HIVgp120 Activates Autoreactive CD4-specific T Cell Responses by Unveiling of Hidden CD4 Peptides During Processing

By Simonetta Salemi, Anna Paola Caporossi, Laura Bozza, Maria Grazia Longobardi, and Vincenzo Barnaba*

From the Medicina Interna I, Istituto Clinica Medica, Policlinico "Umberto I," Università di Roma "La Sapienza," 00161 Roma, Italy

Summary

T cells are made tolerant only to those self-peptides that are presented in sufficient amounts by antigen-presenting cells. They ignore cryptic self-determinants, such as either those not generated by processing machinery or generated in insufficient amounts. It is anticipated that mechanisms that either change antigen processing or increase the yield of previously "invisible" peptides may be capable of inducing T cell priming and, if they are self-maintained, may sustain autoimmune diseases. Herein, we demonstrate for the first time a mechanism by which the gp120 human immunodeficiency virus-I, by downregulating plasma membrane CD4 and increasing its processing, unveils hidden CD4 epitopes, inducing an autoimmune-specific T cell response.

T cell tolerance to self-antigens is sustained either by elimination of self-reactive T lymphocytes in the thymus (clonal deletion) or through peripheral tolerance mechanisms, such as clonal anergy or downregulation of TCR (1, 2). Both mechanisms are dependent on the ability of APC to process self-peptides, and the capacity of a self-determinant to bind with high affinity the MHC molecules (immunodominant peptide) and to be consequently recognized by specific autoreactive T cells (3). Therefore, normally naive (virgin) T cells, specific for "invisible" self-determinants, ignore them for lack of a productive "meeting." The precise events by which an "invisible" self-antigen become "visible" for the immune system are not clear, although different mechanisms have been implicated in this phenomenon. For example, it has been suggested that either cross-reactive non-self-antigens, complexes of self- and non-self-antigens, infections, or general inflammatory processes may alter the self-antigen processing, facilitating the generation and presentation of previously cryptic T cell epitopes (4-8).

Here, we demonstrate a novel mechanism by which a viral antigen, such as the HIVgp120, unveils hidden CD4 peptides by changing CD4 processing, thus making them "visible" to autoreactive specific T cells.

Materials and Methods

Reagents. Soluble recombinant CD4 and gp120 proteins, expressed in CHO cells and baculovirus, respectively, were purchased from Intracel Corp. (Cambridge, MA). The analogous CD4 peptides were synthesized by solid-phase method on an automated multiple peptide synthesizer (AMS 422; Amed, Langenfeld, Germany) using F-moc chemistry. The purity of peptides was determined by reverse-phase HPLC. Peptides were diluted to a concentration of 2 mg/ml and stored at −20°C.

The following anti-CD4 mAbs were used for CD4 modulation: 6D10 (9), 10A12 (10, 11), and OKT4B (12) are specific for V1, V1-2, and V2-3 domains of CD4, respectively. The following anti-class I mAbs were used for the blocking experiments: anti-DP (IgG1, B7.21), anti-DQ (IgG2a, SPVL3), anti-DR (IgG2a, L243). FITC-labeled second antibodies and OKT4-FITC were purchased from Southern Biotechnology Associates (Birmingham, AL) and Ortho Diagnostic Systems, Inc. (Raritan, NJ), respectively.

Cell culture: Antigen-specific T cell clones and EBV-transformed B cell lines were isolated and maintained as previously described (12). EBV-B cells or T cell clones, used as APC, were pulsed (4 h) with different concentrations of recombinant soluble CD4, washed, and irradiated (13,000 and 3,000 rad, respectively). Alternatively, APC were pulsed overnight with increasing concentrations of either recombinant gp120 or different anti-CD4 mAbs. APC (5 × 10⁴) were then cultured for 72 h with 3 × 10⁴ antigen-specific cloned T cells in 200 μl RPMI-10% FCS (Hyclone Laboratories Inc., Logan, UT) in flat-bottomed microtiter plates (Falcon Plastics, Cockeysville, MD) in triplicate. 18 h before harvesting the cultures, 1 μCi [3H]thymidine (Amersham Corp., Amersham, UK) was added and the radioactivity incorporated by cells determined as described (12). Cloned T cells, used as APC, were taken up no more than 6-10 d after the last stimulation, when they can efficiently present soluble antigens (13). In some experiments, the presenting T cells were pulsed with either anti-CD4 mAbs or gp120, washed, stained with OKT4-FITC, and analyzed with direct immunofluorescence using a FACScan®.

Results and Discussion

Recently, we demonstrated that peripheral blood T lymphocytes of some HIV-infected patients proliferate in response...
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to a soluble form of the CD4 molecule (data not shown). Fig. 1 shows representative T cell clones, generated from one such CD4-responsiveness individual, proliferating in response to increasing concentrations of CD4 (Fig. 1, A–C) in the context of HLA-DR11 allele (Fig. 1, D–F). Indeed, CD4 presentation is strongly inhibited only by anti-DR mAb in blocking experiments, and particularly among a large panel of homozygous EBV-B cell lines with known haplotype, only those DR1101* or DR1104* are able to present soluble CD4. In addition, the fine specificity study, using overlapping synthetic peptides spanning the entire CD4 molecule, demonstrates that the clones recognize two adjacent peptides (amino acids 73–88 and 81–96, respectively; Fig. 1, G–I). We wondered whether these clones were able to recognize not only CD4 peptides generated by processing of exogenous CD4 molecules, but also those derived from plasma membrane CD4 since it may be internalized by CD4+ cells and sorted to the endocytic pathway (14–16).

Fig. 2 A shows that irrelevant autologous CD4+ T cell clones, used as APC, are able to present soluble CD4, as well as autologous EBV-B cells (Fig. 1), confirming that human class II+ T cells, particularly when recently activated, can efficiently capture and present soluble antigens (13). On the contrary, activated T cells, although expressing CD4, are unable to present it and can present only exogenous CD4, demonstrating that in normal conditions the epitopes derived from both plasma membrane and endogenous CD4 are not generated during processing (Fig. 2 B). It is possible, however, that in conditions in which membrane CD4 is downregulated, the immunogenic CD4 peptides may be generated in sufficient amounts and be efficiently presented on class II molecules to CD4-specific T cells. To evaluate the possibility that membrane CD4 processing could be altered by amplifying CD4 internalization (3, 6, 17–20), we tested the APC efficiency of autologous activated CD4+ T cells, which were previously pulsed with increasing concentrations of either soluble HIVgp120 antigen or anti-CD4 mAbs, both of which can drastically downregulate plasma membrane CD4 on cells (Fig. 3, a and b). More important, Fig. 3 shows that CD4+ APC pulsed with either gp120 or anti-CD4 mAb effectively acquire the capacity to stimulate a CD4-specific T cell clone in a dose-dependent fashion, indicating that the modulation

Figure 1. Antigen-specific proliferative response of class II-restricted T cell clones to a soluble form of CD4 molecule and fine specificity studies. (A–C) Proliferative response of three representative T cell clones (CP12, CP20, and CP50) derived from a CD4 responder individual (HLA A3, 10 [26]; B8, 51; Cw5; DR5 [11]; DQ3) to increasing concentrations of soluble CD4, presented by an autologous EBV-B cell line. (D–F) HLA restriction of CD4-specific T cell clones, using as APC either autologous EBV-B cells (in the presence of absence of different anti-class II mAbs) or a series of homozygous EBV-B cell lines with known haplotype, previously pulsed (solid bars) or not (open bars) with soluble CD4. (G–I) Fine specificity of CD4-specific T cell clones. CD4-specific T cell clones were cultured with autologous EBV-B cells in the presence or absence of synthetic overlapping 16-mer peptides spanning the entire CD4. The sequence of stimulatory peptides are as follows: peptides 73–88 (PSKLNDRADSRSLWD); peptides 81–96 (DSRRSLWDQGNPLLI).
of membrane CD4 by both polyvalent and monovalent specific ligands leads to a more efficient internalization and to changes in CD4 processing. In contrast, autologous CD4+ APC, like EBV-B cells or cloned CD8+ T cells, although pulsed with the same reagents, are incapable of inducing the proliferation of CD4-specific T cells, ruling out the possibility that the response was caused by cross-reactive peptides generated by processing of gp120 or anti-CD4 mAbs (Fig. 3). In addition, CD4+ APC, which were previously pulsed with an irrelevant mAb, such as the anti-transferrin receptor (CD71), which is known to modulate its own membrane ligand on cells (19), do not stimulate the CD4-specific T cells (not shown).

Experiments on antigen processing reveal that both chloroquine treatment and glutaraldehyde fixation of presenting CD4+ T cells drastically abrogate the capacity of APC function, supporting the notion that membrane CD4 is processed through the endocytic pathway (Table 1). Recently, it has been reported that class II molecules available for peptide loading are selectively confined to specialized class II-containing vesicles (CIIV) of APC, which appear only partially accessible to endosomal content (21-24). Since membrane CD4 has been suggested to spontaneously enter CIIV, presumably directed by a dileucine motif (15, 25-27), the enhanced internalization of membrane CD4 by its own ligands may more efficiently dictate its localization to CIIV, increasing the efficiency of antigen processing and presentation.

In conclusion, our results indicate that in normal conditions, the immunogenic CD4 peptides derived from both membrane and endogenous CD4 are not generated in sufficient amount during processing, and the CD4-specific T cells remain in a state of ignorance in the periphery. In contrast, either gp120 or anti-CD4 mAb, increasing the delivery of membrane CD4 into the endocytic pathway of activated CD4+ T cells, and maybe of more specialized CD4+ APC (28), allow the generation of an increased yield of previously invisible CD4 peptides available to bind class II molecules and to prime naive CD4-specific class II-restricted T cells.

These data suggest a new pathogenetic mechanism in some AIDS patients in which both the high blood concentrations of gp120 (29) and the anti-CD4 autoAbs (30-32) may operate as previously discussed, triggering an autoimmune T cell response against CD4+ cells (e.g., T cells, dendritic cells, macrophages) taking part in the severe dysfunction of these cells (33, 34). The T cell response to CD4 could be started by HIVgp120 and self-perpetuated, irrespective of the presence of gp120, by a vicious circle maintained by anti-CD4 autoAbs sensitizing CD4+ APC to activate CD4-specific T cells, which in turn may boost the production of anti-CD4 autoAbs.

Finally, our results may represent a general model of autoimmune, suggesting that a non-self-antigen, such as gp120, altering the processing of a self-antigen (i.e., the CD4), can unveil hidden immunogenic self-peptides, awakening specific autoreactive T and B cells from their lethargy.

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Address correspondence to Vincenzo Barnaba, Medicina Interna I, Istituto I Clinica Medica, Policlinico "Umberto I," Università di Roma "La Sapienza," via del Policlinico n. 155, 00161 Roma, Italy.

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