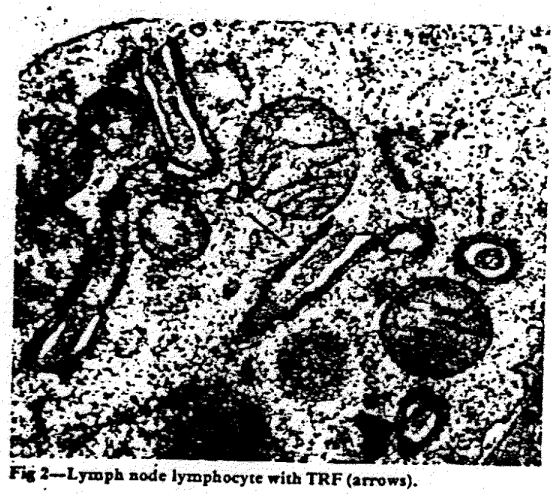

**Fig 2—Lymph node lymphocyte with TRF (arrows).**
(×20 000.)

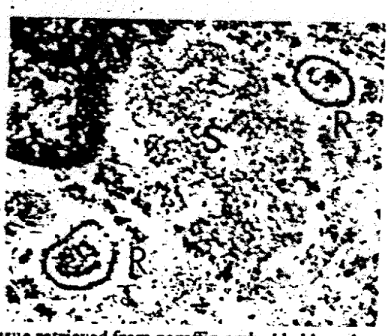

**Fig 3—Tissue retrieved from paraffin-embedded lymph node.**
Note a TRS (S) and two TRF (R). (×35 750.)

TRF have been described only once before, in a Japanese case with adult T cell leukaemia.[7] Patients with this disease harbour the human T leukaemia virus (HTLV).[8] The nature of TRF and their relation to this or any other agent is unknown.

TRS and TRF appear to be ultrastructural markers of AIDS.

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## FACTOR VIII PRODUCTS AND DISORDERED IMMUNE REGULATION

SIR,—Altered distribution of T-lymphocyte subpopulations in young haemophiliacs under treatment with factor VIII concentrate in Washington, DC, has lately been reported in *The Lancet*.[1] Similar observations in other haemophiliacs in Ohio and Wisconsin were reported earlier this year[2,3] and a recent report from Iowa describes similar observations.[4] By March 3, 1983, eleven cases of clinical acquired immunodeficiency syndrome (AIDS) in haemophiliacs had been reported to the Centers for Disease Control; all had received factor VIII concentrate. These observations are consistent with the hypothesis that AIDS is caused by a transmissible agent, presumably a virus, that can be included in blood products, and that some recipients of the agent have not (at least not yet) developed the complete clinical syndrome with its devastating complications. They are also compatible, however, with the possibility that repeated administration of factor VIII concentrate from many varied donors induces a mild disorder of immune regulation by purely immunochemical means, without the intervention of an infection. Such a mild immunosuppression could predispose to subsequent infection with a biological agent.

These alternative hypotheses might be distinguished through a study of T-lymphocyte subpopulations among similarly treated haemophiliacs in a geographical area to which AIDS has not yet been introduced. The resolution of this question by a timely investigation in some country, where cases of AIDS have not yet been reported would be an immense help to public health workers worldwide. In this situation "negative results" would be of great significance.

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## ABNORMAL T-LYMPHOCYTE SUBPOPULATIONS ASSOCIATED WITH TRANSFUSIONS OF BLOOD-DERIVED PRODUCTS

SIR,—The T-lymphocyte abnormalities which accompany the acquired immunodeficiency syndrome (AIDS) have also been observed in some patients with haemophilia A, in the presence or absence of opportunistic infections.[5-8] Because homosexuality and intravenous drug abuse were not associated with these cases, repeated exposure to lyophilised factor VIII concentrates[7,8] with possible transfer of an undefined blood-borne agent(s)[9] has been an implicated aetiological factor for these patients' immune dysfunction. Menitove et al[7] and Lederman et al[8] suggest that the risk of developing impaired T-lymphocyte function may be negligible in haemophiliacs treated only with cryoprecipitate or fresh frozen plasma.

We have studied groups of patients repeatedly exposed to lyophilised FVIII concentrates or to other blood products (table). All the patients had a good performance status and were evaluated in the absence of concurrent illnesses. T-lymphocyte subpopulations were counted by flow cytometry and indirect immunofluorescence with monoclonal antibodies OKT3 (pan T cell), OKT4 (helper/inducer T-cells), and OKT8 (suppressor/cytotoxic T-cells).

Haemophiliacs treated with lyophilised FVIII concentrates had a significantly reduced mean T4/T8 ratio compared with age and sex-matched controls. Similarly, T4/T8 ratios were much depressed in von Willebrand's disease, mild haemophilia A, and in hyper-transfused patients with sickle cell anaemia. These groups received cryoprecipitate, fresh frozen plasma, or packed red cells exclusively. Mildly decreased T4/T8 ratios have been noted in hypertransfused subjects with β-thalassaemia (P. Gaseon, N. S. Njaung, and others, unpublished), Diamond-Blackfan syndrome, and congenital dyserythropoietic anaemia (table), but numbers are too small for statistical testing. In contrast, those treated with prothrombin

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T-CELL VALUES (MEAN ±SEM) IN PATIENTS TREATED WITH BLOOD DERIVED PRODUCTS

| Group | % of total lymphocytes | | | OKT4/OKT8 | % with OKT4/OKT8≤1·1 |
|---|---|---|---|---|---|
| | OKT3 | OKT4 | OKT8 | | |
| *Haemophilia A, severe* | | | | | |
| Adolescents (n=13) | 71·0±3·7 | 34·2±3·7 (p<0·01) | 33·8±3·7 | 1·1±0·2 (p<0·005) | 46 |
| Adults (n=23) | 74·2±2·0 | 31·5±2·9 | 37·2±3·4 (p<0·025) | 0·8±0·1 (p<0·005) | 74 |
| *Haemophilia A, mild (n=3)* | 67·5±5·3 | 32·3±3·2 | 34·5±2·8 | 0·9±0·2 (p<0·05) | 67 |
| *Classic von Willebrand's, severe (n=3)* | 75·1±6·0 | 38·4±2·1 | 36·3±3·1 | 1·1±0·2 (p<0·05) | 67 |
| *Patients on PCC* | | | | | |
| Haemophilia B (n=9) | 65·6±3·2 | 46·0±4·8 | 28·2±2·2 | 1·9±0·2 | 11 |
| Haemophilia A with antibodies to AHF (n=3) | 67·2±8·2 | 43·1±2·9 | 25·4±3·4 | 1·8±0·4 | 0 |
| *Chronic hypertransfusion states* | | | | | |
| Sickle cell anaemia (n=11) | 66·4±3·2 | 41·5±4·0 | 30·9±3·6 | 1·3±0·1 (p<0·025) | 27 |
| Diamond-Blackfan syndrome (n=3) | 75·6±3·5 | 46·0±3·1 | 31·3±4·1 | 1·5±0·2 | 0 |
| Congenital dyserythropoietic anaemia (n=3) | 78·0±7·0 | 48·0±3·0 | 30·0 | 1·4±0·1 | 0 |
| *Controls* (n=63) | 69·3±5·9 | 44·7±1·7 | 29·5±1·6 | 1·8±0·1 | 10 |

complex concentrates ('Konyne') for circulating anticoagulants against antihaemophilic factor or for haemophilia B had normal T4/T8 ratios (table).

Lyophilised FVIII concentrate and prothrombin complex concentrates were examined for presence of thymosin,[10] which induces differentiation of T-cells[11] and stimulates lymphokine production.[12] No thymosin $\alpha_1$ was detected in samples from five separate lots of lyophilised FVIII concentrate from two manufacturers; however, 1000 pg/ml thymosin $\alpha_1$ was measured in samples from two separate lots of prothrombin complex concentrates. These immunological determinations[10] are comparable with concentrations in normal sera, but functional activities were not evaluated. We do not know whether thymosin in prothrombin complex concentrates protects individuals from the development of T-cell dysfunction, whether it reverses pre-existing imbalances of immunoregulatory T-cell activity associated with use of blood products, or simply whether the undefined agent(s) responsible for inducing T-cell dysfunction is absent in the material tested.

Repeated exposure to many blood products can be associated with development of T4/T8 abnormalities. Exclusion of lyophilised FVIII concentrates from the products available for the management of haemophilia A may reduce the incidence of AIDS, but the risk may not be eliminated altogether.

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## AIDS IN HAEMOPHILIA PATIENTS IN SPAIN

SIR,—An acquired immunodeficiency syndrome (AIDS) has been observed in homosexual males, intravenous drug abusers, Haitian immigrants, and haemophiliacs.[1] We describe three haemophilia patients, treated with commercial concentrates of factor VIII, with severe opportunistic infections who are the first cases of AIDS in Spain. None of them were homosexual or drug abusers or had been under immunosuppressive treatment. Patients 1 and 2 were brothers.

*Case 1.*—9-year-old male. He had been in good health until 18 months before admission when he had frequent episodes of fever and cough with progressive weight loss. He was admitted for persistent fever in November, 1982. Physical examination revealed cachexia, oropharyngeal candidiasis, and acinar consolidation of right middle lobe in the chest X-ray. Laboratory data revealed severe lymphopenia, polyclonal hypergammaglobulinaemia, *Candida albicans* in urine culture and serological markers for previous infection by cytomegalovirus (CMV). He was anergic to skin-test antigens. Despite antibiotics and amphotericin B therapy the patient died of respiratory distress. Necropsy showed bilateral intra-alveolar haemorrhage with CMV and *Aspergillus* infection.

*Case 2.*—16-year-old male. He had good health until age 15 when he began to have fever, respiratory symptoms, diarrhoea, and weight loss. He was admitted in March, 1982. Physical examination revealed emaciation with oropharyngeal candidiasis, and a chest X-ray showed lesions which suggested bronchiectasis. As in case 1 there was severe lymphopenia and polyclonal hypergammaglobulinaemia. He also had disaccharidase deficiency (intestinal biopsy), oesophageal candidiasis (endoscopy), and serological markers for previous hepatitis A and B. With antibiotic and 5-flucytosine therapy the fever was controlled. Recently he has been readmitted, seriously ill.

*Case 3.*—38-year-old male. He had been in good health until June, 1982, when fever and progressive weight loss began. 1 week before his admission he had a generalised convulsive crisis, being sent to our hospital in February, 1983. He also complained of non-productive cough and increasing dyspnoea. Physical examination showed mental stupor without other neurological findings, herpes labialis, hepatosplenomegaly, and oropharyngeal candidiasis. Chest X-ray revealed a bilateral diffuse interstitial infiltrate and *Pneumocystis carinii* was identified from lung tissue and bronchial secretions. Other data were severe lymphopenia, polyclonal hypergammaglobulinaemia, serological markers for previous infections by Epstein-Barr virus, *Aspergillus*, *Candida albicans*, hepatitis A and B, and abnormal liver function tests with cirrhosis on needle biopsy. He was anergic to skin-test antigens. The

LYMPHOCYTE SUBPOPULATIONS IN PATIENTS AND CONTROLS

| Case | SIG (B cells) | OKT3 (pan T) | OKT4 (T helper) | OKT8 (T suppressor) | OKT4/OKT8 (ratio) |
|---|---|---|---|---|---|
| 1 | 27 | 50 | 9 | 52 | 0·17 |
| 2 | 46 | 38 | 9 | 35 | 0·26 |
| 3 | 4 | 86 | 20 | 64 | 0·31 |
| Controls (n=10) | 9·5±3·5 | 81·5±6·6 | 55·0±4·4 | 26·6±5·4 | 2·1±0·3 |

SIG = surface immunoglobulin-bearing lymphocytes. Monoclonal antibodies studied by indirect immunofluorescence.
All results significantly different from control values at p<0·05 (Student's t test; quantiles test for SIG) except for SIG and OKT3 in case 3.

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