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ANALYSIS OF THE ANTIGEN- AND MITOGEN-INDUCED DIFFERENTIATION OF B LYMPHOCYTES FROM ASYMPTOMATIC HUMAN IMMUNODEFICIENCY VIRUS-SEROPOSITIVE MALE HOMOSEXUALS

Discrepancy between T Cell-Dependent and T Cell-Independent Activation[1]

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Five asymptomatic human immunodeficiency virus (HIV)-seropositive male homosexuals were immunized with the recall antigens tetanus toxoid (TT) and the three types of poliovirus present in diphtheria, tetanus, and polio vaccine. Four weeks after immunization, the in vivo response to booster immunization, the in vitro pokeweed mitogen (PWM)-induced IgG secretion, and the in vitro T cell-dependent and T cell-independent antigen-induced antibody response were assayed. Increase in serum antibody titer to TT and poliovirus was low and normal, respectively. In all five subjects studied, a high rate of spontaneous IgG production, including antibodies directed toward HIV was observed. Addition of PWM to the cultures induced suppression of the spontaneous IgG secretion. Only one donor showed a slightly increased IgG production after stimulation with PWM. Peripheral blood mononuclear cells of four of the five HIV-seropositive individuals did not produce TT, or poliovirus-specific antibodies when stimulated with the respective T cell-dependent antigens. However, stimulation of these peripheral blood mononuclear cells with TT coupled to agarose beads, which was shown to be T cell-independent, resulted in the generation of IgG anti-TT antibody-forming cells.

Human immunodeficiency virus (HIV)[2] is a recently discovered member of the Lentivirinae subfamily of the Retroviridae family, and is cytotropic and cytopathic for T lymphocytes with the cluster designation (CD) 4 (helper/inducer) phenotype (1–3). HIV has been implicated as the cause of acquired immunodeficiency syndrome (AIDS), of AIDS-related complex (ARC), and of a degeneration of the central nervous system (1, 2, 4–7). After an asymptomatic period of some weeks to several years, infection with HIV may result in disease symptoms caused by immunosuppression. The immune system of patients with AIDS or ARC is characterized by a decrease in CD4/CD8 (T helper/T suppressor) cell ratio, predominantly due to decreased numbers of circulating T lymphocytes with the CD4 phenotype (8). Besides the decrease in the number of $CD4^+$ lymphocytes, an intrinsic functional defect in the surviving T cell population is also responsible for the variety of immunologic abnormalities that have been described in this syndrome (9, 10). The most prominent abnormalities are a decreased blast transformation in response to mitogens and antigens, a decreased lymphokine production, a diminished expression of interleukin 2 receptors, a decreased alloreactivity, an impaired T cell clonability, the inability to provide help to B lymphocytes, a defective B lymphocyte function, and a defective monocyte function (9–21).

Since the introduction of serologic assays for the demonstration of antibodies to HIV, it has become apparent that the immunologic abnormalities, regularly observed in healthy male homosexuals during recent years, could be attributed to infection with HIV (22–24). However, little is known about the nature of the malfunction of the immune system in these asymptomatic HIV-seropositive individuals. In the present study we have investigated antigen-specific antibody production in vivo and in vitro of peripheral blood mononuclear cells (PBMC) isolated from asymptomatic HIV-seropositive male homosexuals who had recently been boosted with the antigens tetanus toxoid (TT) and the three types of poliovirus. In vitro antigen-specific T and B cell functions were studied by stimulating PBMC with pokeweed mitogen (PWM) or with soluble (T cell-dependent) and insolubilized (T cell-independent) antigens. Results of our study demonstrate that asymptomatic HIV-seropositive male homosexuals, as compared with age-matched heterosexual control individuals, have a subnormal increase in serum antibody titer after booster immunization, a severely impaired in vitro antigen-specific T cell function and/or accessory cell function, but an antigen-specific B cell function which appears normal.

MATERIALS AND METHODS

Subjects. Five 25- to 35-yr-old male homosexuals seropositive for HIV in several enzyme-linked immunosorbent assay (ELISA) systems

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[1] This study was supported by Grant 28-1271, Praeventiefonds, The Hague, The Netherlands.
[2] Abbreviations used in this paper: HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cells; TT, tetanus toxoid; iTT, insolubilized TT; sTT, soluble TT; PWM, pokeweed mitogen; AIDS, acquired immunodeficiency syndrome; ARC, AIDS-related complex; ELISA, enzyme-linked immunosorbent assay.

including Vironostika (see below), who had been diagnosed as clinically healthy, volunteered in this study. These men had been HIV-seropositive for at least 2 yr and so far exhibited a normal or slightly decreased CD4/CD8 ratio. Each HIV-seropositive male homosexual had more than 0.7×10^6 CD4 cells/ml. The observed decreased CD4/CD8 ratios were always the result of an increased $CD8^+$ cell population. All of the HIV-seropositive volunteers remained clinically healthy during this study. Age-matched HIV-seronegative heterosexual male donors served as normal controls. Both HIV-seropositive and control donors had been immunized with diphtheria, tetanus toxoid, and polio vaccine during childhood and they had not been reboosted during at least the last 2 yr.

Immunization and sampling. All subjects, unless otherwise stated, received an intramuscular booster immunization of one dose of diphtheria (D), tetanus (T), and polio vaccine routinely produced in the National Institute of Public Health, The Netherlands. The vaccine contained: 2.5 flocculation units (LF) D toxoid, 5 LF T toxoid (TT), and killed poliovirus type 1 (Mahoney) 20 antigen units (DU), type 2 (MEF) 2 (DU), and type 3 (Saukett) 3.5 (DU) per dose. Blood samples were collected before and 4 wk after immunization. PBMC were isolated from heparinized blood by density gradient centrifugation on Ficoll-Isopaque at $1000 \times G$ for 20 min and were then washed twice with RPMI 1640 (GIBCO, Grand Island, NY) supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml). PBMC were depleted of T cells by two cycles of rosette formation and separation on Ficoll-Isopaque with 2-aminoethylisothiouronium bromide-treated sheep red blood cells as described elsewhere (25). Nonrosetting cells were treated with RIV9, a CD3 monoclonal antibody of the IgG3 subclass (gift from Dr. J. G. Kreeftenberg, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands) plus complement (Cedarlane Lab., Inc., Hornby, Ontario, Canada) and depleted of monocytes by differential adherence to plastic as described elsewhere (26). These enriched B cell fractions contained less than 0.1% T cells as determined by immunofluorescence microscopy using a fluoresceinated CD2 reagent (Leu-5: Becton Dickinson, Sunnyvale, CA).

Culture conditions. For antigen-induced B cell differentiation, PBMC were cultured at 2×10^6 cells/well and for PWM-induced B cell differentiation, PBMC were cultured at 10^6 cells/well. Cultures were performed in 24-well, flat-bottomed plates (Falcon 3074: Becton Dickinson, Oxnard, CA) in RPMI 1640 containing penicillin (100 IU/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and 2×10^{-5} M β-mercaptoethanol (complete RPMI). For insolubilized TT (see below) induced B cell differentiation PBMC or purified B cells were cultured at 4×10^{-5} cells/well in 96-well round-bottomed microtiter plates (Greiner No. 650180) in complete RPMI. Depending on the antigen or mitogen used for stimulation, the culture medium contained 10% fetal calf serum (Rehatuin Pharmaceutical Co., Kankakee, IL) (poliovirus and PWM-stimulated cultures) or 10% pooled human AB serum (TT-stimulated cultures). After 10 days stimulation with different concentrations of poliovirus type 1, 2, or 3 (a gift from Dr. A. L. van Wezel, National Institute of Public Health and Environmental Hygiene, The Netherlands), culture supernatants were collected and tested in the poliovirus neutralization assay (see below). After 10 days of stimulation with PWM (80 μg/ml), culture supernatants were collected and tested in ELISA (see below). After 6 days of stimulation with different concentrations of TT or insolubilized TT (iTT), cultured cells were washed twice with RPMI 1640 and tested in the spot-ELISA (see below). For the antigen- and mitogen-induced proliferation, PBMC (10^5 cells/well) were cultured in round-bottomed microtiter plates with TT (1 LF/well) or PWM (80 μg/ml), respectively. All cultures were performed in triplicate. After 5 days of culture, cells were pulsed with 1 μCi [^{3}H]thymidine and after 18 hr harvested and counted in a scintillation counter.

iTT. Tetanus toxoid (a gift from Dr. J. Nagel, National Institute of Public Health and Environmental Hygiene, the Netherlands) was dialyzed against 0.1 M $NaHCO_3$ (pH 8.0) and adjusted to a concentration of 0.5 mg/ml. One milliliter of this solution was mixed with 1 ml of sedimented, washed, *n*-hydroxysuccinimide ester of agarose (Affi-Gel 10 beads; Bio-Rad Laboratories, Richmond, CA) and incubated for 4 hr at room temperature while shaking continuously. Free binding sites were blocked by the addition of 100 ml of 1 M ethanolamine, and after 30 min the beads were washed twice with 0.1 M $NaHCO_3$ (pH 8.0) and minimal essential medium (MEM)-Tris. Finally this iTT was resuspended in MEM-Tris at a 1:1 ratio (v/v) and stored at 4°C. From this stock solution, all subsequent dilutions were made.

Determination of poliovirus type-specific neutralizing antibody. The content of poliovirus type-specific neutralizing antibodies in culture supernatant fluids or in sera was determined by a micro virus neutralization test as described in detail elsewhere (27). Titers of neutralizing antibody were expressed as reciprocals of dilutions of supernatant fluids neutralizing 100 TICD50 in the virus neutralization test.

ELISA. Anti-HIV-specific IgG was assayed by ELISA (Vironostika anti-HIV-III micro-elisa system, Organon Teknika BV, Boxtel, The Netherlands). TT-specific serum titers were assayed by ELISA as described in detail elsewhere (28). Total IgG in supernatant fluids was assayed as previously described (29).

Spot-ELISA. B cells secreting IgG or IgM class antibodies to TT were enumerated by a modification of the spot-ELISA test as described by Sedgwick and Holt (30). Flat-bottomed 96-well microtiter plates (Greiner No. 655180) were coated with 100 μl/well 0.02% glutaraldehyde in phosphate buffer (pH 5) by incubation at 4°C for 18 hr. After washing with phosphate-buffered saline (PBS), wells were filled with 100 μl of TT (4 LF/ml) in phosphate buffer, pH 5. After 2 hr incubation at 37°C, plates were washed with PBS and were incubated for 45 min at 37°C with PBS, 1% bovine serum albumin (BSA) (Boseral, Organon Teknika, Turnhout, Belgium), to block remaining binding sites. PBMC stimulation in vitro with TT or iTT and nonstimulated control PBMC were washed and added to TT-coated wells in 100 μl RPMI 1640, 1% BSA, at a cell density of 10^6 cultured cells/ml. After 18 hr of incubation in a vibration-free incubator in a humidified atmosphere of 5% CO_2 in air at 37°C, wells were washed with PBS, 0.05% Tween-20, and incubated for 2 hr at 37°C with affinity-purified goal anti-human IgM or IgG conjugated to alkaline phosphatase (Tago Inc., Burlingame, CA) in PBS, 1% BSA, 0.05% Tween-20. After washing with PBS, 0.05% Tween-20, the substrate 5-bromo-4-chloro-3-indolylphosphate (5-BCIP) was added in gelling agarose. The substrate was a 2.3 mM 5-BCIP (Sigma Chemical Co., St. Louis, MO) solution in 0.1 M 2-amino-2-methyl-1-propanol buffer (Sigma) containing 5 mM $MgCl_2 \cdot 6H_2$, 0.01% Triton X-405 (Sigma), 0.01% NaN_3, and 0.6% 36°C gelling agarose (Sigma). After incubation at 37°C, IgG anti-TT-secreting cells became visible as blue spots which were counted after 18 hr using an inverted microscope.

RESULTS

In vivo response to booster immunization. To investigate the in vivo capacity to respond upon recall antigens, five asymptomatic HIV-seropositive male homosexuals were immunized with TT and the three types of poliovirus. Before and 4 wk after immunization, tetanus and poliovirus antibody serum titers were determined in an ELISA and in virus neutralization assay, respectively. As shown in Table I, all five donors had protective levels of tetanus antibodies (>0.01 IU/ml) before immunization (31). Four of the five donors also had protective levels of poliovirus type 1, 2, or 3 neutralizing antibodies (≥2) (32). After immunization with TT, the increase of anti-TT

TABLE I

TT and poliovirus type 1, 2, or 3 antibody serum titers before and 4 wk after booster immunization with a diphteria toxoid, tetanus toxoid, and poliovirus vaccine

Donors		Tetanus Antibody Titer (IU/ml)	Poliovirus-Neutralizing Antibody Titer (2 log)		
			I	II	III
I[a]	Pre[b]	0.45	≤2	≤2	≤2
	Post	0.95	5	9	≤2
II[a]	Pre	0.95	8	10	8
	Post	1.05	11	12	11
III[a]	Pre	1.40	5	5	5
	Post	1.65	7	8	12
IV[a]	Pre	1.05	11	5	3
	Post	1.90	12	11	10
V[a]	Pre	1.90	9	11	8
	Post	4.95	10	11	10
I[a]–V[a]	Pre	1.15 ± 0.48	7 ± 4	7 ± 4	5 ± 3
	Post	2.00 ± 1.47	9 ± 3	10 ± 2	9 ± 4
Age-matched controls[c]	Pre	3.16 ± 2.55[d]	7 ± 2[e]	7 ± 4	4 ± 3
	Post	27.53 ± 18.54	11 ± 2	11 ± 3	12 ± 3

[a] Asymptomatic HIV-seropositive male homosexual.
[b] Pre- or post-booster vaccination.
[c] HIV-seronegative heterosexuals.
[d] n = 9.
[e] n = 8.

serum titers proved significantly less than the increase observed in age-matched control individuals ($p < 0.01$, F-test). The differences in increase of virus-neutralizing serum antibody titers against poliovirus type 1, 2, or 3 between the asymptomatic HIV-seropositive donors, on the one hand, and, on the other hand, the age-matched controls were less evident (Table I). However, one seropositive donor (I) had an anti-poliovirus type 3 serum titer ≤2 before and after immunization.

In vitro mitogen-induced IgG secretion. PBMC of the five asymptomatic HIV-seropositive male homosexuals, isolated 4 wk after booster immunization and PBMC of five nonimmunized healthy male heterosexuals were stimulated with PWM. After 10 days of culture, supernatants from unstimulated and PWM-stimulated cultures were assayed for the presence of total IgG and IgG anti-HIV. Unstimulated PBMC of the seropositive donors, but not of the heterosexual control individuals, spontaneously secreted IgG (Fig. 1*A*). Part of this spontaneously secreted IgG consisted of antibodies directed to HIV, as measured in ELISA (Fig. 1*B*). In PWM-stimulated cultures of PBMC from four out of five HIV-seropositive donors, total IgG secretion had decreased, whereas PBMC from only one seropositive donor showed a slightly increased IgG secretion as compared with the elevated IgG production by PWM-stimulated PBMC from the control individuals (Fig. 1*A*). Moreover, in PWM-stimulated cultures of PBMC from all five HIV-seropositive donors, HIV-specific antibody production had decreased (Fig. 1*B*). None of the control individuals produced HIV-specific antibodies after stimulation with PWM (Fig. 1*B*). In addition poliovirus neutralizing antibodies and antibodies directed to TT were not detected in supernatants of unstimulated or PWM-stimulated cultures of PBMC from HIV-seropositive donors.

Antigen-induced T cell-dependent antibody production in vitro. Four weeks after the booster immunization with TT and the three types of poliovirus, PBMC of the five asymptomatic HIV-seropositive male homosexuals and of five HIV-seropositive male heterosexuals were assayed for their capacity to produce specific antibody in vitro. To that extent PBMC were cultured with different concentrations of soluble TT, or poliovirus type 1, 2, or 3. PBMC from five HIV-seronegative control individuals produced anti-TT IgG (no anti-TT IgM, data not shown) and poliovirus type 1, 2, and 3 neutralizing antibody upon stimulation with the respective antigens (Figs. 2 and 3). This in vitro induced antibody response proved to be specific since, in cultures of PBMC, anti-poliovirus-producing cells were not detected after stimulation with TT; similarly after stimulation with poliovirus no production of anti-TT was observed (data not shown). After culturing of PBMC from the five HIV-seropositive donors with soluble TT, no TT-specific IgG or IgM (data not shown) production could be detected in a spot-ELISA (Fig. 2). After 10 days of culturing of PBMC from the HIV-seropositive donors with poliovirus type 1, 2, or 3, respectively, cells from four out of five donors failed to produce detectable levels of poliovirus-neutralizing antibody as measured in the virus neutralization assay. PBMC from the other seropositive donor did produce neutralizing antibodies against poliovirus type 2 and 3, but not against type 1 when stimulated with the respective antigens (Fig. 3).

Mitogen- and antigen-induced lymphocyte proliferation. Given the impaired T cell-dependent antigen or mitogen-induced B cell activation, proliferative responses upon stimulation with soluble TT (sTT) or PWM of PBMC from three of the HIV-seropositive individuals were evaluated. As shown in Table II, proliferative responses upon stimulation with PWM or TT were low compared with the responses of PBMC from HIV-seronegative controls, except for donor III who showed a response upon stimulation with PWM. This donor also demonstrated an increase in total IgG production after stimulation with PWM, as shown in Figure 1.

Antigen-induced T cell-independent antibody production in vitro. It has previously been shown that the

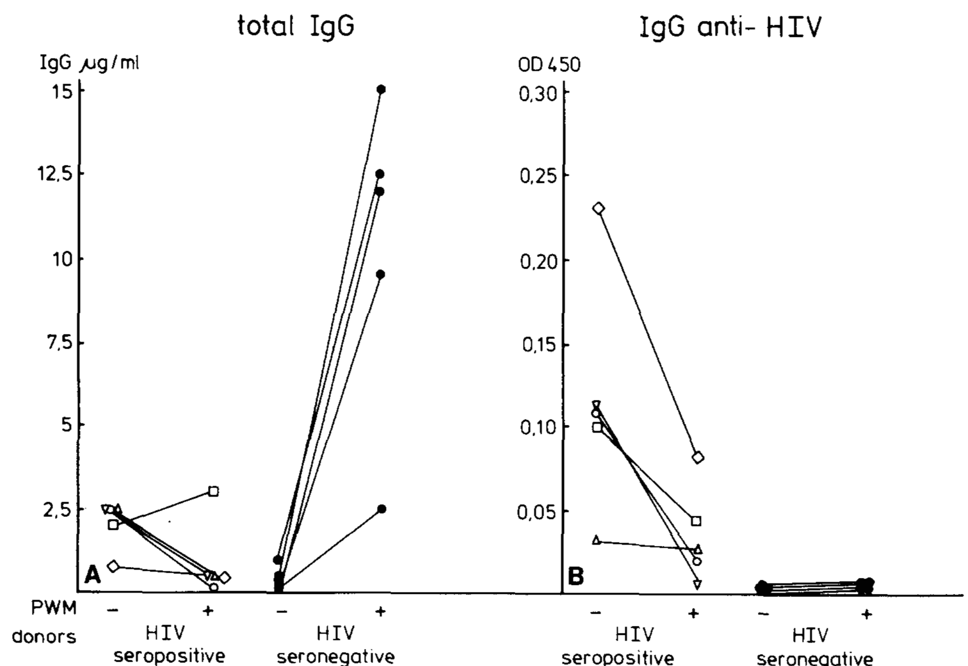

Figure 1. Spontaneous total IgG and IgG anti-HIV production and the influence of PWM stimulation on the total IgG and IgG anti-HIV production of PBMC from five booster-immunized asymptomatic HIV-seropositive male homosexuals and five nonimmunized HIV-seronegative heterosexual control individuals. Cultures were set up as described in *Materials and Methods.* After 6 days of culture, culture supernatants were collected and assayed for total IgG and IgG anti-HIV production in ELISA. ○(I), △(II), □(III), ▽(IV), and ◇(V) = asymptomatic HIV-seropositive male homosexuals; ● = HIV-seronegative male heterosexual control individuals.

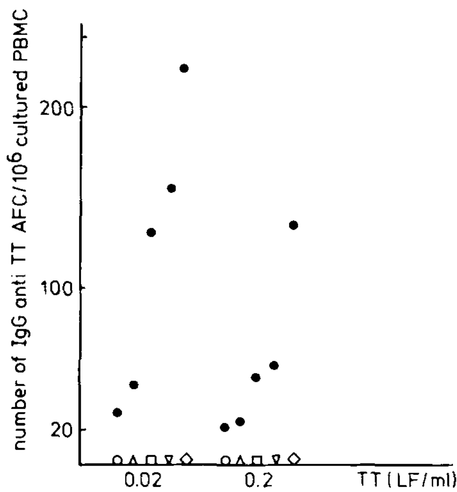

Figure 2. Number of IgG anti-TT-producing cells after stimulation of PBMC from TT booster-immunized individuals with two different doses of TT. PBMC were isolated 4 wk after booster immunization and cultured as described in *Materials and Methods*. After 6 days of culture, cells were washed and assayed for specific antibody production in a spot ELISA. ○(I), △(II), □(III), ▽(IV), and ◇(V) = asymptomatic HIV-seropositive male homosexuals; ● = HIV-seronegative male heterosexual control individuals.

autoantigen thyroglobulin coupled to a solid phase can be used to induce antigen-specific secondary type B cell responses in vitro, independently of T cells (26, 33). In analogy, we coupled TT to Affi-Gel 10 (iTT) to examine whether secondary anti-TT responses independently of T cells could be raised. To demonstrate the capacity of B cells to respond upon stimulation with iTT without the involvement of T cells and adherent cells, B cells of TT booster-immunized control individuals were purified as described in *Materials and Methods*.

PBMC and purified B cells were stimulated with serial dilutions of iTT and sTT and after 6 days of culture assayed for IgG anti-TT antibody-forming cells in a spot ELISA. Representative data from one control donor are shown in Table III. Stimulation of purified B cells and unseparated PBMC with iTT resulted in the production of IgG anti-TT. In contrast to PBMC, purified B cells did not respond upon stimulation with sTT. These data show that iTT activates human B cells independently of T cell help to the production of TT-specific antibody.

We used this method to analyze the T cell-independent responsiveness of B cells to TT from the HIV-seropositive donors. PBMC were stimulated with serial dilutions of iTT and examined for production of TT-specific antibody in comparison with production by PBMC from the HIV-seropositive controls. As shown in Figure 4, PBMC from both HIV-seropositive and HIV-seronegative men could be stimulated equally well to IgG TT-specific antibody production. No IgM TT-specific antibody-forming cells were detected (data not shown).

DISCUSSION

Previous studies on the function of the immune system in HIV-infected individuals have mainly been focused on patients with AIDS or ARC. These studies demonstrated a severely impaired antibody response induced by antigen or mitogen, without elucidating the underlying mechanisms (14–18). In the present report we have studied both T cell-dependent and T cell-independent activation of B cells from asymptomatic HIV-seropositive male homosexuals. To this end in vivo and in vitro antigen-specific immune responses were measured in TT and poliovirus (type 1, 2, and 3) booster-immunized asymptomatic HIV-seropositive male homosexuals and compared with those of similarly booster-immunized HIV-seronegative heterosexual male individuals.

Four weeks after immunization, all five seropositive donors showed a relative low and normal increase in serum antibody titers to TT and polioviruses respectively, suggesting B cell activation in vivo. Despite this in vivo observed responsiveness, T cell-dependent antigen- and mitogen-induced B cell differentiation in vitro proved to

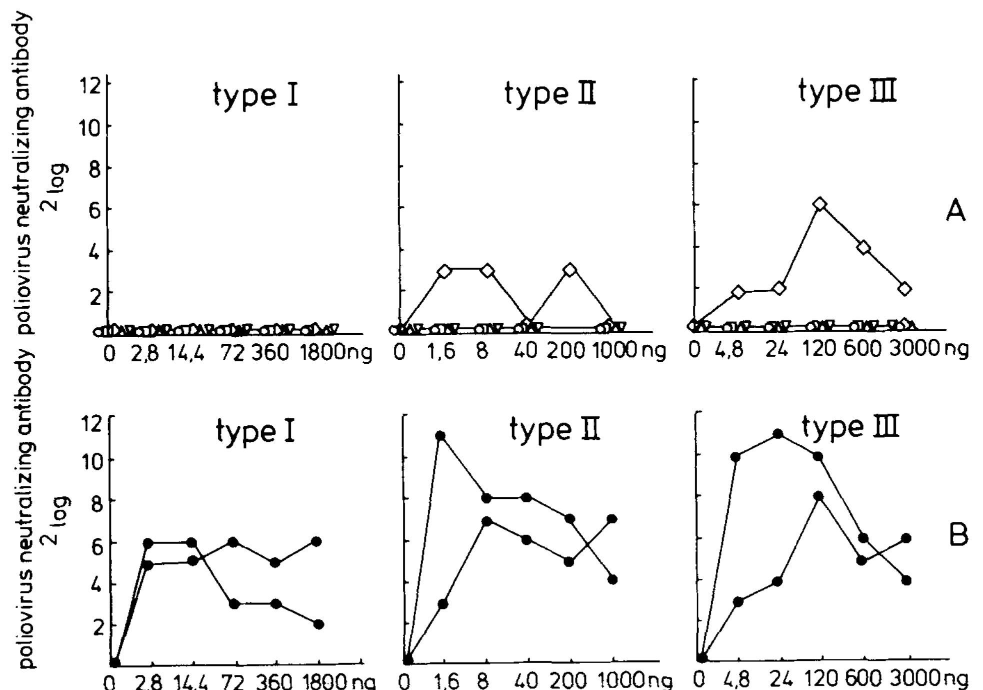

Figure 3. Poliovirus-neutralizing antibody after stimulation of PBMC from poliovirus type 1, 2, and 3 booster-immunized individuals with different doses of poliovirus type 1, 2, or 3. PBMC were isolated 4 wk after booster immunization and cultured as described in *Materials and Methods*. After 10 days of culture, supernatants were collected and assayed for poliovirus type 1, 2, and 3 neutralizing antibodies in the poliovirus neutralization assay. ○(I), △(II), □(III), ▽(IV), and ◇(V) asymptomatic HIV-seropositive male homosexuals (A); ● = HIV-seronegative male heterosexuals (B).

TABLE II

Mitogen- and antigen-induced lymphocyte proliferation of PBMC from TT booster immunized HIV-seropositive and HIV-negative individuals[a]

Donors	Unstimulated	PWM	TT
I[b]	66 ± 9[c]	2,471 ± 128	626 ± 130
III[b]	363 ± 7	11,710 ± 916	2,135 ± 188
V[b]	272 ± 174	3,883 ± 94	4,820 ± 372
Control[d]	407 ± 49	13,487 ± 2,343	19,934 ± 1,099
Control[d]	712 ± 338	12,080 ± 1,913	19,537 ± 2,924

[a] Four weeks after booster immunization. PBMC (10^5 cells/well) were stimulated with TT (1 LF/well) or PWM (80 μg/ml). After 5 days of culture proliferative responses were assayed as described in *Materials and Methods*.
[b] Asymptomatic HIV-seropositive male homosexual.
[c] Values represent cpm and are expressed as the mean ± SD of triplicate cultures.
[d] HIV-seronegative heterosexual.

TABLE III

Number of IgG anti-TT-producing cells after stimulation of PBMC and purified B cells from a booster-immunized control individual with serial dilutions of insolubilized TT and different doses of soluble TT[a]

Stimulus	Numbers of Ig Anti-TT Antibody-Forming Cells/10^6 Cultured Cells	
	Purified B cells	PBMC
iTT[b]		
0	0	0
1/100	30	60
1/200	110	145
1/400	180	140
sTT		
0	0	0
0.02 (LF/ml)	0	210
0.2 (LF/ml)	0	225

[a] PBMC and purified B cells were cultured and assayed as described in *Materials and Methods*.
[b] Dilutions of the Affi-Gel-bound TT stock solution prepared as described in *Materials and Methods*.

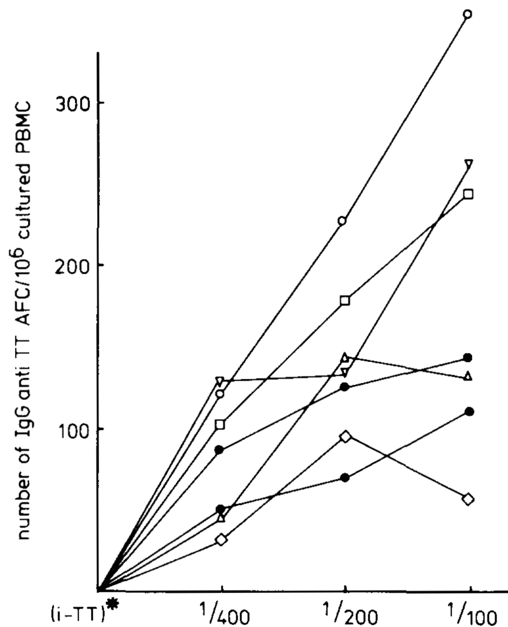

Figure 4. Number of IgG anti-TT-producing cells after stimulation of PBMC from TT booster-immunized individuals with different dilutions of iTT. PBMC were isolated 4 wk after booster immunization and cultured as described in *Materials and Methods*. The number of IgG anti-TT antibody-forming cells were assayed as described in the legend of Figure 2. ○(I), △(II), □(III), ▽(IV), and ◇(V) = asymptomatic HIV-seropositive male homosexuals; ● = HIV-seronegative male heterosexuals. (* Dilutions of the Affi-Gel-bound TT stock solution prepared as described in *Materials and Methods*.)

be severely impaired in these individuals. PBMC from the asymptomatic HIV-seropositive individuals in contrast to those of control individuals failed to respond upon stimulation with the soluble protein antigen TT or the three types of poliovirus. For this impaired in vitro antigen-induced antibody synthesis, the following explanations may be considered: the absence of functionally active antigen-specific memory B cells, an insufficient T helper cell activity, or an impaired accessory cell function.

The absence of functionally active antigen-specific B cells could be excluded, since here we demonstrated that PBMC of both HIV-seropositive and -seronegative individuals could be triggered equally well to anti-TT IgG production by iTT, which has shown to stimulate human B cells, independently of T cell help and probably without the interference of adherent cells. The observed impaired antigen-induced T cell-dependent B cell responses, but normal T cell-independent B cell responses suggest that T cell function and/or accessory cell function rather than B cell function is disturbed in these asymptomatic HIV-seropositive male homosexuals. Consistently, proliferative responses of PBMC from the HIV-seropositive individuals upon stimulation with sTT proved relatively low when compared with those from HIV-seronegative control individuals. This finding confirms recently published data showing low antigen-induced responses of T cells from both patients with AIDS and asymptomatic HIV-seropositive individuals (9, 34).

The possibility that accessory cells rather than, or in addition to, T helper cells themselves, may have accounted for the observed impaired secondary immune responses cannot be ruled out from the data presented. Interestingly, it has been shown that both monocytes and macrophages are permissive for HIV infection and it is conceivable that a defective monocyte function as described in AIDS patients may also play a role in the unresponsiveness of *asymptomatic* HIV-seropositive individuals as reported here (35–37).

Patients with AIDS or ARC often show hyperimmunoglobulinemia with increased numbers of circulating immunoglobulin-secreting cells (14, 16, 17). Here we showed that the PBMC of asymptomatic HIV-seropositive male homosexuals also spontaneously produced elevated levels of IgG, including antibodies directed toward HIV. Poliovirus-neutralizing antibodies or antibodies directed to TT were not detected in the culture supernatants of these PBMC isolated 4 wk after the booster immunization. Recently, Yarchoan et al. (18) demonstrated in a very sensitive precursor frequency determination that in patients with AIDS or ARC part of the spontaneously produced antibodies comprised antibodies toward HIV and to at least one recall antigen and frequencies observed for recall antigen-specific B cells were lower than those for HIV-specific B cells. The failure to detect spontaneously secreted antibodies directed to recall antigens may be inherent to culture conditions and sensitivity of the assays used, but is in agreement with the observation that lymphoblastoid B cells, spontaneously secreting antibodies to an immunizing agent, independent of T cell help, circulate only between 5 and 10 days after immunization (38, 39). The detection of spontaneously produced IgG directed to HIV may reflect recent or continuous activation of HIV-specific B cells by the expression of HIV antigens in vivo. Furthermore, HIV has been

shown to be a T cell-dependent polyclonal stimulator in vitro, which may also have accounted for the spontaneous production of IgG, including antibodies directed to HIV (18, 40, 41). In four out of five seropositive donors, addition of PWM to the cultures resulted in a decreased spontaneous IgG production, whereas HIV-specific antibody production was decreased in all five donors tested. PWM-induced suppression of the spontaneous Ig secretion of in vivo activated B cells, as has been demonstrated in several systems, has been explained by the involvement of a subset of PWM-inducible, in vivo preactivated $CD8^+$ suppressor T cells (42, 43). This may represent a nonspecific physiologic control system on activated B cell stages. More recently it was observed that in HIV-seropositive asymptomatic hemophiliacs, abnormalities of in vitro PWM-induced Ig secretion coincided with HIV seropositivity (24, 44). Thus, an increased Ig production and in vitro anergy to mitogens such as PWM not only appears to be valid for individuals with AIDS or ARC but seems a general phenomenon for individuals seropositive for HIV.

Considering the immune-stimulating and immune-suppressive consequences of HIV infection, we conclude that the impaired in vivo and in vitro immune functions of HIV-seropositive male homosexuals described in this paper are the result of persistent HIV infection.

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REFERENCES

1. **Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugevre, S. Chamaret, J. Gruest, C. Dauget, C. Axler-Blim, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T lymphotropic retrovirus from a patient at risk for AIDS. *Science 220:868.*
2. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLVIII) from patients with AIDS and at risk for AIDS. *Science 224:500.*
3. **Klatzmann, D., F. Barré-Sinoussi, M. T. Nugeyre, C. Daugnet, F. Brun-Vezinet, C. Rouzioux, J. C. Gluckman, J. C. Chermann, and L. Montagnier.** 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-induced lymphocytes. *Science 225:59.*
4. **Sarngadharan, M. G., M. Popovic, L. Bruch, J. Schüpbach, and R. C. Gallo.** 1984. Antibodies reactive with human T-lymphotropic retroviruses (HTLVIII) in the serum of patients with AIDS. *Science 224:506.*
5. **Brun-Vezinet, F., C. Rouzioux, F. Barré-Sinoussi, D. Klatzmann, A. G. Saimot, W. Rozenbaum, J. C. Gluckman, L. Montagnier, and J. C. Chermann.** 1984. Detection of IgG antibodies to lymphadenopathy associated virus (LAV) by ELISA, in patients with acquired immunodeficiency syndrome or lymphadenopathy syndrome. *Lancet 1:1253.*
6. **Shaw, G. M., M. E. Harper, B. H. Hahn, L. G. Epstein, D. C. Gajdusek, R. W. Price, B. A. Navia, C. K. Petito, C. J. O'Hara, J. E. Groopman, E. S. Cho, J. M. Oleske, F. Wong-Staal, and R. C. Gallo.** 1985. HTLVIII infection in brains of children and adults with AIDS encephalopathy. *Science 227:177.*
7. **Ho, D. D., T. R. Rota, R. T. Schooley, J. C. Kaplan, J. D. Allan, J. E. Groopman, L. Resnick, D. Felsenstein, C. A. Andrews, and M. S. Hirsch.** 1985. Isolation of HTLVIII from cerobrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *N. Engl. J. Med. 313:1493.*
8. **Fahey, J. L., H. Prince, M. Weaver, J. Groopman, B. Visscher, K. Schwartz, and R. Detels.** 1984. Quantitative changes in T helper or T suppressor/cytotoxic lymphocyte subset that distinguish acquired immune deficiency syndrome from other immune subset disorders. *Am. J. Med. 76:95.*
9. **Lane, H. C., J. M. Depper, W. C. Greene, G. Whalen, T. A. Waldmann, and A. S. Fauci.** 1985. Qualitative analysis of immune function in patients with the acquired immuno deficiency syndrome. *N. Engl. J. Med. 313:79.*
10. **Margolick, J. B., D. J. Volkman, H. C. Lane, and A. S. Fauci.** 1985. Clonal analysis of T lymphocytes in the acquired immunodeficiency syndrome. *J. Clin. Invest. 76:709.*
11. **Ebert, E. C., D. B. Stoll, B. J. Cassens, W. H. Lipshutz, and S. P. Hauptman.** 1985. Diminished interleukin 2 production and receptor generation characterize the acquired immunodeficiency syndrome. *Clin. Immunol. Immunopathol. 37:283.*
12. **Gupta, S.** 1986. Study of activated T cells in man. II. Interleukin 2 receptor and transferrin receptor expression on T cells and production of interleukin 2 in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC). *Clin. Immunol. Immunopathol. 8:93.*
13. **Epstein, J. S., W. R. Frederick, A. H. Rook, L. Jackson, J. E. Manischewitz, R. E. Mayner, H. Masur, J. C. Enterline, J. Y. Djeu, and G. V. Quinnan, Jr.** 1985. Selective defects in cytomegalovirus- and mitogen-induced lymphocyte proliferation and interferon release in patients with acquired immunodeficiency syndrome. *J. Infect. Dis. 152:727.*
14. **Lane, H. C., H. Masur, L. C. Edgar, G. Whalen, A. H. Rook, and A. S. Fauci.** 1983. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med. 309:453.*
15. **Ammann, A. J., G. Schiffman, D. Abrams, P. Volberding, J. Ziegler, and M. Conant.** 1984. B-cell immunodeficiency in acquired immunodeficiency syndrome. *JAMA 251:1447.*
16. **Pahwa, S. G., M. T. J. Quilop, M. Lange, R. N. Pahwa, and M. H. Grieco.** 1984. Defective B lymphocyte function in homosexual men in relation to the acquired immunodeficiency syndrome. *Ann. Intern. Med. 101:757.*
17. **Katz, I. R., S. E. Krown, B. Safai, H. F. Oettgen, and M. K. Hoffmann.** 1986. Antigen specific and polyclonal B-cell responses in patients with acquired immunodeficiency disease syndrome. *Clin. Immunol. Immunopathol. 39:359.*
18. **Yarchoan, R., R. R. Redfield, and S. Broder.** 1986. Mechanisms of B cell activation in patients with acquired immunodeficiency syndrome and related disorders. Contribution of antibody-producing B cells, of Epstein-Barr Virus-infected B cells, and of immuno globulin production induced by human T cell lymphotropic virus, type III/lymphadenopathy-associated virus. *J. Clin. Invest. 78:439.*
19. **Smith, P. D., K. Ohura, H. Masur, H. C. Lane, A. S. Fauci, and S. M. Wahl.** 1984. Monocyte function in the acquired immunodeficiency syndrome. Defective chemotaxis. *J. Clin. Invest. 74:2121.*
20. **Prince, H. E., D. J. Moody, B. I. Shubin, and J. L. Fahey.** 1985. Defective monocyte function in acquired immunodeficiency syndrome (AIDS): evidence from a monocyte-dependent T cell proliferative system. *J. Clin. Immunol. 5:21.*
21. **Hersh, E. M., P. W. A. Mansell, J. M. Reuben, A. Rios, and G. R. Newell.** 1984. Immunological characterizations of patients with acquired immunodeficiency syndrome, acquired immunodeficiency syndrome-related symptom complex, and a related life style. *Cancer Res. 44:5894.*
22. **Nicholson, J. K. A., J. S. McDougal, H. W. Jaffle, T. J. Spira, M. S. Kennedy, B. M. Jones, W. W. Darrow, M. Morgan, and M. Hubbard.** 1985. Exposure to human T lymphotropic virus type III/lymphadenopathy-associated virus and immunologic abnormalities in asymptomatic homosexual men. *Ann. Int. Med. 103:37.*
23. **Dobozin B. S., F. N. Judson, D. L. Cohn, K. A. Penley, P. E. Rickmann, M. J. Blaser, P. S. Sarin, S. H. Weiss, and C. H. Kirkpatrick.** 1986. The relationship of abnormalities of cellular immunity to antibodies to HTLV-III in homosexual men. *Cell. Immunol. 98:156.*
24. **Daniel V., G. Opelz, A. Schäfer, Kl. Schimpf, I. Wendler, and G. Hunsmann.** 1986. Correlation of immune defects in hemophilia with HTLV-III antibody titer. *Vox Sang. 51:35.*
25. **Saxon A., J. Feldhaus, and R. A. Robins.** 1976. Single step separation of human T and B cells using AET treated SRBC rosettes. *J. Immunol. Methods 12:285.*
26. **Logtenberg T., A. Kroon, F. H. J. Gmelig-Meyling, and R. E. Ballieux.** 1986. Antigen-specific activation of auto reactive B cells in normal human individuals. *Eur. J. Immunol. 16:1497.*
27. **Dömök I., and D. I. Magrath.** 1979. Guide to poliovirus isolation and serological techniques for poliomyelitis surveillance. *WHO Offset Publ. 13:1.*
28. **Hagenaars, A. M., R. W. van Delft, and J. Nagel.** 1984. Comparison of ELISA and toxin neutralization for the determination of tetanus antibodies. *J. Immunoassay 5:1.*
29. **UytdeHaag, F. G. C. M., A. D. M. E. Osterhaus, H. G. Loggen, R. H. J. Bakker, J. A. A. M. van Asten, J. G. Kreeftenberg, P. van der Marel, and B. van Steenis.** 1983. Induction of antigen-specific antibody response in human peripheral blood lymphocytes in vitro by a dog kidney cell vaccine against rabies virus (DKCV). *J. Immunol. 131:1234.*
30. **Sedgwick, J. D., and P. G. Holt.** 1983. A solid-phase immunoenzymatic technique for the enumeration of specific antibody secreting cells. *J. Immunol. Methods 57:301.*
31. **Scheibel, I.** 1955. The uses and results of active tetanus immunization. *Bull. WHO 13:381.*
32. **Salk, J.** 1960. Persistence of immunity after administration of for-

malin-treated poliovirus vaccine. *Lancet 2:715.*

33. **Logtenberg, T., A. Kroon, F. H. J. Gmelig-Meyling, and R. E. Ballieux.** 1986. Production of anti-thyroglobulin antibody by blood lymphocytes from patients with autoimmune thyroiditis, induced by the insolubilized autoantigen. *J. Immunol. 136:1236.*
34. **Antonen, J., and K. Krohn.** 1986. Interleukin 2 production in HTLVIII/LAV infection: evidence of defective antigen-induced but normal mitogen-induced Il-2 production. *Clin. Exp. Immunol. 65:489.*
35. **Ho, D. D., T. R. Rota, and M. S. Hirsch.** 1986. Infection of monocytes/macrophages by human T lymphotropic virus type III. *J. Clin. Invest. 77:1712.*
36. **Gartner, S., P. Markovitz, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III infection. *Science 233:215.*
37. **Nicholson, J. K. A., G. D. Cross, C. S. Callaway, and J. S. McDougal.** 1986. In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *J. Immunol. 137:323.*
38. **Stevens, R. H., E. Macy, C. Morrow, and R. H. Stevens.** 1979. Characterization of circulating subpopulation of spontaneous antitetanus toxoid antibody producing B cells following in vitro booster immunization. *J. Immunol. 126:1146.*
39. **UytdeHaag, F. G. C. M., H. G. Loggen, T. Logtenberg, R. A. Lichtveld, G. van Steenis, J. A. A. M. van Asten, and A. D. M. E. Osterhaus.** 1985. Human peripheral blood lymphocytes from recently vaccinated individuals produce both type-specific and intertypic cross-reacting neutralizing antibody on in vitro stimulation with one type of poliovirus. *J. Immunol. 135:3094.*
40. **Pahwa, S., R. Pahwa, C. Saxinger, R. C. Gallo, and R. A. Good.** 1985. Influence of the human T lymphotropic virus/lymphadenopathy-associated virus on function of human lymphocytes: evidence for immunosuppressive effects and polyclonal B cell activation by banded viral preparations. *Proc. Natl. Acad. Sci. USA 82:8198.*
41. **Pahwa, S., R. Pahwa, R. A. Good, R. C. Gallo, and C. Saxinger.** 1986. Stimulatory and inhibitory influences of human immunodeficiency virus on normal B lymphocytes. *Proc. Natl. Acad. Sci. USA 83:9124.*
42. **Arneborn, P., G. Biberfeld, M. Forsgren, and L. U. van Stedingk.** 1983. Specific and non-specific B cell activation in measles and varicella. *Clin. Exp. Immunol. 51:165.*
43. **Brieva, J. A., and R. H. Stevens.** 1983. Inhibition of human antigen-induced lymphoblastoid B cell function by an *in vivo*-induced suppressor T cell. *Cell. Immunol. 77:109.*
44. **Biagiotti, R., M. G. Giudizi, F. Almerigogna, M. Mazzetti, A. Alessi, G. F. del Prete, D. Rafenelli, M. Fiorilli, M. Morfini, and S. Romagnani.** 1986. Abnormalities of *in vitro* immunoglobulin production in apparently healthy haemophiliacs: relationship with alterations of T cell subsets and with HTLV-III seropositivity. *Clin. Exp. Immunol. 63:354.*