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# Inhibition of lymphocyte IL2-receptor expression by factor VIII concentrate: a possible cause of immunosuppression in haemophiliacs

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**Summary.** Factor VIII concentrate inhibits T-cell function *in vitro* and *in vivo*. The mechanisms underlying this phenomenon were investigated. Factor VIII concentrate has a direct effect on lymphocytes, uninfluenced by haemophilic monocyte dysfunction, since it inhibited lymphocyte transformation with phorbol myristate acetate, a reaction unaffected by monocyte depletion. Inhibition of lymphocyte transformation by factor VIII concentrate is not corrected by the addition of exogenous IL2, suggesting that it does not inhibit lymphocyte function by suppression of IL2 secretion alone. Factor VIII concentrate causes profound inhibition of IL2-receptor expression (CD25); with an 89% reduction in CD25-positive CD4 cells and a 50% reduction in CD25-antigen molecules per cell. CD8 lymphocytes are similarly affected. Smaller reductions in CD71 and HLA-DR expression are also observed. Down modulation of CD25-antigen may explain the reduced IL2 secretion observed by others, and may be an important cause of immunodeficiency in HIV-seronegative haemophiliacs.

Treatment with factor VIII concentrate is associated with reduced CD4 lymphocyte counts and abnormal lymphocyte transformation with lectins and in the mixed lymphocyte reaction (Moffatt *et al*, 1985; Hay & McEvoy, 1990). This is thought to be caused by a constituent of the clotting-factor concentrate, and occurs independently of HIV infection (Carr *et al*, 1984). The mechanisms underlying these defects are poorly understood, although several groups have suggested that inhibition of lymphocyte IL2 secretion by factor VIII concentrate may be the major cause of immunosuppression in HIV-seronegative haemophiliacs (Lederman *et al*, 1986; Thorpe *et al*, 1989). Other inhibitory mechanisms are probably also important. Factor VIII concentrate has been shown to decrease monocyte Fc receptor expression (Eible *et al*, 1987), and may also modulate lymphocyte cell surface receptors. The effects of factor VIII concentrate on monocytes also include impaired antigen presentation, bacterial killing and oxygen radical production (Mannhalter *et al*, 1986; Eible *et al*, 1987). These abnormalities may have an indirect effect on lymphocyte mitogenesis, since lymphocyte transformation with lectins is dependent on monocytes (Rosenstreich *et al*, 1976; De Vries *et al*, 1979). These interactions were investigated further using mononuclear cells taken from normal individuals.

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## METHODS

420 ml of blood, from each of five normal subjects, was taken into standard CPD-adenine blood transfusion bags. Buffy coats were prepared from each donation by centrifugation at 1800 *g* for 3½ min. Blood taken from the plasma interface was mixed 1:1 with normal saline, and further separated by density gradient centrifugation on Ficol/Hypaque at 400 *g* for 30 min (Lymphoprep, Nycomed). Mononuclear cells removed from the interface with a pasteur pipette were washed three times in RPMI 1640, and stored in liquid nitrogen prior to use.

Phorbol-myristate-acetate (PMA) or phytohaemagglutinin (PHA) was used to stimulate lymphocyte transformation of mononuclear cells taken from five normal subjects. These cells were incubated for 3 d with PMA 8 ng/ml or PHA 2 μg/ml in RPMI 1640 (Flow Laboratories) with 10% AB plasma, in the presence or absence of factor VIII concentrate (Profilate, Alpha) reconstituted in RPMI 1640 at a final concentration of 4 u/ml. The cells were pulsed with tritiated thymidine after 72 h and harvested 6 h later. Each experiment was conducted in triplicate, with appropriate negative controls. These experiments were also conducted with and without

$5 \times 10^4$ per well. Cell viability was found to be greater than 95% after 3 d by staining with 0·5% trypan-blue.

Monocytes were removed prior to culture, using monoclonal murine Leu M3 (Becton Dickinson) and magnetic beads (Dynabeads) coated in sheep anti-mouse IgG, using standard methods (Funderud *et al*, 1987), and with appropriate isotypic controls (Becton Dickinson). Flow-cytometry, using anti-Leu M3 and anti-Leu M5, with isotypic controls, indicated that fewer than 1% of monocytes remained after this procedure.

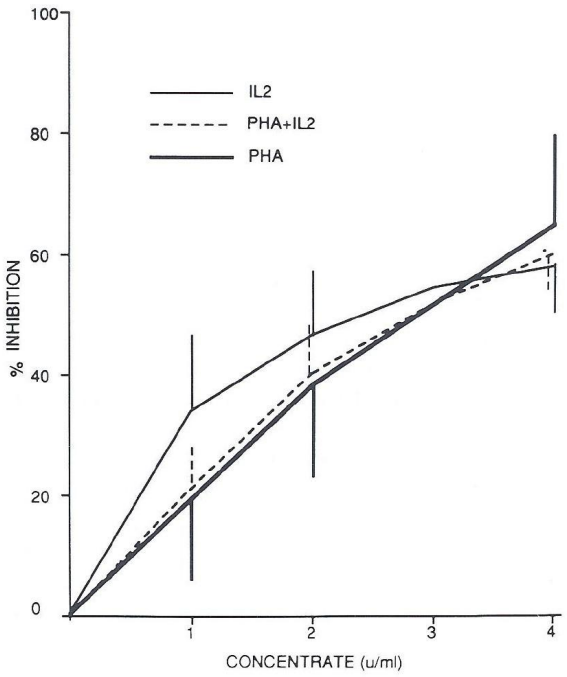


**Fig 1.** The percentage inhibition (mean ±SEM) of lymphocyte transformation by factor VIII concentrate, using PHA, PHA and IL2, and IL2 alone as stimulators.

We attempted to correct the inhibitory effect of factor VIII concentrate on lymphocyte transformation by adding exogenous IL2 to the culture; since this effect has been attributed to reduced IL2 secretion, the addition of IL2 might be expected to correct the defect. Mononuclear cells from five normal subjects were cultured with PHA 2 μg/ml, as before, with factor VIII concentrate at final concentrations of 0, 1, 2 and 4 u/ml, in the presence or absence of IL2 at an optimal concentration of 250 u/ml. This concentration was chosen by titration. The results were expressed as percentage inhibition of tritiated thymidine uptake taking the 0 u/ml factor VIII wells as 0% inhibition.

The effect of factor VIII on IL2 receptor (CD25), HLA-DR, and transferrin receptor (CD71) expression of CD4 and CD8 lymphocytes was investigated following culture of mononuclear cells with PHA 2 μg/ml. Mononuclear cells at a concentration of $2 \cdot 5 \times 10^5$ cells per ml were incubated in 24-well flat-bottomed 'space-saver' plates. These wells were divided into four groups containing: (a) PHA 2 μg/ml and factor VIII 4 u/ml; (b) PHA 2 μg/ml; and as negative controls, (c) factor VIII 4 u/ml; and (d) medium alone. After 3 d incubation, the cells from each group of wells were pooled and adjusted to a concentration of $2 \times 10^7$ cells/ml. The cells were analysed using an EPICS C flow cytometer, using dual-staining with either anti-CD4 or anti-CD8 directly conjugated to fluorescein isothiocyanate (FITC) and anti-CD25, anti-HLA-DR or anti-CD71 (transferrin receptor) directly conjugated to phycoerythrin (Becton Dickinson). Appropriate isotypic controls were also used. The number of receptors per cell was calculated from a standard line derived by flow-cytometry of calibrated beads. This data was log transformed and analysed using a two-tailed paired *t*-test. *P*-values of $<0 \cdot 01$ were considered significant.

## RESULTS

*IL2 fails to correct the inhibitory effect of factor VIII concentrates on lymphocyte mitogenic responses*

Factor VIII concentrate caused a dose-related inhibition of

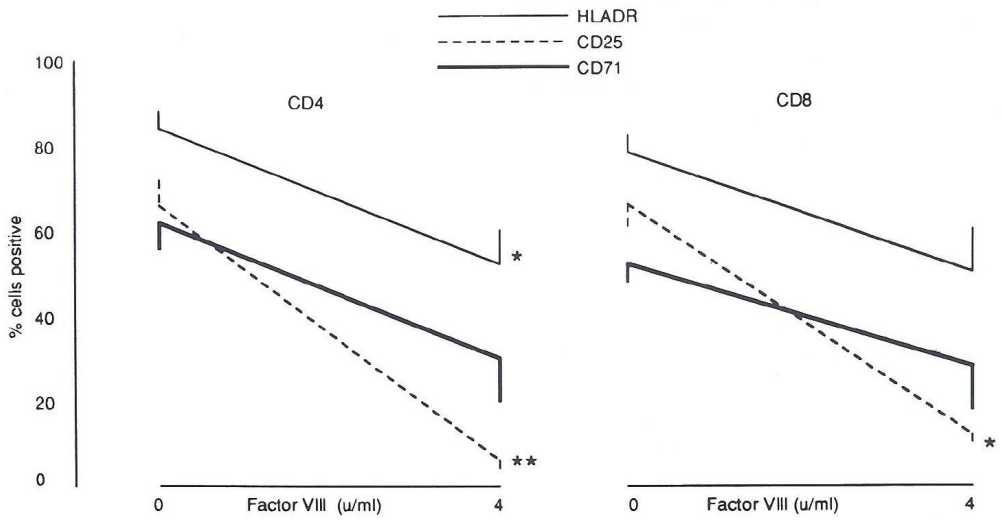


**Fig 2.** The effect of factor VIII concentrate on the percentage of CD4 and CD8 lymphocytes expressing HLA-DR, CD71 and CD25 following

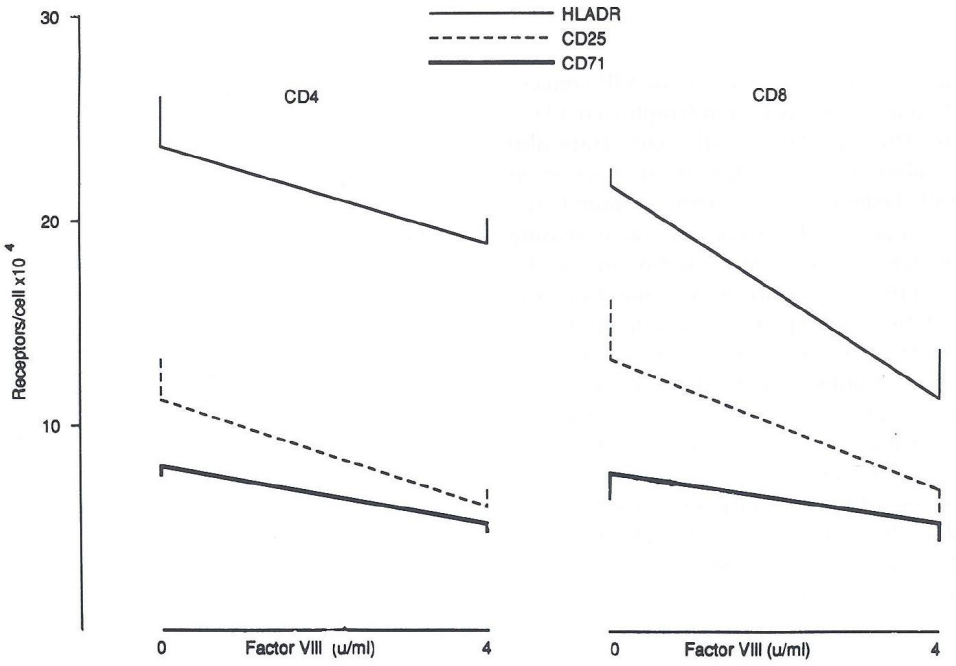


Fig 3. The effect of factor VIII concentrate on the expression of HLA-DR, CD71, and CD25 (receptors/cell) on CD4 and CD8 lymphocytes afte incubation with PHA (mean ±SEM).

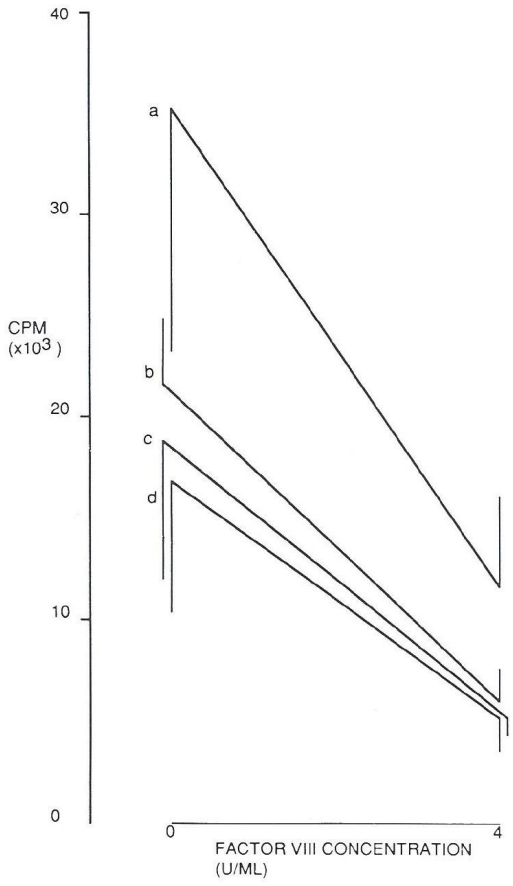


Fig 4. Inhibition of lymphocyte transformation (mean ±SEM) by factor VIII using PHA 2 μg/ml (a and d) and PMA 8 μg/ml (b and c) as

lymphocyte transformation with PHA (Fig 1). Although the addition of 250 u/ml of IL2 increased lymphocyte mitogenic responses at all concentrations of factor VIII, it failed to abrogate the inhibitory effect. The percentage inhibition o lymphocyte transformation at each concentration of factor VIII concentrate was similar whether IL2 alone, PHA alone or both IL2 and PHA were used (Fig 1).

*Factor VIII concentrate causes down-modulation of CD25 antigen HLA-DR, and CD71*

Flow cytometric analysis of mononuclear cells incubated with PHA in the presence or absence of factor VIII concentrate (4 u/ml) showed that concentrate caused a reduction in the percentage of CD4 lymphocytes expressing CD25 antigen, HLA-DR, and CD71 of 89% ($P<0{\cdot}001$), 37% ($P<0{\cdot}01$) and 53% ($P<0{\cdot}02$, not significant) respectively (Fig 2). CD8 lymphocytes were similarly affected. The CD4/CD8 ratio was unaffected by incubation with factor VIII. The number o CD25, HLADR and CD71 antigens on each CD4 lymphocyte was reduced in the presence of factor VIII concentrate by 50%, 21% and 35% respectively (not significant) (Fig 3). CD8 lymphocytes were similarly affected.

*Inhibition of lymphocyte function by factor VIII concentrates is probably independent of monocyte interaction*

Factor VIII concentrate, 4 u/ml caused a 70% inhibition o lymphocyte transformation with PMA. The degree of inhibition was unchanged by monocyte depletion (Fig 4). Flow cytometry indicated that fewer than 1% of monocytes remained. Similar monocyte depletion reduced PHA induced lymphocyte transformation by over 50% but failed to abrogate the inhibitory effect of factor VIII concentrate (Fig

## DISCUSSION

Incubation of normal lymphocytes with factor VIII concentrate caused a profound 95% reduction in lymphocyte CD25 antigen expression. Although factor VIII concentrate also causes marked inhibition of lymphocyte IL2 secretion (Thorpe *et al*, 1989; Lederman *et al*, 1986), optimal IL2 secretion depends, in a positive-feedback loop, on increasing IL2-receptor expression (Smith, 1984). Inhibition of IL2 secretion by factor VIII concentrate may result from the down-modulation of the IL2-receptor observed in our experiments, since decreased IL2-receptor expression will result in reduced IL2 secretion. It is unlikely that reduced IL2 secretion causes the decrease in CD25 antigen expression observed, since the addition of exogenous IL2 failed to correct the inhibitory effect of concentrate on lymphocyte function.

The IL2-receptor consists of a 75 kDa and 55 kDa chain, the latter recognized by anti-CD25. Although signalling through the 75 kDa chain alone has been reported, optimal responses require expression of both chains and CD25 is thus a reasonable reflection of IL2-receptor expression under most circumstances. T-cell activation results in the modulation of several cell-surface receptors and it is therefore not surprising that factor VIII concentrate should also inhibit HLA-DR and transferrin-receptor (CD71) expression. Although these changes were very much less marked than the changes in CD25 antigen expression, they do indicate that the effect of factor VIII concentrate is not specific for CD25 and suggest that it acts by interfering with an early activation event.

The effect of factor VIII concentrate on lymphocytes appears to be uninfluenced by monocyte interaction, since factor VIII concentrate inhibits lymphocyte transformation with PMA. This is a reaction which does not require the presence of monocytes and which is unaffected by monocyte depletion.

Haemophilic patients have been reported to suffer an increased incidence of tuberculosis (Bedall *et al*, 1985), recurrent hepatitis B (Williams *et al*, 1988) and an increased susceptibility to HIV infection (Ludlam *et al*, 1985), which suggest that these immune defects are clinically significant. Further evidence of the clinical importance of these abnormalities should be sought, however, since some authors consider the evidence for the immunosuppressive effects of factor VIII to be controversial (Cash, 1988).

In summary, our *in vitro* data suggest that factor VIII concentrate inhibits lymphocyte function by interference with an early activation event, associated with inhibition of IL2 secretion and decreased CD71, CD25 and HLA-DR expression. Down-regulation of the IL2-receptor could account for most of the abnormalities of lymphocyte function observed following treatment with pooled blood products. Immune-modulation is not a property of all factor VIII concentrates (Brettler & Levine, 1989). High purity, non-immunosuppressive concentrates are now readily available, although these are still expensive and in limited supply. The potential immunosuppressive effects of factor VIII concentrate should be considered carefully by manufacturers and prescribers alike.

## ACKNOWLEDGMENT

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