Identification of Transforming Growth Factor-β as a Contaminant in Factor VIII Concentrates: A Possible Link With Immunosuppressive Effects in Hemophiliacs

By Meenu Wadhwa, Paula Dilger, Jill Tubbs, Anthony Mire-Sluis, Trevor Barrowcliffe, and Robin Thorpe

In previous studies, we have shown that some, but not all low-, intermediate-, and high-purity factor VIII concentrates inhibit interleukin-2 (IL-2) secretion from phytohemagglutinin (PHA)-stimulated T lymphocytes. We now present evidence that this inhibitory action of concentrates is, at least in part, due to contamination with transforming growth factor-β (TGF-β). Originally identified in platelets, TGF-β is a 25-kD homodimer that has been shown to be a natural and potent inhibitor of many immunologic responses. Using a specific bioassay, we have measured TGF-β in various factor VIII concentrates. While some concentrates contained substantial amounts of the cytokine, there was a wide variation in concentrations of TGF-β in different products. These levels correlated with the degree of inhibition of IL-2 secretion from T cells exhibited by each product (P < .0001). Noninhibitory concentrates contained no detectable TGF-β. Addition of a specific TGF-β1 antibody reversed the inhibitory effect of some concentrates on IL-2 secretion by PHA-stimulated Jurkat T cells and interleukin-5 (IL-5)-induced proliferation of an erythroleukemic cell line. These findings suggest that TGF-β contamination is a major contributory factor to the inhibitory activity of some factor VIII concentrates on cytokine secretion or activity, and may partially explain the reported immunosuppressive effects in recipients of these blood products.

© 1994 by The American Society of Hematology.

MATERIALS AND METHODS

Factor VIII concentrates. The details of the concentrates used in the study are as follows: Intermediate-purity concentrates (specific activity, 0.5 to 10 IU/mg protein) consisted of dry-heated (80°C, 72 hours) A, from Bio-Products Laboratory (Elstree, UK; 8Y); B (68°C, 72 hours) from Miles Inc., Pharmaceutical Division, Biological Products (Berkeley, CA; Koate HT); solvent/detergent-treated C from Centre Regionale de Transfusion de Sang (Lille, France). High-purity concentrates, prepared by conventional chromatography (specific activity, 50 to 250 IU/mg protein) consisted of Solvent/detergent-treated E from CRTS, Lille, France; F from Octapharma, Vienna, Austria (Octuri); G from Alpha Therapeutic Corp (Los Angeles, CA; Profilate-SD); and D from Centre Regionale de Transfusion de Sang (Lille, France).

Very-high-purity concentrates, prepared by monoclonal antibody

From the Divisions of Immunobiology and Haematology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK.

Submitted October 25; accepted May 3, 1994.

Supported in part by the Scottish National Blood Transfusion Service, Edinburgh, UK.

Address reprint requests to Meenu Wadhwa PhD, Division of Immunobiology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.

Blood, Vol 84, No 6 (September 15), 1994; pp 2021-2030

2021
Fig 1. Inhibitory effect of two different factor VIII concentrates, (A) concentrate A and (B) concentrate C, on IL-5-induced proliferation of TF-1 cells is neutralized by the antibody specific for human TGF-β1. TF-1 cells were incubated with different doses of IL-5 either alone (○), in conjunction with a factor VIII concentrate (2.5 U/mL) (■), or with IL-5 and factor VIII concentrate in the presence of antibody (△).

Absorption (specific activity, ~3,000 IU/mg protein before addition of an albumin stabilizer) consisted of solvent/detergent-treated J from BPL (Elstree, UK; 8SM); and pasteurized K from Armour Pharmaceuticals (Kankakee, IL; Monoclate P).

Cytokines. Recombinant human IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) were gifts of Schering-Plough Corp (Kenilworth, NJ). Recombinant human TGF-β1 was donated by R & D Systems (Minneapolis, MN) and Bristol-Myer Squibbs (Seattle, WA).

Origin and maintenance of cell lines. The human erythroleukemia cell line, TF-1, was a gift of Dr T. Kitamura, University of Tokyo (Tokyo, Japan). This line was maintained at a density of 10^4 to 10^5 cells/mL in RPMI 1640 medium containing 2 ng/mL recombinant human GM-CSF (rhGM-CSF), and 5% heat-inactivated fetal calf serum (FCS).

The human T-cell lymphoblastoid line, Jurkat, was maintained at a density of 10^5 to 10^6 cells/mL in RPMI 1640 containing 10% heat-inactivated FCS.

The murine T-cell line, CTLL-2, was maintained at a density of approximately 2 × 10^4 cells/mL in RPMI 1640 supplemented with 10% FCS and partially purified rat IL-2 (15 to 20 IU/mL). The cultures were maintained on a 3-day feeding schedule. All cell lines and cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

Separation of peripheral blood mononuclear cells from whole blood. Peripheral blood mononuclear cells (PBMCs) were separated from freshly collected heparinized blood by centrifugation over Histopaque-1077 (density, 1.077; Sigma, Poole, UK) at 400g for 30 minutes. Mononuclear cells were removed from the interface, washed three times with RPMI 1640, and immediately used for experiments.

Antibody to human TGF-β1. This antibody was generated by immunization of white leghorn chickens with recombinant human (rh) TGF-β1 using the immunization schedule described elsewhere. The antibody purified from egg yolk by the dextran sulfate method was specific for TGF-β1, the isoform of TGF-β abundant in platelets, lymphocytes, and monocytes. At a dilution of 1:200 (~15 μg/mL IgY), the antibody was capable of neutralizing the biologic activity of 5 ng/mL of both recombinant DNA and platelet-derived TGF-β1.

In neutralization experiments, purified chicken anti-TGF-β1 (1:200 dilution) was incubated with the appropriate samples for 1 hour at 37°C before the addition of cells. Controls using the Ig derived from eggs of nonimmunized chickens were also included.
FACTOR VIII CONCENTRATES CAN CONTAIN TGFβ

Fig 2. Inhibition of IL-5-induced proliferation of TF-1 cells by TGF-β1 (A) and factor VIII concentrate, C (B). The TGF-β1 specific antibody neutralized the inhibitory effect in both instances. (A) TF-1 cells were incubated either with TGF-β1 alone (○), or with TGF-β1 and antibody to TGF-β1 (□). (B) TF-1 cells were incubated with factor VIII concentrate (■) or with concentrate and antibody to TGF-β1 (□).

Bioassay for IL-2. IL-2 was measured using a bioassay based on the IL-2-dependent mouse cytotoxic lymphocyte cell line, CTLL-2. For this, 50 μL of cell suspension (5 × 10^6 cells) was added to 50 μL of test samples or control medium, and cultured for 18 hours in 96-well microtiter plates. A titration of an IL-2 working standard calibrated directly against the World Health Organization First International Standard for human IL-2 (86/504) was included in each assay. The cultures were then pulsed for 4 hours with [3H]-thymidine (0.5 μCi/well), harvested onto filter mats, and the radioactivity incorporated into DNA estimated by scintillation counting. The percentage inhibition was calculated using parallel-line analysis. The proliferation of CTLL-2 cells was unaffected by the presence of factor VIII concentrates.

Bioassay for TGF-β. TGF-β was assessed using a bioassay based on the ability of TGF-β to inhibit IL-5-induced proliferation of the erythroleukemic cell line, TF-1. For this, a dilution series of samples, ie, factor VIII concentrates or TGF-β1 standard, was prepared in 100-μL volumes in 96-well microtiter plates. TF-1 cells were washed three times, resuspended to 10^5 cells/mL in RPMI 1640 containing 5% FCS and 10 ng/mL IL-5, and 100-μL aliquots added to each well. The plates were incubated for 48 hours, pulsed for 4 hours with [3H]-thymidine, harvested onto filter mats, and the radioactivity incorporated into DNA estimated by liquid scintillation counting. A dose-response curve of cpm versus dilution of standard or unknown was plotted, and the amounts of TGF-β in the samples estimated by comparison of the sample dilution series to the standard curve, using parallel-line analysis.

Activation of TGF-β in factor VIII concentrates. In biologic samples, a large proportion of TGF-β is often present in a latent form. Conversion to the active form is necessary if total TGF-β levels are to be estimated. Factor VIII concentrates were activated by addition of 10 μL of 1.2N HCl to a 100-μL aliquot of the sample. The samples were mixed thoroughly, incubated at room temperature for 15 minutes, and neutralized by addition of 25 μL of 0.5-mol/L HEPES/0.72-mol/L NaOH. The activated samples were then assayed at appropriate dilutions.

Fractionation of factor VIII concentrates. Intermediate-purity factor VIII concentrates were fractionated using Sepharose CL-4B (Pharmacia, St Albans, UK), as described previously. The fractions were monitored for absorbance at 280 nm, and for inhibitory effects on IL-2 secretion by Jurkat cells. In addition, the fractions were assayed for the presence of TGF-β. In some experiments, the fractions were acid-activated for quantitation of total TGF-β levels.

RESULTS

Identification and characterization of TGF-β as an inhibitory component in some factor VIII concentrates. To establish the identity of the inhibitory component(s), different factor VIII concentrates were analyzed for any inhibitory
Table 1. Levels of TGF-β Detectable in Different Factor VIII Concentrates

<table>
<thead>
<tr>
<th>Concentrate Type</th>
<th>Product Code</th>
<th>Batch</th>
<th>% Inhibition of IL-2 Secretion</th>
<th>Active TGF-β (ng/ml)</th>
<th>Total TGF-β (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate-purity</td>
<td>A</td>
<td>1</td>
<td>38</td>
<td>0.00</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>24</td>
<td>0.51</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>29</td>
<td>0.24</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>25</td>
<td>0.00</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>15</td>
<td>0.26</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>85</td>
<td>5.80</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>71</td>
<td>3.20</td>
<td>10.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>40</td>
<td>2.40</td>
<td>12.00</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1</td>
<td>99</td>
<td>2.70</td>
<td>5.00</td>
</tr>
<tr>
<td>High-purity</td>
<td>E</td>
<td>1</td>
<td>70</td>
<td>1.20</td>
<td>7.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>82</td>
<td>0.80</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>68</td>
<td>2.80</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>63</td>
<td>1.00</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>49</td>
<td>0.40</td>
<td>10.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>32</td>
<td>0.16</td>
<td>7.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>44</td>
<td>0.06</td>
<td>4.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>60</td>
<td>0.00</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1</td>
<td>ND</td>
<td>0.40</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>55</td>
<td>0.38</td>
<td>3.80</td>
</tr>
<tr>
<td>Very-high purity</td>
<td>J</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

0 = Below detection limit of assay. Active TGF-β - TGF-β levels before acid activation. Total TGF-β = active TGF-β + TGF-β levels following acid activation. % inhibition of IL-2 secretion represents values obtained at 2.5 IU/mL of each factor VIII concentrate. Sensitivity of assay, ~0.005 ng/mL.

Abbreviation: ND, not done.

effect on cytokine-induced proliferation of the erythroleukemic cell line TF-1. This cell line proliferates in response to GM-CSF, interleukin-3 (IL-3), IL-4, IL-5, and the stimulatory activity of the latter two (but not the former two) cytokines is susceptible to inhibition by TGF-β.22 Factor VIII concentrates did not downmodulate the GM-CSF or IL-3 effects, but the IL-4- or IL-5-stimulated proliferation of TF-1 cells was significantly inhibited by some concentrates. A dose-response curve of IL-5–induced proliferation of TF-1 cells, and inhibition of this proliferation with the addition of two different factor VIII concentrates is shown in Fig 1A and B.

The above suggests that the inhibitory component of factor VIII concentrates may be TGF-β and, to confirm this, we used a neutralizing polyclonal antibody specific for TGF-β1.23 This antibody was able to reverse the inhibitory effect of factor VIII concentrates on IL-5–induced proliferation of TF-1 cells either completely or partially, depending on the concentrate used (Fig 1A and B), whereas nonimmune chicken egg IgY had no effect (data not shown). This suggested that the inhibitory effect of factor VIII concentrates could be, at least partially, attributed to the presence of TGF-β1 in these products.

![Fig 3. Analysis of correlation between total TGF-β content of different factor VIII concentrates, and inhibition of IL-2 secretion from PHA-stimulated Jurkat cells. The correlation coefficient of these two variables using Spearman rank correlations is 0.804 (P = .0001).](image-url)

![Fig 4. Dose-response curve for neutralization effect of the TGF-β1 antibody on the inhibitory effect of a factor VIII concentrate. In this experiment, Jurkat cells were incubated with product D at 1.25 U/mL either alone (○) or in conjunction with different amounts of antibody for 1 hour before stimulation with PHA.](image-url)
FACTOR VIII CONCENTRATES CAN CONTAIN TGFβ

Fig 5. Representative experiment shows differential effect of anti-TGF-β1 on factor VIII concentrate-induced inhibition of IL-2 secretion from PHA-stimulated Jurkat cells. All concentrates were used at a concentration of ~2.5–3.0 IU/mL. Jurkat cells were stimulated with PHA in the presence of concentrate alone (●) or in the presence of concentrate and anti-TGF-β1 (○).

less than 0.5 pg/mL of TGF-β, and recognizes different recombinant forms, as well as the natural protein derived from human and bovine platelets. A typical dose-response curve of TGF-β1 and a factor VIII concentrate causing inhibition of IL-5-induced proliferation is shown in Fig 2A and B, respectively. Results showed that concentrates that exerted significant inhibition of IL-2 secretion using Jurkat cells and IL-5-induced proliferation of erythroleukemic cells also contained relatively large amounts of TGF-β. Intermediate-purity concentrates contained either intermediate or very low levels of TGF-β, depending on the product. All chromatographically purified concentrates contained detectable levels of TGF-β; some contained higher levels than intermediate-purity products (Table 1). The very-high-purity noninhibitory concentrates prepared by monoclonal antibody affinity purification contained no detectable TGF-β (Table 1). The ability of concentrates to inhibit IL-2 secretion correlated with the TGF-β content of the concentrates (Fig 3). The correlation coefficient of the two variables using Spearman rank correlation was 0.804 for total TGF-β and 0.829 for active TGF-β, taking each observation as an independent result (in both cases, P = .0001). Product G caused appreciable inhibition of IL-2 secretion, but contained no detectable active TGF-β. However, this concentrate contained a significant amount of latent TGF-β, which may become active during cell culture and induce inhibition of IL-2 secretion.

Reversal of inhibitory effect of some factor VIII concentrates on IL-2 secretion from stimulated Jurkat cells or PBMCs by antibody specific for TGF-β1. To determine if TGF-β1 in the concentrates was responsible for the inhibitory effects of concentrates on IL-2 secretion from stimulated Jurkat cells, a neutralizing antibody against TGF-β1 was included in experiments involving either Jurkat cells or PBMCs. The TGF-β1 antibody neutralized the inhibitory effect of factor VIII concentrates in a dose-dependent manner (Fig 4).

Addition of the TGF-β1 antibody abrogated almost all of the inhibition exerted by product A at 2.5 IU/mL on IL-2 secretion from stimulated Jurkat cells (Fig 5). However, with products C and D at 2.5 to 3 IU/mL, only 5% to 30% of the inhibitory activity was removed; these products required further dilution to either 1.25 IU/mL or even 0.625 IU/mL to obtain complete neutralization. Almost all inhibitory activity observed with products C and D at concentrations of 0.625 IU/mL in the IL-2 secretion Jurkat cell system was abolished in the presence of the TGF-β1 antibody (Fig 6A and B), while the nonimmune chicken egg IgY had no effect (Fig 6C). These products contained large amounts of TGF-β1, as determined by bioassay (Table 1), and also caused total inhibition of IL-2 secretion. With high-purity products, 30% to 50% of the inhibitory activity was removed by the TGF-β1 antibody, despite a comparable or even higher TGF-β1 content in these products relative to some intermediate-purity products. The amount of antibody required for neutralization of the inhibitory activity of TGF-β1 varied between products. As expected, there was no effect of the antibody on IL-2 secretion in the presence of noninhibitory concentrates, J and K (Fig 5). These results provide evidence for TGF-
β1 as a component responsible for the inhibitory effect of concentrates on IL-2 secretion from stimulated T cells.

IL-2 secretion inhibition experiments with factor VIII concentrates in conjunction with the neutralizing antibody to TGF-β1 were also performed using PBMCs. As with Jurkat cells, the addition of neutralizing antibody reversed the inhibitory potential of some factor VIII concentrates on IL-2 secretion by PHA-stimulated PBMCs (data not shown). As expected, the nonimmune chicken egg IgY did not reverse the inhibitory effect of the concentrates.

Inhibition of IL-2 secretion from Jurkat cells by spiking a noninhibitory factor VIII concentrate with active human TGF-β1. PHA-stimulated Jurkat cells were incubated with an affinity-purified noninhibitory concentrate in the presence or absence of different concentrations of rhTGF-β1. While the concentrate itself was noninhibitory, exogenous addition of TGF-β1 induced an inhibition profile similar to that obtained using TGF-β1 alone as a control (Fig 7). These results illustrate that addition of TGF-β1 to a previously noninhibitory factor VIII concentrate mimics the inhibition of IL-2 secretion exhibited by inhibitory factor VIII products.

Inhibitory fractions from factor VIII concentrate contain TGF-β1. Having identified TGF-β as a contaminant of some factor VIII concentrates, gel-filtration analysis was used to establish the molecular form(s) of the cytokine present and the relationship of these with the substances responsible for inhibition of IL-2 secretion from T cells. The protein profile of an intermediate-purity factor VIII concentrate C is shown in Fig 8A. As previously shown, evaluation of the fractions for effect on IL-2 secretion from Jurkat cells indicated a major peak (fraction 50) of inhibition at approximately 200 kD, and a minor peak (fraction 77) at a molecular weight of approximately 60 kD. These fractions, as well as a noninhibitory fraction, were acid-activated and tested, both in the presence or absence of the TGF-β1 specific antibody for effect on IL-2 secretion. Both peaks contained inhibitory activity, which was significantly neutralized by the TGF-β1 specific antibody and contained significant amounts of TGF-β.

Following acid activation, the biologic activity was substantially increased, and was neutralized by the antibody to TGF-β1, confirming the presence of TGF-β1 in both fractions (Fig 8B). The noninhibitory fraction contained no detectable TGF-β (data not shown). Therefore, it seems most likely that the 200-kD inhibitory peak contains latent TGF-β, whereas the 60-kD peak contains the cytokine complexed with some other plasma-derived component(s) or self-aggregated TGF-β.

DISCUSSION

TGF-β is a multifunctional polypeptide that influences a diverse range of cell types by either stimulating or inhibiting
FACTOR VIII CONCENTRATES CAN CONTAIN TGFβ

Inhibition of IL-2 secretion from Jurkat cells by spiking a very-high-purity factor VIII concentrate with active human TGFβ1. In this experiment, Jurkat cells were incubated with different concentrations of TGFβ1 alone (A) or in conjunction with an affinity-purified concentrate, K, at 2.7 U/mL (B). PHA control (Φ); TGFβ1—1 ng/mL (○); 0.5 ng/mL (●); 0.1 ng/mL (△); 0.05 ng/mL (▲); and product K (□).

Three structurally and functionally related isoforms of TGFβ—β1, β2, and β3—have been described in man. The β1 form is abundant in platelets, lymphocytes, and macrophages. TGFβ is produced as a latent 210- to 235-kD complex. This is composed of a 135-kD modulator protein, a 75-kD dimeric latency-associated glycoprotein derived from the TGFβ precursor, and mature dimeric 25-kD TGFβ. This complex can be activated by transient exposure to extremes in pH, chaotropic agents, repeated freeze thawing, or proteases to yield the biologically active 25-kD dimer capable of binding to specific cell-surface receptors to cause the appropriate biologic action on the target cell.

TGFβ is a potent immunomodulatory molecule exerting profound immunosuppressive effects. In vitro studies have shown that TGFβ inhibits proliferation of T lymphocytes, as well as secretion of some cytokines. TGFβ exerts differential effects on CD4+ and CD8+ cells; it can completely inhibit cell-mediated immune functions of CD4+ cells, but it is less inhibitory for CD8+ cells, and may even promote their growth. It can also enhance secretion of some other cytokines, eg, IL-4 and interleukin-10 (IL-10), which, in turn, can exert immunosuppressive effects, either alone or in concert with TGFβ. TGFβ also inhibits the generation of cytotoxic T lymphocytes, induction of IL-2—induced lymphokine-activated killer cell activity, and interferon-gamma (γ-IFN) augmentation of natural-killer cells. Additionally, TGFβ inhibits the growth of B cells, and compromises their responsiveness to antigens by decreasing membrane immunoglobulin expression and the ability to secrete antibodies following IL-2— or IL-6—induced stimulation. This cytokine also exhibits diverse effects on monocytes, induces chemotaxis, and enhances production of cytokines, such as IL-1, while impairing respiratory burst activity.

Our studies show that some factor VIII concentrates diminished the proliferative response of TF-1 cells to IL-5. Addition of antihuman TGFβ reversed this inhibitory effect, suggesting the presence of TGFβ in the concentrates. Furthermore, the inhibitory effect of some concentrates on IL-2 secretion from stimulated Jurkat cells was completely or partially reversed in the presence of anti—TGFβ, providing further evidence for TGFβ in some concentrates. Some concentrates may contain biologically compromised degraded TGFβ that binds the antibody or even other protein molecules, which impair the binding of TGFβ to the antibody, and so more antibody is required to neutralize the biologic effects of TGFβ. We have previously shown that the inhibitory activity of concentrates involves more than...
one component, with the amounts of these varying between different concentrates. Therefore, the proportion of the inhibitory activity removed by the TGF-β1 antibody can vary between products, and also between batches, depending on the amounts of the different components in the concentrates. In some products, e.g., those that were affinity-purified, there was no detectable TGF-β, while other concentrates contained substantial amounts of the cytokine sufficient to cause total inhibition of IL-2 secretion from T lymphocytes in the experimental systems used. Other concentrates contained intermediate amounts of TGF-β, which closely correlated with the ability of these products to inhibit IL-2 secretion from T cells. There was no obvious relationship between the levels of TGF-β in the concentrates and the purity of the products, as has been shown for inhibition of IL-2 secretion. In particular, levels of TGF-β in some of the chromatographically purified products were higher than in some intermediate-purity products, despite their much higher specific activity (Table 1).

The presence of TGF-β in both active and latent forms in the factor VIII concentrates is perhaps not surprising. Human plasma is the starting material from which all the concentrates studied here are prepared, and may contain considerable amounts of TGF-β, predominantly derived from platelets, although lymphocytes and monocytes are also capable of secreting the cytokine. Handling of plasma and storage conditions before cryoprecipitation could cause lysis of the residual platelets in plasma and release of latent TGF-β, which on exposure to chemical and/or thermal treatment for extraction and manufacture of the final product may yield biologically active TGF-β.

Some biologic effects mediated by TGF-β on cells of the immune system show resemblance to those observed in vitro following incubation with some factor VIII concentrates. Inhibition of mitogen-induced lymphocyte proliferation and IL-2 secretion, impaired natural-killer cell activity, and impaired monocyte function have been documented. In hemophiliacs undergoing concentrate therapy, infusion of
concentrates that contain considerable amounts of TGF-β may cause a transient impairment of IL-2 secretion and several other related effects, such as γ-IFN production, inhibition of the activity of some cytokines, and inhibition of B- and T-cell expansion, followed by a gradual normalization as TGF-β and induced substances are cleared from the blood. Pharmacokinetic studies in rats have shown that biologically active TGF-β is cleared from the circulation within minutes, but the latent form circulates with a half-life of more than 90 minutes. Although the physiologic mechanisms whereby TGF-β is activated in vivo are unknown, cleavage of the latent form by proteolytic enzymes may yield the active molecule, which in turn may induce yet another secondary episode of immunologic disturbance in hemophiliacs undergoing therapy. Alterations in immune function, which can be induced by TGF-β, may contribute to the susceptibility of such patients to infections.

A marked depression of inflammatory and immunologic responses has been observed in animals systemically administered TGF-β1 for treatment of autoimmune diseases, such as experimental allergic encephalomyelitis. In acquired immune deficiency syndrome (AIDS), endogenous overproduction has been implicated in the development of immunodeficiency. Kekow et al have shown that defective responses of CD4+ lymphocytes to the recall antigens purified protein derivative or tetanus toxoid were partially restored on addition of antibody to TGF-β in PBMC cultures from HIV-infected donors. Furthermore, purified TGF-β or PBMC culture supernatant (containing TGF-β) from HIV-positive individuals preferentially inhibited proliferation of CD4+ lymphocytes, as compared with CD8+ cells. In HIV-seropositive hemophiliacs, administration of concentrates that contain high levels of TGF-β (eg, some intermediate-purity and conventional high-purity) could exacerbate the disease and alter the clinical outcome. There is some evidence for this from recent clinical studies, showing a decline in CD4+ counts in patients given some intermediate-purity or conventional high-purity products, but not in patients treated with monoclonal antibody affinity-purified products.

Our results clearly show significant variation between products, even within the same purity groups with respect to TGF-β content or inhibition of IL-2 secretion by T cells. Thus, conclusions cannot be drawn concerning inhibitory effects of products of a specific purity group from results with one particular factor VIII concentrate.

ACKNOWLEDGMENT
We thank Alan Heath for statistical analysis, and Deborah Kirk for processing the manuscript.

REFERENCES


Identification of transforming growth factor-beta as a contaminant in factor VIII concentrates: a possible link with immunosuppressive effects in hemophiliacs

M Wadhwa, P Dilger, J Tubbs, A Mire-Sluis, T Barrowcliffe and R Thorpe