CLINICAL RESEARCH

Immunological abnormalities in haemophilia: are they caused by American factor VIII concentrate?

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Abstract

Scottish patients with haemophilia, most of whom had received no American factor VIII concentrate for over two years, were found to have immunological abnormalities similar to those in their American counterparts—that is, a reduced proportion of T helper cells, an increased proportion of T suppressor cells, and a reduced response to concanavilin A. Factor VIII from both the United States and Scotland severely inhibited the in vitro lymphocyte response to mitogens in patients and controls. The American and Scottish concentrates could not be distinguished in terms of either patient usage or their effect in vitro.

These results argue against a disease vector specific to American blood products.

Introduction

The finding that the acquired immune deficiency syndrome may be transmitted by a blood borne agent[1 2] has serious implications for patients with haemophilia, whose ability to lead a normal life depends on regular replacement treatment with concentrates of blood products.[3 4] The incidence of the disease in the United States may be due in part to the contamination of commercial blood supplies by a virus or some other transmissible agent that affects the immune system. We therefore investigated the cellular immunity of a group of Scottish patients with haemophilia who were treated with factor VIII derived exclusively from Scottish donors and prepared by the Scottish Blood Transfusion Service. All but two of the patients had received commercial (and therefore American) factor VIII or IX at some time during their lifetime, but only five of the 19 had received it during the past two years. We also investigated the effect in vitro of adding factor VIII concentrate in tests of cell mediated immunity.

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Methods

Patients were selected with severe haemophilia type A (n = 17) or type B (n = 2). All were men, aged 19-66 years (mean 36 years), and were clinically well at the time of testing. Controls were healthy members of staff matched for age.

The percentages of total T cells, T helper cells, T suppressor cells, Ia positive cells, and monocytes (in Ficoll-Hypaque preparations of mononuclear cells) were determined using monoclonal antibodies (Ortho) and fluorescence microscopy.[5] Natural killer activity against K562 cells and the proliferative responses of lymphocytes to optimal concentrations of phytohaemagglutinin and concanavilin A were measured as described elsewhere.[6 7]

Factor VIII concentrates from America (kindly supplied by Dr Oscar Ratnoff, Cleveland, Ohio; Profilate, list No 5893, from Alpha Therapeutic Corporation, Los Angeles) and from the Scottish Blood Transfusion Service were added to the in vitro lymphocyte cultures at therapeutic concentrations. The patients were screened for infections with cytomegalovirus and Epstein-Barr virus and tested for antibody to factor VIII.

The results obtained in the patients and controls were analysed with the Minitab statistics package by computer.[8]

Results

Lymphocyte subpopulations—Table I shows the results. The percentage of total T cells (T3 staining cells) was normal in the patients. The percentage of T helper (T4) cells was significantly reduced, with nine out of 18 patients having results below the lowest normal level. Conversely, the percentage of T suppressor (T8) cells was significantly raised, with a different nine patients having results above the normal range. Combining these results showed the patients to have a reduced ratio of T helper to T suppressor cells ($p < 0.01$, figure). The percentages of DR positive cells (which include the B cell population) and of monocytes were normal.

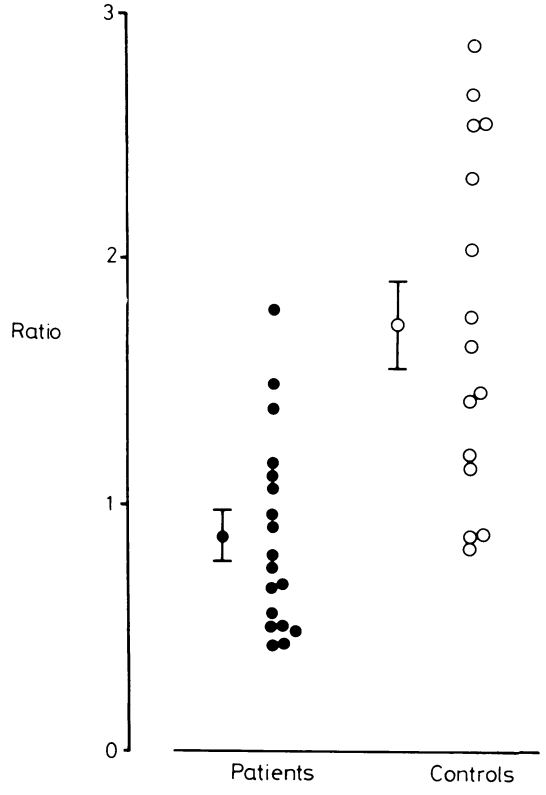
Lymphocyte function tests—Natural killer activity was normal in the patients. The proliferative response to phytohaemagglutinin was normal, but the response to concanavilin A was significantly reduced ($p < 0.02$) in the patients (table II). Addition of either American or Scottish factor VIII inhibited the mitogenic responses to both phytohaemagglutinin and concanavilin A in a dose dependent manner in both patients and controls (table III). Factor VIII had no effect on natural killer activity.

Serology—None of the patients had evidence of a recent infection with either cytomegalovirus or Epstein-Barr virus. One patient had antibody to factor VIII.

TABLE I—*Lymphocyte subpopulations in patients and controls*

Antibody	Mean (SEM) % of cells staining	
	Patients (n = 18)	Controls (n = 15)
OKT3	57·9 (2·8)	55·0 (2·1)
OKT4	25·9 (1·2)*	37·0 (1·9)
OKT8	33·0 (2·2)*	22·1 (1·8)
OKIa1	12·5 (1·2)	13·0 (1·8)
OKm1	8·3 (1·4)	10·8 (2·5)

* Patients significantly different from controls ($p < 0.01$, two sample t test).



Ratio of T helper to T suppressor cells (mean (SEM)) in patients and controls.

TABLE II—*In vitro lymphocyte function in patients and controls (means (SEM))*

	Natural killer (% cytotoxicity)	Phytohaemagglutinin (cpm)	Concanavilin A (cpm)
Patients	32·9 (3·9) (n = 10)	5526 (663) (n = 19)	3153 (496)* (n = 19)
Controls	29·2 (6·0) (n = 10)	7010 (727) (n = 30)	5077 (537) (n = 30)

* Patients significantly different from controls ($p < 0.02$, two sample t test).

TABLE III—*Effect of adding exogenous factor VIII to mitogen cultures. (Results expressed as the percentage of the response to mitogen in the absence of factor VIII)*

	American factor VIII (U/ml)			Scottish factor VIII (U/ml)		
	0·01	0·1	1	0·01	0·1	1
			Phytohaemagglutinin			
Patients (n = 11)	99·3 (9·2)	64·6 (11·4)	41·6 (6·3)	97·7 (6·4)	57·0 (9·5)	36·8 (5·8)
Controls (n = 10)	92·7 (8·27)	48·1 (7·1)	30·8 (2·6)	75·7 (6·7)	30·7 (1·8)	27·0 (2·7)
			Concanavilin A			
Patients (n = 11)	83·8 (11·9)	66·5 (11·3)	51·5 (7·9)	84·2 (12·5)	61·2 (10·2)	47·4 (19·6)
Controls (n = 11)	88·6 (4·5)	66·1 (6·5)	44·8 (3·5)	89·2 (4·4)	59·5 (5·4)	42·1 (2·2)

Discussion

If the acquired immune deficiency syndrome is being transmitted to American patients with haemophilia by a blood borne agent that has contaminated their commercial supplies of concentrate[9] then we might expect to see a difference in similar patients who are treated with replacement factor derived from a different source. All but two of our series of 19 Scottish patients had received the American product at some point in the past six years, although nine had received it on fewer than five occasions and 14 had not received any in the past two years. Two of the patients were known to be practising homosexuals; they were among the five who had both reduced helper cells and increased suppressor cells.

Dividing the subjects on the basis of low (less than 1000 units/kg/year), medium (1000-3000 units/kg/year), or high (more than 3000 units/kg/year) usage of factor VIII showed no relation between usage of factor VIII and lymphocyte abnormality. The results confirm that Scottish patients with haemophilia have T cell abnormalities similar to those of their American counterparts.[1 4 9-11] What is not known is how few helper cells or how many suppressor cells it takes for a person to be clinically immunodeficient, and thus the risk that these patients have of developing the infections or neoplasms associated with the acquired immune deficiency syndrome.

In vitro the Scottish and American factor VIII preparations behaved identically in inhibiting the lymphocyte response to non-specific mitogens. This effect was seen in both patients and controls. If factor VIII concentrate does interact with lymphocytes in such a way as to render them immunologically unresponsive then patients with haemophilia may be assaulting their immune system every time they inject themselves, leading to a gradual diminution in their ability to resist infections or neoplasms. In studies of mortality in haemophilia carried out over the past 25 years, however, there has been no evidence of an increase in deaths from tumours or unexplained infection (C D Forbes, personal communication). At this stage we cannot rule out the possibility that the in vitro results are due to an interaction between factor VIII and mitogen.

Our results, however, argue against a disease vector that is specific to American blood products. In terms of lymphocyte abnormalities, Scottish patients with haemophilia yield results that are consistent with those seen in the acquired immune deficiency syndrome and in acute viral infection.[12-15] Whether these abnormalities in the T cell ratios and in the response to concanavilin A are sufficient to render the patients immunodeficient and therefore, possibly, in a prodromal stage of the acquired immune deficiency syndrome, will become apparent as the patients are followed up clinically.

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Gonadal function in men treated for acute leukaemia

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Abstract

Gonadal function was assessed in eight men in remission of leukaemia who had completed treatment eight months to eight years previously. All four men treated for acute myeloid leukaemia had normal sperm counts and motility, compared with only one of the four with acute lymphatic or undifferentiated leukaemia. Hormonal studies indicated that the sterility resulted from gonadal failure rather than pituitary dysfunction after cranial irradiation.

These findings are important in the counselling of patients with leukaemia.

Introduction

Complete remission may now be induced in most young adults with acute leukaemia, and the proportion continuing without recurrence at five years is roughly one third. Thereafter relapse is rare and cure must be considered to be probable. As with other malignancies for which the rate of cure is appreciable, attention must be given to long term complications. We performed this study to assess gonadal function after the treatment of acute leukaemia in adults.

Patients and methods

We investigated eight of 21 men aged under 50 in remission of leukaemia who had completed treatment. Their mean age at presentation was 23·6 years (range 15 to 33 years) and at follow up 29·2 years (range 20 to 38 years). The treatment free interval ranged from eight months to eight years. Table I gives details of treatment.

Seminal analysis was performed after a minimum of three days' abstinence. At 9 am, after basal blood specimens had been obtained for estimation of concentrations of prolactin (mean of three measurements), sex hormone binding globulin, testosterone, and 17β-oestradiol, a standard test was performed in which luteinising hormone releasing hormone (100 μg) was given. Follicle stimulating hormone, luteinising hormone, and prolactin concentrations were measured by specific double antibody radioimmunoassay using Medical Research Council standards 69/104, 68/40, and 71/222 respectively. After ether extraction testosterone and oestradiol concentrations were measured by tritiated radioimmunoassay. Progesterone concentration was measured by tritiated radioimmunoassay after hexane extraction. Concentrations of sex hormone binding globulin were measured by saturation radioimmunoassay.[1]

TABLE I—*Details of treatment*

Case No	Leukaemia	Induction agents and total dosage	Maintenance agents and dosage
1	Acute myeloid	Adriamycin 620 mg Cytarabine 5·6 g Thioguanine 16·8 g	
2	Acute myeloid	Daunorubicin 1·68 g Cytarabine 27·6 g Thioguanine 9·2 g	
3	Acute myeloid	Adriamycin 900 mg Cytarabine 15·18 g Thioguanine 15·18 g	
4	Acute myeloid	Adriamycin 900 mg Cytarabine 8·4 g Thioguanine 13·4 g	
5*	Acute lymphatic	Vincristine 12 mg Adriamycin 270 mg Colaspase 210 × 10^3 IU	Cyclophosphamide 18·75 g Methotrexate 1·875 g Mercaptopurine 43·75 g
6*	Acute lymphatic	Vincristine 8 mg Adriamycin 200 mg Colaspase 16·5 × 10^3 IU	Cyclophosphamide 22·5 g Methotrexate 2·34 g Mercaptopurine 52·5 g
7*	Acute lymphatic	Vincristine 12 mg Adriamycin 640 mg Colaspase 252 × 10^3 IU Cyclophosphamide 3·6 g	Cyclophosphamide 4·5 g Methotrexate 4·5 g Mercaptopurine 78·75 g
8*	Acute undifferentiated	Vincristine 8 mg Adriamycin 200 mg Colaspase 102 × 10^3 IU	Cyclophosphamide 18·75 g Methotrexate 2·5 g Mercaptopurine 43·5 g

*Cranial irradiation of 2400 rads given.

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