HGP-30, a synthetic analogue of human immunodeficiency virus (HIV) p17, is a target for cytotoxic lymphocytes in HIV-infected individuals

Ammar Achour*, Odile Picard*, Daniel Zagury*, Prem S. Sarin†, Robert C. Gallo†, Paul H. Naylor‡, and Allan L. Goldstein‡

*Physiologie Cellulaire, Universite Pierre et Marie Curie, Unité Enseignement et Recherche 61, 4 Place Jussieu (Tour 32), 75005 Paris, France; †Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892; and ‡Department of Biochemistry and Molecular Biology, The George Washington University Medical Center, 2300 1 Street, NW, Washington, DC 20037

Communicated by Ludwik Gross, June 20, 1990

ABSTRACT
Evaluation of the immune response of individuals exposed to human immunodeficiency virus (HIV) is an important component of any plan designed to lead toward the development of an AIDS vaccine. Since the levels of antibodies to HIV p17 and the synthetic p17 peptide HGP-30 correlate with stages of progression to AIDS, studies were initiated to determine whether cytotoxic lymphocytes directed toward target cells pulsed with HGP-30 and radioactive chromium were present in seropositive individuals. The significance of such cells in controlling HIV viral infection has recently been enhanced by reports that HIV p17 is on the surface of infected cells and that an inactivated virus vaccine depleted of viral envelope appears to be effective in controlling expression. The selection of HGP-30 as the p17 peptide to be evaluated in early studies is based on the presence of both T-cell and B-cell epitopes as predicted by computer modeling and mouse studies and the demonstration of in vitro neutralization activity by antibodies to the epitope. By using B-lymphoblastoid cells pulsed with HGP-30 and radioactive chromium as autologous targets and mixed leukocyte culture-expanded peripheral blood lymphocytes as effectors, CD8+ cytotoxic T lymphocytes against HGP-30-coated targets were identified in seropositive individuals. In this report we demonstrate that a synthetic p17 epitope can be a target for major histocompatibility complex-restricted cytotoxic T lymphocytes in HIV-infected individuals.

Several approaches to the development of an AIDS vaccine are currently being explored, but to date a consensus of what is the appropriate protective response required for protection has not developed (1). An important part of such studies is the characterization of the host immune response, both humoral and cell-mediated, to human immunodeficiency virus type 1 (HIV-1). Defining the immune response is important since disease stabilization can be contrasted with disease progression and immunodominant epitopes for a large number of individuals can be defined (2–4).

With respect to the cell-mediated immune protection, cytotoxic T lymphocytes (CTLs) may be extremely important in the host defense mechanism against this infection since HIV transmission is often cell-associated, and the viral particles in that instance may not be easily accessible to neutralizing antibodies. CTL responses specific for the major viral proteins, env, gag, and pol, have been reported, and to date all could be of potential significance for protection since no correlation between CTLs against specific epitopes has been demonstrated (6–10).

Early studies have shown that a 30-amino acid synthetic HIV-1 gag p17-related peptide (HGP-30) is immunogenic in a variety of species and contains both T-cell and B-cell epitopes and that antibodies directed against the HIV-1 p17 epitope neutralize HIV-1 in culture (11–16). The antibodies directed against HGP-30 cross-react with HIV p17 as defined by Western blot (12). Also, monoclonal antibodies to HIV p17 bind to the surface of HIV-infected cells. These studies are confirmed by reports of HIV p17 on the cell surface of infected cells by using monoclonal antibodies (mAbs) whose epitope recognition has not been defined (18). In this report, cells from HIV-1-infected individuals were examined for the presence of CTLs specific for the HGP-30 epitope of HIV-1 p17.

MATERIALS AND METHODS
Antibodies. mAbs to human lymphocyte surface markers CD4 (OKT4) and CD8 (OKT8) were obtained from Ortho Diagnostics.

HLA Typing. HLA transplantation antigens were identified serologically at the tissue-typing laboratories of Hospital Saint-Louis (Paris) and Centre National de Transfusion Sanguine.

Lymphocyte Donors. Healthy HIV-seronegative controls were volunteers who received an HIV envelope vaccine (subjects 1 and 2) or HGP-30 core vaccine (subject AG) and an HIV-negative individual (subject 3) who was a donor from the Centre National de Transfusion Sanguine blood bank. Subjects 4–7 were asymptomatic HIV carriers; subjects 8 and 9 had AIDS-related complex.

Cells. Peripheral blood lymphocytes (PBLs) were isolated by Ficoll/Hypaque gradient centrifugation (Seromed, Berlin) from heparinized blood derived from healthy seronegative donors or HIV-positive individuals. All cells were stored in liquid nitrogen after slow freezing in fetal calf serum containing 10% dimethyl sulfoxide. Autologous Epstein–Barr virus (EBV)-transformed target cells were frozen and stored in a similar manner.

Lymphocyte Cultures. Human HIV-specific CTLs were generated in autologous mixed leukocyte culture (MLC) with

Abbreviations: CTL, cytotoxic T lymphocyte; HGP-30, 30-amino acid synthetic HIV gag p17-related peptide; HIV, human immunodeficiency virus; mAb, monoclonal antibody; MHC, major histocompatibility complex; MLC, mixed leukocyte culture; PBL, peripheral blood lymphocyte; EBV, Epstein–Barr virus.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
PBL from donors by mixing $1 \times 10^6$ PBL with $2 \times 10^5$ x-ray-irradiated (10,000 rad; 1 rad = 0.01 Gy) HIV-1-infected autologous phytohemagglutinin-stimulated blasts (15–30% of them expressed cell surface HIV-1 antigens as detected by immunofluorescence). Cultures were established in 24-well culture plates (Falcon, Becton Dickinson) in 2 ml of RPMI supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, penicillin at 100 units/ml, streptomycin at 100 $\mu$g/ml, and 10% fetal calf serum.

**HGP-30 Peptide.** The synthetic p17 peptide termed HGP-30 was provided by Viral Technologies (Washington, DC). This analogue is identical to the HIV-1 (ARV) p17 amino acid sequence from residue 88 to 115 (12). The peptide length was determined by the requirement that it contain the naturally occurring tyrosine in the viral protein sequence for subsequent labeling studies, that it be small enough for rapid and efficient synthesis, and that it be symmetrical around the region of homology with thymosin $\alpha_1$, where neutralizing antibodies were directed in initial studies (11).

**Transfected Mouse Tumor Cells.** P815 mouse tumor cells and P815 cells transfected with HLA-A2, P815-A2 cells (20), were grown in Dulbecco’s minimal essential medium supplemented with 10% fetal calf serum.

**Cytotoxicity assay.** Lymphocyte suspensions were tested for cytotoxicity after a 6-day culture in autologous MLC with a standard 6-hr $^{51}$Cr release assay (21). One hour after contact with peptide HGP-30 at a final concentration of 10 $\mu$g/ml ($=31$ $\mu$M), $4 \times 10^6$ EBV-transformed B cells were suspended with 200 $\mu$Ci of Na$_2^{51}$CrO$_4$ (1 Ci = 37 GBq) for 90 min at 37°C, washed extensively, and used as targets. Percent specific cytotoxicity was determined from the formula: $100 \times$ (release in assay − spontaneous release)/(maximum release − spontaneous release). Maximum release was determined by lysis with 1 M HCl. Average spontaneous chromium release for all assays was 20% of the maximum.

**RESULTS**

**Cytotoxic T-Cell Responses in HIV-Seropositive Individuals.** Evaluation of cytotoxic T cells in seropositive individuals to gag epitopes is generally difficult unless populations are expanded prior to utilization and autologous (histocompatible) targets are used. B lymphoblastoid cells were used as targets in an autologous chromium release assay, where lysis of labeled target cells results in release of chromium into the culture medium as an indirect measure of cytotoxicity. Immortalized B-cell lines were established from six HIV-1-seropositive and three seronegative controls, pulsed with HGP-30, and used as CTL targets. HIV-specific CTLs were generated in autologous MLC with irradiated HIV-1-infected autologous phytohemagglutinin-stimulated blasts and used as effector cells in the assay. The results for each subject group by clonal and serological status are presented in Fig. 1 at a representative effector-to-target ratio of 30:1. Table 1 shows the results obtained with different effector :target ratios. No HGP-30-specific responses were observed in seronegative individuals (Fig. 1A, subject 1) and in healthy controls receiving a HIV envelope vaccine (Fig. 1A, subjects 2 and 3). In contrast, cytotoxic responses were observed in a healthy seronegative volunteer receiving a HGP-30 subunit vaccination (Table 1, subject AG). In addition p-14, a control peptide corresponding to the HIV-1 gp160 envelope sequence 252–273, did not elicit cytotoxic responses (22).

In each of the seropositive subjects (Fig. 1 B and C), the HGP-30-specific cytotoxic response was 3- to 16-fold higher than that observed for the control target cells. Similarly, low background cytotoxicity of the EBV-transformed control cells were found in all cases except one of the seropositive subjects (Fig. 1C, subject 9). The reason for higher cytotox-

---

**Fig. 1.** Specific cytotoxicity for each subject at a fixed effector-to-target ratio (30:1). Subjects are grouped by serological status and disease category. (A) Seronegative controls. (B) Healthy HIV seropositive subjects. (C) AIDS-related complex and AIDS subjects. The solid bars represent the cytotoxicity against EBV-transformed subject lymphocytes alone. The crosshatched bars represent the cytotoxicity against the peptide HGP-30-pulsed targets. HLA serotypes of the subjects whose cells were used in these experiments were subject 4: A9(24), A29; B21(50), B12(44); subject 5: A9(23), A32; B21(50), B8; subject 6: A2, A11; B27, B37; C2, C6 BW4; subject 7: A2, A11; B60; subject 8: A9, A19-2, B8, B21, subject 9: A3, A9(23), B7, B12(40). ARC, AIDS-related complex.
Table 1. Elicitation of T-cell-mediated cytotoxic lymphocyte response to the synthetic p17 epitope (HGP-30) by PBLs in HIV-negative and HIV-positive individuals

<table>
<thead>
<tr>
<th>Subject no.*</th>
<th>Disease status (vaccine received)</th>
<th>% lysis at effector:target cell ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBS control†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30:1</td>
</tr>
<tr>
<td>1 (DZ)#</td>
<td>Healthy, HIV- (HIV envelope)</td>
<td>8.7</td>
</tr>
<tr>
<td>2 (G 90)#</td>
<td>Healthy, HIV- (HIV envelope)</td>
<td>11</td>
</tr>
<tr>
<td>AG‖</td>
<td>Healthy, HIV- (HGP-30 p17 subunit)</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Healthy, HIV-</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>HIV+</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>HIV+</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>HIV+</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>HIV+</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>ARC/AIDS</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>ARC/AIDS</td>
<td>50</td>
</tr>
</tbody>
</table>

HIV-specific human CTLs were generated in autologous MLC with PBLs from the seronegative or seropositive subjects as follows. Autologous phytohemagglutinin-stimulated blasts (2 × 10⁶) were incubated with live HIV, irradiated with x-rays (10,000 rad), and mixed with 1 × 10⁶ PBLs from the subjects. Cultures were used 6 days after initiation. Spontaneous ⁵¹Cr release by targets in medium alone varied between 15% and 20% of the cpm released by 1 M NaCl. Significant cell-mediated lysis response: experimental (HGP-30) – PBS control = 10% lysis of target cells. ARC, AIDS-related complex; ND, not determined; PBS, phosphate-buffered saline.

*Volunteer identification number (Paris).
†The target cells were EBV-transformed B cells that were incubated with phosphate-buffered saline.
‡The target cells were EBV-transformed B cells that were incubated with HGP-30 (10 µg/ml).
§The target cells were EBV-transformed B cells that were incubated with p-14 (10 µg/ml). p-14 corresponds to the HIV-1 gp160 envelope sequence (residues 252–273), which is not a CTL epitope (cf. ref. 22).
‖The individual was vaccinated and boosted with the gp160 envelope protein and has a cell-mediated lysis response against this protein but not p17 (HGP-30).

Conformation of the Histocompatibility Requirement of the Response. The ability of effector cells from seropositive subjects to kill HLA class I nonidentical target cells pulsed with HGP-30 was investigated with cells from three HIV-1 seropositive subjects. As shown in Fig. 4, lysis of autologous HGP-30-pulsed target cells was 40%, 38%, and 35%. In these studies, lysis of the allogeneic HGP-30-pulsed target cells was significantly lower—18%, 22%, and 15%. The fact that effector cells from three of the subjects tested caused preferential killing of the HGP-30-pulsed autologous target cells (compared to heterologous targets) indicates that the major histocompatibility complex (MHC) may be important in the cytotoxic response against the HIV-1 p17 peptide HGP-30.

To verify that HLA-A2 can be a restricting determinant for CTL to the HGP-30 peptide, we used the P815 mouse cell line, which is HLA-A and -B negative and its HLA-A2-transfected cell analogue, P815-A2, which is HLA-A2 positive. PBLs from subject 7, after stimulation with HIV antigens, were retested on the p815 and p815-A2 cell lines pulsed with or without the HGP-30 peptide. As shown in Table 2, P815-A2 cells, but not P815 cells, preincubated with HGP-30 were lysed by effector cells from the HLA-A2 subject 7.

DISCUSSION

To our knowledge, these results are the first to demonstrate that an epitope on the synthetic HIV-1 p17 peptide HGP-30 can be a target for MHC-restricted CD8+ cytotoxic T lymphocytes in HIV-infected individuals. The HIV-specific CTLs recognized the peptide HGP-30 in association with common class I HLA antigens. These studies are consistent with other investigations that have also reported gag-specific CTL responses in cells of some patients (8–10).

HIV-1 gag-specific CTLs defined with synthetic peptides have been described (9), and an epitope in the envelope glycoprotein that is recognized by H-2Dd murine CTLs has also been reported (19). It is likely that there are several other CTL-specific epitopes in other HIV gene products (6, 16, 18, 19, 21, 22).

Although Plata et al. (6) and Walker et al. (7) have reported the detection of circulating HIV-specific CTLs in asymptomatic HIV-positive patients and in patients with AIDS, no clear correlation can be established between the existence of circulating HIV-immune CTLs and resistance to AIDS. This was also true for recent studies identifying additional HIV targets such as gag, nef, and vif (10). Circulating HIV-immune CTLs also vary in number from one time to the next.
in the same patient. In order to have access to a stable source of active CTLs, we have used HIV-immune CTLs expanded from PBLs by using an autologous MLC system. Our results demonstrate the presence of HIV-specific cytotoxic T cells in persons infected with HIV. Despite the fact that the seropositive subjects are infected with heterologous HIV-1 genotypes (23), all exhibited an HIV-specific cytotoxic response against HGP-30, a synthetic HIV-p17 epitope of 30 amino acids.

Virus-specific CTLs directed against internal viral antigens have been reported in other viral infections (5, 17, 20) and presumably occur as a result of processing antigen, since it must be in the context of MHC class I. Less clear, however, is our understanding of the nature of viral epitopes recognized by CTLs on the surface of an infected target cell. The results presented in this paper are consistent with the view that all cells bearing class I molecules may be capable of degrading and presenting newly synthesized viral proteins to autologous CTLs.

In the studies of Nixon et al. (9), CTL responses were mapped to HIV p24 peptides. In more recent studies, CTL activity against the p14 region as well as the p24 region was reported. In that study using gag synthetic peptides, an epitope on HIV p14 was recognized by an HIV-1-specific HLA-A2-restricted human cytotoxic T-cell line. To our knowledge, this is the first study to use an HIV p17 peptide. Our data showing HLA-A2 restriction demonstrates that the synthetic peptide HGP-30 is MHC class restricted. Furthermore, the high incidence of HLA-A2 expression in the population indicates that this class I determinant for HIV CTL could occur in a high proportion of individuals. Further delineation of the role of MHC in the cytotoxic response as well as definition of alleles permissive for CTL lysis needs further investigation. These studies are especially significant since all subjects to date have demonstrated lysis of the identical histocompatibility matched autologous lymphocytes and antibodies to CD8 significantly reduced the cytotoxic activity.

These studies demonstrate that HIV-infected individuals make a potent CTL response to HIV antigens and that these CTLs may be important in controlling HIV infection. These

Table 2. HLA-A2 restriction of cytotoxic T-cell responses with transfected P815 tumor cells and PBLs

<table>
<thead>
<tr>
<th>Target cells</th>
<th>30:1</th>
<th>10:1</th>
<th>3:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>P815-A2</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>P815-A2 + HGP-30</td>
<td>70</td>
<td>53</td>
<td>25</td>
</tr>
<tr>
<td>P815 + HGP-30</td>
<td>12</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

PBLs from an HIV-seropositive patient (subject 7 in Table 1) were stimulated for 6 days in autologous MLC with x-ray irradiated HIV-infected lymphoblasts.

*The percent specific 51Cr release was calculated at three different lymphocyte-to-target cell ratios. Spontaneous 51Cr release ranged from 10% to 20%.
studies suggest that the HIV-1 p17 peptide HGP-30 may be an important candidate subunit vaccine either alone or in combination with other subunit vaccines designed to induce cell-mediated immunity against HIV infection by means of the induction of MHC-restricted cytotoxic lymphocytes.

These studies were supported in part by grants and/or gifts from The World Laboratory subproject MCD 2, the ARC-Villejuif, Viral Technologies Inc., and The Institute for Advanced Studies in Immunology and Aging.
