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$CD4^+$ lymphocyte function with early human immunodeficiency virus infection

(AIDS-related complex/gp120/soluble CD4)

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ABSTRACT **The pathogenesis of cellular immune deficiency following human immunodeficiency virus (HIV) infection could result from quantitative and/or qualitative dysfunction of the $CD4^+$ lymphocyte population. To better characterize the T-cell response to soluble antigen with HIV infection, we have isolated peripheral blood lymphocytes and purified populations of $CD4^+$ lymphocytes from healthy HIV antibody-positive subjects, patients with acquired immunodeficiency syndrome (AIDS)-related complex (ARC), and healthy HIV antibody-negative controls. T-lymphocyte function was determined by proliferative response to lectin (phytohemagglutinin), phorbol 12-myristate 13-acetate (PMA), calcium ionophore, purified recombinant HIV envelope gp120, tetanus toxoid antigen, and tetanus toxoid antigen in the presence of recombinant gp120 or purified recombinant soluble CD4. PBLs and $CD4^+$ lymphocytes from asymptomatic HIV-infected subjects responded equally well to lectin, PMA, and/or calcium ionophore and to tetanus toxoid as cells from uninfected control subjects. The cells that proliferated in response to a soluble antigenic stimulus did not respond to gp120. Cells from subjects with ARC had a selective antigen recognition defect independent of the number of $CD4^+$ lymphocytes. Recombinant gp120 inhibited $CD4^+$ lymphocyte proliferation to antigenic stimulus by 30–40%. Recombinant soluble CD4, a proposed therapeutic for HIV, had no effect on T-cell response to antigen. A selective antigen recognition response was not compromised early in HIV infection but was compromised in subjects with ARC. Inhibition of proliferation to tetanus toxoid by gp120 suggests that HIV may affect major histocompatibility complex II restricted antigen recognition independent of $CD4^+$ cell loss.**

Initial descriptions of the acquired immunodeficiency syndrome (AIDS) included the recognition of a profound depletion in the $CD4^+$ T-lymphocyte population and inversion in the ratio of $CD4^+$ to $CD8^+$ T lymphocytes in the peripheral blood (1–3). Isolation of the human immunodeficiency virus (HIV) facilitated the development of methods for the identification of persons infected prior to the development of AIDS. Much has been learned about the long incubation time prior to the onset of clinical manifestations and some of the subtle symptoms of impaired host defenses prior to the onset of AIDS. Significant gaps remain, however, in our understanding of the pathophysiology of disease progression with HIV infection. The issue of whether HIV-infected hosts develop underlying defects in $CD4^+$ lymphocyte function prior to, or in addition to, a decrease in $CD4^+$ cell number remains unresolved. Such a functional immunologic defect in asymptomatic carriers, manifest as impaired lymphocyte proliferation in response to mitogenic or antigenic stimuli, may provide insight into the immunopathogenesis of AIDS.

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Although several studies have attempted to characterize lymphocyte dysfunction in patients who have developed AIDS, few have utilized purified populations of $CD4^+$ cells to evaluate whether an inherent functional defect exists independent of a decrease in $CD4^+$ lymphocyte number (4, 5). Though relatively little work exists on lymphocyte function early in HIV infection, a selective defect in soluble antigen recognition has been proposed as an early sign of T-lymphocyte dysfunction with HIV infection (6, 7). Prior studies have focused on generation of cytotoxic lymphocytes in response to antigen or evaluation of proliferative response to antigen (but not mitogen) in peripheral blood lymphocyte (PBL) cultures (8, 9).

The present study, designed to address the immunological effects of HIV infection prior to the development of AIDS, indicates that specific antigen recognition does occur early in HIV infection but is impaired in lymphocyte cultures from subjects with AIDS-related complex (ARC). We also demonstrate that T lymphocytes from HIV-infected subjects that are capable of an antigen-specific response to tetanus toxoid do not proliferate in response to purified recombinant HIV envelope glycoprotein gp120. To better understand the possible mechanisms of a selective antigen recognition defect, we tested the effects of the HIV envelope gp120 and its receptor, CD4, in this system. Recombinant gp120 can inhibit antigen recognition in purified populations of normal $CD4^+$ T lymphocytes. Soluble CD4, a candidate anti-HIV agent, had no effect on tetanus toxoid antigen recognition in PBLs or purified $CD4^+$ T cells from either normal or HIV-infected subjects. HIV infection may interfere with antigen recognition independent of $CD4^+$ T-lymphocyte depletion.

MATERIALS AND METHODS

Patient Selection. Subjects in the study were classified into three groups. All participants were vaccinated with tetanus toxoid within 1 year of donation and were evaluated by ELISA (Electro-Nucleonics) for the presence of antibody to HIV. Study subjects were (*i*) healthy HIV antibody-negative volunteers ($n = 9$); (*ii*) asymptomatic HIV antibody-positive volunteers ($n = 8$) with risk factors for HIV exposure being homosexuality ($n = 4$), heterosexual contact with an HIV-infected partner ($n = 2$), transfusion recipient ($n = 1$), and hemophilia A ($n = 1$); and (*iii*) patients who met the Centers for Disease Control–National Institutes of Health definition of ARC ($n = 6$) and AIDS ($n = 1$). Risk factors for patients in the ARC and AIDS group were homosexuality ($n = 5$),

Abbreviations: HIV, human immunodeficiency virus; PBL, peripheral blood lymphocyte; ARC, AIDS-related complex; APC, antigen-presenting cell; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; MHC, major histocompatibility complex.

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intravenous drug abuse (n = 1), and heterosexual contact of an antibody-positive individual (n = 1).

Preparation of Cell Populations. *PBLs.* PBLs were isolated from heparinized whole blood (240 ml) by density gradient centrifugation (Ficoll–Paque). PBLs were then depleted of adherent cells by overnight incubation in polystyrene flasks (Corning no. 25110). Nonadherent cells were washed and resuspended at 2 × 10^6 cells per ml in RPMI 1640 complete medium, which included 5% fetal calf serum (GIBCO), L-glutamine (2 mM), and penicillin/streptomycin (2 units/ml and 2 μg/ml).

$CD4^+$ T lymphocytes. Following density gradient centrifugation of PBLs, purified $CD4^+$ populations of T lymphocytes were isolated by normal sheep erythrocyte rosetting, followed by negative selection through $CD8^+$ lymphocyte depletion of the rosette-positive fraction (10). Equal volumes of PBLs and 5% sheep erythrocytes were incubated at room temperature and then centrifuged at 100 × g. Following centrifugation, the pellet was gently resuspended in RPMI 1640 medium, and erythrocyte rosette-positive and -negative fractions were separated by density gradient centrifugation. Sheep erythrocytes in the rosette-positive fraction were removed by lysis in a hypotonic ammonium chloride solution; the lymphocytes were then washed twice and incubated overnight. Erythrocyte rosette-positive cells were then incubated in monoclonal antibody (10 μg/ml) to CD8 (anti-Leu2a antibody, Becton-Dickinson, no. 6310) in phosphate-buffered saline (PBS). Polystyrene Petri dishes (Fisher, no. 8-757-12) were coated with 10 μg of goat $F(ab')_2$ anti-mouse IgG antibody per ml (Tago, no. 4240) for 40 min at room temperature in a solution of 0.05 M Tris buffer (pH 9.5) and then rinsed twice with PBS. Anti-CD8-labeled cells were then washed, plated onto the Petri dish in 1% fetal calf serum in PBS, and incubated at 4°C for 2 hr. Nonadherent cells were removed by washing gently with PBS. These cells were washed and resuspended in complete medium at 2 × 10^6 cells per ml prior to addition to experimental cultures. The $CD4^+$ T-lymphocyte populations were of 95% purity as analyzed by immunofluorescence with anti-Leu3a (Becton-Dickinson) monoclonal antibody.

Antigen-presenting cells (APCs). The erythrocyte-negative fraction of cells was used as APCs. Following erythrocyte rosetting, negative cells were washed, irradiated [4000 rads (1 rad = 0.01 Gy)], and resuspended in complete medium at a concentration of 6.5 × 10^5 cells per ml. Cells at a concentration of 5 × 10^4 per well were added to a 96-well plate (Costar, no. 3596) and allowed to adhere in the presence of tetanus toxoid for 1 hr at 37°C prior to the addition of responder cells.

Mitogen Stimulation of Cells. PBLs and $CD4^+$ T cells (1 × 10^6 cells per well) from each subject were incubated in 24-well plates (Costar, no. 3424) containing 1 ml of complete medium with one of the following mitogens: phorbol 12-myristate 13-acetate, 5 ng/ml (PMA; Sigma, no. P8139); phytohemagglutinin, 1 μg/ml (PHA, Burroughs-Wellcome, no. HA16); calcium ionophore, 1 μM (Ionomycin, Calbiochem, no. 407-952); PMA and PHA; or calcium ionophore and PMA. Duplicate wells of each of the above mitogen stimulants were incubated for 72 hr.

After 72 hr, cells were transferred to a 24-well plate (Costar, no. 3424) and pulsed for 6 hr with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine to measure proliferation. Cells were harvested by using a Cambridge Technologies P.H.D. cell harvester (Cambridge, MA) onto glass filter paper. Incorporation of radioactivity was assessed by liquid scintillation counting, and results from duplicate cultures were averaged.

Tetanus Toxoid Stimulation of Cells. The responding cells (PBLs or $CD4^+$ T lymphocytes at 2 × 10^6 cells per ml) from donors were incubated in 96-well plates (Costar, no. 3596) containing 0.2 ml of complete medium with one of the following combinations: (*i*) responding cells alone, (*ii*) responding cells and APCs, (*iii*) responding cells and 12.5 μg of tetanus toxoid per ml (Massachusetts Public Health Biologic Laboratory, lot no. LP-457PR), and (*iv*) responding cells, 12.5 μg of tetanus toxoid per ml, and APCs. Tetanus toxoid was extensively dialyzed prior to use and an optimal proliferative concentration was determined by prior titration using normal responder cells. Replicate wells were incubated for 7 days. At 6 days, duplicate wells were pulsed with 1 μCi of [^{3}H]thymidine and harvested on day 7 after 18 hr to test for proliferation. For the evaluation of the effects of purified recombinant gp120 (gift of Genentech) (11), purified soluble recombinant CD4 (12), and anti-Leu3a (Becton-Dickinson, no. 6320) on soluble antigen recognition, various concentrations of protein were added to the responding cells and incubated for 30 min at 37°C prior to the addition of tetanus toxoid. Cells were then incubated 7 days in the same combinations of tetanus toxoid and APCs as for detection of antigen stimulation.

Statistical Analysis. The nonparametric Mann–Whitney test of statistical significance was used in evaluating the results of mitogen and antigen stimulation. This test was chosen due to the sample size and the relatively large variance among individual donors' proliferative responses. HIV-infected populations were compared to normal controls only.

RESULTS

Mitogen Stimulation of PBLs and $CD4^+$ Cells. PBLs incubated for 3 days with mitogens proliferated equally well in asymptomatic HIV-infected individuals (n = 8) and ARC/AIDS patients (n = 5) as compared to HIV antibody-negative normal controls (n = 8, Fig. 1). Purified $CD4^+$ T lymphocytes incubated for 3 days demonstrated similar responses. Interestingly, a significant (P = 0.05) increased response to PHA was found in asymptomatic HIV-infected subjects when compared with normal controls. Increased responsiveness of PBLs from these asymptomatic HIV-infected subjects to

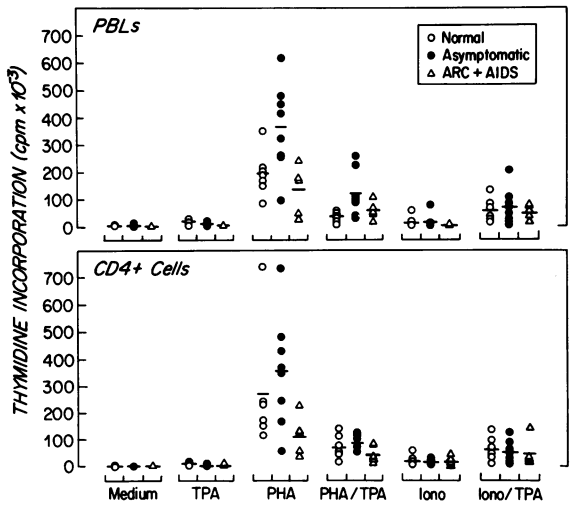

FIG. 1. Proliferation of PBL and $CD4^+$ lymphocytes in HIV antibody-negative (normal) subjects, asymptomatic HIV antibody-positive subjects, and ARC/AIDS patients in response to mitogens. Open or filled symbols represent independent donor responses; each bar represents the mean of all values. Proliferation is measured by the uptake of [^{3}H]thymidine for 6 hr on day 3. Cells (1 × 10^6) were stimulated in 1 ml of medium with one of the following combinations of mitogens: PMA (TPA), 5 ng/ml; PHA, 1 μg/ml; PMA and PHA; calcium ionophore (Iono), 1 μM; calcium ionophore and PMA; or medium alone. Sample numbers: normal, n = 8; asymptomatic, n = 8; and ARC and AIDS, n = 5.

PMA plus PHA was not found to be significant. No defect in response to PHA, PMA, and/or calcium ionophore was observed.

Tetanus Toxoid Stimulation of PBLs and CD4$^+$ T Lymphocytes. PBLs and purified CD4$^+$ T lymphocytes were tested for proliferative responses to soluble antigen with addition of medium alone, tetanus toxoid, APCs, and APCs with tetanus toxoid (Fig. 2). No significant difference was found in unstimulated proliferation between the patient groups. Asymptomatic HIV-infected individuals (n = 6) were found to have PBL or CD4$^+$ T-lymphocyte proliferative responses to tetanus toxoid antigen similar to those of normal controls (n = 7). ARC and AIDS patients' PBLs (n = 7) were found to have a lower response to tetanus toxoid with and without APCs as compared to normal controls (P = 0.05). A markedly decreased proliferative response was also seen in purified CD4$^+$ cell populations from ARC and AIDS patients (P = 0.01). PBL cultures from ARC and AIDS patients also displayed a significant decrease in response to APCs alone (autologous mixed lymphocyte response) (4), when compared to normal controls (P = 0.05).

Effects of Anti-Leu3a, CD4, and gp120 on Tetanus Toxoid Stimulation. Cells from normal controls and from HIV-infected subjects were incubated with several dilutions of recombinant purified gp120, soluble CD4, or anti-Leu3a (Figs. 3–5). Anti-Leu3a (Fig. 3) was found to inhibit proliferative responses to tetanus antigen by 50–80% in normal and HIV-infected subjects. Purified, soluble CD4 (Fig. 4) was found to have no effect on either PBL cultures or purified CD4$^+$ T-cell cultures from normal or HIV-infected subjects. Recombinant gp120 (Fig. 5) was found to decrease proliferative responses to tetanus by purified populations of CD4$^+$ T cells in normal and HIV-infected subjects, and the inhibitory effects on proliferation were found to diminish in a dose-dependent manner. gp120 alone, in medium control cultures, did not stimulate proliferation in control or HIV-infected subjects. Recombinant gp120 had no effect on antigen proliferative response with PBL cultures. HIV-infected subjects demonstrated either minimal or no proliferative response to recombinant gp120 in the absence of tetanus toxoid in either cultures of PBLs or purified CD4$^+$ T lymphocytes (Fig. 5). Proliferative responses of PBL and CD4$^+$ lymphocytes from patients with ARC (n = 3) were also tested for inhibition by soluble CD4, anti-Leu3a, and gp120 (results not shown). These patients all showed reduced antigen-specific proliferative responses, as compared to control or asymptomatic groups, making evaluation of further inhibition by the agents such as CD4 difficult. The patients did, however, appear to follow the same pattern of dose-dependent inhibition by anti-Leu3a and gp120 and no inhibition by CD4 as observed in the asymptomatic group.

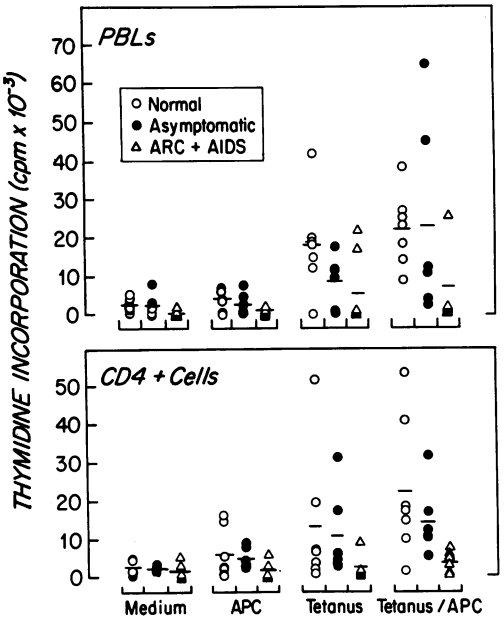

FIG. 2. Proliferative responses to tetanus toxoid of PBL and CD4$^+$ lymphocytes in HIV antibody-negative (normal) subjects, asymptomatic HIV antibody-positive subjects, and ARC/AIDS patients. Open or filled symbols represent independent donor responses; each bar represents the mean of all values. Proliferation is measured by the uptake of [^{3}H]thymidine for 18 hr on day 7. Cells (2 × 10^5) were stimulated in 0.2 ml of medium with one of the following combinations of antigen stimulation: responding cells alone; responding cells and APCs; responding cells and 12.5 μg of tetanus toxoid per ml; or responding cells, APCs, and tetanus toxoid. Autologous APCs were obtained from the erythrocyte rosette-negative fraction, irradiated with 4000 rads, and used at a 1:4 ratio to responding cells. Sample numbers: normal, n = 7; asymptomatic, n = 6; and ARC and AIDS, n = 7.

DISCUSSION

In evaluating the effects of HIV infection on lymphocyte function prior to the onset of AIDS, we included in our study asymptomatic HIV antibody-positive subjects, ARC patients, and HIV-negative control subjects. Test cell populations consisted of PBLs and purified CD4$^+$ T lymphocytes. Purified populations of CD4$^+$ T lymphocytes were obtained to comparatively evaluate their functional capacity independent of donor differences in cell number or the effects of other cell types. CD4$^+$ lymphocytes were obtained by using a combination of sheep erythrocyte rosetting and CD8$^+$ lymphocyte removal by panning to avoid potential effects of monoclonal antibody binding to the CD4 protein. PHA, PMA, calcium ionophore, and combinations of mitogens were used to determine whether a defect in response to mitogenic stimulation might exist. A single-agent stimulus or combinations of stimuli, such as calcium ionophore and lectins, have been shown in murine T-cell clones to differentially stimulate proliferative responses and/or lymphokine responses (13). In evaluating whether tetanus-immunized subjects early in the course of HIV infection might have a defect in recognition of tetanus toxoid, a combination of stimuli was also used. PBLs and purified CD4$^+$ T lymphocytes were exposed to medium, tetanus toxoid alone, APCs alone, and APCs with tetanus toxoid. In contrast to what has been reported elsewhere (6, 7), the proliferative responses of PBLs and purified populations of CD4$^+$ lymphocytes did not demonstrate a defect in T-lymphocyte function with early HIV infection. PBLs and CD4$^+$ lymphocytes from HIV-infected asymptomatic subjects exhibited similar responses to those of normal subjects with tetanus toxoid antigen stimulation. In response to PHA, PMA, and/or calcium ionophore, PBLs and CD4$^+$ T lymphocytes from asymptomatic HIV-infected subjects also demonstrated similar proliferative responses compared with normal subjects.

Production of HIV was also measured in supernatants from mitogenically or antigenically stimulated cells using the Abbott p24 enzyme immunoassay kit. No HIV production was found with this detection method in either PBL or CD4$^+$ T lymphocytes (results not shown). Perhaps, a more sensitive HIV detection system, such as polymerase chain reaction (14), might reveal a relationship between lymphocyte stimulation and viral activation.

In contrast to our findings with asymptomatic HIV-infected subjects, PBLs or purified CD4$^+$ T-lymphocyte cultures from ARC patients displayed a number of significant differences compared to normal controls. PBL responses to PHA, PMA, and calcium ionophore were similar to those of normal controls with prolonged exposure but were signifi-

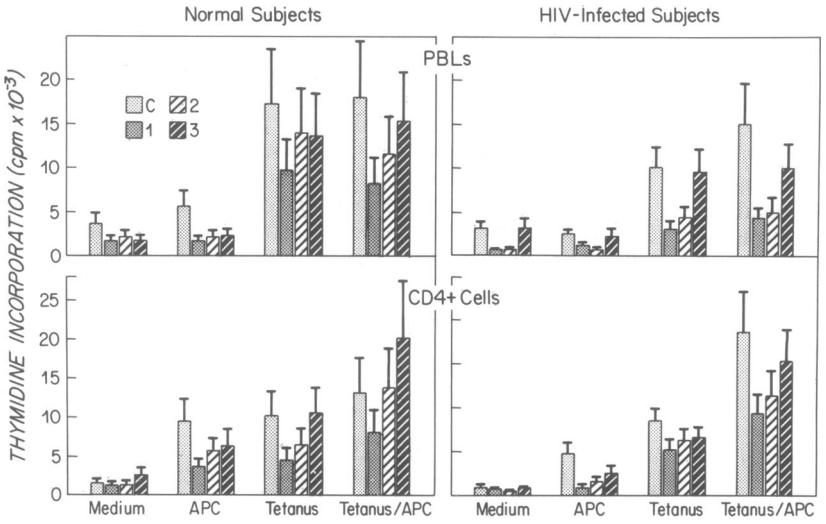

FIG. 3. Effect of anti-Leu3a on proliferative responses to tetanus toxoid antigen by PBL and $CD4^+$ cells in HIV antibody-negative (normal) subjects and asymptomatic HIV antibody-positive subjects. Striped bars represent the mean of all values, with standard errors (normal subjects, $n = 2$; asymptomatic HIV antibody-positive subjects, $n = 3$). Proliferation is measured by the uptake of [^{3}H]thymidine and expressed in terms of cpm per culture. See text for concentrations of tetanus toxoid and descriptions of APCs employed. Dilutions of anti-Leu3a: 1, 0.02 μM; 2, 4 nM; 3, 0.032 nM. C, control responses without the addition of anti-Leu3a.

cantly decreased on brief exposure to PHA (results not shown). Interestingly, PBLs from ARC patients, but not purified $CD4^+$ T-lymphocyte cultures, in medium alone demonstrated a significantly decreased level of resting proliferation compared to normal controls. This observation may indicate a baseline suppression of proliferation in ARC patients that could be diminished or negated with maximal stimulation (as with PHA). PBL cultures from ARC patients were also significantly different from normal PBLs with respect to tetanus antigen recognition. Proliferative responses were significantly diminished with APCs alone, tetanus toxoid alone, and APCs with tetanus toxoid. This decreased responsiveness was not, however, corrected in cultures adjusted to a constant number of purified $CD4^+$ T cells, arguing in favor of an intrinsic selective defect in antigen recognition.

Among the possible causes of a selective antigen recognition defect in HIV infection is the interference by gp120 with binding between the major histocompatibility complex (MHC) II and the CD4 protein. High-affinity binding by gp120 could interfere with MHC II restricted stimulation and diminish T-cell responses to antigen presentation (15). Studies of monoclonal antibody to CD4 that block HIV binding or that inhibit MHC II restricted antigenic T-cell stimulation show a close correlation between these two effects (7, 16–19). Thus, effective blockade of HIV binding and MHC II restricted proliferation is seen with anti-Leu3a, OKT4a, and OKT4b; no HIV blocking occurs with OKT4; and neither HIV blocking nor proliferation inhibition occurs with OKT4c. The possible role of HIV envelope protein in inhibiting antigenic response was evaluated by using recombinant gp120 and tetanus toxoid in normal PBL and purified $CD4^+$ lymphocyte cultures. Maximal inhibition of 30–40% of antigen-specific proliferation occurred with addition of gp120 to purified populations of normal $CD4^+$ T lymphocytes. Interestingly, this inhibition was not seen in PBL cultures. Similar levels of inhibition have been reported with monoclonal antibody binding to CD4 (17, 20).

Purified recombinant gp120 was also evaluated to determine its ability to induce a proliferative response in subjects previously exposed to virus (Fig. 5, medium and APC controls, and results not shown). Purified recombinant gp120 did not stimulate a proliferative response and inhibited tetanus-specific proliferation in these infected, asymptomatic subjects.

Soluble recombinant CD4, a proposed therapeutic agent for HIV infection, was evaluated for its possible inhibitory effects on MHC II-mediated antigenic stimulation (12, 20). Anti-Leu3a, a monoclonal antibody to CD4, was used as a positive control. Although anti-Leu3a was found to have inhibitory effects on our system similar to those reported elsewhere (17, 20), purified, soluble CD4 did not suppress tetanus toxoid antigen recognition by PBLs or $CD4^+$ T lymphocytes in either normal or HIV-infected subjects. This could be due to differences in the affinity of soluble versus membrane-bound CD4 for the MHC II complex protein.

Analysis of our study groups revealed few significant differences, and those were only between ARC patients and normal subjects. Such an analysis, however, might tend to invalidate small differences that could become statistically

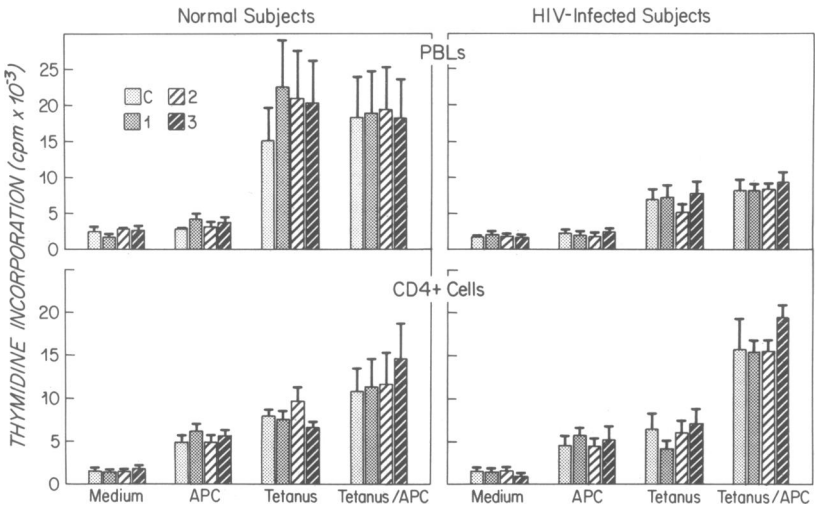

FIG. 4. Effect of soluble recombinant CD4 on proliferative responses to tetanus toxoid antigen by PBL and $CD4^+$ cells in HIV antibody-negative (normal) subjects and asymptomatic HIV antibody-positive subjects. Striped bars represent the mean of all values, with standard errors (normal subjects, $n = 2$; asymptomatic HIV antibody-positive subjects, $n = 3$). Proliferation is measured by the uptake of [^{3}H]thymidine and expressed in terms of cpm per culture. See text for concentrations of tetanus toxoid and descriptions of APCs employed. Dilutions of purified soluble CD4: 1, 0.1 μM; 2, 4 nM; 3, 0.032 nM. C, control responses without the addition of CD4.

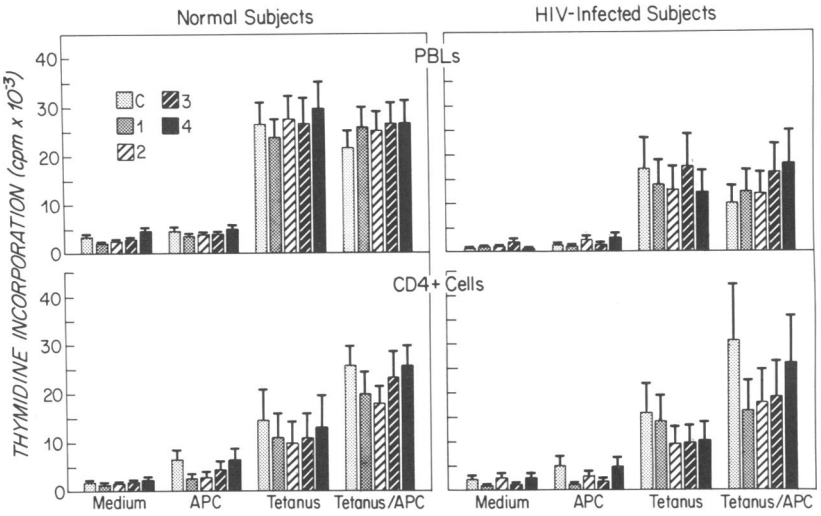

FIG. 5. Effect of recombinant gp120 (HIV envelope glycoprotein) on proliferative responses to tetanus toxoid by $CD4^+$ cells in HIV antibody-negative (normal) subjects and asymptomatic HIV antibody-positive subjects. Striped bars represent the mean of all values, with standard errors (normal subjects, $n = 6$; asymptomatic HIV antibody-positive subjects, $n = 3$). Proliferation is measured by the uptake of [^{3}H]thymidine and expressed in terms of cpm per culture. See text for concentrations of tetanus toxoid and descriptions of APCs employed. Dilutions of purified, recombinant gp120: 1, 0.1 μM; 2, 4 nM; 3, 0.16 nM; 4, 6.4 pM. C, control responses without the addition of gp120.

significant with larger sample sizes. Further testing with larger patient groups, therefore, may reveal subtle functional differences between T lymphocytes from asymptomatic HIV-infected subjects and those from normal controls.

Our data do not support a functional T-lymphocyte defect early in HIV infection. Functional defects in antigen recognition, however, do seem to exist in ARC patients. These antigen recognition defects were present in purified populations of $CD4^+$ T lymphocytes from the patients. Defects in antigen recognition, therefore, seem to develop with the progression of chronic HIV infection from asymptomatic stage to ARC.

A selective defect in antigen recognition, but normal response to mitogen stimulation, would suggest that the cellular response to activation was intact at a level following protein kinase C activation and inositol 1,4,5-triphosphate release of free calcium (21–24). Recently, normal T cells, infected by HIV *in vitro*, were shown to have a selective CD3/antigen receptor complex signaling defect (25). This type of defect is similar to one that we observed in ARC patient lymphocytes, presumably exposed to HIV *in vivo*. It is not clear whether this effect is due to dissociation of CD4 from the CD3/antigen receptor complex, as proposed by Linette *et al.* (25), but our observation of reduced antigen-specific proliferation in the presence of gp120 would be consistent with such a mechanism.

Our experiments on the effects of recombinant gp120, recombinant soluble CD4, and anti-Leu3a on the response to antigen support the possibility that HIV may interfere with immune recognition independent of $CD4^+$ T-lymphocyte depletion. These *in vitro* observations must be interpreted with caution because of the lack of data on the concentration or distribution of gp120 in different tissues in infected patients. Conversely, purified, soluble CD4 was shown to have no effect on *in vitro* tetanus toxoid proliferative responses in either normal or HIV-infected subjects. Further work should elucidate the roles in antigen recognition of MHC II molecule binding, gp120 binding, CD4-dependent cell membrane signal transduction, and CD4 modulation in the immunopathogenesis of HIV infection.

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