Brief Definitive Report

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

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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 bacolovirus, 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; Aimed, 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.
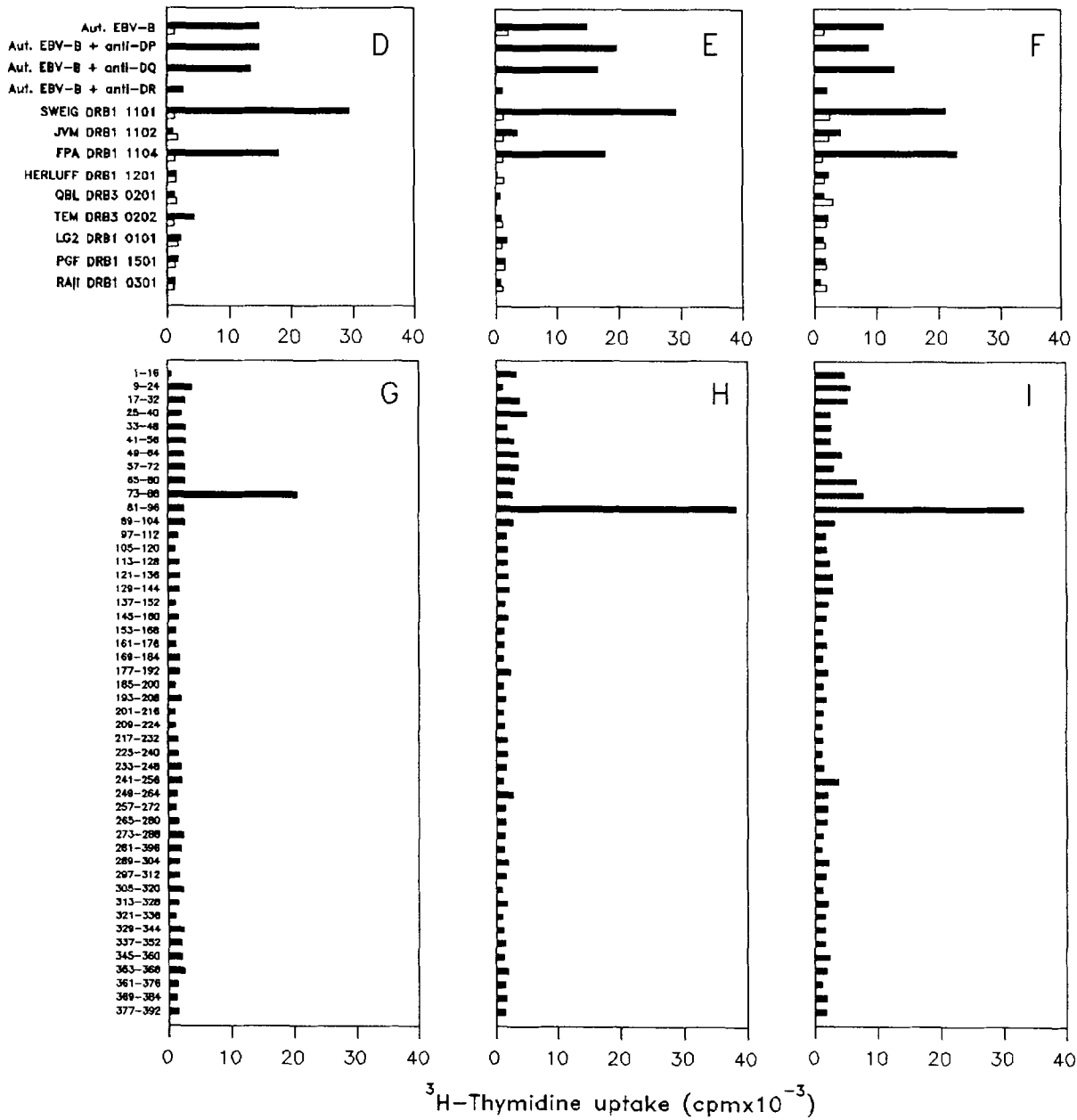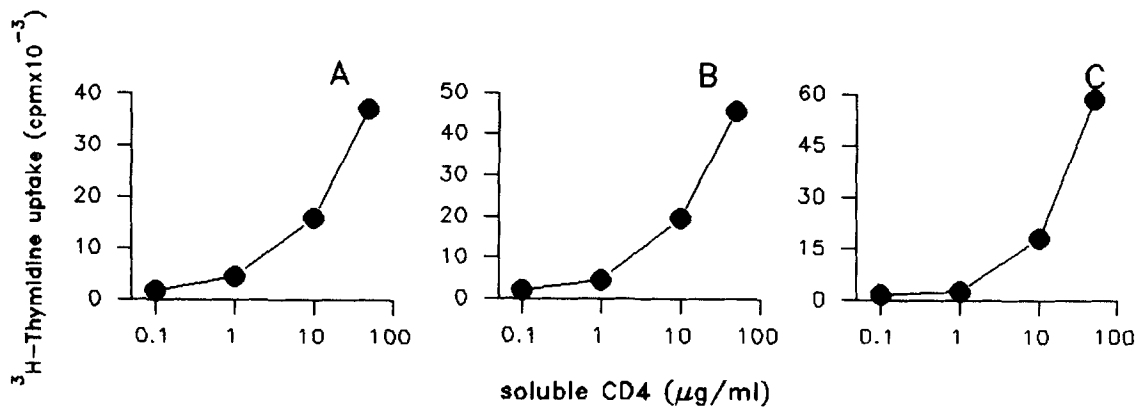
The following anti-CD4 mAbs were used for CD4 modulation: 6D10 (9), 10A12 (10, 11), and OKT4B (12) are specific for V1, V1-2, and V2-3 domains of CD4, respectively. The following anti–class II mAbs were used for the blocking experiments: anti-DP (IgG1, B7.21), anti-DQ (IgG2a, SPVL3), anti-DR (IgG2a, 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 fluorescence was analyzed with the FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA).

Cell Cultures. 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

³H-Thymidine uptake (cpmx10⁻³)

^{3}H-Thymidine uptake (cpmx10^{-3})

A   B   C

soluble CD4 (μg/ml)

D   E   F

Aut. EBV-B
Aut. EBV-B + anti-DP
Aut. EBV-B + anti-DQ
Aut. EBV-B + anti-DR
SWEIG DRB1 1101
JVM DRB1 1102
FPA DRB1 1104
HERLUFF DRB1 1201
QBL DRB3 0201
TEM DRB3 0202
LG2 DRB1 0101
PGF DRB1 1501
RAJI DRB1 0301

0   10   20   30   40

G   H   I

1-16
9-24
17-32
25-40
33-48
41-56
49-64
57-72
65-80
73-88
81-96
89-104
97-112
105-120
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257-272
265-280
273-288
281-396
289-304
297-312
305-320
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321-336
329-344
337-352
345-360
353-368
361-376
369-384
377-392

0   10   20   30   40

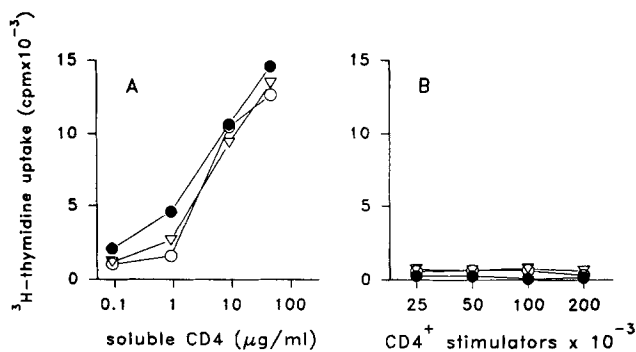^{3}H-Thymidine uptake (cpmx10^{-3})

Figure 2. APC endogenously expressing CD4 can efficiently present soluble CD4, but are unable to spontaneously stimulate CD4-specific T cells. Proliferative response of CD4-specific T cell clone CP12 (A) to three independent autologous CD4+ T cell clones (●, ▽, O), previously pulsed with increasing concentrations of soluble CD4; (B) to increasing numbers of the same CD4+ APC, which were not pulsed with antigen. Cultures were set up and T cell proliferation measured after 48 h as described in Materials and Methods.

to a soluble form of the CD4 molecule (data not shown). Fig. 1 shows representative T cell clones, generated from one such CD4-responsive 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 gener-
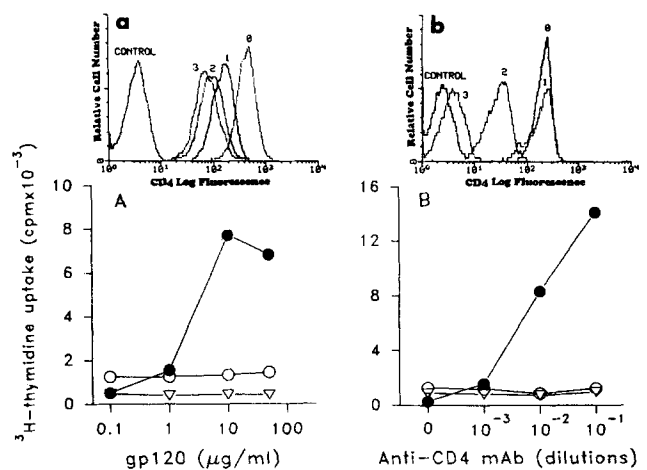

Figure 3. Modulation of membrane CD4 on presenting T cells leads to productive CD4 processing and presentation. The figure shows a representative experiment on proliferative responses of CD4-specific T cell clone CP12 to (A) autologous cloned CD4+ T cells (●), cloned CD8+ T cells (O), or CD4- EBV-B cells (▽), previously pulsed (overnight) with increasing concentrations of recombinant gp120; (B) autologous cloned CD4+ T cells (●), cloned CD8+ T cells (O), or CD4- EBV-B cells (▽), previously pulsed (overnight) with increasing dilutions of anti-CD4 mAb supernatant (IgG1, 6D10) recognizing the V1 domain of CD4. Analogous results were obtained using either 10A12 or OKT4B mAbs recognizing V1-2 and V2-3, respectively, or the CD4-specific CP50 clone as effector cells. Cultures were set up and T cell proliferation measured after 48 h as described in Materials and Methods. The upper half of the figure shows the flow cytometric analysis of CD4 modulation on cloned CD4+ T cells, previously pulsed with (a) 0 (1), 1 (2), or 10 (3) µg/ml gp120; (b) 10⁻³ (1), 10⁻² (2), or 10⁻¹ (3) dilutions of anti-CD4 mAb (6D10). Then, cells were stained with OKT4-FITC mAb and analyzed by direct immunofluorescence using a FACScan®.

ated 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]; B18, 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 or 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 (PSKLNDRADSRRSLWD); peptides 81–96 (DSRRSLWDQGNFPLII).

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 antitransferrin 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.

Table 1. *Processing Requirement for Membrane CD4 Presentation by CD4⁺ APC*

Time after pulsing and before fixation	[³H]Thymidine uptake by T cells
min	*cpm*
0	280
30	325
120	11,625
120 + chloroquine	774

Cloned CD4⁺ T cells, as APC, were pulsed at 0°C with anti-CD4 mAb for 30 min, washed, and cultured at 37°C for the times indicated before fixation with 0.05% glutaraldehyde (Sigma Chemical Co., St. Louis, MO). In some experiments, prepulsed APC were incubated at 37°C with 100 μM chloroquine (Sigma Chemical Co.). APC were then cultured with CP12 T cell clones, and T cell proliferation was measured after 48 h, as described in Materials and Methods.

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 autoimmunity, 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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References

1. Nossal, G.J.V. 1994. Negative selection of lymphocytes. *Cell.* 76:229–239.
2. Schonrich, G., U. Kalinke, F. Momburg, M. Malissen, A.-M. Schmitt-Verhulst, B. Malissen, G.J. Hammerling, and B. Arnold. 1991. Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. *Cell.* 65:293–304.
3. Sercarz, E.E., P.V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T cell antigen determinants. *Annu. Rev. Immunol.* 11:729–766.

4. Gammon, G., and E.E. Sercarz. 1989. How some T cells escape tolerance induction? *Nature (Lond.).* 342:183–185.

5. Lipham, W.J., T.M. Redmond, H. Takahashi, J.A. Berzofsky, B. Wiggert, G.J. Chader, and I. Gery. 1991. Recognition of peptides that are immunogenic but cryptic: mechanisms that allow lymphocytes sensitized against cryptic peptides to initiate pathogenic autoimmune processes. *J. Immunol.* 146: 3757–3762.

6. Lehmann, P.V., E.E. Sercarz, T. Forsthuber, C.M. Dayan, and G. Gammon. 1993. Determinant spreading and dynamics of the autoimmune T-cell repertoire. *Immunol. Today.* 14:203–208.

7. Mamula, M.J. 1993. The inability to process a self-peptide allows autoreactive T cells to escape tolerance. *J. Exp. Med.* 177:567–571.

8. Dong, X., K.J. Hamilton, M. Satoh, J. Wang, and W.H. Reeves. 1994. Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. *J. Exp. Med.* 179:1243–1252.

9. Traunecker, A., W. Luke, and K. Karjalainen. 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. *Nature (Lond.).* 331:84–86.

10. Cammarota, G., A. Scheirle, B. Takacs, D.M. Doran, R. Knorr, W. Bannwarth, J. Guardiola, and F. Sinigaglia. 1992. Identification of a CD4 binding site on the β_2 domain of HLA-DR molecules. *Nature (Lond.).* 356:799–801.

11. Lamarre, D., D.J. Capon, D.R. Karp, T. Gregory, E.O. Long, and R.-P. Sékaly. 1989. Class II MHC molecules and the HIV gp 120 envelope protein interact with functionally distinct regions of the CD4 molecule. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3271–3277.

12. Barnaba, V., A. Franco, A. Alberti, C. Balsano, R. Benvenuto, and F. Balsano. 1989. Recognition of hepatitis B virus envelope proteins by liver-infiltrating T lymphocytes in chronic HBV infection. *J. Immunol.* 143:2650–2655.

13. Barnaba, V., C. Watts, M. de Boer, P. Lane, and A. Lanzavecchia. 1994. Professional presentation of antigen by activated human T cells. *Eur. J. Immunol.* 24:71–75.

14. Lanzavecchia, A. 1990. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. *Annu. Rev. Immunol.* 8:773–793.

15. Shin, J., R.J. Dunbrack, S. Lee, and J.L. Strominger. 1991. Phosphorylation-dependent down-modulation of CD4 requires a specific structure within the cytoplasmic domain of CD4. *J. Biol. Chem.* 266:10658–10665.

16. Germain, R.N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell.* 76:287–299.

17. Siliciano, R.F., T. Lawton, C. Knall, R.W. Karr, P. Berman, T. Gregory, and E.L. Reinherz. 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism of CD4$^+$ cell depletion. *Cell.* 54:561–575.

18. Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman, and S. Abrignani. 1988. T cells can present antigens such as HIVgp120 targeted to their own surface molecules. *Nature (Lond.).* 334:530–532.

19. Franco, A., M. Paroli, U. Testa, R. Benvenuto, C. Peschle, F. Balsano, and V. Barnaba. 1992. Transferrin receptor mediates uptake and presentation of hepatitis envelope antigen by T lymphocytes. *J. Exp. Med.* 175:1195–1205.

20. Watts, C., and A. Lanzavecchia. 1993. Suppressive effect of antibody on processing of T cell epitopes. *J. Exp. Med.* 178:1459–1463.

21. Amigerona, S., J.R. Drake, P. Webster, and I. Mellman. 1994. Transient accumulation of new class II MHC molecules in a novel endocytic compartment in B lymphocytes. *Nature (Lond.).* 369:113–120.

22. Tulp, A., D. Verwoerd, B. Dobberstein, H.L. Ploegh, and J. Pieters. 1994. Isolation and characterization of the intracellular MHC class II compartment. *Nature (Lond.).* 369:120–126.

23. West, M.A., J.M. Lucocq, and C. Watts. 1994. Antigen processing and class II MHC peptide-loading compartments in human lymphoblastoid cells. *Nature (Lond.).* 369:147–151.

24. Qui, Y., X. Xu, A. Wandinger-Ness, D.P. Dalke, and S.K. Pierce. 1994. Separation of subcellular compartments containing distinct functional forms of MHC class II. *J. Cell Biol.* 125:595–605.

25. Schmid, S.L., and M.J. Jackson. 1994. Making class II presentable. *Nature (Lond.).* 369:103–104.

26. Verhey, K.J., and M.J. Birnbaum. 1994. A leu-leu sequence is essential for COOH-terminal targeting signal of GLUT4 glucose transporter in fibroblasts. *J. Biol. Chem.* 269:2353–2356.

27. Trowbridge, I.S., J.F. Collawan, and C.R. Hopkins. 1993. Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu. Rev. Cell Biol.* 9:129–161.

28. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271–296.

29. Ameisen, J.C., and A. Capron. 1991. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. *Immunol. Today.* 12:102–105.

30. Kowalski, 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.* 86:3346–3350.

31. Chams, V., T. Idziorek, and D. Klatzmann. 1991. Biological properties of anti-CD4 autoantibodies purified from HIV-infected patients. *AIDS.* 5:565–569.

32. Callahan, L.N., G. Roderiquez, M. Mallinson, and M.A. Norcross. 1992. Analysis of HIV-induced autoantibodies to cryptic epitopes on human CD4. *J. Immunol.* 149:2194–2205.

33. Pantaleo, G., C. Graziosi, and A.S. Fauci. 1993. The immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med.* 328:327–335.

34. Meyaard, L., H. Schuitemaker, and F. Miedema. 1993. T cell dysfunction in HIV infection: anergy due to defective antigen presenting function? *Immunol. Today.* 14:161–164.