63,330
Addition for superannuation and National Insurance calculated as 26% of salaries in (a), (b), and (c)		9,628	10,044	10,646	30,218
(d) Visiting senior scientist (salary/subsistence, airfare)					
(e),(f),(g) Expenses		19,795	19,100	19,300	58,195
	Total recurrent	66,457	67,778	70,413	204,648
(h) Apparatus		1,400			1,400
	Total support requested	67,857	67,778	70,413	206,048

(PG5)

8. Does the project require Local Ethical Committee approval? Yes/No Has been obtained, see letter

9. (a) Is your related research currently being supported by any outside body (other than the MRC)?
If so, please indicate the topic, supporting organisation, value and tenure
NO

(b) Are you currently applying elsewhere for support for work relating to the present proposal?
If so, please give details as for 9(a)
NO

(c) Is this application currently being submitted elsewhere?
If so, to which organisation; and by what date is a decision expected?
NO

(d) Has this application been submitted elsewhere over the past year?
If so to which organisation and what was the result?
NO

(e) Is the proposed research likely to lead to patentable or otherwise commercially exploitable results? If so, please give brief details
NO

10. Full official postal address of applicant
Dr. C.A. Ludlam
Department of Haematology
Royal Infirmary, Edinburgh

Telephone number of applicant (please give STD code from London and extension)
031-229-2477 Ext GRO-C

11. Acceptance of regulations and conditions

I have read the conditions set out in the Council's current Project Grants booklet and, if my application is successful, I agree to abide by them. I shall be actively engaged in, and in day-to-day control of, the project.

Signature of applicant(s): Date:

12. This application should be submitted by/through (i) the Head of Department and (ii) the officer who will be responsible for administering any grant that may be awarded. Each should sign the following declaration:

I confirm that I have read this application and that, if granted, the work will be accommodated and administered in the Department/Institution in accordance with the conditions in the Council's current Project Grants booklet. The **staff gradings and salaries** quoted are correct and in accordance with the normal practice of this Institution.

(i) Signature of **Head of Department**

GRO-C

Title Professor of Medicine.

To be appended in typescript or block capitals

Name and initials (of (i) above)
Professor I.A.D. Bouchier

Institution
University of Edinburgh

Address (if different from 10 above)
Dept. of Medicine
Royal Infirmary
Edinburgh.

Date: 8 02 89

(ii) Signature of **Administrative Authority**

GRO-C

J.T. Suddaby, Deputy Secretary
~~Finance Officer/Bursar/Registrar~~/Secretary of Institution
(delete as appropriate)

To be appended in typescript or block capitals

Name and initials (of (ii) above)
J.T. Suddaby,

Institution
University of Edinburgh

Address and telephone number
(including STD code from London and extension)
University of Edinburgh
Old College, South Bridge,
EDINBURGH
031.667.1011 Ext. GRO-C

Date: 9.02.89

13. Name, address and telephone number (including STD code from London and extension) in typescript (or block capitals) of the officer who should be contacted regarding the administration of the grant if awarded, if different from (ii) above:

PROPOSED INVESTIGATION **APPENDIX I page 1**

1. Title of project
2. Purpose of proposed investigation
3. Background of the project
4. Plan of investigation
5. Detailed justification for support requested

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CLINICAL ,IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH PARTICULAR REFERENCE TO HIV INFECTION

PURPOSE OF PROPOSED INVESTIGATION

To investigate the effect of HIV infection we wish to continue our longitudinal study of a cohort of 32 haemophiliacs who were transfused with a single HIV infected batch of NHS factor VIII concentrate in the Spring of 1984 (1). Studies of this cohort so far have indicated factors which predispose to HIV infection and to clinical and immunological decline following infection. We wish to continue to characterise in detail changes in the virus itself, in specific anti-viral immune responses and in non-specific immunological parameters in the patients because such studies will provide insight into the mechanism by which HIV causes clinical decline. The studies will be both prospective and retrospective using our unique collection of serial serum and leukocyte samples stored over a period of 6 years (ie predating exposure to HIV). Almost half the patients who received the infected batch of factor VIII have not demonstrated any markers of HIV infection and we propose to use the polymerase chain reaction (PCR) technique with its greatly enhanced sensitivity to try and detect HIV RNA/DNA in these anti-HIV negative individuals. We have also further non-cohort patients who have received factor VIII concentrates and are HIV antigen, antibody and culture negative but have been exposed to HIV and it is proposed to seek the HIV genome by PCR also in these individuals (4).

One of the risk factors we have identified which predisposes to HIV infection and subsequent clinical decline is possession of the HLA haplotype A1 B8 DR3 (2). To study this further collaboration has been established with Haemophilia Centres at the Royal Free Hospital London, Newcastle and Glasgow which have apparently different prevelance rates of HIV induced clinical disease. We have also agreed to participate in an EEC Commission sponsored study of the immunogenetics of AIDS co-ordinated by Dr. Arne Srejgaard, Copenhagen.

HIV is transmitted heterosexually and some couples, where the man is infected, wish to have children but do not wish the wife to be exposed sexually to HIV. One possible solution to this difficulty is to separate sperm from semen and offer artificial insemination. A technique for separating sperm from semen has been deeloped by Dr. F. Wu at the MRC Reproductive Biology Unit in Edinburgh and it is proposed to assess partition of HIV in the fractionated semen with a view to developing a technique that could be used to offer artifical insemination to couples.

The other major aim of the grant application is to investigate the mechanism by which factor VIII concentrates (independent of HIV)modulate the immune system. Our original studies demonstrated immune abnormalities, eg depressed cell mediated immunity and reduced CD4 counts, in some instances as severe as those induced by HIV (3). This may be important for two principal reasons. Firstly such immune modulation may predispose to infection by both bacteria and viruses (26). Secondly it has become very important to assess further which components of the factor VIII concentrate are responsible because it may be possible to manufacture concentrates with reduced levels of these. At present only very high purity concentrates, eg ones manufactured with the use of monoclonal antibodies, have low levels of these components (unpublished observations) but in the manufacture of these products there are large losses of factor VIII which is contributing to the present world shortage of factor VIII. Furthermore such studies may be informative in identifying candidate blood components which modulate the immune system in a beneficial way prior to renal transplant and to the deteriment of the patient with colonic cancer.

BACKGROUND OF THE PROJECT

In the study are a group of 32 originally HIV negative haemophiliacs who were inadevertently transfused with an HIV "infected" batch of SNBTS factor VIII concentrate in the spring of 1984; at least 18 became infected with HIV (1). This cohort has been followed up clinically and detailed virological, immunological and neurological investigations have been supported by a current MRC Project Grant. This further application is to continue our studies of these and other HIV positive and negative haemophiliacs. Immunological studies were initiated in many of the patients in 1983 prior to HIV infection and these data has greatly added to the value of the study.

Clinical Status of Patients

Of the 18 infected individuals two have died of AIDS and a third of HIV related problems; five individuals have CDC Group 4 disease and have therefore started on zidovudine. All patients have been followed in detail and the results of the studies have been published (1,2,5) or are outlined below (manuscripts 2,4,5,6). The 14 haemophiliacs in the cohort who remain anti-HIV negative despite receiving the HIV infected factor VIII are clinically well as are the recipients of the factor IX concentrate which was made from the same HIV infected donor plasma. In addition there are 7 haemophiliacs who were infected by blood products (both NHS and commercial) for whom we have an accurate date of seroconversion (from stored serum samples) and they have also been followed up in a similar manner to the cohort group. All other HIV antibody negative haemophiliacs are clinically well despite a number being exposed to HIV from two further infected batches of SNBTS concentrate (4).

The Table summarises the present patients and their clinical status.

	NO.	ANTI-HIV	TRANSFUSION GROUP	HIV CLINICAL STATUS
Group Ia	16	Positive	Infected cohort	3 dead; 5 ARC
Group Ib	7	Positive	Non-cohort infected from other blood products	Asymptomatic
Group II	14	Negative	"Non-infected" cohort	Asymptomatic
Group III	15	Negative	Christmas Disease	Asymptomatic
Group IV	50	Negative	Exposed to Factor VIII concentrate	Asymptomatic

Virological Studies

We have followed in detail the tine course of HIV antibody (total; both IgM and IgG, anti-env and anti-core) as well as antigen from infusion of the infected batch, through seroconversion (5,6). We wish to continue these studies and perhaps extend the serology to include analysis of the specific antibody response, to synthetic peptides as well as correlate the changes with alteration in tests of immune function (vide infra). Similarly we have followed serum HIV antigen levels and demonstrated antigenaemia in half the patients prior to seroconversion and the later reappearance of antigen associated with immunological and clinical decline(5). Virus has been identified by culture of lymphocytes with PHA and IL2 and current studies indicate that virus isolation can be improved by selective removal or enrichment of mononuclear cell subsets (manuscript 1). We have observed that virus is more readily detected in patients (7/7) with CDC Group IV compared to asymptomatic (4/9) CDC Group II and III (manuscript 2) and this is consistent with observations on p24 antigenaenia and transmission risk in other studies in homosexuals (7) haemophiliacs and other recipients of blood products (8).

The polymerase chain reaction (PCR) has greatly increased the sensitivity of viral

detection (9) and we have therefore recently set up this technique with a pair of gag specific primers. Additionally we have markedly increased both the sensitivity and specificity of the technique by using a sceond set of internal primers (manuscript 3). We have started to investigate the HIV negative patients, both those in the cohort (Group II) and others who have definitely or may have been exposed to HIV (Group III and IV) to determine whether viral genome is detectable. It is proposed to search for viral RNA as well as transcribed or integrated DNA both in cell free plasma as well as peripheral blood lymphocytes and monocytes separately. We also propose to use the PCR reaction to attempt to detect HIV genome in the batch of HIV contaminated factor VIII which infected our cohort as well as other non-heat treated and heat treated factor concentrates of both NHS and commercial origin. We shall use various primer pairs for major viral genes for the PCR of seronegative patients to check if incomplete provirus may be present.

There are a small group of haemophiliacs, who despite large use of non-heat treated factor VIII concentrate prior to 1984, have remained seronegative. In conjunction with the Royal Free Haemophilia Centre we should like to investigate whether using the PCR virus is detectable and further explore why such individuals may be resistant to the virus.

As the 18 HIV positive individuals in the cohort may have been infected by a single strain of virus a collaborative study under the direction of Dr. J.O. Bishop and Dr. A. Leigh -Brown (MRC funded) has been established to examine genomic mutation and drift. As differing viral strains have different infectivities for particular cell types and detailed knowledge of the genomic characteristics of the virus that infected the cohort will become available,a more detailed examination of the inter-action between virus and host may be possible and lead to an explanation of the differing rates of immunological and clinical decline. A related study of the infectivity of lymphocytes and macrophages for various isolations of HIV has been previously funded by the MRC (Drs. J.F. Peutherer and D.W.J. Aw).

We propose to test sequential stored serum samples for evidence of infection with CMV, EBV and human herpes virus^{6} to see if infection with these viruses either before or after HIV infection affects the rate of clinical progression. At the same time, serum levels of anti-HBs and anti-HBC will be measured as any decrease could lead to a risk of hepatitis B(10). These sudies will be performed on both the HIV positive and negative patients.

Immunological Investigations

These were begun in the Edinburgh patients in 1983 prior to infection by HIV (3) and follow up studies have continued since then and the findings correlated with clinical status (1,2,5 and manuscript 4,5 and 6). Our results reveal that in the group as a whole there is a steady decline in CD4 numbers of those infected but that there may be marked fluctuation within each individual. The HLA haplotype A1 B8 DR3 (vide intra) is associated with a more rapid decline in CD4 counts. We propose to continue the longitudinal assessment of CD4 and CD8 subsets along with enumeration of other subsets including NK cells, activated T cells, monocytes and null cells (12).

In view of the known changes in B2 microglobulin, and neopterin in HIV infection we have measured these on stored serum samples in all members of the cohort. Marked and progressive changes have been observed which correlate with immunological and clinical status (manuscript 6). Shed IL2 receptor levels in serum may reflect the extent of T cell activation (13,14) (as does identification of MHC class II +ve T cells). Both of these are yielding results that appear interesting and will be included in the future programme. Anti-EBV antibody levels (VCA) have been correlated with the risk of developing EBV-related Burkitt's lymphoma, a finding which is attributed to the inverse relationship between effectiveness of T cell function and EBV-induced B cell proliferation (15,16). Perhaps surprisingly,

anti-EBV antibody levels have been reported as not raised in AIDS patients with reduced circulating T4 cells, but we propose to examine this question in the Haemophilia cohort, both in relation to the known rates of decline of T4 cells and to the likely increased risk of lymphoma in these patients (17).

The lymphocyte membrane enzyme ecto-5-nucleotidase (E.C.3.5.7.1) is a passive degradative enzyme,activity of which is related to lymphocyte maturity and function. Two studies have recorded reduced expression of the enzyme in male homosexuals though whether it is a cause or a consequence of T cell defects associated with HIV infection is unclear (18,19). A single study in haemophilia patients with and without antibody to HIV did not show abnormalities of ecto-5-nucleotidase levels (20) and again the issue requires to be addressed in a well-characterised group of subjects. We propose to carry out at least a pilot investigation in Edinburgh and the Haemophilia cohort will form one of the groups to be studied.

Recent studies have demonstrated a plasma factor in HIV positive individuals that inhibits mitogen and antigen induced lymphocyte proliferation in vitro. The inhibitory activity is associated with low molecular weight plasma fractions and it increases with falling CD4+ cell counts (25). As we have established a microtechnique to assess lymphocyte proliferative response (vide intra) we propose to seek evidence for plasma inhibitory factors in our patients.

Skin tests to 7 recall antigens have been repeatedly assessed since 1983 as a measure of cell mediated immunity. We have demonstrated (21) as have others that haemophiliacs are relatively anergic, due to the effect of factor VIII concentrate per se, and that HIV causes a further progressive decline in cell mediated immunity.

HLA studies

As several reports indicated that particular HLA antigens were associated with HIV disease progression we tissue typed all 32 patients in the cohort. We have reported that the haplotype A1 B8 DR3 is associated with succeptibility to infection as well as a more rapid decline in CD4 numbers and progression to CDC Group IV disease (2). This has been possible only because we know accurately when the patients were infected and because we have been able to HLA type all patients. The lack of confirmation by some other investigations of our observation may be because individuals with the succeptible haplotype have already died prior to study or because the duration of infection is not accurately known (22,23). (All three deaths in our cohort to date, have been in patients with the HLA, A1, B8,DR3 haplotype).

To investigate the relationship between HLA type and disease progression collaborative studies are in hand with other Haemophilia Centres with particularly high and low incidence of HIV related disease. In addition we have a collaborative study with Sheffield Haemophilia Centre where liver biopsies are being performed prior to interferon therapy for non-A non-B hepatitis to investigate the possibility of an association between HLA and progression of liver disease or response to interferon therapy. We are co-operating with other groups where special expertise in particular areas of immunology can be applied appropriately to samples from our patients.

Studies in HIV Negative Haemophiliacs

Blood transfusion can profoundly influence the clinical course in several groups of patients by modifying immune competence, eg renal transplant engraftment and survival of individuals with colonic cancer. Factor VIII concentrates per se also produce immune changes which mimic those induced by HIV, eg skin anergy and depressed CD4 and high normal CD8 counts (1). Furthermore relatively low purity concentrates depress lymphocyte reactions in vitro compared to more highly purified concentrates (unpublished observations) and this has been associated with uncharacterised high molecular weight component of the concentrate (24). Initial studies are in hand to gel filter factor VIII concentrate to assess the ability of components to inhibit PHA stimulation of normal lymphocytes. Studies to elucidate the mechanism by which factor VIII influences lymphocyte numbers and function may also shed light on the mechanism by which other blood products profoundly affect patients with other disorders.

Neurological Studies

Progressive neurological derrangement is a characteristic feature of HIV infection although relatively little is known about its early manifestations. Detailed neurological and neuropsychological studies have been undertaken in both our HIV positive and negative haemophiliacs on a single occasion. Haemophiliacs are prone to intrcranial bleeds and hence HIV negative haemophiliacs may have impaired neurological function and are therefore an important control group. Baseline data is available on approximately 12 patients which is fewer than we had originally intended. The investigations are very time consuming and the haemophilacs have been reluctant to give up yet more timefor this research project. Despite our difficulties it is our intention to serially follow up the patients with the aim of characterising which investigations are most sensitive at detecting early HIV encephalopathy.

PROPOSED INVESTIGATION

1. Title of project
2. Purpose of proposed investigation
3. Background of the project
4. Plan of investigation
5. Detailed justification for support requested

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CLINICAL, IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH PARTICULAR REFERENCE TO HIV INFECTION.

PLAN OF INVESTIGATION

The project will be based at the Edinburgh Haemophilia Centre, Department of Haematology under the direction of Dr. C.A. Ludlam. Laboratory and office space is available within the Department for the project. The immunological investigations are undertaken by Dr. C.M. Steel and the virology by Dr. J.F. Peutherer.

1. All patients will be seen clinically and examined in detail at least every six months. Those who are anti-HIV positive will be reviewed at least every three months. Many will need to be seen medically much more frequently because they are unwell and/or on Ziudovudine.

2. Neurological and neuropsychological examinations will be attemped annually on as many anti-HIV positive and negative haemophiliacs as possible. It is hoped to continue with the following investigations which have been set up and applied to many haemophiliacs during the past three years.

a) Clinical neurological examination (Dr. R.E. Cull, Consultant Neurologist).

b) Peripheral nerve conduction studies - motor and sensory in right median nerve, motor in right lateral popliteal nerve and sensory in right sural nerve.

c) CT/NMR scanning (Professor J.J.K. Best).

d) Clinical neurophysiological tests:

   i) EEG (with FM recording for fast Fourier transformation).
   ii) Auditory evoked potentials.
   iii) Visual evoked response using patern reversal.
   iv) P300 (as measure of long latency).

e) Psychological tests (Mr. W. McKinlay, Principal Clinical Psychologist).

   i) Reaction times.
   ii) Short term memory tests.
   iii) Tests of concentration - PASAT.
   iv) Verbal reasoning IQ test.
   v) Simple tests of language and comprehension.

3. Blood samples for the following investigations will be collected at least every six months but in many this will be as frequently as every two weeks depending on HIV status and symptomatology. Investigations will include:

1. Full blood count
2. Liver function tests
3. Makers of immunological function (see below)
4. Makers of HIV infection (see below)
5. Antibodies to EBV, CMV and HHV6

4. Cell mediated immunity will continue to be measured annually using Multitest device which assesses recall to 7 antigens.

5. Assessment of HIV status. The following investigations are currently set up and it is proposed to continue to test the patients at least at six monthly intervals.

(i) HIV antigen in plasma

(ii) HIV culture by co-cultivation using HIV antigen assay and dot blot hybridisation to detect positive cultures. The technique is also currently being developed to increase its sensitivity by enrichment with CD4 cells.

(iii) Polymerase chain reaction has been set-up in conjunction with Dr. J.O. Bishop, Dr. A. Leigh-Brown and Dr. P. Simmonds. The specificity and sensitivity of this have been greatly enhanced by the use of a second set of nested primers (Simmonds, Balfe and Ludlam. Br J. Haematol, 71, Suppl 1, 19).

This technique will be important to apply to individuals who are known to have been exposed to HIV but who remain anti-HIV negative.

(vi) (a) Antibodies to total HIV, env and gag will be quantitated using recombinant antigens.

(b) ADCC activity - this is being undertaken in conjunction with Dr. A.G. Dalgleish. (AIDS 2 465, 1988, Koup, R.A. et al. J. Virol 63 584, 1989).

(c) Antibodies to HIV env peptides. It is proposed to set up Elisa assays for these (Klasse P.J. et al. Proc Natl Acud Sci USA 85 5225, 1988, Kennedy R.C. et al. Science 231 1556, 1986, Dalgleish A.G. et al. Virology 165 209, 1988). The corresponding synthetic peptides have been prepared at our request in conjunction with the AIDS Directed Programme.

(d) Antibodies titres to Nef may be of prognostic significance and these will be measured on samples in our serum store. Furthermore there is preliminary evidence that some individuals possess anti-Nef activity in the absence of other evidence of HIV infection and it is therefore proposed to study samples from anti-HIV negative subjects.

6. Assessment of Immune Status (in vitro)

1. Quantitation of lymphocyte subsets (Becton and Dickinson FACS already provided by MRC funds).

2. Serum B_2 microglobulin

3. Serum neopterin

4. Soluble IL2 receptors

5. Ecto-5-nucleotidase (in plasma and on cell surfaces)

6. Lymphocyte responses to antigens and to mitogens and the influence of factor VIII preparations on these responses in conjunction with Miss Alison Batchelor (MRC Studentship supervisors Dr. C.A. Ludlam and Dr. C.M. Steel).

7. HLA typing by standard microcytoxicity test on samples from other Haemophilia Centres (Royal Free Hospital, Glasgow & Newcastle) is being carried out to investigate further the relationship between A1, B8, DR3 and clinical status. An RFLP technique has been set up to genotype for A1 B8 DR3. An attempt is also underway to HLA type individuals, from stored serum samples, who have died. It is important to have such data if a realistic assessment is to be made as to the importance of HLA in determining susceptibility to HIV.

8. To study partition of HIV in semen this will be fractionated to separate sperm from lymphocytes on a percoll gradient in conjunction with Dr. F. Wu at the MRC Reproductive Biology Unit, Edinburgh. HIV will be detected by culture and PCR. Although there have been two recent publications on this topic suggesting that it may be difficult to separate sperm from HIV the reports describe artificial systems. Furthermore, even although it may not be possible to completely eliminate HIV from sperm it may be feasible to greatly reduce the quantity of HIV and particularly to remove infected lymphocytes which may be more likely to transmit virus than free HIV.

9. Intermediate purity SNBTS factor VIII concentrate will be fractionated and the fractions will be assessed for their ability to modulate PHA, Pokeweed mitogen and tetanus toxoid stimulation of lymphocytes. A range of more highly purified concentrates produced by monoclonal technology e.g. Monoclate, or conventional fractionation techniques e.g. Octapharma, and new SNBTS factor VIII concentrates are also available for study. Miss Alison Batchelor (MRC Research Student) is assisting with this part of the project. It is proposed to examine the immunoassay results for each patient in relation to documented usage of Factor VIII to see whether the properties of locally-produced Factor VIII may be relevant to the observed high rate of progression of HIV-related disease in one cohort.

DETAILED JUSTIFICATION FOR SUPPORT REQUEST

CLINICAL LECTURER

A medically qualified clinical lecturer is required to be responsible for the following:

1. Reviewing the patients both for routine appointments and acutely when they are unwell. It is very important for the patients to relate to a single doctor to provide continuity of care for HIV related problems as well as their haemophilia. Most of these haemophiliacs have taken part in many different research projects over the years and, whilst they are generally very willing to help, they will only do so if they receive prompt and sympathetic medical attention for the research investigations as well as treatment for HIV related problems and haemophilic bleeding episodes.

2. Detailed clinical records are essential for the success of the project because a major aim is to relate the results of the in vitro test to clinical status. By having a single observer to record clinical status the same standards will be applied to all the patients uniformly. Our collaborative study with Dr. J.O. Bishop, and Dr. A. Leigh-Brown (ADP funded) to study the HIV genome in the various members of our cohort is also dependent on good clinical data.

3. To reduce the burden on the patients careful and sympathetic co-ordination of clinical investigations is essential to maximise patient acceptability. This is particularly important for the neurological assessments as it has been harder for the patients to accept these.

4. The Lecturer will be responsible for undertaking the neuropsychological assessments.

5. The annual skin tests will be the responsibility of the Lecturer. These will be carried out on both HIV positive and negative individuals. To ensure consistency these must be performed, and read two days later, by a single observer. Such a procedure can only be accomplished by a medically qualified

individual.

6. A large amount of data will be generated which needs to be collected, collated and analysed statistically.

Technician 1

(Mrs. D. Beatson) This individual will work under the supervision of Dr. C.M. Steel and undetake the measurement of immune activity i.e. lymphocyte subsets, HLA type, B_2 microglobulin, neopterin, IL2 receptors, ecto-5-nucleotidase. She will also be responsible for performing the Elisa assays for quantitating antibody activity against env peptides. Mrs. Beatson will also help with the studies to fractionate factor VIII concentrate and assess the immunodepressant activity of the components.

Technician 2

(Mrs. S. Rebus) Under the direction of Dr. J.F. Peutherer this person will continue studies to investigate HIV culture techniques to imporve the yield of positives as well as culture separate cell fractions of PBMC's. The work will include assays for HIV antigen and anti-HIV (both total and specific assays) as well as using the PCR technique for detecting HIV in plasma, lymphocytes, monocytes and semen in both HIV sero-positive and sro-negative subjects. She will be responsible for the measurement of antibodies to EBV, CMV and HHV6.

Materials and Consumables

Materials for HIV cultures (approx. 200 per annum), HIV antigen and antibody tests as well as PCR reagents are needed to undertake the virological studies. Reagents for quantitating specific antibodies to CMV, EBV, HH6, HTLVI and HBV are also required. For the immunological investigations IL2 receptor kits, B_2 microglobulin, neopterin along with monoclonal antibodies to quantitate T cell subsets are needed. Pipettes disposable plastic and gloves are required because of the "risk of infectio Liquid nitrogen is for the storage tanks. All the equipment for the neurological investigations was purchased with the previous MRC Project Grant but further funding is required for CT/NMR scanning. We estimate two further scans will be required on each of 20 patients over the three year project.

Equipment

An additional liquid nitrogen storage facility for plasma and cells is needed as the present store is almost full. A Dewar flask is required for the transport of samples between laboratories.

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CURRICULUM VITAE OF APPLICANT **APPENDIX III page 1**

1. Surname LUDLAM Forename(s) CHRISTOPHER A. Age d.o.b. GRO-C 46

2. Degrees, etc. (subject, class, university, and date)

BSc. (1st Class Honours, Biochemistry) Edinburgh 1968
MB. ChB. Edinburgh 1971
MRCP(UK) 1973
PhD Edinburgh 1977
MRCPath 1977
FRCP 1982 FRCPath 1989

3. Posts held (with dates). Where personal support is requested please identify tenure and source of funding of present post.

Present: Director, Edinburgh Haemophilia Reference Centre
Consultant Haematologist, Royal Infirmary, Edinburgh
Senior Lecturer, Dept. of Medicine, University of Edinburgh (Part Time)

Past: House Physician and House Surgeon, Royal Infirmary Edinburgh 1971-2
MRC Research Fellow, Edinburgh 1972-75
Senior Registrar in Haematology, University Hospital of Wales 1975-78
Lecturer in Haematology, Welsh National School of Medicine 1979

4. Recent publications; also papers in press

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6. C.V. Prowse and C.A. Ludlam. Mechanism of Action and Clinical Use of DDAVP (In Press)

RICULUM VITAE OF APPLICANT 1

APPENDIX III page 1

1. Surname PEUTHERER Forename(s) John Forrest Age 52
d.o.b. GRO-C 36

2. Degrees, etc. (subject, class, university, and date)

BSc (ordinary, Chemistry & Physics) Edinburgh 1957;
MBChB Edinburgh 1962;
MD (Gold Medal) 1975;
MRCPath. 1975;
MRCPE elected 1986;
FRCPath. 1987;
FRCPE 1988

3. Posts held (with dates). Where personal support is requested please identify tenure and source of funding of present post.

Oct 1962 - Sept 1963: Resident House Officer;
Oct 1963 - Dec 1984: Research Fellow, Bacteriology Dept, Edinburgh University;
Jan 1965 - Aug 1976: Lecturer, " " " " ;
Sept 1976 - date : Sen. Lecturer, " " " "
and Honorary Consultant, Lothian Health Board.
Permanent position.

4. Recent publications; also papers in press

Peutherer JF, Edmond E, Simmonds P, Dickson JD & Bath GE (1985) HTLV-III antibody in Edinburgh Drug Addicts. Lancet, ii, 1129-1130.

Robertson JR, Bucknall ABV, Welsby PD, Roberts JJK, Inglis JM, Peutherer JF & Brettle RP (1986) An epidemic of HTLV-III/LAV infection amongst intravenous drug abusers in a general practice. Brit. med. J., 282, 527-529.

Simmonds P, Peutherer JF & McClelland DBL (1987) LAV/HTLV-III antibody testing: confirmation methodologies and future prospects. In: AIDS: The safety of blood and prood products. Ed. JC Petricciani, ID Gust, PA Hoppe & HW Krignen. WHO.

Simmonds P, Smith IW & Peutherer JF (1987) Detection of antibody to viral proteins following primary infection with herpes simplex virus. J. med. Virol., 23, 191-205.

Simmonds P, Lainson FAL, Cuthbert R, Steel CM, Peutherer JF & Ludlam CA (1988) HIV antigen and antibody detection; variable responses to infection in the Edinburgh haemophilia cohort. Brit. med. J., 296, 593-598.

Sleigh JD & Peutherer JF (1988) Changing patterns of bacterial and viral infections in surgery. Brit. med. Bull., 44 403-422

Johnston FD, McAllan L, Brettle RP, Inglis JM & Peutherer JF (1988) Does HIV seropositivity affect the outcome of pregnancy? Brit. med. J. 296 467

CURRICULUM VITAE OF APPLICANT

1. Surname STEEL Forename(s) CHRISTOPHER MICHAEL *Age d.o.b. GRO-C 1940

2. Degrees, etc. (subject, class, university, and date)

BSc (1st Class Hons Physiology)	Edinburgh 1962
MB ChB (Hons)	Edinburgh 1965
PhD (Cell Biology)	Edinburgh 1972
DSc (Cell Biology/Genetics)	Edinburgh 1988
MRCP Ed (Paediatrics)	Edinburgh 1968
MRC Path	Edinburgh 1981

3. Posts held (with dates). Where personal support is requested please identify tenure and source of funding of present post.

SHO Medicine, Western General Hospital, Edinburgh 1966-68
Faculty of Medicine Research Fellow, Edinburgh 1968-71
MRC Travelling Research Fellow (Univ Nairobi, Kenya) 1972/73
Member of Clinical Scientific Staff, MRC Clinical and Population Cytogenetics Unit 1973-present
Assistant Director of Unit from 1979.

4. Recent publications; also papers in press

Steel CM, Ludlam CA, Beatson D, Pentherer JF et al.
HLA Haplotype A1 B8 DR3 as a risk factor for HIV-related disease.
Lancet 1, 1185-1188 (1988).
Mackay J, Steel CM, Elder PA, Forrest APM and Evans MJ.
Allele loss on short arm of chromosome 17 in breast cancers.
Lancet ii, 1384-1385, 1988.
Steel CM and Hutchins D.
Soluble factors and cell surface molecules involved in human B lymphocyte activation, growth and differentiation.
Biochem Biophys Acta. In Press, 1989.

GMR/RC

Lothian Health Board — South Lothian District

Your ref :
Our ref : MCO/85
If calling ask for: Mr. Redmond
Date : 9th October, 1985

Astley Ainslie Hospital
Grange Loan
Edinburgh EH9 2HL
Tel. 031-447-3399

Dr. C. A. Ludlam,
Department of Haematology,
Royal Infirmary of Edinburgh,
Lauriston Place,
Edinburgh,
EH3 9YW

Dear Dr. Ludlam,

CLINICAL AND IMMUNOLOGICAL STUDY OF HAEMOPHILIACS TREATED EXCLUSIVELY WITH NHS FACTOR VIII/IX CONCENTRATE

With reference to your application for ethical approval of the above project, I am pleased to advise you that this has been granted by the Ethics of Medical Research Sub-Committee for Medicine and Clinical Oncology.

Yours sincerely,

GRO-C

G. M. Redmond,
Secretary,
Ethics of Medical Research Sub-Committee
(Medicine and Clinical Oncology)

Appendix 4

LOTHIAN HEALTH BOARD

Royal Infirmary of Edinburgh and Associated Hospitals

Lauriston Place Edinburgh EH3 9YW Telephone 031-229 2477
Fax 031-228 2189

HAEMATOLOGY DEPARTMENT
Dr A.C. Parker
Dr C.A. Ludlam
Senior Clinical MLSO's
Mrs F. Turner
Mr I. Abbott

Dr. A.C. Peatfield,
Medical Research Council,
20 Park Crescent,
London.
W1N 4AL

Your Ref
Our Ref CAL/AT
Date 31st May, 1989.
Enquiries to
Ext. No. GRO-C

rec'd 6/6

Dear Dr. Peatfield,

CLINICAL IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH-PARTICULAR REFERENCE TO HIV INFECTION

Thank you for your helpful letter of the 15th May and for enclosing the reports of the referees. You know from discussions that we had, along with Dr. Peutherer and Dr. Steel, whilst you were in Edinburgh recently, that we were very disappointed that the study was not fully funded.

May I respond to the points raised in your letter as follows.

1. The "Plan of Investigation" has been expanded to give greater detail of our proposals. These were given in outline because to be too specific might make the project unnecessarily rigid and deny flexibility in such a fast moving field. Since submission of our original application there have been several developments which we have incorporated in the more detailed enclosure.

2. We have specifically not attempted to isolate virus from the offending batch of factor VIII because there are very few vials remaining and we would want to be certain of our methods before opening these. It is likely we would have to begin by "spiking" factor VIII concentrates to validate the detection method. Furthermore we would need to be able to distinguish between potentially viable and inert (dead) virus.

3. We are already undertaking a study to examine the variability of the virus in the different individuals of the cohort (Dr. J.O. Bishop and Dr. A. Leigh-Brown; ADP funded).

4. It is suggested that we might investigate why our patients appear to have declined further than similar groups. This, in essence, is what the project has been about for the past5 years and is one of the major aims of the current application.

5. None of the asymptomatic participants wishes to take part in

PNM

the Concorde Study so it will not be necessary to take into account the effect of zidovudine on asymptomatic haemophiliacs.

Referee Reports

I have read with interest the referee reports and I was delighted that they were so supportive. May I respond to several points raised:

Referee A

It is suggested that we seek funding for an individual of Registrar grade; this is in fact the level of funding we applied for to employ a Lecturer.

Referee B

No criticisms to answer.

Referee C

The project has been evaluated by a virologist who has supported our proposal. He is less enthusiastic about our immunological proposals making the unsubstantiated criticism that the effect of factor VIII concentrates on the immune system is insignificant. There is substantial evidence to the contrary which has been cited in the application. We already have an MRC Research Student working on this. The results of these investigations may well have importance beyond haemophilia within the field of blood transfusion induced modulation of the immune system, e.g. renal transplantation. It is difficult to disentangle the immunology from the virology in a project such as ours, for example is the specificity of antibody to a particular epitope or peptide of env an immunological or virological investigation?

The issue of what determines rate of progression of HIV-related disease also demands serial immunological (as well as virological) assessments of these patients. It should be understood that one of the great strengths of this study is the ability to carry out longitudinal studies on patients whose rate of progress is very well defined. Most of the immuno-assays detailed later will be carried out on sequential stored plasma samples from each patient, spanning the period 1983 to the present.

Referee D

The referee raises the difficulty of the specificity of the PCR. We are very well aware of this and in conjunction with Dr. Bishop

and colleagues a unique double system of nested primers has been devised which is exquisitely sensitive as well as being much more specific and robust than the use of a single pair of primers (Simmonds P., Leigh-Brown A and Ludlam C.A.. British Journal of Haematology, suppl 1, 19, 1989).

We are aware of two publications in relation to HIV in semen which both appeared after our original grant application was drawn up. The studies describe artificial systems for infecting semen. In one study virus was added to semen in vitro and after fractionation was found associated with sperm. In reality it may be the lymphocytes in semen which are responsible for the infection and these can readily be separated from sperms on percoll.

General Comments

We note the offer of support for the two technicians for one year along with consumables at a reduced level and that you do not consider it necessary to employ a Clinical Lecturer. The two technicians in post are highly competent and dedicated to the project. The granting of only a single year's support gravely undermines the study and it is quite possible that one or both may leave, particularly as they are well qualified and have very topical marketable skills. Certainly if either did it would put the project back more than a year because there would be insufficient funds to employ a further individual. Even if money was available, new technicians would require 6-12 months to become as proficient as Mrs. Rebus and Mrs. Beatson. Meanwhile at least a years follow up would be lost for ever.

Without the Clinical Lecturer the project would lose much and I suspect would be unsustainable. Certainly without a dedicated individual to run the study of the 100 patients it is certain that much of the value of the laboratory investigations would be lost. Many of the investigations require a clinician e.g. skin tests and neuropsychological tests. The responsibilities of the lecturer have been clearly set out in the revised application. In the original application a misunderstanding may have occurred about the amount of time myself and Dr. Peutherer were spending on the project. I hope page 1 of the amended application now clears up any misunderstanding.

Consumables

I note your comments about the estimate for the costs of consumables; HIV cultures are expensive to perform and we feel our estimate is reasonable.

On your advice and that of Dr. Jane Cope we have revised our application and in particular expanded the "Plan of Investigation" and the "Justification for the Clinical Lecturer". I have also updated the salary scale to take into account this

years pay awards.

We were heartened by the very positive assessment of the referees and hope that it will be possible for the application to be reconsidered in the near future.

Yours sincerely,

GRO-C

C.A. Ludlam,
Consultant Haematologist.

c.c. Dr. J.F. Peutherer
Dr. C.M. Steel

MRC
Medical Research Council

89/5062 ANNEX 2

Application for a project grant

Please type throughout and return this form with nine photocopies

1. Applicant(s)	Applicant 1	Applicant 2	Applicant 3
Surname	LUDLAM	PEUTHERER	STEEL
Forename(s)	CHRISTOPHER A.	JOHN F.	C. MICHAEL
Age	42	52	48
Title	Dr.	Dr.	Dr.
Post held	Director, Haemophilia Centre	Senior Lecturer	Assistant Director, MRC Human Genetics Unit
No. of hours p.w. on project	Daily 4-6	Daily 5-10	5

2. Institution/Authority (administering grant if approved)
Univeristy of Edinburgh

Department accommodating project (and institution if other than opposite)

City or Town Edinburgh

3. Title of project Clinical, immune and virological investigation of haemophiliacs with particular reference to HIV infection

4. Type of grant sought
Project Grant

5. Abstract of research This application is for support to continue investigations into HIV virology and immune function in both anti-HIV positive and negative haemophiliacs. The study will include the long term follow up of 32 haemophiliacs, of whom at least 18 became infected by HIV following transfusion of a single batch of NHS factor VIII concentrate in 1984. To date the study has enabled the positive identification of risk factors to infectivity and progression of clinical disease. It is proposed to continue detailed clinical and laboratory follow up including serial determinations of viral, immune and neurological parameters. The project will also include evaluation of anti-HIV negative haemophiliacs who are known to have been exposed to HIV, by the polymerase chain reaction to determine whether they too may be infected. As our previous studies have demonstrated that factor VIII concentrates are also directly immunosuppressive (apart from the effect of HIV), we wish to continue our studies to identify the concentrate components responsible.

For Office use											

6. Proposed starting date 1st August 1989 Proposed duration (in months) 36

7. Summary of support requested

		First year £	Second year £	Third year £	Total over period £
(a) Personal support of applicant					
(b) Research staff (medical/scientific)	Number of posts: 1 Whole/part-time	15,510	16,330	17,150	48,990
(c) Technical/other assistance	Number of posts: 2 Whole/part-time	17,560	18,181	18,928	54,669
Addition for superannuation and National Insurance calculated as 26% of salaries in (a), (b), and (c)		8,597	8,971	9,380	26.948
(d) Visiting senior scientist (salary/subsistence, airfare)					
(e),(f),(g) Expenses		19,795	19,100	19,300	58,195
Total recurrent		61,462	62,582	64,758	188,802
(h) Apparatus		1,400			1,400
Total support requested		62,862	62,582	64,758	190,202

(PG5)

8. Does the project require Local Ethical Committee approval? Yes/No Has been obtained, see letter

9. (a) Is your related research currently being supported by any outside body (other than the MRC)?
If so, please indicate the topic, supporting organisation, value and tenure
NO

(b) Are you currently applying elsewhere for support for work relating to the present proposal?
If so, please give details as for 9(a)
NO

(c) Is this application currently being submitted elsewhere?
If so, to which organisation; and by what date is a decision expected?
NO

(d) Has this application been submitted elsewhere over the past year?
If so to which organisation and what was the result?
NO

(e) Is the proposed research likely to lead to patentable or otherwise commercially exploitable results? If so, please give brief details
NO

10. Full official postal address of applicant
Dr. C.A. Ludlam
Department of Haematology
Royal Infirmary, Edinburgh

Telephone number of applicant (please give STD code from London and extension)
031-229-2477 Ext GRO-C

11. Acceptance of regulations and conditions

I have read the conditions set out in the Council's current Project Grants booklet and, if my application is successful, I agree to abide by them. I shall be actively engaged in, and in day-to-day control of, the project.

Signature of applicant(s): Date:

12. This application should be submitted by/through (i) the Head of Department and (ii) the officer who will be responsible for administering any grant that may be awarded. Each should sign the following declaration:

I confirm that I have read this application and that, if granted, the work will be accommodated and administered in the Department/Institution in accordance with the conditions in the Council's current Project Grants booklet. The **staff gradings and salaries** quoted are correct and in accordance with the normal practice of this Institution.

(i) Signature of **Head of Department**

Title

To be appended in typescript or block capitals

Name and initials (of (i) above)
Professor I.A.D. Bouchier

Institution
University of Edinburgh

Address (if different from 10 above)
Dept. of Medicine
Royal Infirmary
Edinburgh.

Date:

(ii) Signature of **Administrative Authority**

Finance Officer/Bursar/Registrar/Secretary of Institution (delete as appropriate)

To be appended in typescript or block capitals

Name and initials (of (ii) above)

Institution

Address and telephone number (including STD code from London and extension)

Date:

13. Name, address and telephone number (including STD code from London and extension) in typescript (or block capitals) of the officer who should be contacted regarding the administration of the grant if awarded, if different from (ii) above:

PROPOSED INVESTIGATION

1. Title of project
2. Purpose of proposed investigation
3. Background of the project
4. Plan of investigation
5. Detailed justification for support requested

Please leave margins blank

CLINICAL ,IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH PARTICULAR REFERENCE TO HIV INFECTION

PURPOSE OF PROPOSED INVESTIGATION

To investigate the effect of HIV infection we wish to continue our longitudinal study of a cohort of 32 haemophiliacs who were transfused with a single HIV infected batch of NHS factor VIII concentrate in the Spring of 1984 (1). Studies of this cohort so far have indicated factors which predispose to HIV infection and to clinical and immunological decline following infection. We wish to continue to characterise in detail changes in the virus itself, in specific anti-viral immune responses and in non-specific immunological parameters in the patients because such studies will provide insight into the mechanism by which HIV causes clinical decline. The studies will be both prospective and retrospective using our unique collection of serial serum and leukocyte samples stored over a period of 6 years (ie predating exposure to HIV). Almost half the patients who received the infected batch of factor VIII have not demonstrated any markers of HIV infection and we propose to use the polymerase chain reaction (PCR) technique with its greatly enhanced sensitivity to try and detect HIV RNA/DNA in these anti-HIV negative individuals. We have also further non-cohort patients who have received factor VIII concentrates and are HIV antigen, antibody and culture negative but have been exposed to HIV and it is proposed to seek the HIV genome by PCR also in these individuals (4).

One of the risk factors we have identified which predisposes to HIV infection and subsequent clinical decline is possession of the HLA haplotype A1 B8 DR3 (2). To study this further collaboration has been established with Haemophilia Centres at the Royal Free Hospital London, Newcastle and Glasgow which have apparently different prevelance rates of HIV induced clinical disease. We have also agreed to participate in an EEC Commission sponsored study of the immunogenetics of AIDS co-ordinated by Dr. Arne Srejgaard, Copenhagen.

HIV is transmitted heterosexually and some couples, where the man is infected, wish to have children but do not wish the wife to be exposed sexually to HIV. One possible solution to this difficulty is to separate sperm from semen and offer artificial insemination. A technique for separating sperm from semen has been deeloped by Dr. F. Wu at the MRC Reproductive Biology Unit in Edinburgh and it is proposed to assess partition of HIV in the fractionated semen with a view to developing a technique that could be used to offer artifical insemination to couples.

The other major aim of the grant application is to investigate the mechanism by which factor VIII concentrates (independent of HIV)modulate the immune system. Our original studies demonstrated immune abnormalities, eg depressed cell mediated immunity and reduced CD4 counts, in some instances as severe as those induced by HIV (3). This may be important for two principal reasons. Firstly such immune modulation may predispose to infection by both bacteria and viruses (26). Secondly it has become very important to assess further which components of the factor VIII concentrate are responsible because it may be possible to manufacture concentrates with reduced levels of these. At present only very high purity concentrates, eg ones manufactured with the use of monoclonal antibodies, have low levels of these components (unpublished observations) but in the manufacture of these products there are large losses of factor VIII which is contributing to the present world shortage of factor VIII. Furthermore such studies may be informative in identifying candidate blood components which modulate the immune system in a beneficial way prior to renal transplant and to the deteriment of the patient with colonic cancer.

BACKGROUND OF THE PROJECT

In the study are a group of 32 originally HIV negative haemophiliacs who were inadevertently transfused with an HIV "infected" batch of SNBTS factor VIII concentrate in the spring of 1984; at least 18 became infected with HIV (1). This cohort has been followed up clinically and detailed virological, immunological and neurological investigations have been supported by a current MRC Project Grant. This further application is to continue our studies of these and other HIV positive and negative haemophiliacs. Immunological studies were initiated in many of the patients in 1983 prior to HIV infection and these data has greatly added to the value of the study.

Clinical Status of Patients

Of the 18 infected individuals two have died of AIDS and a third of HIV related problems; five individuals have CDC Group 4 disease and have therefore started on zidovudine. All patients have been followed in detail and the results of the studies have been published (1,2,5) or are outlined below (manuscripts 2,4,5,6). The 14 haemophiliacs in the cohort who remain anti-HIV negative despite receiving the HIV infected factor VIII are clinically well as are the recipients of the factor IX concentrate which was made from the same HIV infected donor plasma. In addition there are 7 haemophiliacs who were infected by blood products (both NHS and commercial) for whom we have an accurate date of seroconversion (from stored serum samples) and they have also been followed up in a similar manner to the cohort group. All other HIV antibody negative haemophiliacs are clinically well despite a number being exposed to HIV from two further infected batches of SNBTS concentrate (4).

The Table summarises the present patients and their clinical status.

	NO.	ANTI-HIV	TRANSFUSION GROUP	HIV CLINICAL STATUS
Group 1a	18	Positive	Infected cohort	3 dead; 5 ARC
Group 1b	7	Positive	Non-cohort infected from other blood products	Asymptomatic
Group II	14	Negative	"Non-infected" cohort	Asymptomatic
Group III	15	Negative	Christmas Disease	Asymptomatic
Group IV	50	Negative	Exposed to Factor VIII concentrate	Asymptomatic

Virological Studies

We have followed in detail the time course of HIV antibody (total; both IgM and IgG, anti-env and anti-core) as well as antigen from infusion of the infected batch, through seroconversion (5,6). We wish to continue these studies and perhaps extend the serology to include analysis of the specific antibody response, to synthetic peptides as well as correlate the changes with alteration in tests of immune function (vide infra). Similarly we have followed serum HIV antigen levels and demonstrated antigenaemia in half the patients prior to seroconversion and the later reappearance of antigen associated with immunological and clinical decline(5). Virus has been identified by culture of lymphocytes with PHA and IL2 and current studies indicate that virus isolation can be improved by selective removal or enrichment of mononuclear cell subsets (manuscript 1). We have observed that virus is more readily detected in patients (7/7) with CDC Group IV compared to asymptomatic (4/9) CDC Group II and III (manuscript 2) and this is consistent with observations on p24 antigenaemia and transmission risk in other studies in homosexuals (7) haemophiliacs and other recipients of blood products (8).

The polymerase chain reaction (PCR) has greatly increased the sensitivity of viral

detection (9) and we have therefore recently set up this technique with a pair of gag specific primers. Additionally we have markedly increased both the sensitivity and specificity of the technique by using a sceond set of internal primers (manuscript 3). We have started to investigate the HIV negative patients, both those in the cohort (Group II) and others who have definitely or may have been exposed to HIV (Group III and IV) to determine whether viral genome is detectable. It is proposed to search for viral RNA as well as transcribed or integrated DNA both in cell free plasma as well as peripheral blood lymphocytes and monocytes separately. We also propose to use the PCR reaction to attempt to detect HIV genome in the batch of HIV contaminated factor VIII which infected our cohort as well as other non-heat treated and heat treated factor concentrates of both NHS and commercial origin. We shall use various primer pairs for major viral genes for the PCR of seronegative patients to check if incomplete provirus may be present.

There are a small group of haemophiliacs, who despite large use of non-heat treated factor VIII concentrate prior to 1984, have remained seronegative. In conjunction with the Royal Free Haemophilia Centre we should like to investigate whether using the PCR virus is detectable and further explore why such individuals may be resistant to the virus.

As the 18 HIV positive individuals in the cohort may have been infected by a single strain of virus a collaborative study under the direction of Dr. J.O. Bishop and Dr. A. Leigh -Brown (MRC funded) has been established to examine genomic mutation and drift. As differing viral strains have different infectivities for particular cell types and detailed knowledge of the genomic characteristics of the virus that infected the cohort will become available, a more detailed examination of the interaction between virus and host may be possible and lead to an explanation of the differing rates of immunological and clinical decline. A related study of the infectivity of lymphocytes and macrophages for various isolations of HIV has been previously funded by the MRC (Drs. J.F. Peutherer and D.W.J. Aw).

We propose to test sequential stored serum samples for evidence of infection with CMV, EBV and human herpes virus 6 to see if infection with these viruses either before or after HIV infection affects the rate of clinical progression. At the same time, serum levels of anti-HBs and anti-HBC will be measured as any decrease could lead to a risk of hepatitis B(10). These sudies will be performed on both the HIV positive and negative patients.

Immunological Investigations

These were begun in the Edinburgh patients in 1983 prior to infection by HIV (3) and follow up studies have continued since then and the findings correlated with clinical status (1,2,5 and manuscript 4,5 and 6). Our results reveal that in the group as a whole there is a steady decline in CD4 numbers of those infected but that there may be marked fluctuation within each individual. The HLA haplotype A1 B8 DR3 (vide intra) is associated with a more rapid decline in CD4 counts. We propose to continue the longitudinal assessment of CD4 and CD8 subsets along with enumeration of other subsets including NK cells, activated T cells, monocytes and null cells (12).

In view of the known changes in B2 microglobulin, and neopterin in HIV infection we have measured these on stored serum samples in all members of the cohort. Marked and progressive changes have been observed which correlate with immunological and clinical status (manuscript 6). Shed IL2 receptor levels in serum may reflect the extent of T cell activation (13,14) (as does identification of MHC class II +ve T cells). Both of these are yielding results that appear interesting and will be included in the future programme. Anti-EBV antibody levels (VCA) have been correlated with the risk of developing EBV-related Burkitt's lymphoma, a finding which is attributed to the inverse relationship between effectiveness of T cell function and EBV-induced B cell proliferation (15,16). Perhaps surprisingly,

anti-EBV antibody levels have been reported as not raised in AIDS patients with reduced circulating T4 cells, but we propose to examine this question in the Haemophilia cohort, both in relation to the known rates of decline of T4 cells and to the likely increased risk of lymphoma in these patients (17).

The lymphocyte membrane enzyme ecto-5-nucleotidase (E.C.3.5.7.1) is a passive degradative enzyme,activity of which is related to lymphocyte maturity and function. Two studies have recorded reduced expression of the enzyme in male homosexuals though whether it is a cause or a consequence of T cell defects associated with HIV infection is unclear (18,19). A single study in haemophilia patients with and without antibody to HIV did not show abnormalities of ecto-5-nucleotidase levels (20) and again the issue requires to be addressed in a well-characterised group of subjects. We propose to carry out at least a pilot investigation in Edinburgh and the Haemophilia cohort will form one of the groups to be studied.

Recent studies have demonstrated a plasma factor in HIV positive individuals that inhibits mitogen and antigen induced lymphocyte proliferation in vitro. The inhibitory activity is associated with low molecular weight plasma fractions and it increases with falling CD4+ cell counts (25). As we have established a microtechnique to assess lymphocyte proliferative response (vide intra) we propose to seek evidence for plasma inhibitory factors in our patients.

Skin tests to 7 recall antigens have been repeatedly assessed since 1983 as a measure of cell mediated immunity. We have demonstrated (21) as have others that haemophiliacs are relatively anergic, due to the effect of factor VIII concentrate per se, and that HIV causes a further progressive decline in cell mediated immunity.

HLA studies

As several reports indicated that particular HLA antigens were associated with HIV disease progression we tissue typed all 32 patients in the cohort. We have reported that the haplotype A1 B8 DR3 is associated with succeptibility to infection as well as a more rapid decline in CD4 numbers and progression to CDC Group IV disease (2). This has been possible only because we know accurately when the patients were infected and because we have been able to HLA type all patients. The lack of confirmation by some other investigations of our observation may be because individuals with the succeptible haplotype have already died prior to study or because the duration of infection is not accurately known (22,23). (All three deaths in our cohort to date, have been in patients with the HLA, A1, B8,DR3 haplotype).

To investigate the relationship between HLA type and disease progression collaborative studies are in hand with other Haemophilia Centres with particularly high and low incidence of HIV related disease. In addition we have a collaborative study with Sheffield Haemophilia Centre where liver biopsies are being performed prior to interferon therapy for non-A non-B hepatitis to investigate the possibility of an association between HLA and progression of liver disease or response to interferon therapy. We are co-operating with other groups where special expertise in particular areas of immunology can be applied appropriately to samples from our patients.

Studies in HIV Negative Subjects

Blood transfusion can profoundly influence the clinical course in several groups of patients by modifying immune competence, eg renal transplant engraftment and survival of individuals with colonic cancer. Factor VIII concentrates per se also produce immune changes which mimic those induced by HIV, eg skin anergy and depressed CD4 and high normal CD8 counts (1). Furthermore relatively low purity concentrates depress lymphocyte reactions in vitro compared to more highly purified concentrates (unpublished observations) and this has been associated with uncharacterised high molecular weight component of the concentrate (24). Initial studies are in hand to gel filter factor VIII concentrate to assess the ability of components to inhibit PHA stimulation of normal lymphocytes. Studies to elucidate the mechanism by which factor VIII influences lymphocyte numbers and function may also shed light on the mechanism by which other blood products profoundly affect patients with other disorders.

Neurological Studies

Progressive neurological derrangement is a characteristic feature of HIV infection although relatively little is known about its early manifestations. Detailed neurological and neuropsychological studies have been undertaken in both our HIV positive and negative haemophiliacs on a single occasion. Haemophiliacs are prone to intrcranial bleeds and hence HIV negative haemophiliacs may have impaired neurological function and are therefore an important control group. Baseline data is available on approximately 12 patients which is fewer than we had originally intended. The investigations are very time consuming and the haemophilacs have been reluctant to give up yet more time for this research project. Despite our difficulties it is our intention to serially follow up the patients with the aim of characterising which investigations are most sensitive at detecting early HIV encephalopathy.

PLAN OF INVESTIGATION

The project will be based at the Edinburgh Haemophilia Centre, Department of Haematology under the direction of Dr. C.A. Ludlam. Laboratory and office space is available within the Department for the project. The immunological investigations are undertaken by Dr. C.M. Steel and the virology by Dr. J.F. Peutherer.

The study will be executed as follows:

1. All patients will be seen and examined in detail clinically at least every six months. Those who are anti-HIV positive but asymptomatic will be reviewed every three months and individuals in CDC Group IV on zidovudine will be seen every 2-4 weeks. Several patients are being enrolled in the MRC/INSERM Concorde Trial.
2. Serial neurological and neuropsychological examinations at yearly intervals on as many HIV positive and negative haemophiliacs as possible will be undertaken using the following techniques; CT scanning, EEG, VER, AER, P300 and neuropsychological assessments under the supervision of Dr. R.E. Cull Consultant Neurologist and Mr. W. McKinlay (Principal Psychologist). CT scanning is supervised by Professor J.J. Best.
3. At least every six months blood will be taken for full blood count, liver function tests, lymphocyte subsets (Total T cells, CD4, CD8, NK etc) B2 microglobulin, neopterin, IL2 receptors, ecto-5-nucleotidase, HIV antigen, anti-HIV, HIV culture and extraction of RNA/DNA for detection of HIV genome as well as EBV, HH6 and CMV serology.
4. Cell mediated immunity will be assessed at yearly intervals to 7 recall antigens (Multitest).
5. HIV detection will be by HIV antigen capture assay (Dupont) in plasma polymerase chain reaction, lymphocyte culture and co-culture using HIV antigen assay and dot blot hybridisation to detect positive cultures. Antibodies to total HIV,

env and gag will be quantitated using recombinant antigens (Abbott) and Western Blots. Antibodies to other viruses will be measured by ELISA or immunofluorescence assays. This will be supervised by Dr. J.F. Peutherer and carried out in appropriate containment facilities at the Department of Bacteriology, University of Edinburgh.

6. Lymphocyte subsets will be quantitated using a Becton-Dickinson FACS(already provided for the study from MRC funds) under the supervision of Dr. C.M. Steel.

7. HLA type to be assessed by standard microcytotoxicity test. HLA AI B8 DR3 to be determined by RFLP on samples from other Haemophilia Centres (Royal Free Hospital, Newcastle and Glasgow).

8. To study partition of HIV in semen this will be fractionated to separate sperm from lymphcoytes by percoll using a technique developed at the MRC Reproductive Biology Unit, Edinburgh and HIV detected in the sperm and lymphocyte fractions by culture and PCR.

9. Intermediate purity SNBTS factor VIII concentrate will be fractionated and the fractions will be assessed for their ability to inhibit PHA stimulation of lymphocytes. A range of more highly purified concentrates produced by monoclonal technology eg Monoclate, or conventional fractionation techniques eg Octapharma, and new SNBTS factor VIII concentrates are also available for study. Miss Alison Batchelor (MRC Research student is assisting with this part of the project).

DETAILED JUSTIFICATION FOR SUPPORT REQUESTED

Clinical Lecturer: A medically qualified individual is needed to co-ordinate the project. He/she will be required to take careful detailed clinical histories and examine the patients every six months. He/she will also be responsible for co-ordinating the neurological investigations and CT scanning. The collection of blood samples for appropriate investigations will be in collaboration with the technicians and the appropriate laboratories. Skin tests will have to be undertaken at twelve monthly intervals. Results of investigations will be collected, collated and analysed using standard statistical techniques.

Technician 1(Mrs. D. Beatson) This individual will work under the supervision of Dr. C.M. Steel and undertake the measurement of immune activity ie lymphocyte subsets, HLA type, B2 microglobulin neopterin, IL2 receptors, ecto-5-nucleotidase. The technician will also help with the studies to fractionate factor VIII concentrate and assess the immunodepressant activity of the components

Technician 2 (Mrs. S. Rebus) Under the direction of Dr. J.F. Peutherer this person will continue studies to investigate HIV culture techniques to improve the yield of positives as well as culture separate cell fractions of PBMCs. The work will include assays for HIV antigen and anti-HIV (both total and specific assays) as well as using the PCR technique for detecting HIV in plasma, lymphocytes, monocytes and semen in both HIV positive and negative subjects. She will be responsible for the measurement of antibodies to EBV, CMV and HH6.

Materials and Consumables

Materials for HIV cultures (approx. 200 per annum), HIV antigen and antibody tests as well as PCR reagents are needed to undertake the virological studies. Reagents for quantitating specific antibodies to CMV, EBV, HH6, HTLVI and HBV are also required. For the immunological investigations IL2 receptor kits, B2 microglobulin, neopterin along with monoclonal antibodies to quantitate T cell subsets are needed. Pipettes disposable plastic and gloves are required because of the "risk of infection" Liquid nitrogen is for the storage tanks. All the equipment for the neurological investigations was purchased with the previous MRC Project Grant but further funding is required for CT/NMR scanning. We estimate two further scans will be required on each of 20 patients over the three year project.

Equipment: An additional liquid nitrogen storage facility for plasma and cells is needed as the present store is almost full. A Dewar flask is required for the transport of samples between laboratories.

ANNEX 3

ITEM No 12

FILE A

89/5862

PROJECT GRANT G8901910
DR C A LUDLAM

This is a good application from a well known group of research workers in the field of haemophilia who have access to a unique cohort of patients all treated with one known batch of HIV infected Factor VIII Concentrate, but only about one half of whom have HIV seroconverted. Already much useful information has accrued from this cohort and it is important that this group of patients continues to be comprehensively studied.

Timeliness The application of the PCR reaction for HIV has a high chance of producing results within the three year timescale as have most of the other investigations planned.

Pervasiveness Certainly this work can be related and compared to that on other HIV risk groups and on immunological function in haemophilia in general. Wider links are more difficult to perceive.

Scientific Merit This is a proven team. The work is carefully planned and technically sound.

Exploitability This is difficult to foresee unless the PCR for HIV could lead to the development of a kit.

Applicability The work could be important in determining risk of sexual transmission of HIV and the feasibility of separating non infected viable spermatozoa.

Significance for Education and Training This is a scientifically orientated project and not directly related to education and training other than, possibly, of the staff employed.

Level of Support Requested The application is extremely expensive mainly on account of the Medical Researdh Assistant and the materials for virological and immunological studies. If thought to be appropriate the applicants could be invited to seek a more junior assistant e.g. at Registrar level or to trim the scope of the application so that use could be made of existing staff. By reducing the number of projects the expenses could also be trimmed. Work similar to that described, such as the Application of PCR for HIV, is being carried out at other centres, but it is difficult to see how the main objectives of the project could be achieved without this on-site facility. The applicants will no doubt continue with this in any case.

Within these possible constraints I think that it is essential that studies are continued on this cohort of patients and therefore the project should be funded.

G 8902835

CLINICAL, IMMUNE AND VIROLOGICAL INVESTIGATION OF HAEMOPHILIACS WITH PARTICLULAR REFERENCE TO HIV INFECTION : Drs Ludlam, Peutherer and Steel.

(typed from a phone dictation)

This is an application to continue virological, immunological, neurological/ neuropsychological and radiological studies on a very important group of haemophiliac patients whose date of infection is accurately known in 1984.

Particular strengths of the application are :

(i) known date of infection

(ii) existence of prior immunological studies

(iii) collection of much data already from this group

The Steering Committee on HIV and the Nervous System has identified the importance of studies of this group of patients in detail because of the potential elementary differences from homosexual and neonatal groups.

The study is well-defined, but I am not competent to comment on the immunological and virological side.

The HLA Studies proposed, clearly need the confirmation of a large group as the application proposes. On the neurological side, I note the reservations of the applicants about changing the tests in the light of the Steering Group's recommendation, and I accept their point.

The question as to whether additional tests should be added is a difficult one as the applicants point out, many of the patients are very reluctant to devote additional time to the study. However, in order not to miss crucial data I suggest Dr Maria Ron (Chairman of the former Working Group on Psychiatry and Neuropsychology) be asked her views. This need not delay further support, which I strongly recommend.

ITEM No. 12
REFEREE C

I have read through Ludlam and colleagues application twice and it is certainly a comprehensive proposal to continue study of this interesting cohort of haemophilia patients exposed to HIV. The trouble with such a proposal is that there is something there to appeal to every referees but, inevitably, some parts that do not seem especially important.

For me the important parts in this application were those referring to the use of PCR on HIV exposed sero-negative haemophiliacs, and to the possibility of fractionating spermatozoa from 'infectivity' so that haemophiliacs partners might safely become pregnant. The suggestion that PCR might be used separately to amplify viral RNA and DNA is interesting, but its use to detect virus in concentrates of factor VIII is of limited value as it may well be that inactivated HIV is detected in this way.

I am less taken with the proposed work on immune modulation. I am unconvinced that this is due to 'a high molecular weight component' of concentrate (though it could be another virus). In any case there is little evidence that any leucopaenia or other effect due to it is of significance.

I find it hard to deal with this proposal using your criteria. Under (i) it is looking for short term benefits. Under (ii) it's scope is really confined to haemophilia patients' interests and needs. Under (iii) I would say the science is sound and this group has already shown that it does good work. Under (iv) to (vi) nothing to say. Under (vii) and (viii) the level of support sought is realistic for what is proposed, but I feel (from a virologist's standpoint), that the immunologic work proposed is not especially valuable even though I think the virology worthwhile.

ITEM No. 12
REFEREE D

GRANT NO. G8901910 (Ludlam, Peutherer and Steel)

This project grant seeks to continue the longitudinal evaluation of a unique group of haemophiliacs identified as having become infected with HIV from a single contaminated batch of NHS Factor VIII in 1984.

This group has conducted some excellent work on this particular cohort of individuals which presents a remarkable opportunity for following a number of markers of HIV infection and disease longitudinally. It also provides an excellent opportunity for examining a group of exposed but apparently uninfected individuals at the same time, together with other members of the haemophilia cohort in Edinburgh. The investigators have an excellent track record for capitalising on this opportunity and have conducted some excellent work to date. I have no doubt that this work should be supported since it continues the evaluation in the long term to establish natural history in predictive markers and also seeks to capitalise on the advantages of this cohort in pursuing a number of separate but related research questions. These include new virological markers, potential infective cofactors, immunological changes, HLA associations, effects of Factor VIII concentrates on HIV negative subjects and neurological evaluations.

I believe that all of these are worthy extensions of this project and represent realistic objectives in all respects. I have slight reservations about two aspects of this study but they do not undermine my overall approval of the project as a whole. I think regarding the polymerase chain reaction studies, the authors give insufficient attention to the dangers of producing false positive results with this technique. There is, of course, no real way of questioning or confirming a positive result in an otherwise apparently negative person. Given that polymerase chain reaction offers the tremendous potential for amplifying contaminating DNA, one would like to have seen more discussion of this problem and ways in which it would be avoided in this proposal. So long as the investigators remain in touch with other investigators working in this area and are cognizant of the problem, I don't think this needs to be a serious reservation. My other concern relates to the studies on semen where the applicants wish to attempt separation of sperm from semen. While this seemed initially a plausible approach, it is somewhat undermined by a very recent publication in the Journal of Acquired Immune Deficiency Syndrome(suggesting that virus may actually be attached to sperm. Since this publication in October 1988 may have reached the authors after their application was submitted, it is no criticism of them that they did not include this concern in making this aspect of their proposal. Given the relatively preliminary aspects of this part of the study, I do not think it necessarily undermines it totally but it certainly raises some questions about the likely applicability of such a technique.

I believe this project fulfills most of the major criteria which one would wish for projects of this type, including the unique opportunities provided by this particular cohort which make it as a study of haemophiliacs quite pre-eminent in this area world wide. It is certainly timely and contemporaneous in its focus and is extremely purvasive. In the long run it will lead to important applicability although its exploitability in commercial terms is not immediately an issue. The level of support is reasonable and well justified and I believe this project should be supported for funding.

provided at the level of £20.2k over the tenure of the grant and £6.5k has been authorised to date for equipment. The total support awarded when this grant was approved was £59.2k.

4. Present proposals

Dr Ludlam and colleagues have submitted a revised application to continue their study on haemophiliacs. It was submitted with a covering letter (annex 1, appendix 4) explaining the changes that have been made in the light of the referees' and Board's comments.

The applicants are requesting support at the level of one scientific assistant and two technical assistants, £58.2k for expenses over the tenure of the grant and £1.4k for equipment. Total support requested is £206k over three years. Referees' opinions have not been sought again.

The proposals have received local ethical committee approval.

If awarded this grant will be a charge to the special allocation of funds from the Science Vote for AIDS research.

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5. Action required

i) Assessment of the merit of the proposal in the light of such criteria as are relevant.

ii) Decision on the scale and duration of support to be made available, if awarded.