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inspiration. An end-expiratory pause increases this washout and reduces dead space reinspiration and $\bar{F}iCO_2$ (table II). Conversely, abolition of this pause by rapid shallow breathing increases $\bar{F}iCO_2$. A rapid onset of inspiration and a fast finish for expiration has a similar effect (fig 7, table III, patient 1). $\bar{F}iCO_2$ in the patients (table III) is higher than in normal subjects (0·37 versus 0·19 for 28% mask, and 0·6 versus 0·18 for the 40% mask) presumably because tachypnoea leads to more dead-space reinspiration.

The rise of $\bar{F}iCO_2$ with dead space reinspiration must lead to CO_2 retention, a rise in arterial PCO_2, and some increase in minute ventilation. Because $\bar{F}iCO_2$ did not exceed 0·76% (except in patient 1), these effects will be small and not noticed by the subjects.

It is clear (fig 2) that if more is withdrawn from the mask than is supplied $\bar{F}iO_2$ will fall because of additional entrainment of air through and around the mask. This will occur when peak flow during inspiration exceeds mask inflow even if mean flow does not reach this level. During tidal breathing, on the other hand, with reinspiration of mask dead space the situation is more complicated because $\bar{F}iO_2$ falls with increasing ventilation before demand exceeds supply (fig 3). Nevertheless, once mask inflow is exceeded by increasing V_T at a fixed frequency, $\bar{F}iCO_2$ and $\bar{F}iO_2$ fall together (fig 4) which suggests that additional air is being drawn into the mask throughout inspiration. On the other hand, with tachypnoea (increase of frequency at constant V_T)—see table II—the fall of $\bar{F}iO_2$ is accompanied by a rise of $\bar{F}iCO_2$ suggesting that reinspiration of dead space is now more important.

Campbell and Minty[6] tested a prototype of the 60% mask. In model studies (fig 2) the behaviour of this mask differed from that of other masks. Because of its low entrainment ratio and low mask inflow compared with other masks (table I) more dead-space reinspiration occurs and with all patterns of breathing $\bar{F}iCO_2$ is higher than with other masks (table II). The lower the entrainment ratio the larger the effect of small perturbations on $\bar{F}iO_2$. The dead-space problem would be alleviated by increasing total mask inflow but it is hardly practicable in a hospital ward to increase the oxygen supply much above 15 l/min. We conclude that under operating conditions the 60% ventimask is not a fixed performance device, though it will give an $\bar{F}iO_2$ of >45%.

The thrust of earlier publications[2,3,5] was to show that the ventimask was a relatively fixed performance device in terms of $\bar{F}iO_2$ and that masks not using the venturi principle were extremely variable in performance.[9] Recent studies[8,10] have looked more closely at the performance of venturi masks alone. Many designs of venturi mask are now available but most (except the Vickers Medical mask used in this study) are of the tight-fit small volume "aviator" type with reservoir volumes not exceeding 100 ml. In a model study Cox and Gilbe[10] found substantial deviations from nominal concentrations in all the small-reservoir masks ('Blease OEM mixamask', 'Inspiron Accurox' mask, 'Hudson multivent', (Sandoz Lifeline'). The underestimations of $\bar{F}iO_2$ in absolute terms ranged from −1·5% at 24% nominal to −3% at 28% and −10% at 40% nominal. The performance of the large-reservoir mask (Vickers Medical) was ±1% nominal up to 40%. Using flow-weighting Woolner and Larkin[8] found $\bar{F}iO_2$ in the small volume Hudson Multivent mask was substantially below nominal (from the 28% to the 50% settings) in a normal subject breathing quietly. A small reservoir is clearly unsatisfactory; anyway, it is unnecessary with a high-airflow oxygen-enrichment device.

A predictable and constant inspired-oxygen concentration can only be delivered with a mask employing the venturi principle and having an adequate reservoir volume. The Vickers mark III ventimask operates satisfactorily over a range of $\bar{F}iO_2$ from 24% to 40%. These concentrations are sufficient to relieve life-threatening arterial hypoxaemia in nearly all clinical pulmonary conditions except massive alveolar collapse and consolidation or CO poisoning. A 60% mask is available which in practice gives 50%. The manufacturer's recommended oxygen flow rates are satisfactory and economic in terms of oxygen usage. Nevertheless, in patients with tachypnoea (respiratory frequency >30/min) the oxygen supply should be increased by 50%.

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ABNORMALITIES OF CIRCULATING LYMPHOCYTE SUBSETS IN HAEMOPHILIACS IN AN AIDS-FREE POPULATION

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Summary Markers of the immune system were examined in 47 patients with haemophilia A and B who had been treated exclusively with blood products from a population apparently free from acquired immunodeficiency syndrome (AIDS). In haemophilia A the absolute number of T helper cells was depressed, resulting in a reduction in the helper/suppressor ratio in about half the patients. The serum IgG and IgA concentrations were raised and the serum IgG correlated with serum alanine aminotransferase. In haemophilia B, the helper/suppressor ratio was also depressed but this was attributable to a slight increase in the mean suppressor cell number and a slight decrease in the helper cells. These observations suggest that the abnormalities result from transfusion of foreign proteins and not from a specific infective agent and, further, that individuals may differ in susceptibility to the induced disturbances of immunity.

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Introduction

SINCE the acquired immunodeficiency syndrome (AIDS) was recognised in 1981,[1] cases have been reported among homosexuals, haemophiliacs, recipients of blood transfusions, intravenous drug abusers, Haitians, and female partners of patients with AIDS.[2-4] The occurrence of AIDS in haemophiliacs, including 2 born in Britain,[5,6] has emphasised the potential importance of AIDS for patients with congenital bleeding disorders treated with blood products. All recorded cases in haemophiliacs have arisen in patients treated with commercial factor VIII or factor IX concentrates. T lymphocyte subset abnormalities, similar to those found in AIDS, are common in healthy homosexuals[7-9] and haemophiliacs.[10-22] The significance of these abnormalities in symptomless individuals is unclear. Most of the data on cell-mediated immunity in healthy haemophiliacs have come from those exposed to commercial factor VIII. To clarify the relation between the T lymphocyte abnormalities seen in healthy haemophiliacs and those in AIDS, we have studied 47 patients with haemophilia A and B treated since 1978 with factor VIII and IX concentrates, prepared exclusively from donors in Scotland. Only one case of AIDS has so far been reported in Scotland, and this patient probably contracted the disorder when resident in East Africa.[23] He had never been a blood-donor in Scotland.

Methods

Patients and Blood Products

The study group comprised 37 patients (age 13–62 years) with haemophilia A (26 severe, factor VIII ⩽2%; 11 non-severe, factor VIII >2%). They had been treated exclusively with factor VIII concentrate or cryoprecipitate prepared by the Scottish National Blood Transfusion Service (SNBTS FVIII). 2 patients had received only cryoprecipitate. None of the study patients had been exposed to commercial factor VIII concentrate in the previous 5 years and most had never received any during their lives. 10 patients with haemophilia B (3 severe, factor IX ⩽2%; 7 non-severe, factor IX >2%) had been treated exclusively with SNBTS factor IX concentrate (SNBTS FIX). The two patient groups were compared with 22 healthy male controls aged 20–42 yr and with a separate group of 6 patients with severe haemophilia A who had received varying amounts of commercial factor VIII (Com FVIII) as well as SNBTS blood products. All patients were healthy at the time of study: none had lymphadenopathy or clinical splenomegaly, or were drug addicts or homosexual. Mean annual factor VIII use was 33 400 and 3000 units for patients with severe and non-severe haemophilia A, and 33 000 and 3300 units for those with severe and non-severe haemophilia B.

Laboratory

Full blood counts were performed with a Coulter S, and lymphocyte counts were calculated from a visual 200 cell differential. Mononuclear cells were separated on a 'Ficoll'-'Hypaque' gradient. T lymphocytes were measured by E-rosetting (Er).[24] Percentages of T helper and T suppressor/cytotoxic lymphocytes were measured by indirect immunofluorescence with FITC anti-mouse immunoglobulin (Meloy) and commercial monoclonal antisera (Leu 3a, Leu 2a, Becton Dickinson), and scored either by eye (Leitz 'Ortholux' microscope with Ploem's incident fluorescence illumination) or on a Becton Dickinson FACS IV flow cytopherometer. Absolute T helper (Th) and absolute T suppressor (Ts) cell numbers were calculated as follows:

$$\text{Absolute Th or Ts counts} = \frac{\%\,\text{Th or Ts}}{\%\text{Th} + \%\text{Ts}} \times \frac{\text{Er}}{100} \times \text{lymphocyte count}$$

Bilirubin, alanine aminotransferase, gamma-glutamyltransferase, and alkaline phosphatase were measured on an SMAC; IgG, IgA, and IgM by radial immunodiffusion on Partigen plates; viral antibodies to herpes simplex, herpes zoster, and cytomegalovirus by routine complement fixation techniques; antibodies to Epstein-Barr virus by immunofluorescence; and hepatitis B markers by radioimmunoassay. Pneumocystis and toxoplasma titres were estimated by Dr H. Williams, Raigmore Hospital, Inverness.

Non-parametric statistical tests were used throughout since several variables had non-gaussian distributions. Patient groups were compared with normal subjects by Wilcoxon's rank sum test. Associations between pairs of variables were assessed with the Spearman rank correlation coefficient.

Results

Haemoglobin and platelet counts were normal in all patients. The mean total leucocyte count was $5{\cdot}32\times10^9$/l in the haemophilia patients compared with $6{\cdot}62\times10^9$/l in the male controls ($p<0{\cdot}05$)

Lymphocyte Indices

In haemophilia A patients treated exclusively with SNBTS factor VIII, absolute total lymphocyte and total T cell counts were lower than those in controls ($p<0{\cdot}01$) (table I). The T lymphocytopenia was principally due to a deficiency of Th cells ($p<0{\cdot}01$), 13 (35%) patients having absolute Th values below the normal range. Absolute Ts cell counts were similar to those in controls (fig 1). The Th/Ts ratios, with 16 (43%) patients falling below the normal range, seem to be bimodally distributed (fig 2); however, the Wilcoxon rank sum test showed no significant difference from controls because of high Th/Ts ratio in some patients (fig 1). Repeat testing of 10 patients after one month revealed that over this period the Th/Ts ratio was relatively stable (table II). There was no correlation between lymphocyte indices and either severity of haemophilia or units of FVIII received during the previous four months or 2 yr. The 2 patients who had received cryoprecipitate only, had ratios of 0·7 and 2·3.

The group of 6 severe haemophilia A patient exposed to varying amounts of Com FVIII had mean values for total and T lymphocytes, and for Th and Ts subsets, similar to the means in exclusively SNBTS-FVIII-treated patients (table I). Numbers are too small for statistical comparison with

TABLE I—LYMPHOCYTE SUBSETS AND RATIOS IN THE STUDY GROUPS

	N	Cell counts (mean±SE×10⁹/l)				
		Total lymphs	T lymphs	Th	Ts	Ratio Th/Ts
Haemophilia A SNBTS FVIII	37	1·70±0·10†	1·23±0·08†	0·70±0·05†	0·53±0·05	1·7±0·11
Haemophilia B SNBTS FIX	10	2·01±0·19	1·46±0·15	0·83±0·10	0·63±0·09	1·50±0·22*
Haemophilia A Com FVIII	6	1·71±0·25	1·11±0·17	0·63±0·07	0·47±0·11	1·7±0·03
Controls	22	2·18±0·13	1·65±0·10	1·09±0·07	0·56±0·06	2·0±0·14

Lymphs = Lymphocytes.
Difference from normal controls *$p<0{\cdot}05$ and †$p<0{\cdot}01$.

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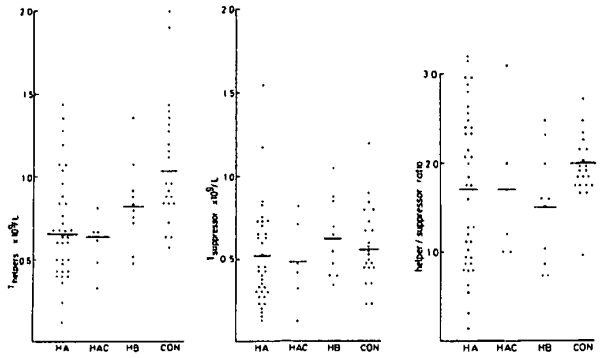

Fig 1—(a) T helper cell counts, (b) T suppressor cell counts, and (c) T helper/suppressor ratio in patients with haemophilia A treated with SNBTS factor VIII (HA), commercial factor VIII (HAC), haemophilia B (HB), and male controls (CON).

TABLE II—REPRODUCIBILITY OF HELPER/SUPPRESSOR RATIOS

Patient	Initial	Repeat (1 mo)
1	0·7	0·8
2	2·1	2·6
3	1·0	0·9
4	2·9	1·8
5	1·0	0·7
6	2·5	1·5
7	0·9	1·5
8	0·7	0·6
9	0·9	0·6
10	1·0	1·4

controls. In haemophilia B patients mean total T lymphocyte and T subset numbers did not differ significantly from those in controls (table I); however, there was a tendency for the Th counts to be lower and for the Ts counts to be higher, so that the mean Th/Ts ratio for the group was significantly ($p<0·05$) lower than that of controls. In 4 of the 10 patients the ratio was below 1·5 (fig 1). There was no correlation between abnormal lymphocyte indices and severity of disease or amount of SNBTS FIX received.

Liver Function Tests and Immunoglobulins

In SNBTS-FVIII-treated haemophilia A patients the serum alanine aminotransferase was significantly raised ($p<0·01$), the values being above normal in 76% (table III). Alkaline phosphatase was also significantly raised ($p<0·01$) after exclusion of patients under 20 years old. IgG and IgA were both increased ($p<0·01$) (table III), and IgG was positively correlated with alanine aminotransferase ($r=0·44$, $p<0·05$). There was no correlation between any of the lymphocyte indices and liver function or immunoglobulin measurements. In haemophilia B patients, the mean alanine aminotransferase was raised ($p<0·01$), 66% of patients having abnormal values. All other indices of liver function were normal as were immunoglobulins.

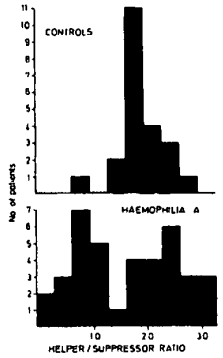

Fig 2—Frequency distribution of helper/suppressor ratio in haemophilia A and control subjects.

Viral and Other Infections

Antibody titres to viruses, pneumocystis, and toxoplasma did not differ from those in the control population. Results of tests for HBsAg, anti-HBs, and anti-HBc, measured regularly in all patients attending the haemophilia centre, yielded evidence of previous HBV infection in 41 patients. There was no correlation between evidence of HBV infection and abnormal lymphocyte indices.

Discussion

Most of the patients reported in this study have been treated exclusively during the past 5 years with locally prepared blood products, and have never received commercial concentrates. This contrasts with the position in other centres, except Australia.[19] The amount of treatment given to the haemophiliacs in this study is in line with therapeutic practice in the United Kingdom. The source plasma from which factor VIII and IX concentrates, as well as cryoprecipitate, are prepared is collected by the SNBTS within Scotland where only 1 case of AIDS has been reported, and that contracted elsewhere. The blood products seem unlikely to contain a "specific" infective agent giving rise to AIDS. It is always possible, of course, that there are blood donors, still unidentified, in the prodromal stage of AIDS. However, at least 1 year has passed since the most recent batch of plasma was collected for preparation of the factor concentrates with which the study patients have been treated. The SNBTS factor VIII is less pure (0·3 IU/mg protein) than some of the commercial products. The factor IX is prepared from cryoprecipitate supernatant after absorption and elution from DEAE cellulose. This concentrate contains 3·0 IU factor IX/mg protein and is therefore substantially purer than factor VIII.

The principal immunological abnormality in our patients was a reduction in the absolute number of T helper cells, in over one-third of those with haemophilia A; T suppressor cell counts were normal. Furthermore, these abnormalities were stable over one month. Workers in Vienna[18] and Sydney[19]

TABLE III—LIVER FUNCTION AND IMMUNOGLOBULINS IN THE STUDY GROUPS (MEANS AND STANDARD ERRORS)

Group		Bilirubin (μmol/l)	ALT	Alk Phos*	GGT	IgG (g/l)	IgA (g/l)	IgM (g/l)
Haemophilia A SNBTS FVIII	34	10·7±0·7	80±5·7‡	96±4·6‡	54±7·1	18±0·7‡	3·1±0·2†	2·4±0·2
Haemophilia B SNBTS FIX	10	8·6±1·4	71±7·6‡	83±9·6	26±12·7	14±1·3	2·9±0·3	2·2±0·3
Controls	15	10·5±1·1	29±6·6	75±6·1	35±10·1	13±1·1	2·4±0·3	2·4±0·2

*Excludes 10 patients with haemophilia A and 4 with haemophilia B of age less than 20 years.
ALT = alanine aminotransferase; alk phos = alkaline phosphatase; GGT = gamma-glutamyltransferase. †$p<0·05$, ‡$p<0·01$.
The results exclude, as outliers, 1 patient with haemophilia A with a bilirubin of 35 μmol/l and another with a GGT of 575 U/l. Samples unavailable on 3 haemophilia A patients and 7 control subjects.

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have reported a similar reduction in Th cells but most other reports, usually on small numbers of patients, record either normal absolute numbers of both Th and Ts cells or an increase in Ts cells. The reduction in Th cells in our patients was not related to severity of the haemophilia, amount of treatment, or abnormalities of liver function or immunoglobulin concentrations. The decreased helper/suppressor ratio accords with many other studies of symptomless haemophilia A patients. A ratio below 1·5 was present in nearly half of our patients compared with 1/22 control subjects. The frequency distribution of Th/Ts ratios suggests that the patients with haemophilia A may be divided into two groups (fig 2). An additional 6 of our patients had received commercial factor VIII as well as SNBTS product. Their immune indices were similar to those of patients treated exclusively with the SNBTS concentrate. The observed reduction in Th cell numbers is similar to that in AIDS victims, "pre AIDS" subjects, and symptomless homosexuals.

In our patients with Christmas disease the results of immunological tests were less abnormal. The mean numbers of Th and Ts cell subsets were within normal limits, although the Th/Ts ratio was reduced by a tendency towards a slight decrease in helpers and increase in suppressors. Other published studies record not only normal absolute numbers of T subsets in haemophilia B but also in some instances normal Th/Ts ratios.[12-15,17,18] We found no correlation between lymphocyte changes and any of the indices measured, but the numbers were small.

We assume that the immunological abnormalities in these haemophiliacs result from intravenous administration of blood products. As regards raised Ig concentrations it is noteworthy that the Ig content of SNBTS FVIII concentrate is about 3 g/l; an average treatment dose is 40 ml containing 120 mg, so the effect on total Ig levels would be minuscule. The differences between haemophilia A and Christmas disease might be associated with the lower antigenic load in Christmas disease, which is treated with a purer product. If this were the case then a correlation would be expected between the amount of factor VIII transfused and the degree of helper cell reduction but this was not observed.

If the immunological disturbances are related to an infective agent then this may be preferentially present in the factor VIII concentrates. Most of our patients have evidence of previous infection with hepatitis B virus, but none of the immunological or liver function test results were related to past infection with this virus. It has been suggested that, because the epidemiology of hepatitis infection is very similar to that of AIDS, the "AIDS agent" may be carried by HBV in a similar way to the delta agent.[25] Our study shows that changes in T cell subsets in haemophiliacs are independent of HBV infection. The titres of other viral, toxoplasma, and pneumocystis antibodies in our haemophiliacs are similar to those found in normal control subjects whereas some patients with AIDS or "pre AIDS" are reported to have very high titres, particularly against cytomegalovirus and Epstein-Barr virus.[26]

As we have reported previously,[27] and in keeping with other studies, three-quarters of our patients have deranged liver function. The degree of abnormality in the tests does not correlate with the amount of concentrate transfused. The correlation of IgG levels with the alanine aminotransferase may therefore merely reflect the presence of chronic liver disease.

This study has not identified the cause of the reduction of Th but it is unlikely to be due to specific AIDS virus in the blood products. It is more likely to result either from an as yet unidentified component of the therapeutic concentrates or from a non-specific effect of foreign protein infused intravenously. Because the reduction in Th is not dose-related and because of its bimodal distribution in patients with haemophilia A it seems that some patients are more susceptible to this immunological disturbance than others. What determines an individual's immunological response to transfusion remain elusive. Furthermore, the relation between the lymphocyte subset abnormalities in symptomless haemophiliacs and the likelihood of eventual frank AIDS remains unclear although it may be connected with HLA status.[28]

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