

REGULATION OF ANTIGEN PRESENTATION
BY ACIDIC pH

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Helper T cells recognize a processed form of antigen bound to class II MHC glycoproteins present on the surface of APC. After ligand recognition and activation, helper T cells modulate the actions of other cell types involved in immune responses, including cytotoxic T cells, B cells, and macrophages. Conditions that affect antigen/class II MHC complex formation therefore have wide ranging effects on immunity. Direct binding of antigenic peptides to purified class II MHC has been demonstrated (1), and kinetic studies (2, 3) performed at neutral pH indicate that the rate of complex formation is very slow. By contrast, the formation of antigenic complexes appears to be rapid in live APC (4, 5). We report here that the formation of functional antigen/class II complexes is accelerated at pH values approximating those found in the acidic intracellular compartments of metabolically active APC.

Materials and Methods

Peptides and T Cells. Peptide antigens were synthesized as previously described (6) by using an automated Applied Biosystems (Foster City, CA) 430A peptide synthesizer. Peptides were constructed on pyridine-2-aldoxime methiodide-polystyrene resin and deprotected in liquid HF after cleavage. Purity was assessed by HPLC and structure was confirmed by microsequencing. Sequences of peptides: OVA(323-339), ISQAYHAAHAEINEAGRY; HEL(104-120), GMNAWVAWRNRCKGTDVY; Myo(106-118), FISEAIIHVLHSR. Esterification of HEL(104-120) carboxyl groups was performed in methanol-HCl (7). The ester was purified and analyzed using reverse-phase HPLC. Hd-1.AC5 and Md-1.D6 T cell hybridomas were derived from BALB/c mice immunized with hen egg lysozyme or sperm whale myoglobin, respectively (6). DO-11.10 T cell hybridomas (2) recognize processed OVA in association with I-A^d.

T Cell Cultures and Lymphokine Assay. The I-A^d and I-E^d-bearing B cell lymphoma M12.4 (8) was used for peptide binding experiments unless otherwise noted. APC were fixed for 20 min at 24°C in 1% paraformaldehyde and extensively washed in RPMI containing 10% FCS (R10). After exposure to peptide antigens under various conditions, fixed M12.4 (4×10^4 cells/well) were cultured with T cells (1×10^5 cells/well) in R10 for 24 h by using flat-bottomed 96-well tissue culture plates. Lymphokine concentration was measured by culturing serial dilutions of culture supernatant with the IL-2-dependent HT-2 cell line (1×10^4 /well) as described (6). Results are expressed in units lymphokine, where 1 U is defined by stimulation of 50% of the maximum response determined by a standard curve, and represent the mean of triplicate cultures. Concentrations less than 10 U/ml could not be measured in the assay.

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Results and Discussion

Acidic compartments are thought to be important in antigen processing because agents that raise the pH of these compartments, such as ammonium chloride and chloroquine, inhibit antigen processing (9). To investigate the effect of pH on functional antigen/class II complex formation, experiments were performed using small peptides, aldehyde-fixed APC, and T cell hybridomas specific for the relevant peptide antigens and class II molecules. The presence of TCR ligand, alone, is sufficient to activate T cell hybridomas (3), and the response, measured by lymphokine production, is proportional to the concentration of peptide/class II complexes (10). A marked increase in the rate and extent of functional complex formation was observed after incubation of fixed APC with peptide at pH 5.0 as compared with pH 7.3 (Fig. 1). This effect was most evident at low peptide concentration, which gave no measurable response after incubation at pH 7.3 for 24 h. Similar results were obtained using three different peptide antigens from ovalbumin, OVA(323-339) (Fig. 1 a); hen egg

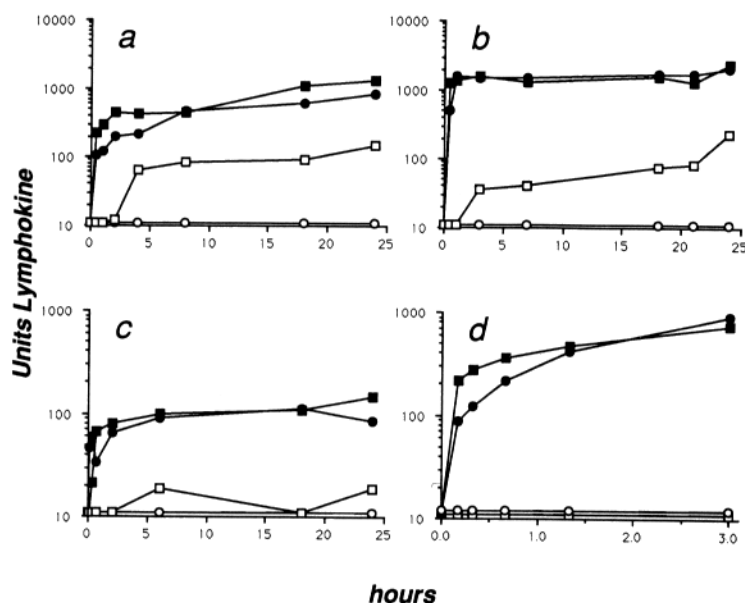


FIGURE 1. (a-c) Effect of pH on the kinetics of functional peptide/MHC complex formation. (a) Fixed M12.4 B cells were incubated with 0.15 M citrate/phosphate buffer solutions containing 1 μ M (O, ●) or 10 μ M (□, ■) OVA(323-339) for the indicated time periods at pH 7.3 (open symbols) or pH 5.0 (closed symbols). After extensive washing, the B cells were cultured with I-A^d-restricted DO-11.10 T cells and lymphokine production was measured. (b) Fixed B cells were incubated with 3.6 μ M (O, ●) or 36 μ M (□, ■) HEL(104-120), washed, and cultured with I-E^d-restricted Hd-1.AC5 T cells. (c) Fixed B cells were incubated with 1 μ M (O, ●) or 10 μ M (□, ■) Myo(106-118) and cultured with I-A^d-restricted Md-1.D6 T cells. (d) Effect of pretreatment at pH 5.0 or pH 7.3. Fixed M12.4 B cells were incubated at 24°C for 18 h in 0.15 M citrate/phosphate buffer, pH 7.3 (O, ●) or pH 5.0 (□, ■). Aliquots of the pretreated cells were washed and incubated for the indicated time period with 3 μ M OVA(323-339) at pH 7.3 (O, □), or pH 5.0 (●, ■). After further washing, treated cells were cultured with DO-11.10 T cells and lymphokine production was measured. Results are representative of at least three independent experiments of similar design.

lysozyme, HEL(104-120) (Fig. 1 *b*); and sperm whale myoglobin, Myo(106-118) (Fig. 1 *c*) and two distinct class II proteins, I-A^d and I-E^d, which are both expressed on the fixed B cells used in this study. HEL(104-120) is recognized in association with I-E^d (6) while the others have no affinity for I-E^d and are recognized in association with I-A^d (11). We were not able to determine whether maximal binding was increased at pH 5.0 versus pH 7.3 because equilibrium was not achieved at pH 7.3 in our experiments.

The pH dependence of complex formation was unchanged even after overnight pretreatment of the fixed cells at pH 5.0 in the absence of peptide antigen (Fig. 1 *d*). This result indicates that pH directly affects the interaction of peptide with the APC membrane, rather than having an indirect effect on peptide binding sites or adhesion molecules. The augmented binding observed is not a result of an increase in available peptide binding sites after dissociation of endogenous peptide from class II MHC exposed to pH 5.0. To a variable extent, we have observed some increase in T cell activation using fixed B cells or MHC-bearing plasma membrane fragments that had been pretreated in acidic buffer (Fig. 1 *d* and unpublished results). The mechanism responsible for this is unclear. No T cell activation is observed with pH 5-treated APC without appropriate antigens (data not shown).

Bell-shaped profiles with optima at pH 4.5-5.0 were observed in experiments using the peptide antigens described above (Fig 2). We considered the possibility that nonspecific electrostatic interactions between peptide and the negatively charged

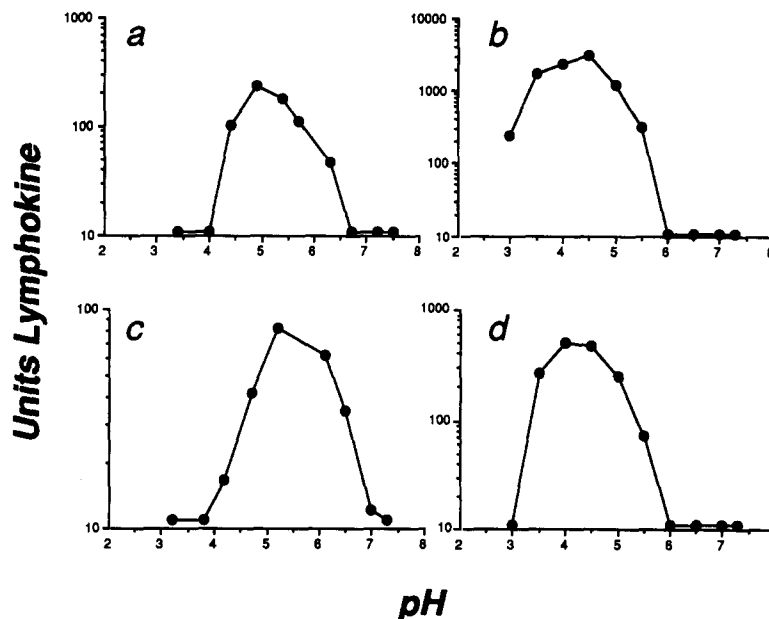


FIGURE 2. Optimal pH for functional antigen binding. Fixed M12.4 were incubated for 2 h at 24°C in 0.15 M citrate/phosphate buffer at various pH with 1 μ M peptide. After washing, treated cells were incubated with appropriate T cells and lymphokine production was measured. (a) OVA(323-339). (b) HEL(104-120). (c) Myo(106-118). (d) HEL(104-120) ester.

plasma membrane are responsible for the observed phenomenon. Peptides containing histidine ($pK'_R \sim 6$) are likely to have a change in net charge between pH 7.3 and pH 5.0. Ova(323–339) and Myo(106–118) each contain two histidine residues (see Materials and Methods). However, a marked pH dependence of binding is also observed using HEL(104–120) (Fig. 2 *b*), which contains no histidine. HEL(104–120) does contain an aspartic acid residue ($pK'_R \sim 4$), which may contribute towards titration of net charge in the relevant pH range. However, esterification of the carboxyl groups of HEL(104–120) did not alter the pH optimum (Fig. 2 *d*). It is therefore less likely that nonspecific electrostatic interactions account for the pH dependence of functional peptide binding, although this possibility cannot be excluded.

The pH dependence of functional complex formation does not require changes introduced in membrane components during fixation with paraformaldehyde. Similar results are obtained with plasma membrane fragments isolated from unfixed B cells (Fig. 3 *a*). Plasma membranes pulsed with peptide at pH 5.0 have increased capacity to activate T cells as compared with those pulsed at pH 7.3. Additionally, membrane components unique to B cells are not required, since the effect is also observed using L cell fibroblast transfectants expressing I-A^d (Fig. 3 *b*). Current efforts are directed towards evaluating the potential requirement for non-class II membrane components using supported artificial membranes and affinity purified class II glycoproteins.

Our results differ from those of Buus et al. (2) who observed a decreased rate of association and an increased rate of dissociation at acidic pH using labeled OVA (323–339) and purified I-A^d in detergent. This difference may result from alterations in class II conformation induced by purification and detergent solubilization.

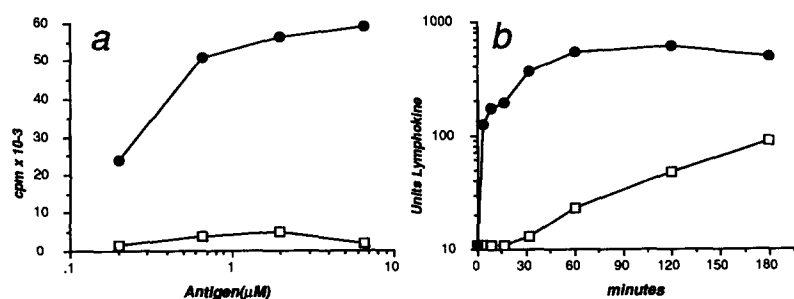


FIGURE 3. pH-dependence using B cell membranes or L cells. (a) M12.4 plasma membrane aliquots were incubated at 37°C for 18 h with various concentrations of a trypsin digest of reduced and carboxymethylated ovalbumin that contains OVA(323–339) at pH 7.3 (□) or pH 5.0 (●). After washing, treated membranes were cultured (50 μg total protein/ml) with DO-11.10 (1×10^5 cells/well). Partially purified plasma membranes were prepared from M12.4 cells after disruption by nitrogen cavitation as described (6). The amount of lymphokine secreted into cultures was assayed by [³H]thymidine uptake of the IL-2-dependent cell line HT-2. Each point represents the mean of triplicate cultures. Increased antigen binding to B cell membranes at pH 5 versus pH 7 was observed in 12 independent experiments using peptides derived from lysozyme or ovalbumin. pH-dependent binding was also observed with membranes that had been extensively pretreated at pH 5 in the absence of peptide. (b) RT 2.3.3H-D6 L cell transfectants (16), which express I-A^d, were fixed with 1% paraformaldehyde and incubated (2×10^6 /ml) at 24°C in pH 7.3 (□) or pH 5.0 (●) citrate/phosphate buffer with 3 μM OVA(323–339). After washing, treated L cells (1×10^5 cells/well) were cultured with DO-11.10 (1×10^5 cells/well) and lymphokine production was measured.

Functionally relevant conformational changes have been documented by Fraser (12), who observed a marked decrease in the affinity of class II toward staphylococcal enterotoxin A after detergent solubilization. It is possible that pH regulates structural transitions in the class II molecule that affect the binding kinetics of peptide antigens. This conformational transition could be entirely intramolecular or may involve interaction with other molecules present in the plasma membrane environment.

Alternatively, a pH-dependent interaction between peptide and other membrane components may facilitate specific binding to class II by increasing the effective local concentration of peptide. Membrane proteins, other than MHC, that bind peptide antigen have been described (13, 14). It is possible that pH-dependent interactions involving such proteins may account for the observed phenomenon.

Whatever the mechanism, the effect of pH on functional association of peptide and MHC is likely to be of physiological importance. The reported rate of antigen/MHC complex formation is relatively slow in experimental systems involving metabolically inert membranes or purified class II as compared with those using live B cells (5). The available evidence indicates that the time period required for antigen processing is limited by the time required for intracellular trafficking rather than peptide/MHC binding and is independent of antigen concentration (4). These general observations may be accounted for by the ability of metabolically active APC to bring antigen and class II together into intracellular compartments with pH between 5 and 6 (15) where peptide/MHC interaction is facilitated. We suggest that this mechanism serves as an important regulator of immunity by sequestering antigen/MHC association and the induction of immunity to selected intracellular compartments in APC.

Summary

The effect of pH on functional association of peptide antigens with APC membranes was investigated by using aldehyde-fixed B cells and class II-restricted T cell hybridomas to assess antigen/MHC complex formation. The results indicated that the rate and extent of functional peptide binding was markedly increased at pH 5.0 as compared with pH 7.3. The pH dependence of binding was preserved after pretreatment of fixed APC with pH 5.0 buffer, suggesting that pH had a direct effect on the interaction of peptide with the APC membrane. Similar results were obtained by using several peptides and I-A^d- and I-E^d-restricted T cells, indicating that pH may be of general importance in regulating the formation of functional antigen/class II MHC complexes.

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References

1. Babbitt, B., P. M. Allen, G. Matsueda, E. Haber, and E. R. Unanue. 1985. The binding of immunogenic peptides to Ia histocompatibility molecules. *Nature (Lond.)* 317:359.
2. Buus, S., A. Sette, S. M. Colon, D. M. Jevis, and H. M. Grey. 1986. Isolation and characterization of antigen-Ia complexes involved in T cell recognition. *Cell* 47:1071.

3. Sadegh-Nasseri, S., and H. M. McConnell. 1989. A kinetic intermediate in the reaction of an antigenic peptide and I-E^k. *Nature (Lond.)* 337:274.
4. Roosneck, E., S. Demotz, G. Corradin, and A. Lanzavecchia. 1988. Kinetics of MHC-antigen complex formation on antigen-presenting cells. *J. Immunol.* 140:4079.
5. Ceppellini, R., G. Frumento, G. B. Ferrara, R. Tosi, A. Chersi, and B. Pernis. 1989. Binding of labeled influenza matrix peptide to HLA-DR in living B lymphoid cells. *Nature (Lond.)* 339:392.
6. Jensen, P. E. 1989. Stable association of processed antigen with antigen-presenting cell membranes. *J. Immunol.* 143:420.
7. Wilcox, P. E. 1967. Esterification. *Methods Enzymol.* 11:606.
8. Kim, K. J., C. Kannelopoulos-Langevin, R. M. Mervin, D. H. Sachs, and R. Asofsky. 1979. Establishment and characterization of BALB/c lymphoma lines with B cell properties. *J. Immunol.* 122:549.
9. Ziegler, H. K., and E. R. Unanue. 1982. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. *Proc. Natl. Acad. Sci. USA.* 79:175.
10. Watts, T. H. 1988. T cell activation by preformed, long-lived Ia-peptide complexes. Quantitative aspects. *J. Immunol.* 141:3708.
11. Buus, S., A. Sette, S. M. Colon, C. Miles, and H. M. Grey. 1987. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science (Wash. DC)* 235:1353.
12. Fraser, J. D. 1989. High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature (Lond.)* 339:221.
13. Nairn, R., M. L. Spengler, M. D. Hoffman, M. J. Solvay, and D. W. Thomas. 1984. Macrophage processing of peptide antigens: Identification of an antigenic complex. *J. Immunol.* 133:3225.
14. Lakey, E. K., E. Margoliash, and S. K. Pierce. 1987. Identification of a peptide binding protein that plays a role in antigen presentation. *Proc. Natl. Acad. Sci. USA.* 84:1659.
15. Mellman, I., R. Fuchs, and A. Helenius. 1986. Acidification of the endocytic and exocytic pathways. *Annu. Rev. Biochem.* 55:663.
16. Germain, R. N., J-D. Ashwell, R. I. Lechler, D. H. Margulies, K. M. Nickerson, G. Suzuki, and J. Y. L. Tou. 1985. "Exon-shuffling" maps control of antibody- and T-cell-recognition sites to the NH₂-terminal domain of the class II major histocompatibility polypeptide A β . *Proc. Natl. Acad. Sci. USA.* 82:2940.