Unresponsiveness to skin testing with bacterial antigens in patients with haemophilia A not apparently infected with human immunodeficiency virus (HIV)

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SUMMARY Unresponsiveness to skin testing with PPD and tetanus toxoid was commonly seen in patients with haemophilia A but not infected with human immunodeficiency virus but was uncommon in controls. Vaccination history indicated that the unresponsive patients had not been immunised in childhood. Other tests of immune competence (skin tests with other antigens, lymphocyte stimulation with mitogens and antigens, and viral serology) showed that the haemophilia A patients had an adequate response to pathogens to which they had been exposed. Five of 12 such patients had a mild T4 lymphopenia, and this may have been related to parenteral administration of large quantities of protein.

The acquired immune deficiency syndrome (AIDS) is now widespread in patients with haemophilia (type A more than type B) due to contamination of blood products with human immunodeficiency virus (HIV).^{1,2} Those infected in this way showed anti-HIV antibodies in their serum,³ T4 lymphopenia,⁴ and cutaneous anergy similar to that seen in patients infected by other routes.⁵ T4 lymphopenia and diminished responsiveness to dinitrochlorobenzene sensitisation occur in such patients not infected with HIV, and it is thought that repeated administration of large amounts of factor VIII or cryoprecipitate may itself be immunosuppressive.⁶

In this study apparent cutaneous anergy to commonly encountered bacterial antigens in some haemophilia A patients reflects lack of prophylactic immunisation rather than immunosuppression: other assessments of immune function were apparently normal.

Material and methods

Fifteen patients with factor VIII deficiency (12 with haemophilia A and three with von Willebrand's disease) and five patients with factor IX deficiency were

studied, along with 12 healthy controls. The study group included more than 95% of the haemophilia patients in the Tayside Region. All patients were treated exclusively with blood products prepared in the Scottish National Blood Transfusion Service Fractionation Centre in Edinburgh. None received treatment from batches now known to have been contaminated with HIV.⁷ Vaccination history, clinical assessment, erythrocyte, leucocyte and platelet counts were obtained at the onset of the study in 1985: total lymphocyte, monocyte, and granulocyte counts were calculated after differential counts on the blood films. Lymphocyte subsets were counted by flow cytofluorimetry after staining with CD4 and CD8 monoclonal antibodies.⁸

Skin tests with the Multitest CMI applicator (Merieux UK Ltd, Slough) and a standard Mantoux test (with 0.1 ml "new tuberculin"⁹) were performed on all subjects. The mean diameter of the response at 48 hours (induration only) was recorded: reactions of >2 mm were regarded as positive.

Lymphocyte stimulation tests were performed separately¹⁰ with phytohaemagglutinin (HA16, Wellcome Diagnostics, Dartford), Concanavalin A (grade IV, Sigma Chemical Company, Dorset) and pokeweed mitogen (061-5360B, Gibco, Paisley), and with tuberculin PPD (Evans Medical Ltd, Beaconsfield)

Table Skin test results and immunisation history of patients with congenital disorders of blood coagulation and healthy controls

Group	Age	Sex	Plasma concentrations of factor VIII/IX (IU/dl)	Supplement of factor VIII/IX	Immunisation history			Tetanus toxoid
					BCG	Polio	Triple vaccine	
Haemophilia A	23	M	19	+	Yes	Yes	*	Yes
	38	M	2	+++	No	No	No	No
	72	M	7.5	+	No	No	No	No
	54	M	3	++	No	No	No	No
	66	M	< 1	++	No	No	No	No
	38	M	< 1	++	No	No	*	No
	14	M	1	++	No	Yes	Yes	Yes
	18	M	< 1	++	Yes	Yes	No	*
	18	M	< 1	+++	No	No	No	Yes
	21	M	< 1	++	Yes	Yes	Yes	Yes
	17	M	< 1	+	Yes	Yes	Yes	Yes
Haemophilia B	28	M	42	+	Yes	Yes	Yes	Yes
	29	M	< 1	++	No	Yes	Yes	No
	26	M	2	++	No	Yes	Yes	Yes
	23	M	2.5	+	Yes	Yes	Yes	Yes
	36	M	2	+	Yes	Yes	Yes	Yes
von Willebrand's disease	17	M	< 1	++	*	*	*	*
	47	F		+	Yes	Yes	Yes	Yes
	21	F		+	Yes	Yes	Yes	Yes
Controls	17	M			No	No	No	No
	53	M			Yes	Yes	Yes	Yes
	35	M			Yes	Yes	Yes	Yes
	41	M			Yes	Yes	Yes	Yes
	33	M			Yes	Yes	Yes	Yes
	28	M			Yes	Yes	Yes	Yes
	26	M			Yes	Yes	Yes	Yes
	51	F			Yes	Yes	No	Yes
	21	F			Yes	Yes	Yes	Yes
	21	F			Yes	Yes	Yes	Yes
	22	M			Yes	Yes	Yes	Yes
	20	M			Yes	Yes	Yes	Yes
	25	M			Yes	Yes	Yes	Yes

*History "don't know", but skin tests positive.

Coagulation factor consumption: +, <10000 IU, p.a.; ++, 10000–40000 IU p.a.; +++, >40000 IU p.a.

and *Candida* extract (Merieux UK Ltd). The cultures were pulsed for four hours with ^3H -thymidine, starting at 68 hours for mitogen and at 116 hours for antigen stimulation.

HIV antibodies were assayed in 1985 by radioimmunoassay and in 1986 by enzyme linked immunosorbent assay (Organon Teknika, Cambridge). Other investigations on serum samples included hepatitis B surface antigen and antibodies; anti-HBc and anti-HBs (both EIA, Abbot Laboratories, Wokingham); cytomegalovirus (CMV) herpes simplex and herpes zoster antibodies complement fixation test¹¹; IgG and IgM antibodies to Epstein-Barr virus capsid (immunofluorescence); and *Toxoplasma gondii* antibodies.

Results were analysed by paired and unpaired *t* tests as appropriate.

Results

Patients with haemophilia A had fewer positive reac-

tions to "old tuberculin" and tetanus toxoid and more positive reactions to *Proteus mirabilis* with the Multitest applicator, and fewer positive Mantoux tests than the other patients or the controls. Positive responses were of comparable size in all groups (table). Vaccination history showed that virtually all controls and patients with haemophilia B or von Willebrand's disease had received "triple vaccine", tetanus toxoid, and BCG immunisation, whereas only four of the 12 patients with haemophilia A were fully vaccinated (table).

No difference was found in the total lymphocyte or T8 cell count between the groups. The mean T4 lymphocyte count in haemophilia A patients was lower than that in haemophilia B patients or controls (figure) ($p < 0.005$). Those haemophilia A patients with T4 lymphocyte counts of $< 1.0 \times 10^9$ g/l had as many positive skin test reactions as the other patients with this disease. Lymphocyte stimulation tests showed no difference in response between the patient groups and controls.

Skin test results (mean diameter mm)

Tetanus toxoid	Multitest CMI						Intradermal infection "new tuberculin"
	Diphtheria toxoid	Streptococcus antigen	Old tuberculin	Candida antigen	Trichophyton antigen	Proteus antigen	
	3	3	5	2	—	2	10
	—	—	—	—	—	3	—
	5	—	—	3	2	—	—
	—	—	1	—	—	1	—
	4	—	—	4	—	—	—
	3.5	2.5	3.5	2.5	—	3	—
	6	—	—	4	—	3	—
	—	—	4	4	—	—	4
	—	—	—	—	—	7	—
	—	5	4	3	—	1.5	—
	2	—	6	4	5.5	—	6.5
	3	2	—	—	—	—	3
	—	—	—	—	—	—	—
	3	4	4.5	—	—	—	—
	3	—	—	—	—	3.5	—
	2	5	4	3	2	4	10
	8	3	8	5	2	—	20
	2	—	4.5	2	1	—	15
	—	—	10	7	—	—	12
	—	2	—	—	—	—	5
	—	—	—	6	—	—	—
	4	3	12	4	—	—	14
	—	—	2	4	4	3	10
	3	2	9	3	3	3	10
	2	—	7	6	3	—	7
	5	4	5	—	—	—	5
	2	4	5	5	—	4	10
	—	—	4	3	—	—	—
	—	—	4	3	—	—	—
	—	2	10	3	—	—	11
	3	—	7	5	4.5	—	7
	2	—	6	2	—	—	5
	—	2.5	5.5	4	—	—	7

No correlation was found between either endogenous factor VIII values, or the dose of therapeutic concentrate required for disease control and T4 lymphocyte count, or lymphocyte stimulation test responses.

The antibody titres to cytomegalovirus, herpes simplex, herpes zoster and *Toxoplasma* in the patients with inherited coagulation defects were not significantly different from those in the controls. Twelve of the 20 patients had antibodies to HBs and HBc as did one of the controls. All controls had positive tests for IgG antibody specific for Epstein-Barr virus (EBV) but were negative to IgM antibody (indicating past infection). Of the 16 patients with IgG antibodies to EBV, six also gave a positive test for IgM antibodies (suggesting initially that these patients had active infection), but these results must be regarded as false positives as the sera became negative after absorption with aggregated human IgG. It is therefore likely that the false positive results were due to the presence of "rheumatoid factor" or related

IgM class anti-IgG antibody.

Tests for antibody to HIV were performed in 1985 on serum samples removed at the time of skin testing (radioimmunoassay by Dr RS Tedder) and samples removed one year later, 1986 (ELISA). All these serum samples were negative for anti-HIV.

Discussion

The absence of skin test responses in the group with haemophilia A occurred in those patients who had not been vaccinated and with those antigens generally only encountered at immunisation. Skin test responses to other antigens were comparable in all groups. Thus the non-reactors were not immunosuppressed, merely unimmunised. The reason why they did not receive immunisation cannot be established at this late date. It may have been due to the natural desire of the parents to protect them from trauma.

The serological studies suggest that all the patients

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