AN IMMUNODOMINANT CLASS I-RESTRICTED CYTOTOXIC T LYMPHOCYTE DETERMINANT OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INDUCES CD4 CLASS II-RESTRICTED HELP FOR ITSELF

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Th cells are involved in induction of CTL (1). As in the case of hapten-carrier conjugates, it is generally assumed that CTL, and the Th cells that help them, respond to different epitopes. This expectation is supported by the fact that in most cases, Th cells express CD4 and are restricted by class II MHC molecules, whereas conventional CTL express CD8 and are restricted by class I MHC molecules. Targeting different classes of MHC molecules increases the likelihood of recognition of different processed antigen fragments. In addition, the class I and class II MHC molecule-restricted antigen processing pathways are thought to be different (2).

The epitopes of proteins can be defined with short synthetic peptides for both class I-restricted CTL (3) and class II-restricted Th cells (4). We have recently identified an immunodominant CTL epitope from the HIV-1-IIIB envelope protein gp160 comprising the 15 amino acids (315–329) (RIQRGPGRAFVTIGK) (18IIIB) and recognized by class I MHC molecule (D*)-restricted murine CD4+CD8+ CTL (5). In our original work, mice were immunized with recombinant vaccinia virus expressing the HIV-1-IIIB gp160 envelope gene (vSC25) (6), and their lymphocytes were restimulated in vitro with a transfected histocompatible cell line expressing gp160IIIB (5). We now show that we can stimulate spleen cells from immune mice to make CTL in vitro with peptide alone, without exogenous lymphokine. We find that during in vitro stimulation with synthetic peptide corresponding to the CTL antigenic determinant, a class II MHC-restricted CD4+ Th cell response against the same peptide contributes to the induction of CTL. Thus, the same peptide structure induces both class II MHC molecule-restricted CD4+ Th cells and class I molecule-restricted CD8+ CTL. We speculate on the significance of this dual function for the immunodominance of this CTL determinant in the HIV envelope.

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Mice. BALB/c, B10.D2, and B10.A/SgSn mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were used at 6-12 wk of age for immunization.

Recombinant Vaccinia Viruses. vSC-8 (recombinant vaccinia vector containing the bacterial lacZ gene), and vSC-25 (recombinant vaccinia vector expressing the HIV env glycoprotein gp160 of the IIIB isolate) have been described previously (6).

Transfectants. BALB/c-3T3 (H-2d) fibroblast transfectants expressing HIV-1 IIIB gp160 and control transfectants with only the selectable marker gene were derived as described (5).

mAbs. The following mAbs were used: anti-L3T4 (CD4) (RL172.4; rat IgM) (7), anti-A4, E4 (M5/114; rat IgG2b) (8), anti-A4 (MK-D6) (9), and anti-E4 (14-4-4) (10).

Peptide Synthesis and Purification. Peptide 18IIIB was synthesized by solid phase techniques by Peninsula Laboratories, Inc. (Belmont, CA), and purified by gel filtration and ion exchange chromatography. The purified peptide was a single peak on reverse phase HPLC on C-18 columns with two different solvent systems, trifluoroacetic acid/water/acetonitrile, and 0.05 M NaH2PO4/acetonitrile, and had the expected amino acid analysis.

CTL Generation. Mice were immunized intravenously with 10^7 PFU of recombinant vaccinia viruses. 3-20 wk later, immune spleen cells (5 × 10^6/ml in 24-well culture plates in complete T cell medium [5]) were restimulated for 6 d in vitro with either 0.5 μM of immunodominant peptide for CTL from HIV-1-IIIB gp160 envelope glycoprotein (18IIIB) (RIQGPGRAFVTIGK) (5) alone, 18IIIB plus 10% rat Con A supernatant-containing medium (rat T cell Monoclonal) (Collaborative Research, Lexington, MA), or 18IIIB plus 10 U/ml of mouse rIL-2 (Genzyme, Boston, MA).

CTL Assay. After culture for 6 d, cytolytic activity of the restimulated cells was measured using a 6-h assay with various ^51Cr-labeled targets (5). Percent specific ^51Cr release was calculated as 100 × (experimental release - spontaneous release)/(maximum release - spontaneous release). Maximum release was determined from cells lysed by addition of 5% Triton-X 100, and spontaneous release from target cells incubated alone. SEMs of triplicate cultures were always <5% of the mean.

Results and Discussion

Peptide 18IIIB Is Sufficient for Induction of gp160- and Peptide 18IIIB–specific CTL from Immune Spleen Cells in H-2d Mice. When we originally induced gp160IIIB-specific CTL, mice were immunized with recombinant vaccinia virus carrying the HIV-1-IIIB gp160 envelope gene (vSC25), and their immune spleen cells were restimulated with gp160-transfected BALB/c.3T3 fibroblasts (5). Here we show that we can elicit CTL specific for the immunodominant site when vSC25-immune BALB/c spleen cells are restimulated in vitro with peptide 18IIIB alone (Fig. 1). Mice immunized with control vaccinia virus (vSC8) showed no significant cytotoxic activity after in vitro restimulation with any of the forms of the antigen (data not shown). Therefore,
immunization with vSC25 is necessary to elicit CTL specific for the immunodominant site, but the synthetic peptide is sufficient for restimulation of CTL in vitro. The CTL induced in this system are CD4+CD8-, based on lysis with anti-CD4 or anti-CD8 and C5(5), and lyse class II fibroblast targets.

Depletion of CD4+ Cells from Immune Spleen Cells Abrogates In Vitro Peptide-stimulated CTL Induction, which Is Restored by IL-2. To determine the role of CD4+ cells in the induction of CTL by peptide 18IIIB, we depleted CD4+ cells from the spleen cells of vSC25-immune BALB/c mice by treatment with anti-CD4 mAb (RL172.4) plus C. CD4+ cell depletion nearly completely abrogated induction of specific CTL by restimulation in vitro with peptide 18IIIB (Fig. 2). However, this activity was completely restored by inclusion of IL-2 in the restimulation culture (Fig. 2). B10.D2 mice gave the same results (data not shown). Thus, CD4+ cells are necessary to induce class I MHC molecule–restricted peptide-specific CTL, and this function can be replaced by exogenous IL-2. Furthermore, vSC25-immune BALB/c spleen cells restimulated in vitro with only peptide 18IIIB secreted IL-2 (data not shown). Without depletion of CD4+ cells, IL-2 produced little or no further enhancement of the response (see Table I, below). Therefore, the function of the CD4+ cells appears to be T cell help. The results also suggest that peptide 18IIIB, the immunodominant site for CTL restricted by the Dd class I MHC molecule, also induces CD4+ Th cells likely to recognize antigen with class II MHC molecules. Therefore, we tested whether mAbs to A and E could block the activation of Th cells by peptide 18IIIB.

The Effect of Anti-A and/or E mAb on CTL Induction. When the vSC25-immunized BALB/c spleen cells were restimulated with peptide 18IIIB in the presence of a mAb (M5/114) specific for both A and E, specific CTL induction was greatly reduced (Table I, Exp. 1). However, little or no inhibition of the CTL induction was produced by M5/114 in the presence of IL-2, indicating: (a) that the antibody was not nonspecifically toxic; and (b) that the effect of M5/114 was to inhibit induction of T cell help, not some other aspect of CTL induction. To further map the class II MHC restriction of Th cells for peptide 18IIIB, we used MK-D6 (anti-I-Ak) and 14-4-4 (anti-I-Ek) mAbs that were restested for specific blocking activity.

**Figure 2.** Spleen cells from BALB/c mice immunized with 10^7 PFU of vSC25 were treated with anti-CD4 mAb (RLI72.4) plus complement and restimulated in vitro with either 0.3 μM of peptide 18IIIB alone (A) or 0.3 μM of 18IIIB plus IL-2 (10% of rat T cell monoclonal or mouse rIL-2 (B). After a 6-d culture, cytotoxic activities were tested against the indicated 51Cr-labeled targets: 1 μM 18IIIB-pulsed BALB/c.3T3 fibroblasts (●); HIV-1-IIB gp160 gene–transfected BALB/c.3T3 (■); and control unpulsed BALB/c.3T3 fibroblasts (○).
(data not shown) with myoglobin-specific T cell clones restricted by I-A<sup>d</sup> and I-E<sup>d</sup>. The results showed that the Th cells stimulated by peptide 18IIIIB were restricted by the class II A<sup>d</sup> molecule (Table I, Exp. 2). Therefore, peptide 18IIIIB, which contains an immunodominant site for class I D<sup>d</sup> molecule-restricted CTL, also contains a Th cell epitope presented by the class II A<sup>d</sup> MHC molecule. Although we cannot be sure at this stage that the residues responsible for binding D<sup>d</sup> and A<sup>d</sup> are the same, the antigenic sites presented by class I and class II MHC molecules are likely to overlap extensively because the peptide is only 15 residues long.

**Peptide 18IIIIB Fails to Stimulate CTL in Immune Spleen Cells from B10.A Mice (A<sup>e</sup>, E<sup>e</sup>, D<sup>e</sup>).** Because we found that H-2<sup>d</sup> mice are CTL high responders, whereas H-2<sup>k</sup> mice are low responders to HIV gp160 (5), it was of interest to test whether congenic B10.A mice, which have the appropriate class I D<sup>d</sup>, but the class II molecules A<sup>k</sup> and E<sup>k</sup> of the low responder strain, can produce specific CTL when their vSC25-immune spleen cells are restimulated in vitro with peptide 18IIIIB. Fig. 3 shows that restimulation with peptide 18IIIIB alone could not elicit specific CTL at all in B10.A mice, in contrast to BALB/c mice (Fig. 1) or B10.D2 mice (data not shown). However, restimulation with 18IIIIB plus IL-2 produced CTL specific for this immunodominant site (Fig. 3). Thus, immune B10.A spleen cells without any CD4 cell depletion behaved identically to BALB/c spleen cells after CD4 cell depletion (compare with Fig. 2). They appear to lack T cell help specific for peptide 18IIIIB.

Phifenzmaier et al. (11) have reported that the CTL response to SV40 tumor-associated specific antigens is associated with K<sup>b</sup>, K<sup>k</sup>, D<sup>b</sup>, and D<sup>d</sup>, but not with K<sup>d</sup> or D<sup>k</sup>, and that B10.D2 mice (A<sup>d</sup>, E<sup>d</sup>, and D<sup>d</sup>) could generate D<sup>d</sup>-restricted CTL, but B10.A mice (A<sup>k</sup>, E<sup>k</sup>, and D<sup>d</sup>) could not. However, B10.A mice could induce K<sup>k</sup>-restricted CTL. Therefore, they speculated that T cell help would be provided preferentially to those CTL recognizing class I molecules of the same haplotype as class
II MHC molecules. Our current data support the speculation, and moreover, we found that the same epitope was seen by both class I and class II MHC molecules from the same haplotype.

There are now a few examples in the literature in which the same peptide is seen by different T cells with multiple alleles of the same class II molecule (12), with both isotypes of class II molecule (13), or even with both classes of MHC molecules (14). However, these various studies do not show a functional importance to this degenerate presentation. In the current study, not only is the same peptide seen by T cells in association with both class I and class II molecules, after in vivo processing of the native protein, but also, these MHC molecules are associated in the same haplotype so that the same small peptide can induce help for a CTL response to itself.

Finally, we would like to speculate that the same antigenic site of pathogens is sometimes presented by both class I and class II MHC molecules. If these class I and class II molecules are shared by the same MHC haplotype, this dual presentation of the same (or closely overlapping) antigenic site may contribute to its immunodominance. Conversely, such relationships between class I and II MHC molecules might provide some selective advantage to the maintenance of certain combinations in haplotypes, and therefore contribute to linkage disequilibrium within the MHC.

Summary

We have observed that a peptide corresponding to an immunodominant epitope of the HIV-1 envelope protein recognized by class I MHC-restricted CD8+ CTL can also induce T cell help for itself. The help is necessary for restimulation of CTL precursors in vitro with peptide alone in the absence of exogenous lymphokines, can be removed by depletion of CD4+ T cells, and can be replaced by exogenous IL-2. Whereas the CTL in BALB/c or B10.D2 mice are restricted by the class I molecule Dd, the Th cells are restricted by the class II molecule A^d, and the help can be blocked by anti-A^d mAb. To examine the genetic regulation of the induction of help, we studied B10.A mice that share the class I D^d molecule, but have different class II molecules, A^k and E^k. Spleen cells of immune B10.A mice behave like CD4-depleted BALB/c spleen cells in that they cannot be restimulated in vitro by the peptide alone, but can with peptide plus IL-2. Therefore, in the absence of exogenous lymphokines, peptide-specific help is necessary for restimulation with this immunodominant CTL epitope peptide, and in H-2^d mice, this peptide stimulates help for itself as well as CTL. We speculate on the implications of these findings.
for the immunodominance of this peptide in H-2d mice, and for the selective advantage of pairing certain class I and class II molecules in an MHC haplotype.

We thank Dr. Gillis Otten for the gift of antibody M5/114, Jeffrey Ahlers for preparation and specificity testing of antibodies MK.D6 and 14-4-4, and Drs. William Biddison and Richard Hodes for critical reading of the manuscript and helpful suggestions.

Received for publication 5 September 1989 and in revised form 30 October 1989.

References

1. Mizuochi, T., H. Golding, A. S. Rosenberg, L. H. Glimcher, T. R. Malek, and A. Singer. 1985. Both L3T4+ and Lyt-2+ helper T cells initiate cytotoxic T lymphocyte responses against allogeneic major histocompatibility antigens but not against trinitrophenyl-modified self. J. Exp. Med. 162:427.

2. Germain, R. N. 1986. The ins and outs of antigen processing and presentation. Nature (Lond.). 322:687.

3. Townsend, A. R. M., J. Rothbard, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael. 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell. 44:959.

4. Livingstone, A., and C. G. Fathman. 1987. The structure of T cell epitopes. Annu. Rev. Immunol. 5:477.

5. Takahashi, H., J. Cohen, A. Hosmalin, K. B. Cease, R. Houghten, J. Cornette, C. Delisi, B. Moss, R. N. Germain, and J. A. Berzofsky. 1988. An immunodominant epitope of the HIV gp160 envelope glycoprotein recognized by class I MHC molecule-restricted murine cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA. 85:3105.

6. Chakrabarti, S., M. Robert-Guroff, F. Wong-Staal, R. C. Gallo, and B. Moss. 1986. Expression of the HTLV-III envelope by a recombinant vaccinia virus. Nature (Lond.). 320:535.

7. Ceredig, R., J. W. Lowenthal, M. Nabholz, and H. R. MacDonald. 1985. Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells. Nature (Lond.). 314:98.

8. Bhattacharya, A., M. E. Dorf, and T. A. Springer. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. J. Immunol. 127:2488.

9. Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible H-2-restricted interleukin 2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. 153:1198.

10. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol. 124:533.

11. Pfizenmaier, K., G. Trinchieri, D. Solter, and B. B. Knowles. 1978. Mapping of H-2 genes associated with T cell-mediated cytotoxic responses to SV40-tumour-associated specific antigens. Nature (Lond.). 274:691.

12. Sinigaglia, F., M. Guttinger, J. Kilgus, D. M. Doran, H. Matile, H. Edlinger, A. Trzcinski, D. Gillessen, and J. R. L. Pink. 1988. A malaria T-cell epitope recognized in association with most mouse and human MHC class II molecules. Nature (Lond.). 336:778.

13. Guillet, J.-G., M.-Z. Lai, T. J. Briner, S. Buus, A. Sette, H. M. Grey, J. A. Smith, and M. L. Gefter. 1987. Immunological self, nonself discrimination. Science (Wash. DC). 235:865.

14. Perkins, D. L., M.-Z. Lai, J. A. Smith, and M. L. Gefter. 1989. Identical peptides recognized by MHC class I- and II-restricted T cells. J. Exp. Med. 170:279.