0022-1767/92/1489-2709$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 148, 2709–2716, No. 9, May 1, 1992
Printed in U.S.A.

# CD4 EPITOPE MASKING BY gp120/ANTI-gp120 ANTIBODY COMPLEXES

## A Potential Mechanism for CD4+ Cell Function Down-Regulation in AIDS Patients[1]

ALBERTO AMADORI,[2]* GIUSTINA DE SILVESTRO,† RITA ZAMARCHI,* MARIA LUISA VERONESE,* MARIA ROSARIA MAZZA,* GIAMPIETRO SCHIAVO,‡ MARINA PANOZZO,*§ ANITA DE ROSSI,* LUCIA OMETTO,* JAN MOUS,‖ ANDREA BARELLI,# ALFREDO BORRI,# LEOPOLDO SALMASO,** AND LUIGI CHIECO-BIANCHI*

*From the *Institute of Oncology, University of Padua, Italy; the †Blood Transfusion Service, Padua General Hospital; the ‡Institute of General Pathology, University of Padua; the §Interuniversity Center for Research on Cancer, IST Biotechnology Section, University of Padua; ‖Hoffmann-LaRoche, Basel, Switzerland; the #Infectious Disease Unit, Mestre General Hospital; and the **Infectious Disease Unit, Padua General Hospital, Padua, Italy*

**The in vitro suppressive effect of gp120 and gp120/anti-gp120 antibody is well known but not yet proven to operate in vivo. We report findings consistent with the presence of gp120/anti-gp120 antibody complexes on CD4+ lymphocytes from HIV-infected patients with advanced disease. PBMC from most AIDS patients showed selective masking of the CD4 epitope associated with the gp120 binding site; immunoprecipitation of PBMC with anti-CD4 mAb disclosed high amounts of IgG bound to CD4 receptors. Antibodies against HIV *env* proteins, but not other HIV products or CD4 Ag, were detected in purified CD4+ cell culture supernatants; in vitro culture was associated with normalization of both CD4 expression in PBMC and the lymphocyte proliferative response to anti-CD3. gp120 presence could not be directly demonstrated, but findings strongly suggested that CD4+ lymphocytes from most HIV-infected patients with advanced disease were covered with gp120/anti-gp120 antibody complexes, which are responsible for down-regulation of surface CD4 expression as well as functional lymphocyte impairment; this event may represent an important mechanism in the pathogenesis of HIV-associated immunodeficiency.**

The immunopathogenesis of AIDS is not yet completely clear. Although a body of virologic evidence indicates that HIV exerts a direct cytopathic effect on CD4+ T cells (1–3), many data indicate that HIV in itself is not entirely responsible for AIDS. The discrepancy between the spread of HIV in the organism (4–6) and the onset of immunodeficiency is thought provoking; moreover, defects in in vivo and in vitro immune function parameters are detected in most HIV-infected patients long before a decrease in the pool of circulating CD4+ cells becomes evident (7).

Viruses very often utilize lymphocyte surface molecules involved in cell function as receptors for infection (8); in particular, HIV binds to CD4 through its envelope protein gp120 (9, 10). CD4 interacts with nonpolymorphic determinants of MHC class II Ag and is associated with CD3 in the Ag response (11, 12). Given the central role of CD4 in immune function, any agent interfering with it will most likely upset the entire immune network; in both mice and humans, the addition of anti-CD4 Ab[3] to lymphocytes in vitro prevents cell activation through the TCR/CD3 complex (13–17).

This novel model of anti-CD4-mediated immunodepression might be relevant to AIDS pathogenesis. Anti-CD4 reactivity in HIV-infected patients may be generated through several mechanisms; we (18) and more recently others (19) advanced that binding of gp120/anti-gp120 Ab complexes could mimic anti-CD4 activity and thus contribute to the pathogenesis of HIV-associated immunodeficiency. Recent in vitro data indicated that incubation of normal CD4+ lymphocytes with gp120 and anti-gp120 Ab induced both CD4 down-regulation and functional cell impairment (20), but no direct demonstration that this mechanism is actually operating in vivo has been yet provided. Our studies to address this issue disclosed that CD4+ lymphocytes from most AIDS/ARC patients are covered in vivo by gp120/anti-gp120 Ab complexes, which cause profound CD4 expression modulation and functional cell impairment.

### MATERIALS AND METHODS

*Study population.* This study is based on 51 seropositive patients (all Caucasians and i.v. drug abusers with documented HIV infection), 12 seronegative patients with autoimmune disorders (SLE, sclerodermia) and circulating IC, and 10 seronegative immunodepressed bone marrow transplant recipients with low numbers (<10%) of circulating CD4+ cells; 33 healthy seronegative donors not at risk for AIDS served as controls. All seropositive patients underwent clinical and laboratory examination and were classified as asymptomatic, ARC, or AIDS. Informed consent was obtained in every case.

Received for publication June 17, 1991.
Accepted for publication February 14, 1992.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported in part by grants from Ministry of Health, AIDS Project; CNR, Target Project on Biotechnology and Bioinstrumentation, and Target Project FATMA; Ministry for University and Scientific and Technologic Research; Italian Association for Research on Cancer (AIRC). M.L.V. is a recipient of a fellowship from AIRC.
[2] Address correspondence and reprint requests to Alberto Amadori, M.D., Institute of Oncology, University of Padua, Via Gattamelata 64, I-35128, Padova, Italy.

[3] Abbreviations used in this paper: Ab, antibody; ARC, AIDS-related complex; IC, immune complex; MFC, mean channel of fluorescence; PCR, polymerase chain reaction; SN, supernatant; WB, Western blot; SPA-Seph, Sepharose-conjugated staphylococcal protein A.

*Cell separation.* PBMC were obtained from EDTA- or preservative-free heparin-anticoagulated peripheral blood as reported (21); cells were isolated by Ficoll-Hypaque (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden) gradient centrifugation, washed three times, resuspended in RPMI medium, and counted. PBMC were used as such or separated further.

To obtain $CD4^+$-enriched lymphocytes, monocytes were removed from PBMC by plastic adherence for 1 h at 37°C; subsequently, T cells were isolated by E-rosetting (22), and $CD8^+$ lymphocytes and residual monocytes were removed by immunomagnetic separation with OKT8 (Ortho Diagnostics, Raritan, NJ) and M3 (Coulter Electronics, Hialeah, FL) mAb, respectively, followed by incubation with anti-mouse IgG-coated magnetic beads (Dynabeads, Dynal A.S., Oslo, Norway), as described elsewhere (23). At subsequent cytofluorographic analysis, the resulting cell populations contained <1% monocytes, $CD8^+$ cells, and B lymphocytes and were ≥99% $CD2^+$ and ≥70% $CD4^+$.

To study the $CD4^+$ lymphocyte proliferative response to anti-CD3, $CD3^+CD8^+$ cell removal was necessary, inasmuch as these cells were predominant in PBMC from most AIDS patients and could mask the $CD4^+$ cell response. To this end, $CD8^+$ lymphocytes were removed from unfractionated PBMC by immunomagnetic separation with OKT8 mAb and Dynabeads as reported above; the resulting cell populations from AIDS patients contained 10 to 35% $CD4^+$ cells and from seronegative controls 35 to 70% $CD4^+$ cells; <1% contaminating $CD8^+$ cells were found throughout.

*In vitro lymphocyte culture.* Proliferative assays were performed in U-bottom microtiter plates (Costar Data Packaging, Cambridge, MA) as described (24) with minor modifications. Briefly, CD8-depleted PBMC were resuspended to $1 \times 10^6$/ml in RPMI medium supplemented with 10% FCS (GIBCO, Grand Island, NY; complete RPMI) and either stimulated immediately with anti-CD3 mAb (courtesy of F. Malavasi (25), 10 ng/ml) or precultured for 24 and 48 h in 24-well tissue culture plates (Costar) without mitogen; at the end of the preculture period, cells were recovered, washed twice, and stimulated as above. Forty-eight h after the initial mitogen exposure, [$^3$H] TdR (Amersham Corp., Buckinghamshire, U.K.; sp. act. 74 TBq/mM, 0.5 GBq/well) was added; plates were harvested 16 h later and processed as described (24).

In control experiments, CD8-depleted PBMC from seronegative donors were incubated for 1 h at 4°C alone and with rgp120 (100 $\mu$g/ml/$1 \times 10^6$ cells), prepared as described previously (26); the cells were then washed and incubated with protein G (Pharmacia)-purified IgG from seropositive donors (anti-HIV IgG; 50 $\mu$g/ml/$1 \times 10^6$ cells). After further washing, the cells were stimulated with anti-CD3 mAb immediately or after preculture as above.

*Supernatant production and WB analysis.* Enriched $CD4^+$ cells from seropositive and seronegative subjects were cultured in vitro ($1–25 \times 10^6$ cells/ml in different cases) for 24 h in complete RPMI in the presence of puromycin (Sigma Chemicals, St. Louis, MO; 10 $\mu$g/ml) to prevent Ig synthesis by possibly contaminating B cells; cell SN were recovered by low speed centrifugation and stored at −20°C until testing. In control experiments, enriched $CD4^+$ cells from seronegative subjects were incubated with rgp120 and anti-HIV IgG as above and kept in culture under the same conditions.

In a set of experiments, HIV-infected H9 cells were mixed in different proportions (from 100 to 0.001%) with noninfected H9 cells; subsequently, aliquots ($2 \times 10^6$ cells) of each cell mixture were incubated with 1 ml of HIV-positive serum for 30 min at room temperature, washed, and cultured in complete RPMI for 36 h. The SN were then collected and analyzed for anti-HIV Ab contents as described below.

To study the specificity of the IgG recovered from culture SN of enriched $CD4^+$ cells, r-CD4 (American BioTechnologies, Cambridge, MA) was run in SDS-PAGE (0.5 $\mu$g/lane) and blotted onto nitrocellulose paper (Hybond, Amersham); for HIV proteins, commercial strips (Du Pont, Wilmington, DE) were employed. Cell SN aliquots (0.5 ml) were incubated with nitrocellulose strips overnight at room temperature followed by incubation for 2 h with $^{125}$I-labeled anti-human IgG (Amersham; sp. act. 19–74 GBq); blots were then exposed to x-ray film with intensifying screen at −70°C for 15 days. Preliminary experiments showed that under these conditions WB assay could detect HIV-specific Ab up to a $4 \times 10^{-6}$ dilution of a standard HIV-positive serum (27).

HIV-specific and total IgG contents in culture SN from purified $CD4^+$ cells were assessed by solid phase RIA, as reported previously (27, 28).

*Cytofluorographic analysis.* Whole blood or isolated PBMC samples were analyzed by standard direct immunofluorescence with FITC-conjugated OKT3, OKT4, OKT8, and OKT11 (Ortho) and Leu-3a (Becton-Dickinson, Mountain View, CA) mAb on an Epics C cytofluorometer (Coulter); Ab were used in saturating conditions (5 $\mu$g/$2 \times 10^5$ cells). To compare results obtained in different experiments, the cytofluorometer was calibrated each time with standard fluorescent beads (Becton-Dickinson) according to the manufacturer's instructions. Results were expressed as MFC intensity on an arbitrary scale.

Unfractionated PBMC from AIDS patients and seronegative donors were also analyzed by simultaneous two-color immunofluorescence using phycoerythrinated anti-CD4 (OKT4) and FITC anti-human IgG (Ortho) mAb; results were represented in a three-dimensional model by computer analysis. In control experiments, PBMC from seronegative donors were incubated with rgp120 and anti-HIV IgG as described above before two-color cytofluorographic analysis.

In a set of experiments, PBMC from seropositive and seronegative subjects were isolated and kept in culture in complete RPMI; CD4 expression was evaluated immediately after isolation and at different culture times with Leu-3a mAb. In control experiments, whole blood samples from seronegative donors were incubated for 2 h at 4°C with rgp120 (50 $\mu$g/ml) and then for a further hour with anti-HIV IgG (50 $\mu$g/ml); subsequently, PBMC were isolated and analyzed immediately and after different times of in vitro culture for CD4 expression.

To quantify CD4 receptors per cell, CD4 expression in PBMC from seropositive patients and controls was analyzed cytofluorometrically using FITC OKT4 and Leu-3a mAb, as reported elsewhere (29) with slight modifications. Briefly, the fluorescence intensity of FITC Ab-stained cells and similarly sized FITC-labeled microspheres of known fluorescein content was compared; the effective fluorochrome/protein ratio for each mAb was calculated using the Simple Cellular Microbeads kit (Flow Cytometry Standards, Research Triangle Park, NC), according to the manufacturer's instructions. Results were expressed as absolute numbers of surface CD4 molecules available to the single mAb.

*Lymphocyte surface labeling and immunoprecipitation.* Enriched $CD4^+$ cells ($5–20 \times 10^6$) were washed twice with 1 ml of HBSS supplemented with 20 mM sodium phosphate, pH 7.5, resuspended in 0.5 ml of the same medium, and then labeled by the lactoperoxidase method (30) using 250 $\mu$Ci of $Na^{125}I$ (Amersham). The reaction was stopped by adding FCS (5% final concentration), and the samples were cooled at 4°C. After several washings with ice-cold HBSS, cells were lysed by adding 50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 100 $\mu$M PMSF, 10 $\mu$M L-p-tosylamino-2-phenylethyl chloromethyl ketone, 1 mM benzamidine, and 100 $\mu$M phenanthroline, pH 7.5. The extraction mixture was kept on ice for 30 min and then clarified by centrifugation at $12,000 \times g$ for 15 min. The resulting SN was immunoprecipitated by adding either 5 $\mu$l of OKT4 mAb and 100 $\mu$l of Sepharose-conjugated goat anti-mouse IgG (kindly provided by Dr. E. Cosulich, Genoa, Italy) or 100 $\mu$l of 10% (w/v) SPA-Seph, incubated for 6 h at 4°C, and centrifuged at $12,000 \times g$ for 2 min. The pellets were washed three times with 1 ml of HBSS and then dissociated by adding 40 $\mu$l of sample buffer (2% SDS, 10% glycerol, 10 mM Tris acetate, 0.1 mM EDTA, pH 6.8) and heated at 100°C for 2 min.

Polyacrylamide gradient slab gels (6–12%), prepared according to Laemmli (31), were run at a constant current of 8 mA for 15 h. The gels were stained and destained, dried on filter paper, and autoradiographed at −70°C using Kodak X-Omat films. Molecular mass standards included human IgG (150 kDa), rgp120 (100 kDa), and human rCD4 (55 kDa).

*Quantitative determination of HIV proviral copies.* The amount of HIV proviral copies in PBMC was determined by the PCR technique, as reported elsewhere (32). A standard reference curve was prepared using 8E51 cells, which contain one proviral copy per cell (33). Samples of standard reference cells and patients' cells were lysed as reported (4); 25 $\mu$l of cell lysate (corresponding to $1 \times 10^5$ cells) were mixed with 75 $\mu$l of PCR buffer containing a 20 nM concentration of each of four deoxynucleotide triphosphates, a 50 pM concentration each of primer SK29 and SK30 (34), and 2 U of Taq polymerase (Perkin-Elmer, Norwalk, CT) and then amplified in a DNA thermal cycler (Perkin-Elmer). Thirty cycles were performed at a denaturation temperature of 94°C, an annealing temperature of 50°C, and an extension temperature of 72°C. Each specimen was prepared and run in duplicate. To control the reaction and quality of DNA to be amplified, we used PCO3/PCO4 primers specific for the $\beta$-globin gene (35). Twenty $\mu$l of each amplified sample were electrophoresed and transferred to Nytran filters. Hybridization was achieved with a 5′ end $^{32}$P-labeled SK31 probe, and filters were exposed to x-ray films for 12 h.

Specific band intensity was determined by densitometer analysis (Ultrascan LX Enhancer Laser Densitometer, LKB, Bromma, Sweden); OD values of reference samples were plotted against the number of HIV-positive cells which corresponded to an equal amount of HIV proviruses. The relationship between OD values and the number of cells (proviruses) was tested by regression analysis. In the present set of experiments, OD values were linearly related up to 1000 HIV copies ($r = 0.99$). OD values of patient samples, compared with the

reference curve, gave the amount of proviruses/$10^5$ PBMC.

*Statistical analysis.* Data were managed by using the Wilcoxon's test and the Mann-Whitney U test where appropriate.

## RESULTS

*Expression of different CD4 epitopes in HIV-infected patients.* Besides the well known reduction in the circulating $CD4^+$ cell number, PBMC from some HIV-infected patients also showed (Fig. 1*A*) a decrease in MFC intensity of the CD4 epitope closely associated with the gp120 binding site, which is recognized by the Leu-3a mAb (36, 37). Significantly reduced Leu-3a MFC intensity values were seen in whole blood samples from 12 AIDS patients and from 7 out of 9 ARC patients but not in 7 asymptomatic subjects (Fig. 1*B*). Six AIDS patients also showed MFC intensity values 2 SD below the mean value in seronegative subjects with the OKT4 mAb, which is directed against a CD4 epitope not related to the gp120 binding site (36, 37); on the other hand, the MFC intensity of CD2, CD3, and CD8 showed no difference compared with control figures (Table I).

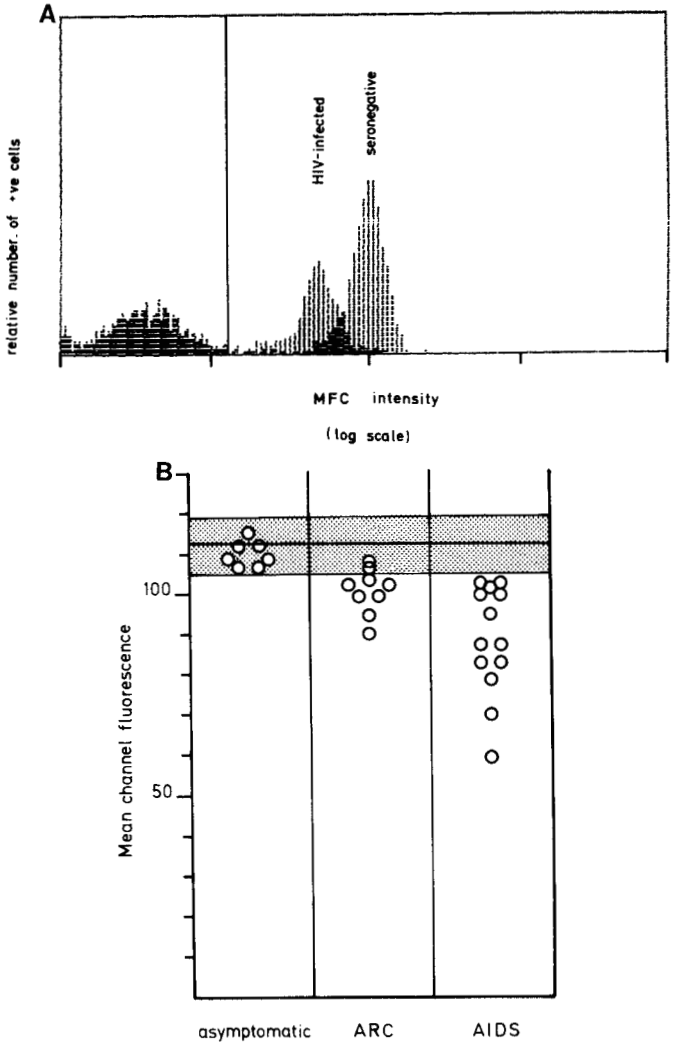


*Figure 1.* Cytofluorographic analysis of Leu-3a epitope expression in HIV-infected and noninfected subjects. *A*, PBMC from an HIV-infected and a seronegative subject were stained with Leu-3a mAb by standard methods; the two fluorograms were then superimposed in a single diagram. The *vertical axis* refers to the percentage of positive cells; the *horizontal axis* denotes the relative channel of fluorescence. The *vertical line* represents the setting of the negative background. *B*, EDTA-anticoagulated samples from 28 seropositive individuals with different stages of disease progression were tested by cytofluorographic analysis with the Leu-3a mAb; results were expressed as MFC intensity on an arbitrary scale. *Circles* denote individual patients; the *hatched area* represents MFC intensity values (mean ± 2 SD) in 26 healthy seronegative controls.

Leu-3a MFC reduction was strictly associated with HIV infection and was not observed in samples from seronegative, immunodepressed subjects with low numbers of circulating $CD4^+$ lymphocytes (Table I). Moreover, it was not caused by nonspecific CD4/IC interactions; even though a binding site for IC in close proximity to the gp120-binding site of CD4 was recently described (38), a decrease in Leu-3a MFC intensity was not observed in autoimmune patients (Table I), nor did in vitro incubation of normal PBMC with aggregated IgG or IC-containing sera change this parameter (not shown). This phenomenon could only be reproduced by incubating PBMC from seronegative donors with rgp120 and anti-HIV IgG. As shown in Figure 2*A*, the addition of as few as 0.3 $\mu$g of rgp120 induced a selective reduction in Leu-3a MFC intensity; on the other hand, higher amounts brought about a decrease in OKT4 MFC intensity (Fig. 2*A*). When rgp120-treated cells were incubated with anti-HIV IgG, virtually 100% of the $CD4^+$ cells were also $IgG^+$ (Fig. 2*B*).

*Quantitation of CD4 receptors per cell.* We then investigated whether the decrease in MFC intensity of CD4 truly reflected a reduction in the number of available CD4 molecules on the cell surface. Although the percentage of circulating $CD4^+$ cells was the same with both OKT4 and Leu-3a mAb, three patterns could be identified when the number of CD4 molecules per cell was calculated with the two different fluorescent probes (Table II). As expected, PBMC from asymptomatic carriers (patients 1, 2, and 3) had the same number of CD4 molecules per cell as control figures with both OKT4 and Leu-3a mAb; in 16 symptomatic individuals (patients 4, 5, and 6) the number of CD4 molecules calculated with OKT4 was within the normal range but was significantly reduced with Leu-3a; finally, in 11 ARC/AIDS patients (patients 7, 8, and 9 in Table II) the number of CD4 receptors per cell with both OKT4 and Leu-3a was decreased, with no apparent difference between the two values. This finding strongly suggested that Leu-3a epitope masking occurred in some individuals and most likely by soluble gp120 molecules or gp120/anti-gp120 Ab complexes. In testing the presence of IgG on $CD4^+$ lymphocytes from AIDS patients by two-color immunofluorescence with OKT4 and anti-human IgG mAb we found that 20 to 100% of the $CD4^+$ lymphocytes were double-positive in most individuals (Fig. 3). This observation is not surprising, however; several reports describe antilymphocyte Ab in AIDS (39–42), but their specificity has been only rarely established (43, 44).

*Immunoprecipitation with solid phase anti-CD4.* Next, we attempted to assess whether IgG were bound to CD4 receptors. Preliminary experiments in which enriched $CD4^+$ lymphocytes were immunoprecipitated with OKT4 and solid phase anti-mouse IgG followed by WB with radiolabeled anti-human IgG disclosed considerable amounts of IgG in samples from seropositive but not seronegative donors (data not shown); however, no 120-kDa bands recognized by anti-HIV IgG could be detected. In a more sensitive approach, $CD4^+$-enriched cells were surface iodinated with $Na^{125}I$ and lactoperoxidase, and the cell lysate was immunoprecipitated with OKT4 mAb and solid phase anti-mouse Ab or SPA-Seph. As shown

TABLE I

*Expression of different CD Ag on PBMC from HIV-infected patients and seronegative controls*[a]

| Patients | CD2 | CD3 | CD4 | | CD8 |
|---|---|---|---|---|---|
| | | | (OKT4) | (Leu-3a) | |
| HIV-infected | 90.4 ± 8.9[b] (71–104) | 113.4 ± 5.2 (104–126) | 84.1 ± 12.3* (54–101) | 97.1 ± 12.6* (57–109) | 121.3 ± 11.2 (105–137) |
| Seronegative donors | | | | | |
| BMT[c] | ND | ND | 102.2 ± 3.7 (97–108) | 106.3 ± 5.1 (102–113) | ND |
| AI[d] | ND | ND | 95.0 ± 5.7 (90–103) | 112.2 ± 7.9 (98–123) | ND |
| Healthy | 91.1 ± 8.2 (73–102) | 115.3 ± 11.1 (94–149) | 92.1 ± 9.3 (77–102) | 111.8 ± 3.1 (105–116) | 123.9 ± 14.9 (88–149) |

[a] Whole blood samples from different subjects were studied by standard immunofluorescence with FITC-labeled mAb against various surface Ag as detailed under *Materials and Methods*.
[b] Mean of MFC intensity value ± SD (range in parentheses). Asterisks denote p, values <0.001 compared with healthy controls.
[c] Bone marrow transplant recipients with low numbers of circulating $CD4^+$ cells.
[d] Autoimmune disease patients.

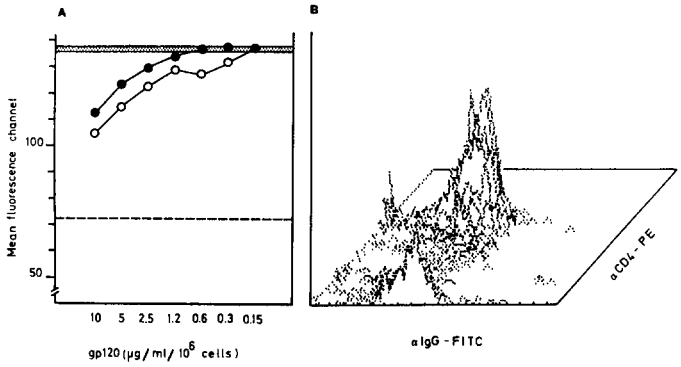


*Figure 2.* Effect of gp120 and gp120/anti-gp120 Ab on MFC intensity of normal $CD4^+$ cells. *A*, PBMC from a seronegative donor were incubated with the indicated concentrations of rgp120 as described under *Materials and Methods*, and CD4 expression was analyzed with OKT4 (*closed symbols*) and Leu-3a (*open symbols*) mAb. Results were expressed as MFC intensity on an arbitrary scale; the *dotted line* represents the setting of the negative background, and the *hatched area* indicates mean values ± 2 SD of six replicate samples of untreated PBMC. *B*, PBMC from a seronegative donor were incubated with rgp120 and anti-HIV IgG as detailed under *Materials and Methods* and analyzed by two-color immunofluorescence with FITC anti-IgG and phycoerythrinated (*PE*) OKT4 mAb. The fluorogram is represented in a three-dimensional form by computer analysis.

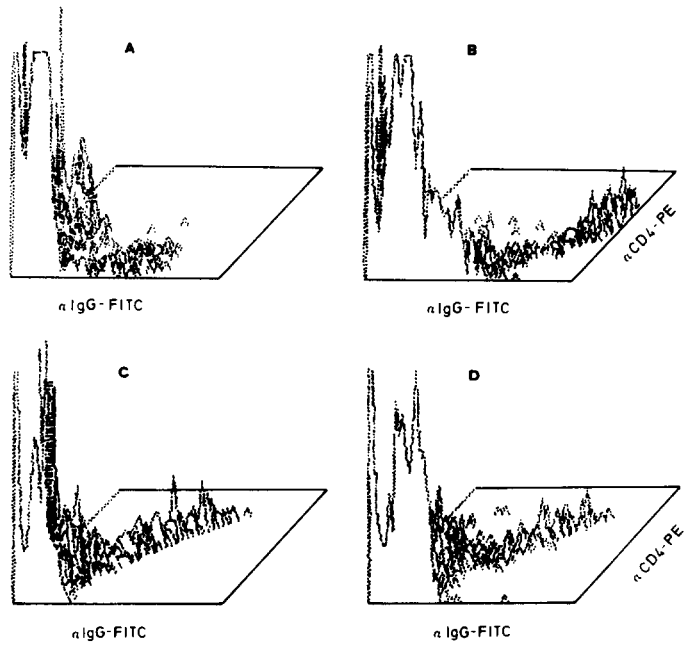


*Figure 3.* Two-color cytofluorographic analysis of PBMC from AIDS patients. PBMC from AIDS patients and controls were studied by two-color cytofluorographic analysis with OKT4 and anti-human IgG mAb. Results are represented in a three-dimensional shape by computer analysis. *A* is the pattern obtained in PBL from seronegative controls; *B*, *C*, and *D* are the patterns obtained in 3 AIDS patients, representative of 15 individuals tested. In these subjects, $CD4^+IgG^+$ lymphocytes accounted for 95, 66, and 50% of the circulating $CD4^+$ lymphocytes, respectively. *PE*, phycoerythrinated.

TABLE II

*Evaluation of CD4 receptors per cell in PBMC from seropositive patients and controls*[a]

| Patient No. | Clinical Status | % Circulating CD4[b] | No. of CD4 Molecules Calculated with | |
|---|---|---|---|---|
| | | | OKT4 | Leu-3a |
| 1 | Asymptomatic | 43.1 | 7.3[c] | 7.7 |
| 2 | Asymptomatic | 38.4 | 7.7 | 7.6 |
| 3 | Asymptomatic | 25.1 | 7.7 | 6.5 |
| 4 | AIDS | 3.0 | 6.0 | 3.9* |
| 5 | ARC | 4.8 | 7.1 | 4.6* |
| 6 | ARC | 21.2 | 7.1 | 5.2* |
| 7 | AIDS | 9.0 | 3.8* | 3.6* |
| 8 | ARC | 2.6 | 4.6* | 4.2* |
| 9 | AIDS | 1.0 | 3.2* | 2.3* |
| Seronegative donors | | 45.3 ± 11.4[d] (24.0–64.4) | 7.5 ± 1.4 (5.4–10.0) | 8.1 ± 1.4 (6.1–11.0) |

[a] PBMC were isolated and examined by cytofluorographic analysis; the number of CD4 molecules per cell was calculated as detailed under *Materials and Methods*.
[b] The results were obtained with the OKT4 mAb; no difference was seen with the Leu-3a mAb.
[c] No. of CD4 receptors per cell ($\times 10^{-4}$). Asterisks denote values <2 SD of the mean values in seronegative controls.
[d] Mean values ± SD (range in parentheses).

in Figure 4 (*left panel*), in cell lysates immunoprecipitated with OKT4 we could demonstrate a radioactive doublet of an apparent molecular mass of about 170 kDa, attributable to human IgG, in the samples from infected subjects (Fig. 4, *lane* $P_2$). This band was present only in trace amounts in samples from seronegative donors (*lane* $C_2$); a comparison of the IgG contents of the samples is shown in Figure 4 (*right panel*). A band of an apparent molecular mass of 120 kDa could also be seen in patients, but its identification as gp120 is uncertain. On the other hand, SPA-Seph immunoprecipitation resulted in the appearance of a much higher amount of material migrating in the region corresponding to human IgG in patients (*lane* $P_1$) but not in controls (*lane* $C_1$). Only a faint image in the region corresponding to human CD4 was observed; this is not surprising as only part of the $CD4^+$ cells was double-positive, and not all of the Leu-3a epitopes were

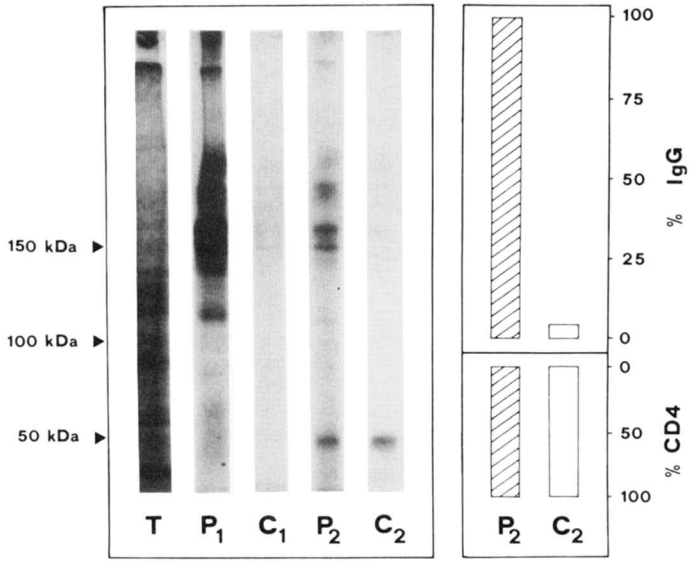


*Figure 4.* Immunoprecipitation of $^{125}$I-labeled PBMC from AIDS patients and seronegative controls with solid phase OKT4. Purified CD4$^+$ lymphocytes ($20 \times 10^6$) from seropositive (*P*) and seronegative (*C*) subjects were surface labeled with $^{125}$I and lactoperoxidase, extracted with detergent, and incubated with SPA-Seph (*lanes* $P_1$ and $C_1$) or with OKT4 mAb and Sepharose-conjugated goat anti-mouse IgG (*lanes* $P_2$ and $C_2$); immunoprecipitates were analyzed by gradient SDS-PAGE followed by autoradiography as detailed under *Materials and Methods*. *Lane T* represents total surface-labeled protein after TCA precipitation (*right panel*). The amounts of CD4 and IgG were evaluated by densitometric scanning and IgG contents normalized on the basis of CD4 concentration (*left panel*).

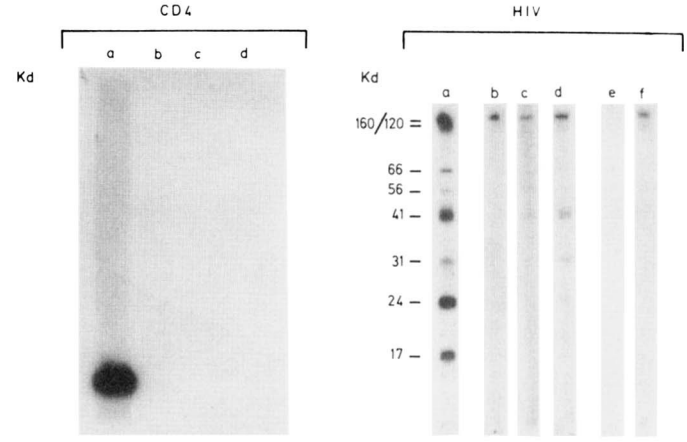


*Figure 5.* Specificity of the IgG recovered in culture SN of purified CD4$^+$ lymphocytes from AIDS patients. CD4$^+$ lymphocytes were purified as reported under *Materials and Methods* and kept in culture in complete RPMI in the presence of puromycin as detailed under *Materials and Methods*. Twenty-four-hour SN were collected by low speed centrifugation and tested for specificity against rCD4 (*left panel*) and HIV proteins (*right panel*). *Left panel: lane a* represents rCD4 decorated with anti-CD4 rabbit antiserum (American BioTechnologies) and $^{125}$I-labeled protein A (Amersham); *lanes b, c,* and *d* are representative of CD4$^+$ cell SN from 12 AIDS patients tested. *Right panel: lane a* shows the pattern of recognition of HIV proteins by pooled serum from HIV-infected subjects (molecular mass is indicated on the *left*); *lanes b, c,* and *d* show the results obtained in SN of cultured CD4$^+$ cells from 3 different AIDS patients out of 15 tested; *lane e* is representative of the pattern of SN of cultured CD4$^+$ cells from seronegative controls ($10 \times 10^6$); *lane f* shows the pattern observed in SN of normal CD4$^+$ lymphocytes ($10 \times 10^6$) preincubated with gp120 (10 $\mu$g/$10^6$ cells/ml) and anti-HIV IgG (50 $\mu$g/$10^6$ cells/ml) and then cultured as above. SN shown in *lanes b, c,* and *d* in both panels were obtained from the culture of 1, 2, and $12 \times 10^6$ CD4$^+$-enriched cells, respectively; the frequency of HIV-infected cells in the three patients, calculated by PCR as described under *Materials and Methods*, was 1/145, 1/190, and 1/490 circulating CD4$^+$ cells, respectively.

masked. A band of an apparent molecular mass of 120 kDa could be also observed, but its identification as gp120 is uncertain. Nonetheless, these findings seemed to indicate that at least part of the IgG present on patient CD4$^+$ cells was associated with CD4.

*Analysis of the specificity of IgG on CD4$^+$ cells.* To assess the specificity of the Ab bound to CD4$^+$ cells, IgG elution from enriched CD4$^+$ cells was attempted. In preliminary experiments variable amounts of IgG (700 pg–2 ng/$10^6$ CD4$^+$ cells) could be demonstrated in SN from 24-h culture of enriched CD4$^+$ cells from different individuals; RIA also revealed anti-HIV Ab presence in these SN (not shown). When SN were tested for reactivity against rCD4 and HIV proteins in a WB assay, no reactivity against CD4 was seen in SN from any of the subjects studied (Fig. 5, *left panel, lanes b, c,* and *d*). However, Ab recognizing gp120/160 were detected in SN from all the 15 AIDS patients tested (Fig. 5, *right panel, lanes b, c,* and *d*); IgG against gp41 were also seen in many subjects (*lanes c* and *d*), but anti-gp120/160 Ab prevailed. Ab against HIV proteins other than *env* (p24, p31), which would otherwise be expected if they were caused by nonspecific IC binding to the contaminating CD16$^+$ cells, were observed in only one SN (*lane d, right panel*), but in this case as well IgG against *env* proteins were predominant.

The possibility that anti-gp120 Ab detected in culture SN could derive from Ab bound to gp120 molecules expressed on the surface of virus-infected cells must be addressed. The percentage of HIV-infected cells in PBMC of the patients studied, determined by PCR analysis, was comparable to that reported (4, 6, 32) and ranged from <1/5000 to 1/145 circulating CD4$^+$ cells; the frequency in three representative patients is given in Figure 5. It is unlikely that these figures could be responsible for our results; in fact, when HIV-infected and noninfected H9 cells were mixed in different proportions, incubated with anti-gp120 IgG, and culture SN analyzed by WB, a faint band corresponding to anti-gp120 Ab could only be demonstrated when infected cells accounted for ≥10% of the cell population (data not shown). Thus, it is conceivable to advance that most anti-gp120 Ab detected in culture SN of enriched CD4 cells from patients probably represent Ab bound to CD4 via gp120 molecules.

*Effect of in vitro culture on CD4 expression and lymphocyte proliferative response to anti-CD3.* After *in vitro* culture, CD4$^+$IgG$^+$ double-positivity was progressively lost (not shown), and PBMC from all AIDS patients showed a rapid rise in Leu-3a MFC intensity which reached control levels within 24 to 36 h in most cases (Fig. 6, *closed symbols*); the time-dependent availability of the Leu-3a epitope also recalls the findings of Weinhold et al. (45) in gp120-treated normal CD4$^+$ lymphocytes. Similar results were obtained when PBMC were cultured in the presence of puromycin (not shown), thus indicating that shedding of molecules masking CD4 or receptor recycling from intracellular stores rather than de novo CD4 synthesis was responsible for the phenomenon.

The in vitro proliferative response to anti-CD3 stimulation of CD4-enriched PBMC from most AIDS patients was significantly reduced compared with seronegative controls (Fig. 7). When these cells were precultured for 24 to 48 h before stimulation the response to anti-CD3 mAb increased progressively and in most cases normalized to the levels of seronegative donors after 48-h preculture (Fig. 7; $p = 0.034$ compared with time 0 values). A similar behavior was observed in control experiments

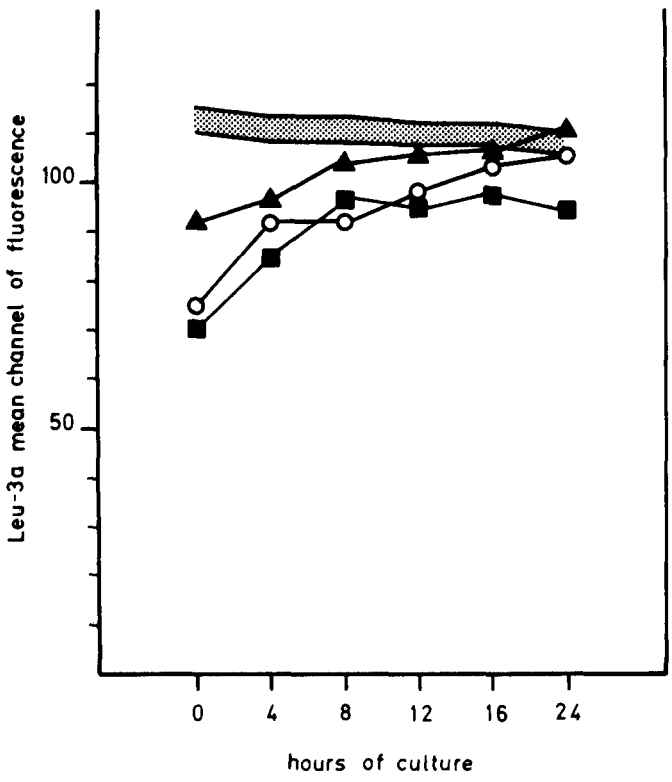


*Figure 6.* Effect of *in vitro* culture on Leu-3a MFC intensity in PBMC from seropositive patients. PBMC from AIDS patients (*closed symbols*) were isolated and cultured in complete RPMI; CD4 expression was evaluated with Leu-3a mAb before and at different intervals from the start of culture. The figure shows results obtained in two patients and is representative of eight consecutive experiments; the *hatched area* indicates the mean value ± 2 SD of PBMC from seronegative subjects, cultured *in vitro* and tested at the same intervals. In control experiments (*open symbol*), peripheral blood from a seronegative donor was incubated with rgp120 and anti-HIV IgG as described under *Materials and Methods*; PBMC were then isolated and tested for Leu-3a MFC intensity at the indicated times.

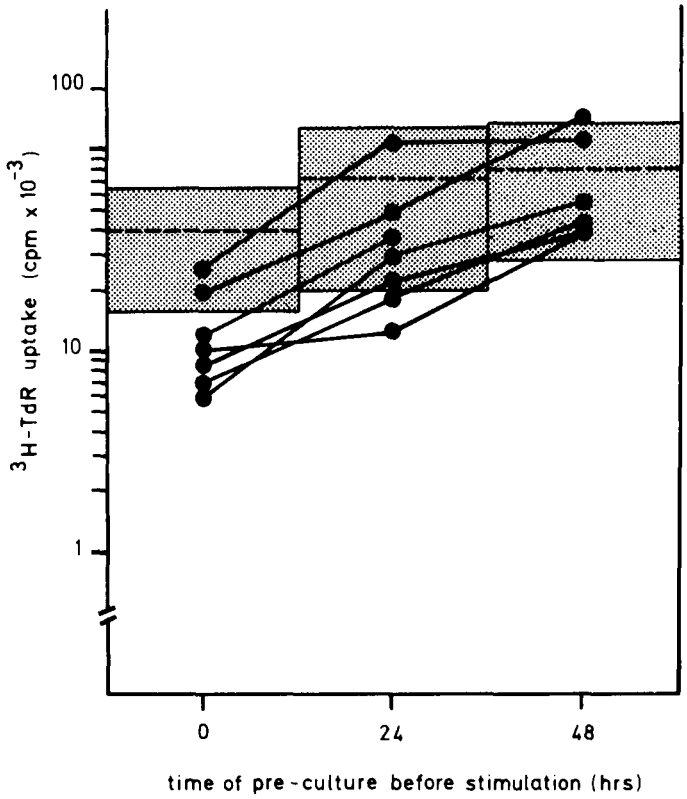


*Figure 7.* Effect of *in vitro* culture on the proliferative response of CD8-depleted PBMC from AIDS patients. PBMC from AIDS patients and controls were depleted in $CD8^+$ lymphocytes as detailed under *Materials and Methods* and either stimulated immediately with anti-CD3 mAb (time 0), or kept in culture for 24 and 48 h in complete RPMI before stimulation. Results were expressed as cpm obtained at the different times of culture; the *hatched area* represents mean values ± 2 SD of cpm observed in seronegative controls; *closed symbols* denote individual seropositive patients, and values obtained at different times in the same subject are connected by a *line*.

(not shown); PBMC from seronegative patients incubated with rgp120 and anti-HIV IgG before stimulation showed a markedly reduced proliferative response to anti-CD3 (35.2 ± 10% compared with untreated PBMC; $p = 0.02$), whereas no difference between treated and untreated cells was seen when PBMC were precultured for 24 to 48 h before mitogenic stimulation.

## DISCUSSION

The in vitro suppressive effect of gp120 on lymphocyte function is well known (45–48). The only study on the combined effect of gp120 and anti-gp120 Ab (20) documented a profound in vitro down-regulating effect of their interaction with CD4; we addressed the occurrence of this event in vivo and here provide evidence suggesting that PBMC from most ARC/AIDS patients are covered with gp120/anti-gp120 Ab complexes that modulate CD4 epitope expression and lymphocyte function.

It is unlikely that HIV infection of T cells is responsible for the modulation of MFC intensity of CD4 by causing intracellular CD4/gp120 association and reduced export of CD4 to the cell surface. Most of our symptomatic patients (patients 4, 5, and 6 in Table II) showed a selective reduction in the availability of the Leu-3a epitope; moreover, HIV infection is also associated with CD2 and CD3 modulation (49, 50), which was not observed in our patients; finally, very few infected cells circulate even in full-blown AIDS patients (4). Nevertheless, because some patients showed an overall reduction in CD4 expression (patients 7, 8, and 9 in Table II), we cannot exclude the selective survival in these subjects of a $CD4^+$ cell subpopulation expressing low CD4 concentrations. Because the HIV cytopathic effect is related to surface CD4 expression, lymphocytes displaying high amounts of this Ag would be exposed first to viral infection, cell-to-cell fusion processes, and eventual cell death (51). However, when PBMC from seronegative donors were incubated in vitro with high gp120 and anti-HIV IgG amounts, modulation of both OKT4 and Leu-3a MFC intensity occurred (Fig. 2); thus, further study is required to discern whether the overall decrease in CD4 expression observed in some patients is a result of a selective survival of a "dim" $CD4^+$ cell subpopulation or of different events such as receptor endocytosis after ligand/receptor interaction.

Our finding that considerable amounts of IgG were associated with CD4 could be a result of either anti-CD4 Ab or anti-gp120 Ab binding via gp120 molecules. The former explanation is improbable, as we did not find any reactivity against CD4 in our SN; although the presence of Ab directed against native CD4 determinants cannot be excluded, such Ab have not yet been found in infected individuals (52–54). The latter possibility is more likely, although we could not detect gp120 in culture SN of enriched $CD4^+$ cells by a sensitive sandwich RIA (data not shown) or by immunoprecipitation of radiolabeled

cells or directly on the cells by cytofluorographic analysis. In fact, very low numbers of $CD4^+$ lymphocytes could be obtained from most AIDS patients; and because only a part of $CD4^+$ cells were double-positive and not all the Leu-3a epitopes were masked, only a minimal amount of gp120 could possibly be recovered. On the other hand, highly glycosylated proteins are difficult to radiolabel, especially if they are coated by Ab. Finally, preliminary experiments showed that cell-bound gp120 was no longer recognized by anti-gp120 mAb when it was covered with anti-gp120 IgG (data not shown). Thus, the sensitivity limits of our detection techniques probably precluded direct demonstration of cell-associated gp120.

Nonetheless, the demonstration of anti-gp120 IgG in enriched $CD4^+$ cell culture SN constitutes compelling evidence for the presence of gp120/anti-gp120 Ab complexes bound to $CD4^+$ cell surface. Our findings seem to rule out that anti-gp120 Ab could derive primarily from Ab recognizing endogenously synthesized gp120 on infected T cells because in control experiments in which the number of infected cells was much higher than that found in our patients, anti-gp120 Ab were not evidenced in culture SN. Nonetheless, we cannot exclude that this phenomenon might possibly contribute in part to the SN anti-gp120 Ab contents.

On the other hand, the exclusive or predominant presence of anti-*env* Ab in the enriched $CD4^+$ cell SN seems to rule out the possibility that our findings could simply reflect nonspecific IC binding via Fc receptor to contaminating $CD16^+$ cells. Indeed, no gp120/anti-gp120 Ab complexes were demonstrated in serum from AIDS patients, but *gag* and *pol* products were found only in circulating IC (55, 56); given its high affinity for CD4 (57), it is probable that gp120 shed in body fluids binds immediately to its cellular receptor. Accordingly, the reduction in Leu-3a MFC intensity was apparently not related to factors present in sera inasmuch as PBMC from seronegative donors incubated with serum from patients displaying Leu-3a epitope modulation showed no MFC intensity change compared with cells incubated with HIV-negative sera (data not presented).

The phenomenon described here is strictly related to disease progression; it may constitute another piece in the AIDS immunopathogenesis mosaic (18, 58) and thus reconcile both virologic and immunologic findings. It is known that different HIV isolates show variable biologic activity, and highly replicating cytopathic strains have been found to be primarily associated with clinical disease (59, 60); moreover, peaks of circulating p24 Ag correspond with a sharp decline in the number of circulating $CD4^+$ cells, and p24 antigenemia is a strong predictor of disease progression (61, 62). The binding of soluble gp120 and anti-gp120 Ab to $CD4^+$ lymphocytes most likely impairs the efficiency of the cellular interactions involved in the immune response; in addition, gp120/anti-gp120 Ab-coated $CD4^+$ lymphocytes are probably exposed to C- and Ab-dependent cellular cytotoxicity (63, 64); finally, these complexes could mimic anti-CD4 Ab activity by causing CD4 receptor cross-linking and cell function impairment (13–17, 65).

*Acknowledgments.* We thank Dino Collavo and Maurizio Zanetti for friendly advice and criticism. We are grateful to E. J. Schlaeger, H. Doebeli and S. Noack for help in preparing recombinant gp120. The invaluable help of Mrs. Patricia Segato in the preparation of this manuscript is gratefully acknowledged.

REFERENCES

1. **Levy, J. A.** 1989. Human immunodeficiency viruses and the pathogenesis of AIDS. *JAMA 261:2997.*
2. **Nabel, G., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature 326:711.*
3. **Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, and E. G. Engleman.** 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature 323:725.*
4. **Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci.** 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science 245:305.*
5. **Graziosi, C., G. Pantaleo, L. Butini, S. Schnittman, P. Pizzo, D. P. Kotler, and A. S. Fauci.** 1991. Heavy viral burden in the lymphoid organs of HIV-infected patients in the early stages of disease. *The Laboratory of Tumor Cell Biology (NCI) 1991 Annual Meeting,* Bethesda, MD, September 1–8.
6. **Schnittman, S. M., J. J. Greenhouse, M. C. Psallidopoulos, M. Baseler, N. P. Salzman, A. S. Fauci, and H. C. Lane.** 1990. Increasing viral burden in $CD4^+$ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapid progressive immunosuppression and clinical disease. *Ann. Intern. Med. 113:438.*
7. **Shearer, G. M., D. C. Bernstein, K. S. K. Tung, C. S. Via, R. Redfield, S. Z. Salahuddin, and R. C. Gallo.** 1986. A model for the selective loss of major histocompatibility complex self-restricted T cell immune responses during the development of acquired immune deficiency syndrome (AIDS). *J. Immunol. 137:2514.*
8. **White, J. M., and D. R. Littman.** 1989. Viral receptors of the immunoglobulin superfamily. *Cell 56:725.*
9. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature 312:763.*
10. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J-C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature 312:767.*
11. **Meuer, S. C., S. F. Schlossman, and E. L. Reinherz.** 1982. Clonal analysis of human cytotoxic T lymphocytes: $T4^+$ and $T8^+$ effector cells recognize products of different major histocompatibility complex regions. *Proc. Natl. Acad. Sci. USA 79:4395.*
12. **Saizawa, K., J. Rojo, and C. A. Janeway.** 1987. Evidence for a physical association of CD4 and the CD3:$\alpha$:$\beta$ T-cell receptor. *Nature 328:260.*
13. **Ledbetter, J. A., C. H. June, P. S. Rabinovitch, A. Grossmann, T. T. Tsu, and J. B. Imboden.** 1988. Signal transduction through CD4 receptors: Stimulatory vs inhibitory activity is regulated by CD4 proximity to the CD3/T cell receptor. *Eur. J. Immunol. 18:525.*
14. **Carrera, A. C., F. Sanchez-Madrid, M. Lopez-Botet, C. Bernabeu, and M. O. De Landazuri.** 1987. Involvement of the CD4 molecule in a postactivation event on T cell proliferation. *Eur. J. Immunol. 17:179.*
15. **Goronzy, J. J., and C. M. Weyand.** 1989. Persistent suppression of virus-specific cytotoxic T cell responses after transient depletion of $CD4^+$ T cells in vivo. *J. Immunol. 142:4435.*
16. **Biasi, G., M. Mazzocchi, A. Facchinetti, M. Panozzo, P. Zanovello, D. Collavo, and L. Chieco-Bianchi.** 1990. Induction of Moloney murine sarcoma virus tolerance in adult mice by anti-CD4 monoclonal antibody treatment. *Cancer Res. 50:5703s.*
17. **Biasi, G., A. Facchinetti, M. Panozzo, P. Zanovello, L. Chieco-Bianchi, and D. Collavo.** 1991. Moloney murine leukemia virus tolerance in anti-CD4 monoclonal antibody-treated adult mice. *J. Immunol. 147:2284.*
18. **Amadori, A., and L. Chieco-Bianchi.** 1990. B-cell activation and HIV-1 infection: Deeds and misdeeds. *Immunol. Today 11:374.*
19. **Ameisen, J. C., and A. Capron.** 1991. Cell dysfunction and depletion in AIDS: The programmed cell death hypothesis. *Immunol. Today 12:102.*
20. **Mittler, R. S., and M. K. Hoffmann.** 1989. Synergism between HIV gp120 and gp120-specific antibody in blocking human T cell activation. *Science 245:1380.*
21. **Amadori, A., G. Faulkner-Valle, A. De Rossi, and L. Chieco-Bianchi.** 1988. Spontaneous in vitro production of virus specific antibody by lymphocytes from HIV-infected subjects. *Clin. Immunol. Immunopathol. 46:342.*
22. **Amadori, A., G. P. Faulkner-Valle, A. De Rossi, P. Zanovello, D. Collavo, and L. Chieco-Bianchi.** 1988. HIV-mediated immunodepression: In vitro inhibition of T-lymphocyte proliferative response by UV-inactivated virus. *Clin. Immunol. Immunopathol. 46:37.*
23. **Amadori, A., R. Zamarchi, M. L. Veronese, M. Panozzo, A. Barelli,**

**A. Borri, M. Sironi, F. Colotta, A. Mantovani, and L. Chieco-Bianchi.** 1991. B cell activation during HIV-1 infection. II. Cell-to-cell interactions and cytokine requirement. *J. Immunol. 146:57.*

24. **Amadori, A., P. Zanovello, E. Cozzi, V. Ciminale, F. Borghesan, U. Fagiolo, and G. Crepaldi.** 1988. Study of some early immunological parameters in aging humans. *Gerontology 34:277.*
25. **Funaro, A., G. C. Spagnoli, C. M. Ausiello, M. Alessio, S. Roggero, D. Delia, M. Zaccolo, and F. Malavasi.** 1990. Involvement of the multilineage CD38 molecule in an unique pathway of cell activation and differentiation. *J. Immunol. 145:2390.*
26. **Dirckx, L., D. Lindemann, R. Ette, C. Manzoni, D. Moritz, and J. Mous.** 1990. Mutation of conserved *N*-glycosylation sites around the CD4-binding site of human immunodeficiency virus type 1 gp120 affects viral infectivity. *Virus Res. 18:9.*
27. **Amadori, A., P. Gallo, R. Zamarchi, M. L. Veronese, A. De Rossi, E. Wolf, and L. Chieco-Bianchi.** 1990. IgG oligoclonal bands in sera of HIV-1-infected patients are mainly directed against HIV-1 determinants. *AIDS Res. Hum. Retroviruses 6:581.*
28. **Amadori, A., R. Zamarchi, V. Ciminale, A. Del Mistro, S. Siervo, A. Alberti, M. Colombatti, and L. Chieco-Bianchi.** 1989. HIV-specific B cell activation: A major constituent of spontaneous B cell activation during HIV-1 infection. *J. Immunol. 143:2146.*
29. **Rivas, A., S. Takada, J. Koide, G. Sonderstrup-McDevitt, and E. G. Engleman.** 1988. CD4 molecules are associated with the antigen receptor complex on activated but not resting T cells. *J. Immunol. 140:2912.*
30. **Ceplak, W., H. M. Gaudin, and L. Eidels.** 1987. Diphteria toxin receptor. *J. Biol. Chem. 262:13246.*
31. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature 277:680.*
32. **De Rossi, A., M. Pasti, F. Mammano, L. Ometto, C. Giaquinto, and L. Chieco-Bianchi.** 1991. Perinatal infection by human immunodeficiency virus type 1 (HIV-1): Relationship between proviral copy number in vivo, viral properties in vitro, and clinical outcome. *J. Med. Virol. 35:283.*
33. **Folks, T. M., D. Powell, M. Lightfoote, S. Koenig, A. S. Fauci, S. Benn, A. Rabson, D. Daugherty, H. E. Gendelman, M. D. Hoggan, S. Venkatesan, and M. A. Martin.** 1986. Biological and biochemical characterization of a cloned Leu-3$^-$ cell surviving infection with the acquired immune deficiency retrovirus. *J. Exp. Med. 164:280.*
34. **Ou, C-Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science 239:295.*
35. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of $\beta$-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science 230:1350.*
36. **Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverley.** 1986. Epitopes of CD4 antigen and HIV infection. *Science 234:1120.*
37. **McDougal, J. S., J. K. A. Nicholson, G. D. Cross, S. P. Cort, M. S. Kennedy, and A. C. Mawle.** 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: Conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J. Immunol. 137:2937.*
38. **Lenert, P., D. Kroon, H. Spiegelberg, E. S. Golub, and M. Zanetti.** 1990. Human CD4 binds immunoglobulins. *Science 248:1639.*
39. **Kloster, B. E., R. H. Tomar, and T. J. Spira.** 1984. Lymphocytotoxic antibodies in the acquired immune deficiency syndrome (AIDS). *Clin. Immunol. Immunopathol. 30:330.*
40. **Dorsett, B., W. Cronin, V. Chuma, and H. L. Ioachim.** 1985. Antilymphocyte antibodies in patients with the acquired immune deficiency syndrome. *Am. J. Med. 78:621.*
41. **Golding, H., G. M. Shearer, K. Hilman, P. Lucas, J. Manischevitz, R. A. Zajac, M. Clerici, R. E. Gress, R. N. Boswell, and B. Golding.** 1989. Common epitope in human immunodeficiency virus (HIV) I-gp41 and HLA class II elicits immunosuppressive autoantibodies capable of contributing to immune dysfunction in HIV I-infected individuals. *J. Clin. Invest. 83:1430.*
42. **Silvestris, F., B. S. Edwards, O. M. Sadeghi, M. A. Frassanito, R. C. Williams, Jr., and F. Dammacco.** 1990. Isotype distribution and target analysis of lymphocyte reactive antibodies in patients with human immunodeficiency virus infection. *Clin. Immunol. Immunopathol. 53:329.*
43. **Stricker, R. B., T. M. McHugh, D. J. Moody, W. J. W. Morrow, D. P. Stites, M. A. Shuman, and J. A. Levy.** 1987. An AIDS-related cytotoxic autoantibody reacts with a specific antigen on stimulated CD4$^+$ cells. *Nature 327:710.*
44. **Ardman, B., K. Mayer, J. Bristol, M. Settles, and E. Levy.** 1990. Surface immunoglobulin-positive T lymphocytes in HIV-1 infection: Relationship to CD4$^+$ lymphocyte depletion. *Clin. Immunol. Immunopathol. 56:249.*
45. **Weinhold, K. J., H. K. Lyerly, S. D. Stanley, A. A. Austin, T. J. Matthews, and D. P. Bolognesi.** 1989. HIV-1 gp120-mediated immune suppression and lymphocyte destruction in the absence of viral infection. *J. Immunol. 142:3091.*
46. **Mann, D. N., F. Lasane, M. Popovic, L. O. Arthur, W. G. Robey, W. A. Blattner, and M. J. Newman.** 1987. HTLV-III large envelope protein (gp120) suppresses PHA-induced lymphocyte blastogenesis. *J. Immunol. 138:2640.*
47. **Manca, F., J. A. Habeshaw, and A. G. Dalgleish.** 1990. HIV envelope glycoprotein, antigen-specific T-cell responses, and soluble CD4. *Lancet 1:811.*
48. **Habeshaw, J. A., A. G. Dalgleish, L. Bountiff, A. L. Newell, D. Wilks, L. C. Walker, and F. Manca.** 1990. AIDS pathogenesis: HIV envelope and its interactions with cell proteins. *Immunol. Today 11:418.*
49. **Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Cederbaum, and J. C. Reed.** 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science 234:1123.*
50. **Stevenson, M., X. Zhang, and D. J. Volsky.** 1987. Down-regulation of cell surface molecules during noncytopathic infection of T cells with human immunodeficiency virus. *J. Virol. 61:3741.*
51. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with human immunodeficiency virus. *N. Engl. J. Med. 317:278.*
52. **Thiriart, C., J. Goudsmit, P. Schellechens, F. Barin, D. Zagury, M. De Wilde, and C. Bruck.** 1988. Antibodies to soluble CD4 in HIV-1-infected individuals. *AIDS 2:345.*
53. **Chams, V., T. Jouault, E. Fenouillet, J-C. Gluckman, and D. Klatzmann.** 1988. Detection of anti-CD4 autoantibodies in the sera of HIV-infected patients using recombinant soluble CD4 molecules. *AIDS 2:353.*
54. **Kovalski, M., B. Ardman, L. Basiripour, Y. Lu, D. Blohm, W. Haseltine, and J. Sodroski.** 1989. Antibodies to CD4 in individuals infected with human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA 86:3346.*
55. **Morrow, W. J. W., M. Wharton, R. B. Stricker, and J. A. Levy.** 1986. Circulating immune complexes in patients with acquired immune deficiency syndrome contain the AIDS-associated retrovirus. *Clin. Immunol. Immunopathol. 40:515.*
56. **Carini, C., I. Mezzaroma, G. Scano, R. D'Amelio, P. Matricardi, and F. Aiuti.** 1987. Characterization of specific immune complexes in HIV-related disorders. *Scand. J. Immunol. 26:21.*
57. **Lasky, L. A., G. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. *Cell 50:975.*
58. **Rosenberg, Z. F., and A. S. Fauci.** 1989. The immunopathogenesis of HIV infection. *Adv. Immunol. 47:377.*
59. **Asjo, B., J. Albert, A. Karlsson, L. Morfeldt-Manson, G. Biberfeld, K. Lindman, and E. M. Fenyo.** 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. *Lancet 2:660.*
60. **Cheng-Meyer, C., D. Seto, D. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. *Science 240:80.*
61. **Murray, H. W., J. H. Godbold, K. B. Jurica, and R. B. Roberts.** 1989. Progression to AIDS in patients with lymphadenopathy or AIDS-related complex: Reappraisal of risk and predictive factors. *Am. J. Med. 86:533.*
62. **Lange, J. M. A., F. deWolf, and J. Goudsmit.** 1989. Markers for progression in HIV infection. *AIDS 3:S153.*
63. **Rook, A. H., H. C. Lane, T. Folks, S. McCoy, H. Alter, and A. S. Fauci.** 1987. Sera from HTLV-III/LAV antibody-positive individuals mediate antibody-dependent cellular cytotoxicity against HTLV-III/LAV-infected cells. *J. Immunol. 136:1064.*
64. **Tyler, D. S., C. L. Nastala, S. D. Stanley, T. J. Matthews, H. K. Lyerly, D. P. Bolognesi, and K. J. Weinhold.** 1989. gp120-specific cellular cytotoxicity in HIV-1 seropositive individuals. Evidence for circulating CD16$^+$ effector cells armed in vivo with cytophilic antibody. *J. Immunol. 142:1177.*
65. **Newell, M. K., L. J. Haughn, C. R. Maroun, and M. H. Julius.** 1990. Death of mature T cells by separate ligation of CD4 and the T-cell receptor for antigen. *Nature 347:286.*