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J Kekow, … , D A Carson, M Lotz

Research Article

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Transforming Growth Factor-β and Suppression of Humoral Immune Responses in HIV Infection

Jörn Kekow,* William Wachsman,‡ J. Allen McCutchan,§ Wolfgang L. Gross,*
Marina Zachariah,* Dennis A. Carson,§ and Martin Lotz§
*Rheumaklinik Bad Bramstedt, and University of Lübeck, Federal Republic of Germany;
‡San Diego Veterans Administration Medical Center and University of California at San Diego School of Medicine, La Jolla,
California 92103; and §University of California at San Diego School of Medicine, La Jolla, California 92093; and Department of
Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, California 92037

Abstract

We reported previously that PBMC from HIV$^+$ patients spontaneously release increased levels of TGFβ1, contributing to defects in cellular immune responses. This study defines the implications of TGFβ overexpression for humoral immunity in HIV infection. We found that upon *Staphylococcus aureus* Cowan I (SAC) stimulation of cells from HIV$^+$ donors, B-lymphocyte proliferative responses were decreased. This deficiency correlated closely ($r = 0.7$, $P < 0.001$) with increased TGFβ secretion by PBMC from HIV-infected donors. Conditioned medium from HIV$^+$ PBMC and purified TGFβ1 had similar inhibitory effects on SAC- or EBV-induced B-cell proliferation, and B cells from HIV-infected donors were as sensitive to inhibition by TGFβ as cells from normal donors. Antibodies to TGFβ1 neutralized the inhibitory effect of HIV$^+$ culture supernatants on normal B cells and increased low proliferative responses by HIV$^+$ cells.

Using PWM as stimulus for B cell differentiation, it was shown that activated TGFβ from HIV$^+$ PBMC is able to significantly reduce the induction of immunoglobulins and this effect was also abrogated by anti-TGFβ.

These studies support the concept that in HIV infection, TGFβ is a potent suppressor, not only of the cellular, but of the humoral immune responses as well. (*J. Clin. Invest.* 1991. 87:1010–1016.) Key words: TGFβ • HIV • B-lymphocytes

Introduction

Infection with HIV results in severe quantitative and qualitative defects of cellular and humoral immune responses (1–3). Humoral immune abnormalities include polyclonal B cell activation with hypergammaglobulinemia, circulating immune complexes, and autoantibodies. B cell function in AIDS is characterized by reduced or absent responses to both T cell-dependent and -independent B cell activators and by an elevation in spontaneous immunoglobulin secretion (4–7). In spite of these signs of spontaneous B cell hyperactivity, antibody responses to both recall antigens and new antigens are frequently deficient (6, 8).

Such B cell dysfunction in HIV$^+$ individuals may increase susceptibility to secondary infections. This impairment of B cell function also has consequences for the diagnosis and management of HIV infection and its sequelae, and may be a significant host factor that could interfere with successful immunization against HIV.

Our recent studies have documented that TGFβ is overexpressed in HIV infection (9). It exerts very potent inhibitory effects on diverse aspects of cellular immunity. In addition, we have shown that TGFβ induces T cell dysfunction that is not related to cytopathic depletion of CD4$^+$ helper cells in HIV infection. Through such effects on T cells, but also through direct effects on B-lymphocytes, TGFβ has the potential to contribute to the, as yet, unexplained humoral immune defects in HIV infection.

This study investigates the consequences of TGFβ overexpression in HIV infection with respect to growth and differentiation of B cells. The data support the concept that this cytokine may also be an important factor in the development of humoral immunodeficiency.

Methods

Patient characteristics. The group of HIV$^+$ donors ($n = 32$) included 10 patients with AIDS (Centers of Disease Control, group IV), 9 patients with CDC II, and 13 patients with CDC III (10). All patients belonged to the risk group of homosexuals and had no history of intravenous drug abuse. Data on current medical treatment are shown in Table I. The control group consisted of 32 age-matched healthy heterosexual men. Measurements of lymphocyte subsets were performed by standard flow cytometry (EPICS coulter counter; Coulter Corp., Hialeah, FL).

Isolation and fractionation of PBMC. Heparinized blood from patients or controls was fractionated using a standard Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) density gradient to isolate PBMC. Purified B- or T-lymphocytes (> 95% pure) were obtained by E-rosetting and monocyte depletion using adherence to polystyrene culture dishes that had been preincubated with autologous plasma (11).

Cell proliferation and differentiation assays. PBMC or purified B cells were cultured at 1×10^6/ml in RPMI 1640 (Whittaker Bioproducts, Wakersville, MD), supplemented with L-glutamine and antibiotics in 96-well flat-bottom microtiter plates. For stimulation, the following reagents were applied: *Staphylococcus aureus* Cowan I (SAC)[1] 1:5,000 vol/vol (Calbiochem-Behring Corp., La Jolla, CA), EBV containing supernatants from B95-8 cells, PWM 1 μg/ml (Serva Fine Biochemicals Inc., Heidelberg, FRG). Proliferation was determined after a 3-d incubation period in serum-free RPMI after a 4-h pulse with [³H]-thymidine (1 μCi/well). For EBV stimulation of B cells, dilutions of

B95-8 supernatants that are optimal for the induction of proliferation were determined for each virus preparation, and a 5-d incubation with 1% FCS was employed. Differentiation was determined in 7-d cultures using synthetic medium (HL-1, Ventrex Corp., Portland, ME) without FCS. All cultures were carried out in triplicate and each experiment included HIV+ and control, HIV− individuals. For analysis of the effects of supernatant (SN) from HIV+ PBMC on Ig production, normal PBMC were stimulated with PWM and cultured in HL-1 medium with 1% FCS in the presence of HIV+ PBMC culture supernatants at the indicated dilutions preincubated with or without TGFβ neutralizing antibodies. The HIV+ SNs were incubated with SAC to remove the rabbit IgG antibodies before culture. Immunoglobulin content in day 7 supernatants was measured by ELISA as previously described (5). In brief, 96-well microtiter plates were coated with the appropriate anti-human Ig antibody using a carbonate buffer at pH 9.8 (anti-IgG and IgA from Tago Inc., Burlingame, CA.; anti-IgM from Dako Corp., Santa Barbara, CA.; all 1:1000 dilutions). The wells were washed, blocked with 1% BSA and, the patient samples (diluted 1:20 or 1:10) or the Ig isotype standards were added. After 12 h at 4°C, the plates were washed and the 1:1,500 diluted peroxidase-labeled conjugate was added (anti-IgG/G/A all from Tago Corp.). After 2 h at RT, the plates were washed again, and the substrate was added in a phosphate buffer at pH 5.6 (OPD, o-phenylenediamine dihydrochloride). Substrate turnover was terminated by adding 1 N sulfuric acid. OD was read at 490 nm. Values were determined in triplicate.

TGFβ determination. Supernatants were collected from 24-h PBMC cultures. Samples were tested for TGFβ activity with and without transient acidification (reduction of the pH in the supernatants to pH 1.5 by the addition of 5 N HCl and neutralization with 1.4 N NaOH in 0.7 M Hepes) (12). Titers of TGFβ are expressed in ng/ml based on a standard curve generated by using purified porcine TGFβ (R&D Systems, Minneapolis, MN). This was performed with each set of assays.

The isoform of TGFβ secreted by PBMC was determined by antibody neutralization. Specificity of the antibodies used to neutralize active TGFβ was demonstrated by comparing their effects in the CCL64 and in the lymphocyte activating factor (LAF) assays (13). TGFβ1 and TGFβ2 were neutralized to the same extent by the antibody recognizing both TGFβ1 and β2 (12.5 μg/ml of antibody completely neutralized 1 ng/ml TGFβ1 or TGFβ2). The antibody to TGFβ2 neutralized TGFβ2 activity, having no effect on TGFβ1 activity. The preimmune rabbit control antibody (IgG-fraction; Sigma Chemical Co.) had no neutralizing effect on either TGFβ1 or TGFβ2.

CCL64 assay. The assay was based on the procedure described by Ikeda et al. and Tucker et al. (14, 15). CCL64 mink lung epithelial cells

Table I. Neutralizing Antibody to TGFβ and SAC-induced Proliferation

Patient	Absence of antibodies	anti-TGFβ		Control antibodies	
		10	1	10	1
1	7742	276	124	83	37
2	451	528	178	121	52
3	3033	282	201	42	11
4	6911	183	93	16	8
5	19414	63	47	21	18
6	8326	202	189	17	23

PBMC from six different HIV+ donors were stimulated with SAC and cultured in the presence of the indicated concentrations (μg/ml) of antibodies. Effects of the antibodies are expressed as a percentage increase in counts per minute induced by SAC in the absence of antibodies. SAC-induced proliferation in PBMC from six normal donors was 16293±2321 cpm and this was increased by 122±43% in the presence of anti-TGFβ (10 μg/ml).
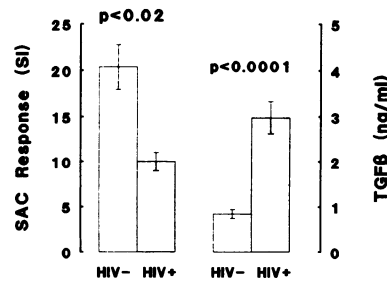

Figure 1. SAC-induced B cell proliferation and TGFβ secretion. PBMC from 32 HIV infected donors (HIV+) and 32 healthy heterosexual controls (HIV−) were stimulated with SAC (1:5,000 dilution) and proliferation was measured after 96 h by [³H]-thymidine uptake during a 4-h incubation period. Results are shown as mean values of the stimulation indices (*SI*, cpm of SAC-stimulated cells/cpm of unstimulated cells). Replicate samples of the same PBMC were cultured in serum-free media and supernatants were collected at 24 h for analysis of TGFβ content in the CCL64 assay. TGFβ levels are expressed in ng/ml as determined on the basis of a standard curve using recombinant human TGFβ1.

were allowed to adhere in 96-well flat-bottomed microtiter plates overnight, using 100,000 cells per well in 200 μl DMEM (Whittaker Bioproducts) containing 1% FCS. After 24 h the medium was removed and replaced with DMEM containing 1% FCS. Standards or conditioned media for TGFβ detection were added in appropriate dilutions to a final total volume of 200 μl/well. Cultures were incubated again at 37°C for 24 h and [³H]thymidine was added during the final 4 h (1 μCi/well). Medium was then removed and the plates with the adherent cells were frozen at −70°C for 2 h. After thawing, the cells were harvested onto glass fiber filters and radioactivity was determined by liquid scintillation counting. Additional experiments showed that the growth of the CCL64 cells was not affected by lymphokines such as IL-1 (up to 100 ng/ml), IL-2 (up to 200 U/ml), or IL-6 (up to 100 ng/ml). No significant difference was observed using TGFβ1 or TGFβ2 as standard, indicating similar sensitivity of the cell line to both TGFβ isoforms. Furthermore, the CCL64 assay was validated by using different TGFβ standards, including platelet-derived human TGFβ from R & D Systems and human recombinant TGFβ1 from Amgen Biologicals (Thousand Oaks, CA).

Statistical methods. All data are shown as arithmetic means±SEM. Analysis of significance was performed with Wilcoxon's rank sum test, and correlations were calculated according to Spearman.

Results

Low B cell responses to SAC correlate with increased TGFβ production in HIV+ PBMC. PBMC from a group of 32 HIV+ individuals, whose clinical characteristics are described in Table I, were stimulated with SAC. SAC-induced proliferation in the HIV+ cohort was significantly ($P < 0.02$) lower than in age-matched healthy heterosexual volunteers ($n = 32$) (Fig. 1).

To investigate the role of TGFβ in this diminished SAC response, patient samples were studied simultaneously for SAC-induced proliferation and TGFβ production. Serum-free supernatants from 24-h cultures of PBMC were tested in the CCL64 assay in which TGFβ inhibits proliferation of lung epithelial cells. The results from these experiments demonstrated that PBMC from HIV+ donors spontaneously released significantly higher amounts ($P < 0.0001$) of TGFβ as compared with the control group (Fig. 1). Only low amounts of TGFβ (< 60 pg/ml) were detected in nonacidified samples, indicating that most of the TGFβ activity secreted by HIV− or HIV+ PBMC was in a latent form. Analysis of randomly selected samples in the LAF assay, where TGFβ inhibits IL-1-induced proliferation of thymocytes, showed comparable levels of TGFβ in the culture supernatants (data not shown). Analysis of these data

revealed a high degree of correlation between SAC stimulation indices (SI) and TGFβ levels ($r = -0.7$, $P = 0.0001$) (Fig. 2). In contrast, there was no discernable correlation between TGFβ levels and the CDC classification of the individual patients (Table I) nor with any medication used by the patients.

While SAC can trigger B cell proliferation in the absence of T cells (16), it is still possible that T cells in PBMC from the different patients can increase the maximal B cell proliferative response. This might occur through the secretion of IL-2 or other cytokines, which could further augment SAC-induced proliferation. Therefore, we investigated the SAC response of HIV$^+$ PBMC as a function of CD4 and CD8 counts from all 32 HIV$^+$ patients. The arithmetic mean±SEM of CD4$^+$ cells was 239±64, and for CD8$^+$ cells 417±151 (cells/μl). Correlation of CD4$^+$ and CD8$^+$ cell numbers and SI of the SAC response was $r = 0.08$ ($P = 0.6$) and $r = -0.003$ ($P = 0.9$), respectively. These data indicate no correlation between SAC responses and CD4$^+$ or CD8$^+$ counts.

TGFβ inhibition of T cell-independent B cell proliferation. To define in more detail the direct effects of TGFβ on B cells, purified (monocyte and T cell depleted) B cell populations were stimulated with SAC or EBV and exogenous TGFβ (either purified or recombinant) was added. Fig. 3 shows the effect of TGFβ on SAC or EBV-induced B cell proliferation. Proliferation induced by either type of B cell activator was strongly inhibited by TGFβ in a dose-dependent manner. In three separate experiments, SAC-induced B cell proliferation was reduced by more than 66% at 1 ng/ml TGFβ. The mean values for suppression of the EBV response were 47 and 56% depending on the dilution of the EBV-containing B95-8 supernatants. Even at 0.1 ng/ml TGFβ, a significant B cell suppression could be observed in the SAC- and EBV-stimulated cultures, which was more than 20% from the values obtained omitting this cytokine. Collectively, these data demonstrate that TGFβ is a very potent and direct inhibitor of B cell proliferation. Furthermore, it is operative even in the setting of T cell-independent stimuli. Additional experiments showed that TGFβ effects of similar magnitude, as described above where TGFβ was present during the entire culture period, are seen when B cells were only exposed to TGFβ during the first 24 h with the B cell activator (not shown).

Inhibition of B-cell proliferation by SN from HIV$^+$ PBMC: neutralization by anti-TGFβ. Having defined the conditions under which purified TGFβ can directly inhibit B cell responses, we analyzed whether the activity in the conditioned

Figure 3. TGFβ inhibition of SAC or EBV-induced B cell proliferation. Monocyte and T cell depleted B cells from normal donors were stimulated with EBV (1:10 or 1:40 dilution of B95-8 supernatant; *left*) or with SAC (1:5,000 dilution) (*right*) and cultured in the absence or presence of TGFβ (1 ng/ml). SAC-induced proliferation was measured after 96 h and the response to EBV after 120 h. Data are shown as mean values of cpm from three separate experiments each performed in triplicate.

medium from HIV$^+$ PBMC can induce a similar effect. EBV stimulation was used for these experiments, since the continuous presence of SAC interfered with the action of both the neutralizing and preimmune antisera. In cultures of EBV-stimulated B cells, the transiently acidified PBMC supernatant (SN) from HIV$^+$ donors reduced proliferation, in all cases, by more than 50% (Fig. 4). The simultaneous addition of a neutralizing antibody, which recognizes both TGFβ1 and TGFβ2, re-
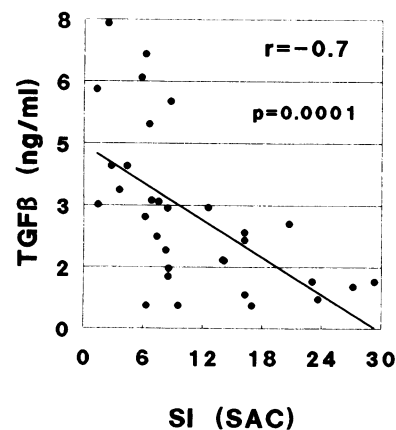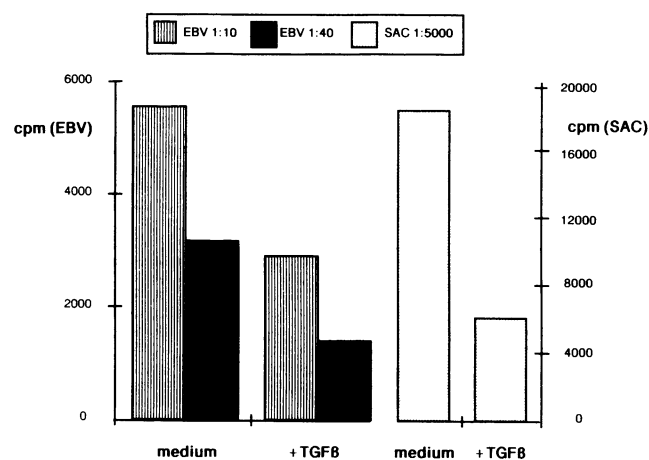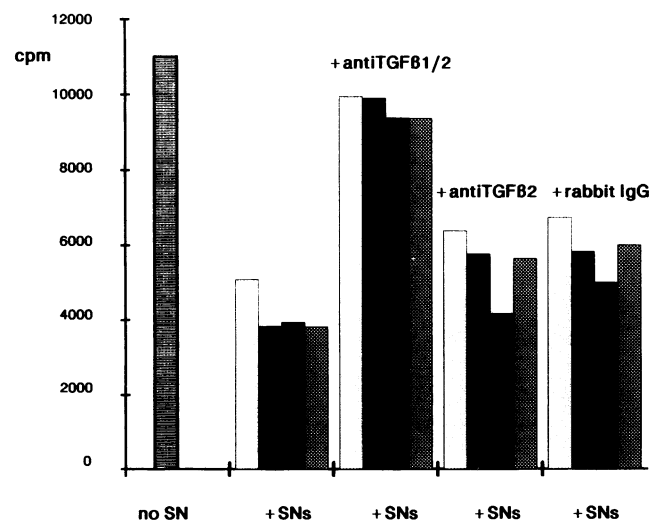

Figure 2. Correlation of TGFβ levels and B cell responses to SAC. Correlation analysis of the TGFβ levels and the SI of the SAC response of 32 HIV-infected donors was performed according to the method of Spearman.

Figure 4. SN from HIV$^+$ PBMC inhibit B cell proliferation: neutralization by anti-TGFβ. Purified B lymphocytes from normal donors were stimulated with EBV (1:10 dilution of B95-8 supernatant) and cultured in the presence of 24-h PBMC culture SN (transiently acidified, 1:10 dilution) from four different HIV$^+$ donors. Aliquots of the SN were treated with antibody to TGFβ1 + 2, with antibody that only recognizes TGFβ2 or with control rabbit IgG (all at 12.5 μg/ml). Proliferation was measured after 5 d by [^{3}H]thymidine uptake.

stored the impaired B cell responses to normal levels. In contrast, a neutralizing antibody to TGFβ2, as well as preimmune rabbit IgG serum, induced only nonspecific B cell stimulation with a rise in incorporated [³H]thymidine between 11 and 14%. These data suggest that most of the TGFβ activity secreted by HIV⁺ PBMC is of the TGFβ1 isoform. Nonacidified SN from HIV⁺ PBMC and acidified supernatants from healthy control donors did not suppress the EBV response, which is consistent with the release of TGFβ predominantly in latent form. Thus, like purified TGFβ1, SN from HIV⁺ PBMC are able to inhibit B cell proliferation and this is due to the presence of TGFβ1.

TGFβ sensitivity of HIV⁺ B cells. To further define the role of TGFβ in humoral immune defects in HIV infection, we examined whether it inhibits proliferation of cells from HIV⁺ donors. PBMC from HIV⁺ and HIV⁻ donors were stimulated with SAC and cultured in the presence of different concentrations of TGFβ1 (Fig. 5 A). There was no apparent difference in the minimal effective doses of TGFβ nor in the levels of inhibition at the highest concentrations of TGFβ tested. B cells were then purified and stimulated with EBV to directly test their TGFβ sensitivity. TGFβ caused similar inhibition of EBV-induced proliferation in cells from HIV⁺ and HIV⁻ donors (Fig. 5 B). The results shown in Fig. 5 represent three separate experiments, each including cells from two HIV⁺ and two HIV⁻ donors. The inhibitory effects of TGFβ2 on cells from HIV⁺ and HIV⁻ donors were similar to those of TGFβ1 (not shown).

Effects of anti-TGFβ on proliferation of HIV⁺ PBMC. The results from the previous experiments suggested that endogenously produced TGFβ may be responsible for impaired B cell responses in HIV⁺ donors. Experiments were then performed to test whether neutralizing antibody to TGFβ can reconstitute the low SAC-induced proliferation in HIV⁺ PBMC. In these studies PBMC were preactivated with SAC for 24 h, washed, and recultured in the presence of IL-2 (100 U/ml) to maintain proliferation which was measured after 3 d. The removal of SAC was necessary because antibodies to TGFβ or control IgG nonspecifically stimulate PBMC proliferation in the presence of SAC. After removal of SAC, rabbit IgG (10 μg/ml) increased
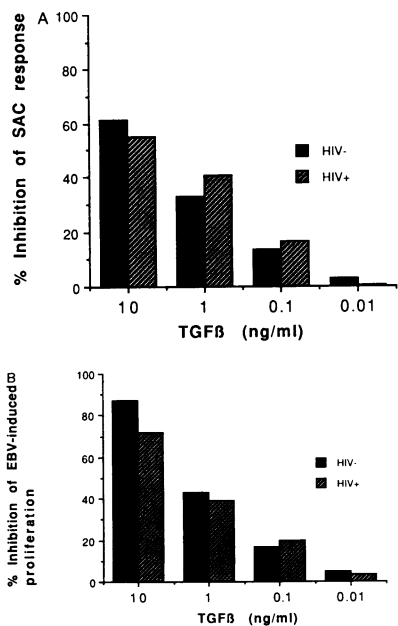

Figure 5. Effects of TGFβ on proliferation of PBMC and B cells from HIV⁺ donors. PBMC and purified B cells from five HIV⁺ and five HIV⁻ donors were stimulated with SAC for 3 d (A) and with EBV for 6 d (B), respectively. Proliferation was measured by [³H]thymidine incorporation during the final 4 h of culture.
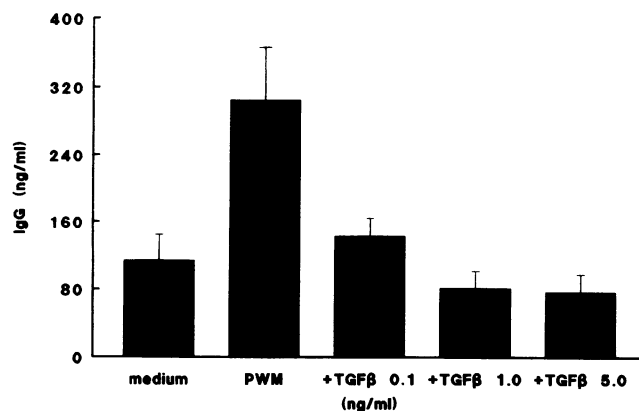

Figure 6. TGFβ effects on PWM-induced B cell differentiation. PBMC from normal donors were stimulated with PWM (1 μg/ml) and cultured for 7 d. Supernatants were collected and IgG content was analyzed by ELISA. Data are shown as IgG in ng/ml (mean±SEM) of eight experiments.
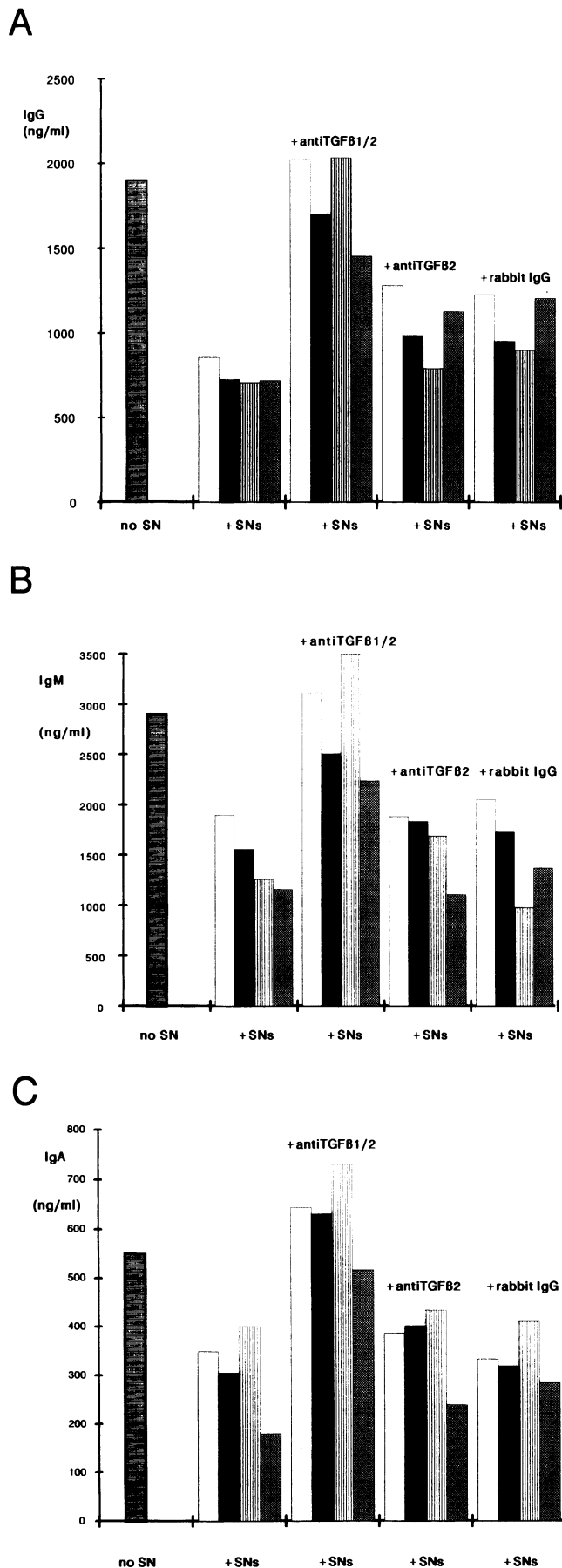
[³H]thymidine uptake by only 47±12%. Antibody to TGFβ increased the proliferation of HIV⁻ PBMC by 122±43%. In cells from HIV⁺ patients, anti-TGFβ caused a significant (P < 0.01) increase (183–528%) in proliferation. The antibody effects were more pronounced in PBMC cultures with lower SAC responses (Table I). These experiments suggest that endogenously produced TGFβ inhibits B cell proliferation in HIV⁺ PBMC.

TGFβ and B cell differentiation. As the effects of TGFβ on B cell function are not restricted to inhibition of antigen or mitogen-induced proliferation, we studied the influence of purified TGFβ and SN from HIV⁺ PBMC on B cell differentiation. Low concentrations of purified TGFβ (100 pg/ml) were able to reduce PWM-induced B cell differentiation (Fig. 6), resulting in a significant reduction in the PWM-induced IgG levels (P = 0.01; n = 8). At 1 ng/ml of TGFβ the PWM stimulation was completely inhibited. This culture system was also chosen for experiments with HIV⁺ culture supernatants because of its high sensitivity to TGFβ. When 24-h SN from HIV⁺ PBMC were tested at a 1:5 dilution in this assay, the PWM-induced production of IgG was completely inhibited. At a 1:20 dilution, IgG levels were still inhibited by more than 60%. This inhibitory effect was completely neutralized by antiserum to TGFβ1 + 2. The TGFβ2-specific antiserum and control rabbit IgG had no significant effects (Fig. 7 A). Furthermore, 24-h SN from HIV⁻ PBMC had no detectable inhibitory effect on IgG production. The same set of conditioned media from HIV⁺ PBMC also inhibited IgM and IgA production (Fig. 7, B and C). These results indicate that the levels of TGFβ1 activity in HIV⁺ PBMC SN are sufficient to inhibit B cell differentiation.

Discussion

HIV infection is often associated with hypergammaglobulinemia, the presence of autoantibodies with diverse specificities and deficient production of antibodies to new antigens. In addition, EBV-associated B-lymphoproliferations occur at increased frequency in these patients (17, 18). This study defines the potential contribution of TGFβ to deficient B cell responses.

Humoral immune responses of cells from HIV⁺ individuals

A

IgG (ng/ml)

+antiTGFB1/2

+antiTGFB2

+rabbit IgG

no SN + SNs + SNs + SNs + SNs

B

IgM (ng/ml)

+antiTGFB1/2

+antiTGFB2 +rabbit IgG

no SN + SNs + SNs + SNs + SNs

C

IgA (ng/ml)

+antiTGFB1/2

+antiTGFB2 +rabbit IgG

no SN + SNs + SNs + SNs + SNs

are characterized in vitro by defects in both B cell proliferation and differentiation. These defects could be the consequence of intrinsic B cell dysfunction, or of impaired interactions between B cells and T cells or monocytes. The well documented abnormalities in $CD4^+$ helper T cell function are only, in part, responsible for this, since coculture of B cells from HIV^+ donors with normal T cells failed to restore B cell function (6). On the other hand, there is evidence that monocytes in HIV infection may be less competent as antigen presenting cells (reviewed in reference 1). However, these cells have been shown to overproduce some cytokines, in particular IL-6, which is critical for the induction of Ig secretion (19–21).

In a recent study we defined a new mechanism for impairment of T cell function in HIV infection. It was shown that PBMC from HIV-infected donors overexpress $TGF\beta_1$ and that this is responsible for some of the defects in T cell function. In currently ongoing work we are identifying the specific cells and stimuli that are responsible for $TGF\beta$ production in HIV infection. Although most of the $TGF\beta$ is secreted in latent form, we have shown that some of this latent factor is activated (9) and suppresses T cell and, as shown in this paper, B cell function. The exact mechanism for $TGF\beta$ activation is not defined, but conversion by plasmin or other monocyte-derived enzymes are mechanisms that have been postulated (22). It would be of interest to obtain evidence for in vivo activation of $TGF\beta$. However, $TGF\beta$ that is activated in vivo binds to cell surface receptors or to carrier proteins such as $\alpha2$-macroglobulin and betaglycan. When bound to carrier proteins, $TGF\beta$ is biologically inactive and can thus not be detected in the CCL64 or radioreceptor assays. Through the suppression of T helper cell function defined in the previous study, $TGF\beta$ can indirectly impair humoral immune responses.

This study implicates $TGF\beta$, which is overexpressed, as a direct inducer of B cell dysfunction in HIV-infected individuals. Low B cell proliferative responses correlate with the production of increased levels of $TGF\beta$. Neutralization of endogenously produced $TGF\beta$ results in a marked increase in B cell proliferation in response to the T cell-independent activators, EBV and SAC. This was demonstrated when antibodies to $TGF\beta$ were used to neutralize the effect of HIV^+ PBMC-conditioned media on normal B cells. In addition, low proliferative responses in HIV^+ PBMC were also restored by antibody neutralization of $TGF\beta$. The induction of immunoglobulin synthesis in vitro by PWM is inhibited in a similar fashion by both HIV^+ PBMC supernatants and by purified $TGF\beta$. Collectively, the findings from this and our previous study demonstrate that $TGF\beta$ has direct and indirect inhibitory effects on B cell function in HIV infection. This concept provides an explanation for the abnormalities in antibody production in response to new antigens.

Paradoxically, however, there are signs of B cell hyperactivity in HIV infection. Analysis of details of the effects of $TGF\beta$ on B cells shows that inhibition by this cytokine is most effec-

Figure 7. Inhibition of Ig production by SN from HIV^+ PBMC: neutralization by anti-$TGF\beta$. PBMC form normal donors were stimulated with PWM (1 μg/ml) and cultured in the presence of a 1:20 dilution of acidified 24-h SN from HIV^+ PBMC. To replicate samples of the SN, the specified antisera were added (all at 12.5 μg/ml). On day 7 SN were collected for quantification of Ig secretion by ELISA. The results for IgG are shown in *A*, IgM in *B*, and IgA in *C*.

tive when the factor is added early and that it has only minor effects on already activated cells (23). In view of these findings, it can be postulated that even in a setting where TGFβ is over-expressed, previously activated B cells might still generate immunoglobulins provided that differentiation factors are available. This indeed seems to be the scenario that best characterizes HIV infection, where in addition to the overexpression of TGFβ, IL-6, the essential and potent inducer of B cell differentiation, is present in elevated titers (19).

A further characteristic of the antibodies produced in HIV infection is that they are predominantly composed of IgG1 and IgA (24–27). In light of the global inhibitory effects of TGFβ on B cell function (28–30), it might not be expected that this particular composition of Ig isotypes is a consequence of TGFβ action. This notion is also supported by our experimental findings that TGFβ equally reduces the production of all IgG subclasses (unpublished results). There is some evidence, however, to indicate that TGFβ may selectively promote the generation of surface IgA$^+$ cells (31) and IgA secretion (32). Other cytokines, such as IL-6 are likely to also be involved in the subclass imbalance, since in transgenic mice that overexpress IL-6 in B cells, IgG1 is the major contributor to the hypergammaglobulinemia (33). Furthermore, in murine B cells from Peyer's patches, IL-6 is the most potent stimulus of IgA secretion (34). These studies suggest that IL-6 and TGFβ have at least the potential to contribute to the imbalance of the subclass distribution of the immunoglobulins that are produced in HIV infection.

A paradox also exists with respect to B cell growth regulation in HIV infection (5, 6). In contrast to the reduced B cell proliferation in vitro reported here and in other studies, a very high frequency of B cell lymphomas is seen in HIV$^+$ patients (17, 18). Two aspects of TGFβ action are significant in this context. A critical determinant in the initial phase of the development of these tumors is a lack of adequate T cell control of reactivated EBV infection. As a potent inhibitor of both T helper cell, and cytotoxic T cell function (35), TGFβ likely contributes to the deficit in T cell surveillance. The role of T cell defects in the development of B cell lymphomas is also supported by the emergence of similar tumors in immunosuppressed transplant recipients (36). Furthermore, recent observations in SCID mice, show that in the absence of normal immune function, injection of blood cells from EBV$^+$ humans leads to the development of EBV-positive B cell proliferation (37). Thus, through the suppression of T cell function, TGFβ may play a similar role in the development of B cell lymphomas in HIV infection. On the basis of the growth inhibitory effects of TGFβ on normal B cells, it might be expected that this cytokine also reduces proliferation of the tumor cells. At least for EBV-induced transformation, it has been shown that B cells become refractory to the growth inhibitory effects of TGFβ, and that TGFβ is also no longer capable of inhibiting spontaneous immunoglobulin synthesis in EBV-transformed B cells (29, 38). B cells that are resistant to TGFβ are probably accumulating in lymph nodes and other tissues where the B lymphoproliferations are localized. The loss of TGFβ responsiveness during EBV-induced B cell activation may explain some aspects of this paradoxical B cell hyperactivity in HIV infection which occurs despite the overexpression of TGFβ.

In summary, abnormal production of TGFβ and changes in B cell responsiveness to this physiological regulator of hu-moral immunity are important components in B cell pathophysiology in HIV infection.

Acknowledgments

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