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Immunology

Immunoregulatory activities of human immunodeficiency virus (HIV) proteins: Effect of HIV recombinant and synthetic peptides on immunoglobulin synthesis and proliferative responses by normal lymphocytes

(immunoregulation/gene products/acquired immunodeficiency syndrome)

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ABSTRACT **Recombinant and synthetic peptides corresponding to envelope proteins of the human immunodeficiency virus (HIV) were examined for their effects on the activities of lymphocytes from normal donors *in vitro*. Although lymphocytes cultured with env–gag peptides produced significant amounts of IgG, addition of env–gag peptides to a pokeweed mitogen-induced B-cell activation system resulted in suppression of immunoglobulin synthesis by normal lymphocytes. Recombinant antigens, env–gag and env-80 dihydrofolate reductase (DHFR), produced a substantial proliferative response by peripheral blood mononuclear cells (PBMC) as determined by [^{3}H]thymidine incorporation. PBMC precultured with HIV synthetic peptide env 578–608 also manifested significant proliferative responses as compared to control cultures. CD3$^+$ lymphocytes precultured with recombinant HIV antigens, env–gag and env-80 DHFR, and synthetic HIV peptide, env 487–511, showed moderate but significant proliferative responses. Both recombinant antigens and synthetic peptides also produced a dose-dependent stimulatory effect on proliferation by CD3$^-$ lymphocytes. Stimulation of CD3$^+$ and CD3$^-$ lymphocyte subpopulations induced by env–gag peptides was specifically inhibited by goat anti-env–gag polyclonal antibodies, demonstrating the specificity of the reaction. These studies demonstrate that recombinant and synthetic peptides of the HIV genome express immunoregulatory T- and B-cell epitopes. Identification of unique HIV epitopes with immunogenic and immunoregulatory activities is necessary for the development of an effective vaccine against HIV infection.**

Infection of CD4$^+$ T cells by the human immunodeficiency retrovirus (HIV) causes profound dysfunction of cellular and humoral immune responses. These impairments include lack of virus and tumor-specific cytotoxicity, a profound decrease in the helper/suppressor T-lymphocyte ratio, and depressed antibody responses to soluble antigens (1–5). However, a paradoxical finding of HIV infection has been the observation of polyclonal B-cell activation leading to increased numbers of B cells and plasma cells in lymphoid tissues and elevated serum levels of immunoglobulins and autoantibodies (1, 3, 5). This suggests that although the CD4 epitope is the receptor for HIV, other lymphocyte subpopulations may be involved in the pathogenesis of the acquired immunodeficiency syndrome (AIDS). Previous *in vitro* studies showed that crude HIV preparations induce polyclonal B-cell activation and proliferative responses by normal lymphocytes (3, 4). Since HIV is a membrane-budding virus, isolated HIV preparations may include host cell membrane antigens possibly altered by virus and thus cause nonspecific stimulation. Through the use of recombinant or synthetic peptides of HIV antigens it is possible to identify the viral epitopes responsible for polyclonal B-cell activation and rule out confounding effects. The present study was undertaken to examine several HIV-specific recombinant and chemically synthesized peptides on immunoglobulin synthesis and proliferative responses by peripheral blood lymphocytes from normal donors *in vitro*.

MATERIALS AND METHODS

HIV Recombinant and Synthetic Proteins. Expression and purification of recombinant proteins of the HIV genome in *Escherichia coli* and preparation of synthetic peptides corresponding to various epitopes of the HIV envelope used in this investigation have been described (6–9). env–gag is a recombinant fusion product that contains an 80-amino acid sequence from the gp41 surface glycoprotein of HIV genome and a 190-amino acid sequence from the HIV internal core protein P24 (Fig. 1). env-80 dihydrofolate reductase (DHFR) is a synthetic oligonucleotide-based recombinant envelope gene product that corresponds to the superconserved stretch of amino acids of gp41 (9) (Fig. 2). In immunoassays, env-80 DHFR showed 100% sensitivity and 99.8% specificity against HIV$^+$ sera that were gag-specific (10). env 578–608 and env 487–511 are chemically synthesized peptides with 10–30 amino acids corresponding to various constant regions of the HIV envelope proteins gp120 and gp41 (7, 8) (Fig. 2). Among the gp41 synthetic peptides, env 578–608 was highly immunoreactive against HIV$^+$ sera by electrophoretic transfer blot assay showing 99.4% specificity and 99.8% sensitivity (10).

Blood Donors. Peripheral blood from healthy, adult, HIV-seronegative donors was drawn into a syringe containing heparin (20 units/ml). Donors were apprised of this study and consents were obtained consistent with the policies of The University of Michigan and the National Institutes of Health.

Isolation of Lymphocytes. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by using a modified method of Boyum (11). Blood was diluted with an equal volume of normal saline and was centrifuged at 400 × *g* for 30 min at 18°C. The mononuclear cell band was harvested, washed three times with saline, and resuspended in RPMI 1640 medium containing 25 mM Hepes buffer

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Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; DHFR, dihydrofolate reductase; PBMC, peripheral blood mononuclear cells; PBL, peripheral blood lymphocytes; PWM, pokeweed mitogen; PHA, phytohemagglutinin; SAC, *Staphylococcus aureus* Cowan strain I.

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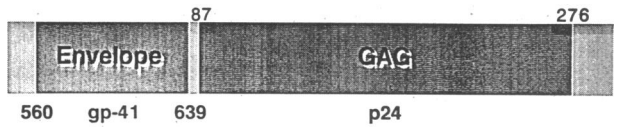

FIG. 1. Selection of fusion product with conserved and antigenic epitopes from env and gag regions of HIV. The sequence of expressed env–gag fusion protein used in the present experiments represents env 560–639 and gag 87–276 amino acid sequences, totaling 270 amino acids. Twenty-one extra amino acids represent coding from the vector region. This protein is expressed in *E. coli* as a single polypeptide without any evidence of premature termination or internal initiation. Expressed proteins were purified and found to be highly conserved and antigenic (10).

supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 80 μg of gentamicin per ml (Schering, Kenilworth, NJ), and 300 μg of fresh glutamine per ml (complete medium).

PBMC in RPMI 1640 with 10% fetal calf serum were depleted of adherent cells by passage through a 7-ml column of Sephadex G-10 beads (Pharmacia Fine Chemicals) equilibrated in the same medium. After 45 min of incubation at 37°C, nonadherent peripheral blood lymphocytes (PBL) were washed through with 1 bed vol of medium at 37°C. Cell recovery was >70% of the total input, and monocyte contamination as indicated by nonspecific esterase staining was <2%.

Lymphocyte Proliferation Assay. PBL (2×10^5) were cultured in RPMI 1640 medium containing 5% fetal calf serum in sterile U-bottom microtitration plates (Dynatech, Alexandria, VA). Cultures received various recombinant or synthetic HIV peptides at concentrations ranging from 5 to 1000 ng/ml and were incubated for 48–120 hr at 37°C in a 5% CO_2 in air atmosphere. [^{3}H]Thymidine (1 μCi; 1 Ci = 37 GBq) was added to each well during the last 24 hr of incubation. The cultures were collected by an automated cell harvester, and incorporation of [^{3}H]thymidine into lymphocyte DNA was determined with a Beckman model LS-700 scintillation counter. Results are expressed as cpm per 2×10^5 cells. As controls, the T-cell mitogens phytohemagglutinin (PHA, Difco) at 2 μg/ml and concanavalin A (Con A, Difco) at 1, 2.5, and 5 μg/ml and whole formalin-treated *Staphylococcus aureus* Cowan strain I (SAC) cells, a B-cell mitogen, at dilutions of 1:1000, 1:2000, 1:5000, and 1:20,000 were also used.

Polyclonal B-Cell Differentiation. PBL (2×10^5) were cultured in 200 μl of complete medium in U-bottom microtitration plates with different concentrations of env–gag proteins for 9 days at 37°C in a 5% CO_2 in air atmosphere. At the end of incubation cultures were centrifuged and the supernates were assayed for IgG as described below. As a positive control for induction of immunoglobulin synthesis and secretion, duplicate cultures received pokeweed mitogen (PWM, Difco) at 5 μg/ml. To measure the effect of env–gag on PWM-induced immunoglobulin synthesis and secretion, cultures also received 5 μg of PWM per ml plus different concentrations of env–gag.

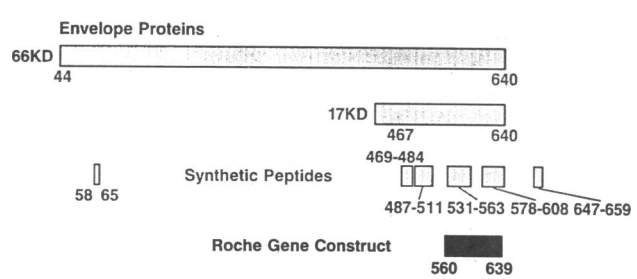

FIG. 2. Selection of viral components with conserved and highly antigenic envelope epitopes. Various peptides of the HIV envelope were cloned or chemically synthesized. Of all peptides cloned and of all chemically synthesized peptides, env-80 DHFR or env 487–511 and env 578–608, respectively, were found to be highly conserved and antigenic; therefore, only those were used in the present experiments. KD, kilodaltons.

ELISA. Immunoglobulin levels of culture supernates were measured by micro-ELISA as described (12). Briefly, wells of polystyrene plates were coated with 0.1 ml of heavy chain-specific rabbit anti-human IgG diluted to 2 μg/ml in 0.05 M sodium carbonate (pH 9.6) containing 0.02% sodium azide. The plates were sealed, incubated at room temperature for 16–20 hr in a humidified chamber, and washed five times with phosphate-buffered saline (PBS) containing polyoxyethylene sorbitan monolaurate (Tween 20) (PBS/Tween) at 4-min intervals. Lymphocyte culture supernates and immunoglobulin standards were diluted in PBS/Tween, and 0.1 ml of each dilution was added to duplicate sensitized wells. After 3 hr of incubation at room temperature, the plates were washed as above. Alkaline phosphatase was conjugated to heavy chain-specific rabbit anti-human immunoglobulin by glutaraldehyde treatment. The conjugate was diluted in PBS/Tween and 0.2 ml was added to each well. After incubation at room temperature for 16–20 hr, the plates were again washed with PBS/Tween and 0.2 ml of alkaline phosphatase substrate (Sigma, no. 104) at 1 mg/ml was added to all wells. After 15 min at room temperature, the plates were measured for absorbance at 405 nm with an automated filter photometer (Multiskan, Flow Laboratories). The concentration of immunoglobulin in the culture supernate was determined by comparing the absorbancy of the samples with absorbancy curves obtained with authentic immunoglobulin standards.

RESULTS

Effect of Recombinant HIV Peptides on Immunoglobulin Synthesis. In view of the findings that patients with AIDS have elevated serum levels of immunoglobulins and autoantibodies (1), studies were carried out to examine the effect of recombinant HIV peptides on immunoglobulin synthesis by normal lymphocytes *in vitro*. Data presented in Fig. 3 demonstrate that human PBMC precultured with 5–200 ng of the env–gag protein per ml produced significant levels of IgG. env–gag at 5, 10, 50, and 100 ng/ml showed a dose-dependent induction of IgG, 381 ($P < 0.025$), 744 ($P < 0.005$), 790 ($P < 0.0005$), and 880 ($P < 0.0005$) ng/ml, respectively, compared to 273 ng of IgG per ml produced by untreated control

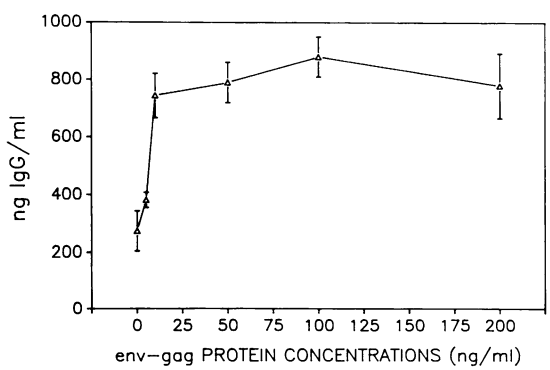

FIG. 3. env–gag peptide-induced IgG production *in vitro*. PBMC (1×10^5) from healthy HIV$^-$ individuals were cultured in 200 μl of RPMI 1640 medium containing 5% fetal calf serum to which varying concentrations of env–gag were added. After 9 days at 37°C in a 5% CO_2 in air atmosphere, supernates were collected and measured for IgG by ELISA. Data are expressed as ng of IgG per ml of culture ± SDM for three separate experiments. A positive control consisted of cells incubated with 5 μg of PWM and a negative control consisted of cells incubated in medium alone.

cultures. Electrophoretic transfer blot analysis of env–gag-induced immunoglobulin showed no specific reaction with env–gag antigens, demonstrating the polyclonality of its induction (data not presented). PBMC precultured with a higher concentration of env–gag, 200 ng/ml, showed a slight decrease in IgG production (779 μg/ml), although no toxicity was observed after 9 days of incubation (data not presented).

As shown in Fig. 4, env–gag protein suppressed PWM-induced immunoglobulin synthesis by normal lymphocytes. PBMC precultured with 5, 10, and 50 ng of env–gag per ml produced a dose-dependent increase in the production of IgG, 381 ($P < 0.001$), 744 ($P < 0.0005$), and 790 ($P < 0.0005$) ng/ml, respectively, compared to 273 ng/ml produced by the control culture. PBMC precultured with 5 μg of PWM per ml, a dose previously determined to yield maximal levels of IgG, produced 4752 ng of IgG per ml ($P < 0.0005$). In coculture experiments, env–gag protein significantly suppressed PWM-induced immunoglobulin production. The suppressive effect was more pronounced at lower concentrations of env–gag and tended to decrease at higher concentrations—i.e., PWM-induced IgG was reduced from a control level of 4752 ng/ml to 875 ($P < 0.0005$), 2000 ($P < 0.0005$), and 3887 ($P < 0.0025$) ng/ml at env–gag concentrations of 5, 10, and 50 ng/ml, respectively.

Lymphocyte Proliferative Response to Recombinant and Synthetic HIV Peptides. We investigated whether recombinant HIV antigens and synthetic peptides could induce the proliferation by normal lymphocytes. Data presented in Fig. 5 demonstrate that env–gag induces significant lymphocyte proliferation as determined by [^{3}H]thymidine incorporation. Proliferation was noted as early as 48 hr and peaked by day 3–5, depending on individual lymphocyte donors. The magnitude of the response was 7639 ($P < 0.0005$), 15,371 ($P < 0.01$), and 16,230 ($P < 0.005$) cpm at days 2, 3, and 5, respectively, of lymphocyte culture when stimulated with 50 ng of env–gag protein. Lymphocytes stimulated with 5 μg of Con A per ml showed a peak response on days 2–3 with a substantial decline by day 5, whereas control cultures without any added stimulant manifested negligible proliferative responses throughout the incubation period. Fig. 6 represents a dose–response study of the effect of env–gag on the proliferation of normal PBMC. env–gag even at 5 ng/ml caused a substantial proliferative response (7842 cpm) by normal PBMC ($P < 0.0005$) and at 10 and 50 ng/ml produced an increased effect [10,436 ($P < 0.0005$) and 10,228 ($P < 0.0005$) cpm, respectively]; the stimulatory activity plateaued

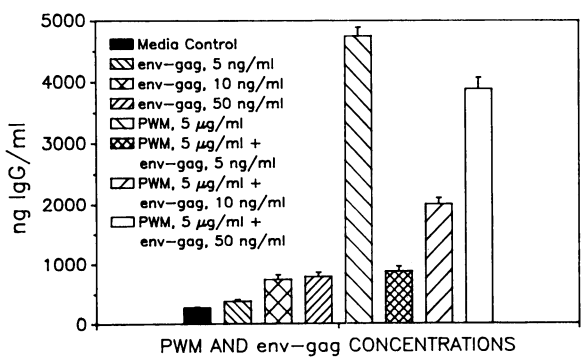

FIG. 4. env–gag peptide suppresses PWM-induced immunoglobulin synthesis. PBMC (1×10^5) from healthy, HIV$^-$ individuals were cultured in 200 μl of RPMI 1640 medium containing 5% fetal calf serum to which either env–gag peptides (5, 10, or 50 ng/ml), PWM (5 μg/ml), or different concentrations of env–gag + 5 μg of PWM per ml were added. Cultures were incubated at 37°C for 9 days and supernates were assayed for IgG as indicated in the legend for Fig. 3. Values are expressed as the mean ± SEM of triplicate determinations from a single experiment. Three other experiments produced similar results.

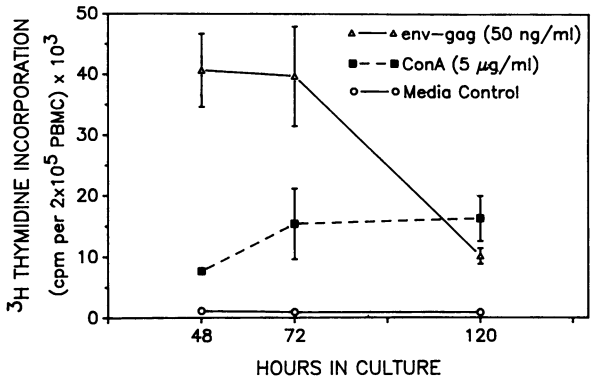

FIG. 5. Kinetics of lymphocyte proliferative responses to env–gag peptides. PBMC (2×10^5) from HIV$^-$ individuals were cultured with either 50 ng of env–gag per ml or 5 μg of Con A per ml for 48–120 hr at 37°C in a 5% CO_2 in air atmosphere. [^{3}H]Thymidine (1 μCi) was added to each well during the last 24 hr of culture and cells were harvested on glass fiber filters. Radioactivity was measured in a liquid scintillation counter and expressed as cpm per 2×10^5 PBMC. Results are expressed as the mean ± SEM of triplicate values from three experiments.

at a concentration of 100 ng of env–gag per ml. Control cultures containing Con A at 1, 2, and 5 μg/ml also produced significant proliferative responses by PBMC compared to controls without any added stimulant. Data presented in Fig. 7 show the proliferative response of lymphocytes to env-80 DHFR peptide at different concentrations. env-80 DHFR at 5 ng/ml caused a significant proliferative response (32,129 cpm, $P < 0.001$) by normal lymphocytes. env-80 DHFR at 10, 50, and 100 ng/ml concentrations produced slightly lower, but significant, proliferative responses [18,847 ($P < 0.025$), 15,471 ($P < 0.01$), and 15,452 ($P < 0.0025$) cpm, respectively] compared to unstimulated control cultures (7449 cpm). Control cultures containing PHA at 2 μg/ml and SAC at a 1:20,000 dilution produced significant proliferative responses (44,631 and 37,736 cpm, respectively). PBMC cultured with synthetic peptide env 578–608, which corresponds to a portion of the conserved region of gp41 of HIV, also showed a significant stimulatory effect on normal lymphocytes (Fig. 8). env 578–608 at 10 and 50 ng/ml resulted in incorporation of 56,585 ($P < 0.0005$) and 62,638 ($P < 0.005$) cpm, respectively, of [^{3}H]thymidine. However, with higher concentrations of pep-

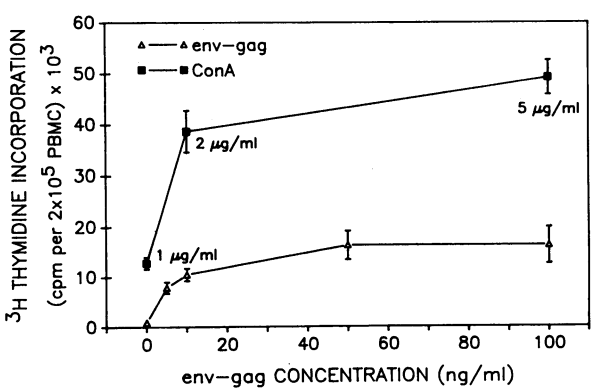

FIG. 6. env–gag peptide induces lymphocyte proliferative responses. PBMC (2×10^5) from normal HIV$^-$ donors were cultured in 200 μl of complete medium containing 5% fetal calf serum to which different concentrations of either env–gag peptides or Con A were added separately and incubated for 72 hr at 37°C in a 5% CO_2 incubator. [^{3}H]Thymidine (1 μCi) was added to each well during the last 24 hr of culture and cells were harvested onto glass fiber filters. Radioactivity was measured in a liquid scintillation counter and expressed as cpm per 2×10^5 PBMC. Results are expressed as the mean ± SEM of triplicate values from three experiments.

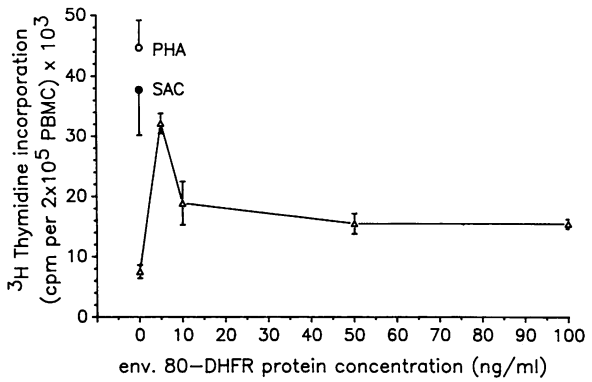

FIG. 7. Lymphocyte proliferative responses to env-80 DHFR peptide. PBMC (2×10^5) from healthy HIV$^-$ individuals were cultured with either PHA (2 μg/ml), SAC (1:20,000 per ml), or env-80 DHFR peptide at different concentrations for 120 hr at 37°C in a 5% CO_2 in air atmosphere. [^{3}H]Thymidine (1 μCi) was added to each well during the last 24 hr and cells were harvested as described in the legend to Fig. 5. Results are expressed as the mean ± SEM of three experiments performed in triplicate.

tide, decreased radioactivities of 5137 and 4633 cpm were obtained with 500 and 1000 ng of peptide per ml, respectively. PHA at a concentration of 2 μg/ml used as a positive stimulant in the assay produced a significant proliferative response (44,631 cpm) compared to the unstimulated control culture (4571 cpm). Fig. 9 shows the proliferative effect of the recombinant HIV antigens env–gag and env-80 DHFR and the synthetic HIV peptides env 487–511 and env 578–608 on CD3$^+$ T cells. CD3$^+$ cells cultured separately with 5 ng of env–gag or env-80 DHFR per ml showed moderate but significant proliferative responses [17,137 ($P < 0.001$) and 24,241 ($P < 0.0005$) cpm, respectively] compared to control cultures (11,289 cpm). Stimulation of CD3$^+$ lymphocytes by env–gag was inhibited by goat anti-env–gag polyclonal antibodies at a 1:10,000 dilution, demonstrating the specificity of the reaction. The known polyclonal B-cell activator, SAC, at a final dilution of 1:1000–1:20,000 did not produce any significant stimulation of CD3$^+$ lymphocytes (12,068–13,591 cpm), supporting the homogeneity of the CD3$^+$ subpopulation used in the assay. The proliferative response of lymphocytes to 2.5 μg of PHA per ml on day 5 of culture was 28,784 cpm. This relatively decreased lymphocyte response to PHA was due to harvesting the culture on day 5, consistent with the maximal response to antigens, rather than day 3, which

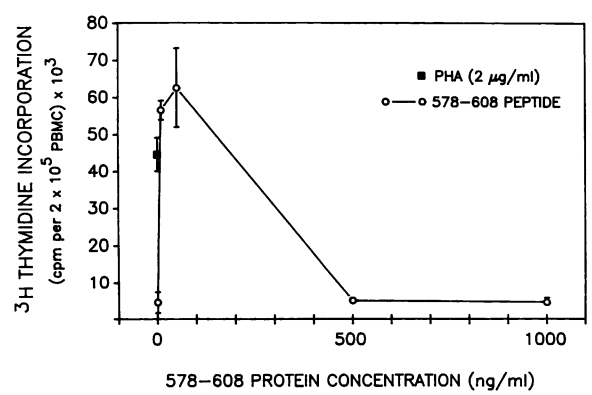

FIG. 8. Lymphocyte proliferative responses to synthetic HIV peptide env 578–608. PBMC (2×10^5) from healthy HIV$^-$ individuals were cultured with either PHA (2 μg/ml) or env 578–608 peptide at different concentrations for 120 hr at 37°C in a 5% CO_2 in air atmosphere. [^{3}H]Thymidine (1 μCi) was added to each well during the last 24 hr and cells were harvested as described in the legend to Fig. 5. Results are expressed as the mean ± SEM of three experiments performed in triplicate.

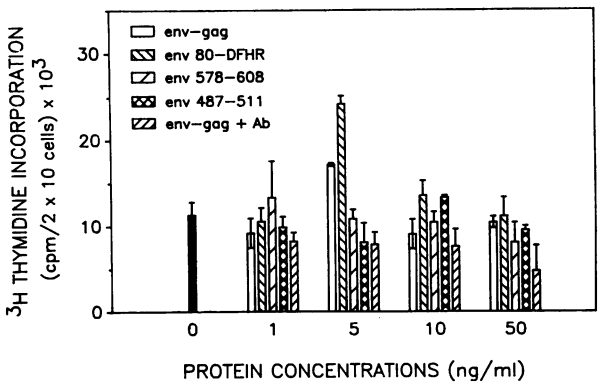

FIG. 9. T-lymphocyte proliferative responses to HIV peptides. CD3$^+$ T cells (2×10^5) separated by panning from the peripheral blood of healthy HIV$^-$ individuals were cultured with different concentrations of HIV peptides for 120 hr. Separate wells received env–gag + goat anti-env–gag antibodies at a 1:10,000 final dilution. As negative controls, a known B-cell mitogen, killed SAC cells, at 1:1000, 1:2000, 1:4000, and 1:20,000 final dilutions was used in separate wells. As positive controls, PHA, a known T-cell mitogen, at 2.5 μg/ml was also included. Values represent the mean ± SEM of triplicate determinations from a single experiment. Two other experiments produced similar results.

is usually the time of peak response to mitogens. The proliferative responses of isolated B lymphocytes (CD3$^-$) to HIV recombinant and synthetic peptides are shown in Fig. 10. B cells precultured with SAC at a 1:20,000 dilution produced 27,736 cpm compared to 6018 cpm manifested by the control culture. B cells precultured with env–gag proteins showed a maximal stimulatory response (17,190 cpm, $P < 0.005$) at a concentration of 5 ng/ml and this response was significantly inhibited (6866 cpm) by a 1:10,000 dilution of polyclonal goat anti-env–gag antiserum. The stimulatory effect of env-80 DHFR was evident in a dose-dependent manner [15,084 ($P < 0.005$), 36,344 ($P < 0.0005$), 40,270 ($P < 0.0005$), and 47,025 ($P < 0.0005$) cpm at 1, 5, 10, and 50 ng/ml, respectively], and the maximum effect was obtained at 50 ng/ml (47,025 cpm, $P < 0.0005$). Both synthetic peptides, env 487–511 and 578–608 also showed a stimulatory effect on CD3$^-$ cells, and the response was maximal at 50 ng/ml and 42,715 ($P < 0.0005$) and 14,375 ($P < 0.005$) cpm, respectively, for 487–511 and 578–608.

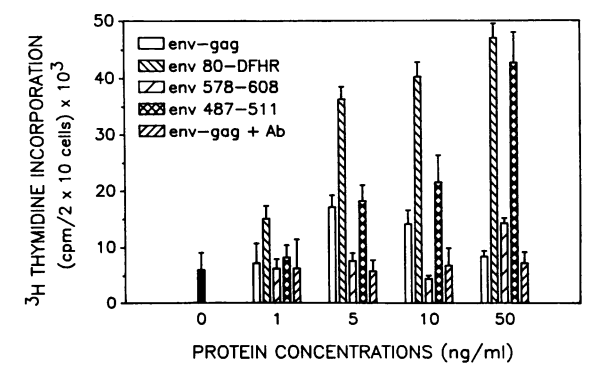

FIG. 10. B-lymphocyte proliferative responses to HIV proteins. CD3$^-$ B lymphocytes (2×10^5) separated by panning from healthy HIV$^-$ individuals were cultured with different concentrations of HIV peptides for 120 hr. Separate culture wells received env–gag + goat anti-env–gag antibodies at a final dilution of 1:10,000. As a positive control, SAC at a 1:20,000 final dilution was included in the assay. Proliferative responses of lymphocytes were measured by [^{3}H]thymidine incorporation. Values represent the mean ± SEM of triplicate determinations of a single experiment. Two other experiments produced similar results.

DISCUSSION

Earlier studies showed up to 30% variability of the genome of different HIV isolates (13). Genomic variation, especially in the hypervariable regions of the envelope glycoproteins of HIV variants, may facilitate evasion of immune recognition resulting in viral persistence. Thus, the likelihood of a single vaccine against all variants of HIV becomes questionable. Indeed, group-specific neutralizing antibodies have demonstrated only limited effect on the progression of AIDS (14). Therefore, an ideal HIV vaccine should contain a mixture of antigenic peptides. Crude extracts of HIV grown *in vitro* have been shown to induce B-cell proliferation and polyclonal immunoglobulin synthesis by normal lymphocytes *in vitro* (3, 5). Since HIV is a membrane-budding virus, the polyclonal B-cell activators may have included virus and host cell membrane antigens. Thus, definitive biochemical characterization of the B-cell-specific moieties remains to be determined. The current investigation demonstrates that certain defined HIV recombinant and synthetic peptides can activate normal lymphocytes to undergo proliferation and immunoglobulin synthesis. No toxicity was manifested by peptide concentrations up to 500 ng/ml, as determined by trypan blue vital dye exclusion, and viability was always comparable with control cultures (data not presented). As these recombinant and synthetic peptides were biologically active *in vitro*, posttranslational modification was not necessary for their effects. In the present experiments, antigens were added at the initiation of the culture and remained throughout the experiment to mimic the *in vivo* situation where virus or their products persist. However, short exposure, up to 1 hr, of lymphocytes with peptides followed by washing also resulted in lymphocyte proliferative responses and immunoglobulin synthesis (data not presented).

It was initially thought that the hypergammaglobulinemia observed in patients with HIV infection was due to coinfection of B lymphocytes with Epstein–Barr virus (EBV), since most AIDS patients are seropositive for EBV (15). However, these studies as well as those of Pahwa *et al.* (5) and Schnittman *et al.* (3) demonstrate that HIV has a direct effect on B lymphocytes, resulting in polyclonal differentiation. Recent studies showed that although $CD4^+$ lymphocytes harbor HIV, other cells may also be involved in virus persistence and dissemination (16). This is evidenced by the observation that only very few $CD4^+$ lymphocytes were infected with HIV (17). B-cell activation by HIV antigens thus indicates involvement of other surface-recognition structures in addition to CD4 antigens. The ability of relatively lower concentrations of env–gag peptides to inhibit PWM-induced proliferative responses of B cells (Fig. 4) suggests that B effects may occur at an early stage of HIV infection before any significant antigenemia.

Although HIV has been shown to directly infect $CD4^+$ T cells, little information is available on other effects of HIV on T-cell functions. Recently Zarling *et al.* (17) demonstrated T-cell responses to gp120 in macaques, and Cease *et al.* (18), employing an emphipathic helix model, demonstrated T-helper sites on env peptides in mice. In an animal model, Krohn *et al.* (19) observed T-cell proliferative responses to native gp120 but not to recombinant env peptides. The lack of T-cell responses to recombinant peptides may be due to the lack of immunogenic epitopes in the preparation. Studies have shown that short HIV peptides, whether recombinant or synthetic, were better T-cell than B-cell immunogens (20, 21). The present investigation demonstrates that T cells make significant proliferative responses to recombinant (env–gag, env-80 DHFR) and synthetic (env 487–511) HIV peptides. In addition to the demonstration of significant T-cell responses (Fig. 9), these recombinant and synthetic HIV peptides also possess immunogenic epitopes for B cells, as demonstrated by B-cell proliferative responses and polyclonal immunoglobulin. Thus, the HIV peptides studied herein may be potential vaccine candidates by virtue of their ability to elicit B- and T-lymphocyte responses. Development of such recombinant constructs of HIV proteins with requisite T-cell and B-cell immunogenic epitopes (multiple epitopes if necessary) is the initial step in formulating an effective vaccine against HIV. Studies are necessary to ensure that these peptides do not have any other biological activities, such as stimulation of suppressor lymphocytes or neuroleukin effects, that may further compromise an immunosuppressed host.

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