

Human T Cells Cannot Act as Autonomous Antigen-presenting Cells, but Induce Tolerance in Antigen-specific and Alloreactive Responder Cells

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Summary

The ability of two HLA-DR-expressing human T cell clones to function as antigen-presenting cells (APC) was investigated using highly purified T cells. The results demonstrated that these T cell clones are unable to act as autonomous APC, and that recognition of nominal or alloantigens on the surface of T cells leads to a state of nonresponsiveness. The first observation was that a T cell clone with specificity for the 306–324 peptide of influenza hemagglutinin (HA), and raised from a DR1 responder, exhibited apparent degeneracy of major histocompatibility complex restriction when cultured with peptide in the presence of peripheral blood mononuclear cells (PBMC) expressing a wide variety of structurally unrelated DR types. However, when the PBMC were pulsed with peptide and washed before coculture with the clone, peptide was exclusively recognized with DR1Dw1. This implied that in the presence of soluble peptide the T cells were displaying ligand to each other, and that the third-party APC were providing costimulatory signals. To test the ability of T cells to act as autonomous APC, accessory cell-free preparations of two DR1-restricted clones were cultured with peptide in the presence or the absence of added B cell APC. T cell purity was established by the absence of proliferation in response to the mitogen phytohemagglutinin (PHA). PHA-nonresponsive T cells were completely unable to proliferate in response to peptide alone; furthermore, preculture of the HA-specific clone, in the complete absence of accessory cells, with the same concentration of peptide (1 $\mu\text{g}/\text{ml}$) that induced optimal proliferation when presented by conventional APC, led to profound nonresponsiveness. The same phenomenon was also observed when two of three anti-DR1 alloreactive T cell clones were precultured with a DR1-expressing T cell clone. The ability of the DR1-expressing clone to induce nonresponsiveness in anti-DR1 clones correlated with recognition of the DR1 alloantigen on the DR1-expressing clone.

CD4-positive T cells recognize antigen in the context of class II MHC molecules on APC (1). Specialized APC such as macrophages, B cells, and dendritic cells constitutively express class II MHC molecules (2). It is well established that a wide variety of other cell types can be induced to express MHC class II molecules, however, the consequences of antigen presentation by such cells may vary widely. Endothelial cells, for example, appear to be capable of functioning as autonomous APC leading to T cell activation (3, 4). In contrast, presentation of antigen by keratinocytes or MHC class II-expressing pancreatic islet cells can lead to T cell nonresponsiveness, or anergy (5–7). Human T cells are MHC class II negative at rest, but can be induced to express all three class II isotypes after activation *in vivo* (8) or *in vitro* (9). Human T cells have been extensively studied as APC, and have been reported to be capable of effective antigen presen-

tion. Mitogen-activated T cells have been shown to stimulate both allogeneic and autologous mixed lymphocyte reactions (10–12), although the opposite result has been described (13). In addition, presentation of exogenous viral antigens (14) and denatured protein antigens by MHC class II-positive T cells has been described (15). More recently, LaSalle et al. (16) have shown that activated T cells can also present peptides of the autoantigen, myelin basic protein, but not the intact antigen, to autologous T cells. The failure to present intact antigen in its native conformation is thought to be due to the inability of T cells to capture and process antigen efficiently. In keeping with earlier observations that antigen presentation by antigen-specific B cells was several orders of magnitude more efficient than by B cells with an irrelevant specificity, Lanzavecchia et al. (17) reported that antigen presentation by T cells occurred when the antigen bound to and

was internalized with cell surface CD4. This suggests that the deficiency of T cells in antigen processing is quantitative, rather than qualitative, and can be overcome when a specific mechanism of antigen capture is operative.

In addition to these reports of T cells as effective APC, apparently conflicting observations have been reported showing that recognition of antigen on the surface of T cells can induce a state of nonresponsiveness. Indeed, the first observation of antigen-specific unresponsiveness by a T cell clone was reported after the incubation of influenza hemagglutinin (HA)-specific human T cells with high concentrations of HA peptide antigen in the absence of accessory cells (18). This state of "anergy" appeared to be the result of peptide recognition by T cells on the surface of other members of the clone, restricted by their expressed DR molecules. Since this observation, many other examples of tolerance induction have been described (19–21). The conditions that lead to the anergic state appear to require an imbalance between TCR occupancy, leading to the delivery of signal 1, and the receipt of poorly defined "second signals." TCR occupancy without cell division appears to have the same result (22).

As a consequence of these contradictory findings, the functional significance of class II MHC molecular expression on T cells is unclear. It could be argued that presentation of antigen by MHC class II-positive T cells leads to the amplification of an immune response; alternatively, it could have the opposite effect due to the induction of anergy. In this study the apparent contradiction between the ability of T cells to present antigen effectively, and their capacity to induce anergy, is addressed.

Materials and Methods

Antigens and Mitogens. The antigens used in this study were formalin-fixed influenza A/Texas virus particles (diluted from a stock preparation of 5 HAU/ml) and whole HA (kindly supplied by Dr. J. Skehel, National Institute of Medical Research, Mill Hill, London). The synthetic HA and matrix (MP) peptides were prepared by solid phase synthesis using standard t-Boc chemistry in "T bags" using commercially available amino acids (Novabiochem, Nottingham, UK). The peptides were cleaved from the resin by treatment with hydrogen fluoride. Purity was determined by HPLC and amino acid analysis.

PHA was obtained from Boroughs Wellcome (Beckenham, UK), and used at a range of concentrations from 0.2 to 20 $\mu\text{g/ml}$. When PHA proliferative responses are quoted, these correspond to the peak proliferative response observed.

mAbs. The L243, anti-DR α mAb (23) was affinity purified from ascites by separation over a protein A-Sepharose column.

Cell Lines. The DAP.3- and DR1-expressing DAP.3 L cell transfectants (24) were maintained in culture in DMEM supplemented with 10% FCS in 25-cm² tissue culture flasks and subcultured, after trypsin treatment, at a 1:10 dilution twice weekly.

EBV-transformed lymphoblastoid B cell lines (B-LCLs), from the 10th International Histocompatibility Workshop, were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 $\mu\text{g/ml}$ streptomycin in 25-cm² flasks, and were regularly passaged.

T Cell Clones. Clone NF4 was derived from PBMC isolated from a DR1Dw1 individual. PBMC (10^7) were cultured with syn-

thetic peptide HA306–324 (10 $\mu\text{g/ml}$) in 10 ml RPMI 1640 supplemented with 10% human serum, 2 mM L-glutamine, 50 U/ml penicillin, and 50 $\mu\text{g/ml}$ streptomycin. After 7 d of culture, the blast cells were purified on Ficoll-Paque density separation medium (Pharmacia, Milton Keynes, UK) and then restimulated twice at weekly intervals with irradiated autologous PBMC and peptide HA306–324. T cell clones were obtained by limiting dilution in Terasaki plates at one cell/well with 10^4 irradiated autologous PBMC, 1 $\mu\text{g/ml}$ HA306–324 peptide, and 20 U/ml rIL-2 (Boehringer Mannheim Biochemicals, Mannheim, Germany). T cell clone HC1 was generated from a DR1,4 individual by stimulating PBMC with purified influenza HA (5 $\mu\text{g/ml}$), and cloning by limiting dilution as for NF4. The clones were maintained in culture by weekly stimulation with autologous PBMC, HA306–324 peptide, and rIL-2.

To obtain accessory cell-free preparations of T cells, cells were reseeded into new 24-well tissue culture plates on days 3 and 6 post-restimulation in order to remove adherent cells. For use in experiments the cells were purified by isolation on Ficoll-Paque 7 d after restimulation and washed four times by slow speed centrifugation (210 g, 5 min) before use.

Alloreactive anti-DR1 T cell clones, G3, G11, and G12, were obtained as described previously (25). The anti-DRw11 clone L5 was obtained from a DRw17 responder using the same protocol. T cell clones were used for functional assays 1 wk after their last stimulation.

T Cell Proliferation Assays. T cell clones (10^4 cells/well) were cultured in the presence of irradiated PBMC or B-LCLs (as indicated in the figure legends) in flat-bottomed microtiter plates, in a total volume of 200 μl . DAP.3 L cell transfectants were treated with mitomycin C (65 $\mu\text{g/ml}$) (Kyowa, Tokyo, Japan). For antigen-specific responses, the APC were either prepulsed with antigen, or soluble antigen was added to the cultures as indicated in the figure legends. Wells were pulsed with 1 μCi of [³H]TdR (Amersham International, Amersham, UK), after 48 h and the cultures harvested onto glass fiber filters 18 h later. Proliferation was measured as [³H]TdR incorporation by liquid scintillation spectroscopy. The results are expressed as the mean cpm for triplicate cultures. Standard errors were routinely <10%.

Two-Stage Cultures for Tolerance Induction. T cell clone NF4 was plated out in wells of 24-well plates in the presence of different concentrations of peptide HA306–324 (as indicated in the figure legends), in a total volume of 500 μl . In the experiments with alloreactive T cell clones, irradiated clone NF4 was cocultured with alloreactive T cells at the ratios indicated in the figure legends in conical 10-ml tubes (Sterilin, Staffordshire, UK). The L243 mAb was added as purified antibody to give a final concentration of 10–100 $\mu\text{g/ml}$ where indicated. After overnight incubation, the cells were harvested, washed extensively, and used in proliferation assay as described above.

Results and Discussion

Apparent Degeneracy in MHC Restriction of an Antigen-specific T Cell Clone. Clone NF4 was raised against HA peptide, as described in Materials and Methods, and responded to inactivated A/Texas influenza virus, purified HA, and HA peptide in a dose-dependent manner. The proliferative responses of clone NF4 to the optimal concentrations, as indicated in parentheses, of these three preparations of antigen are shown in Table 1. The HLA restriction specificity of the clone was mapped using a panel of PBMC expressing different HLA DR molecules in the presence of an optimal concentration

Table 1. T Cell Clone NF4 Exhibits Apparent Degeneracy of MHC Restriction

	Antigen specificity*				
	Med	Flu virus (50 HA U/ml)	HA (5 µg/ml)	HA306-324 (1 µg/ml)	
Clone NF4	177	50,627	31,740	49,860	
	PBMC donor (DR type)†				
	NF (1, -)	RIL (2,4)	MB (3, -)	JRB (4,4)	GL (5, -)
Med	323	159	338	177	217
P306-324	55,244	37,257	41,974	45,420	28,628

Results are expressed as the mean cpm for triplicate cultures.

* 10⁴ T cells (NF4) were cultured with medium alone, influenza virus, intact HA, or HA 306-324 peptide with 10⁵ irradiated DR1-expressing PBMC.

† 10⁵ irradiated PBMC expressing the DR types indicated alone, or with HA peptide 306-324.

of peptide. As shown in Table 1, the T cell clone NF4 responded in the presence of all the PBMC tested, and appeared to be capable of using several unrelated DR molecules as restriction elements.

To investigate further the MHC restriction of clone NF4, PBMC from the same donors shown in Table 1 were preincubated with a range of HA peptide concentrations, washed, and placed in culture with NF4 cells. Under these conditions, only the PBMC expressing DR1Dw1 were able to stimulate a proliferative response, as shown in Fig. 1. These results suggested that the apparent degeneracy of clone NF4 was due to the presentation of peptide by the T cells themselves with their cell surface DR molecules. In this context the third-party PBMC provided a source of the requisite costimulatory signals.

These results suggest that considerable caution needs to be exercised when attempting to define the MHC restriction specificity of human T cells. This is a particular problem when using peptides that require no further processing as antigens. It appears that T cells are inefficient at processing antigen (17), so that the kind of apparent degeneracy observed here is less likely to be seen when intact antigen is used.

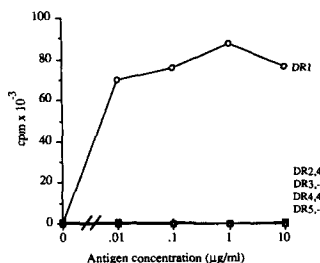


Figure 1. Prepulping of the APC with peptide reveals that the HA-specific T cell clone, NF4, is restricted exclusively by HLA-DR1. PBMC from individuals expressing the DR types indicated were prepulsed with different concentrations of HA 306-324 peptide (2 h, 37°C). PBMC were washed (three times), irradiated, and 10⁵ were cultured with 10⁴ T cells (NF4).

HA-specific T Cells Are Unable to Act as Autonomous APC. Results such as those described above indicate clearly that activated human T cells are fully capable of displaying ligand to other T cells with cell surface MHC class II molecules. Whether or not T cells can provide the necessary "second signals" required for T cell activation is less clear. Accessory cell-free preparations of two HLA-DR1-restricted HA-specific T cell clones, NF4 and HC1, were cultured with specific peptide with or without added APC. NF4 cells prepared in the normal way were included for comparison (Fig. 2). The level of accessory cell contamination of these different preparations is illustrated by their maximal levels of proliferation in response to PHA (0.2–2.0 µg/ml), which were 24,474 (non-purified NF4), 238 (purified NF4), and 188 cpm (purified HC1). As shown in Fig. 2a, the accessory cell-contaminated NF4 cells proliferated in response to soluble peptide in the absence of added APC. In contrast, the PHA-nonresponsive preparations of NF4 and HC1 did not respond to soluble peptide alone, but proliferated strongly in the presence of DR1-B-LCL (Fig. 2, b and c).

This result suggests that purified T cells are unable to act as autonomous APC and is in contrast with the recent data of LaSalle et al. (16), who reported that a human T cell clone specific for the autoantigen myelin basic protein (MBP) was able to present MBP peptide to itself even when contaminating accessory cells had been removed. The difference between these two systems is not clear, but may reflect heterogeneity between T cell clones in their ability to provide costimulatory signals.

Antigen Presentation by Accessory Cell-free T Cells Induces T Cell Tolerance at Low Peptide Concentrations. Previous systems in which T cell anergy has been induced by exposure to specific antigen have used high peptide concentrations (18). Given the inability of T cells to provide the signals necessary for inducing T cell activation, presentation of low peptide concentrations should be sufficient to lead to T cell tolerance.

Accessory cell-free clone NF4 was cultured overnight with four different concentrations of peptide HA306-324 from 0.19 to 50 µg/ml. The T cells were harvested the next day and washed extensively, and transferred to a proliferation assay in the presence of transfectants expressing DR1Dw1 pulsed with different concentrations of HA306-324 peptide. The results are presented in Fig. 3, and show that preincubation with the same concentration of peptide that led to optimal T cell stimulation in the presence of accessory cells (1 µg/ml) caused almost total inhibition of the subsequent response to DR1-expressing DAP.3 transfectants pulsed with a range of peptide concentrations. The effect was antigen-specific in that preincubation with influenza matrix peptide 17-31 did not induce tolerance. Furthermore, preincubation with all of the concentrations of HA peptide led to augmentation of the IL-2 response (data not shown).

Recognition of DR Alloantigens on the Surface of T Cells in the Absence of Accessory Cells Leads to Allospecific Tolerance. The possibility that recognition of alloantigen on the surface of accessory cell-free T cells would lead to tolerance in allospecific responder T cells was tested using three anti-DR1 human

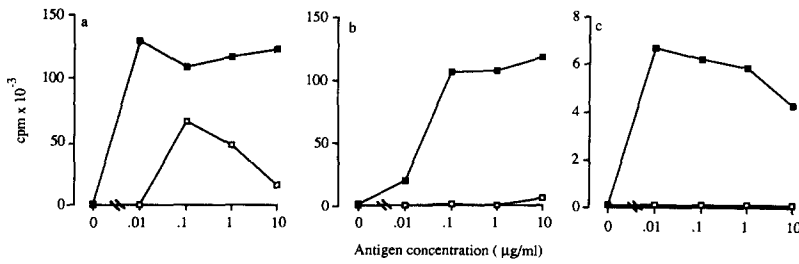


Figure 2. Accessory free T cells are unable to act as autonomous APC. 10^4 T cells, (a) nonpurified NF4, (b) purified NF4, and (c) purified HC1, were cultured with different concentrations of HA 306–324 peptide alone (\square), or with 3×10^4 peptide-pulsed, irradiated, DR1-expressing B-LCL (\blacksquare).

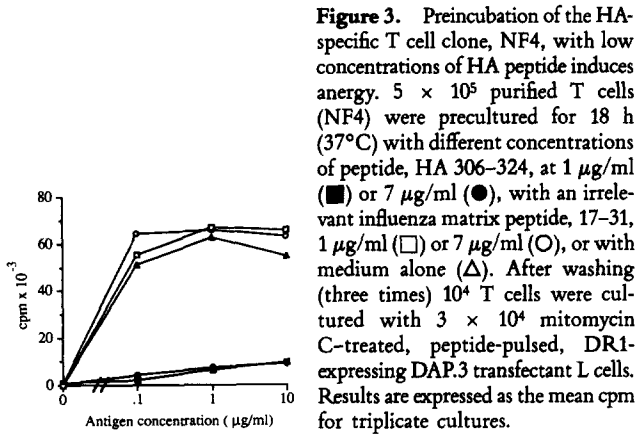


Figure 3. Preincubation of the HA-specific T cell clone, NF4, with low concentrations of HA peptide induces anergy. 5×10^5 purified T cells (NF4) were precultured for 18 h (37°C) with different concentrations of peptide, HA 306–324, at $1 \mu\text{g/ml}$ (\blacksquare) or $7 \mu\text{g/ml}$ (\bullet), with an irrelevant influenza matrix peptide, 17–31, $1 \mu\text{g/ml}$ (\square) or $7 \mu\text{g/ml}$ (\circ), or with medium alone (\triangle). After washing (three times) 10^4 T cells were cultured with 3×10^4 mitomycin C-treated, peptide-pulsed, DR1-expressing DAP.3 transfectant L cells. Results are expressed as the mean cpm for triplicate cultures.

Table 2. The DR1-expressing Clone NF4 Is Recognized by Two of Three Anti-DR1 T Cell Clones

Stimulator cell	Third-party PBMC	Responder clone		
		G3	G11	G12
B-LCL	–	50,903	96,421	166,705
NF4	–	926	1,043	330
NF4	+	18,023	17,282	3,323

10^4 anti-DR 1 T cells (G3, G11, or G12) were cultured with 10^5 irradiated DR1-expressing B-LCL, 10^5 irradiated DR1-expressing purified T cells (NF4) alone, or together with 10^5 irradiated third-party PBMC.

T cell clones. To establish that the DR1-expressing T cell clone, NF4, was displaying the necessary peptide/DR1 complex recognized by the alloreactive clones, a proliferation assay was set up, with and without added third-party DRw17 PBMC. As shown in Table 2, no proliferation occurred in the absence of added accessory cells. In the presence of third-party DRw17 PBMC, however, NF4 induced 35% and 18% of the level of proliferation that was stimulated by DR1-expressing B cell lines for clones G3 and G11, respectively. In contrast, for clone G12, NF4, with added accessory cells, stimulated <2% of the control response. These three clones were then used in a preincubation assay, and the results correlated with the proliferation data, as shown in Fig. 4. The reactivity of G3 and G11 was substantially reduced (Fig. 4, a and b), but no reduction was seen for clone G12 (c). The specificity of this effect and its DR dependence is also illustrated by the lack of effect when G11 was preincubated with a DRw17-expressing clone, or when G3 was preincubated with NF4 in the presence of anti-DR antibody.

These observations raise questions about the functional significance of HLA class II expression by activated T cells. As with MHC class II expression that is induced on any non-professional APC type, two opposite outcomes can be envisaged. One possibility is that the appearance of additional class II-bearing cells leads to amplification of an ongoing immune response, due to the potential for more display of antigen/MHC molecular complexes. This may be limited in the case of T cells due to their apparent inefficiency in the processing of complex antigens. However, the observations made here that allospecific T cells recognize their ligand on the surface of other T cells argues that at least some antigens

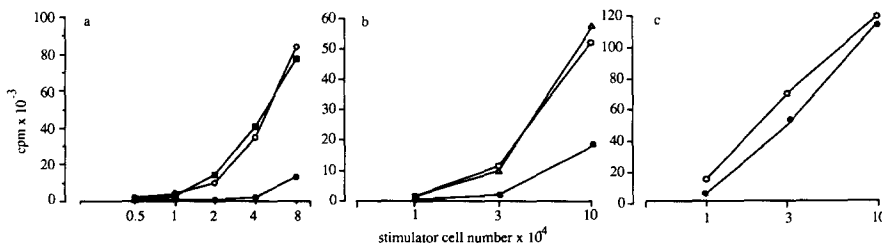


Figure 4. The induction of anergy in anti-DR1 T cells due to preincubation with a DR1-expressing clone correlates with ligand recognition. 4×10^5 purified anti-DR1 T cells, (a) G3, (b) G11, or (c) G12, were precultured for 18 h (37°C) with 8×10^5 irradiated DR1-expressing purified T cells (NF4) (\bullet), medium alone (\circ), or 8×10^5 irradiated DR1-expressing purified T cells (NF4) in the presence of purified L243 anti-DR α mAb ($10 \mu\text{g/ml}$ final concentration) (\blacksquare), or 8×10^5 irradiated DRw17-expressing purified T cells (L5) (\triangle). After washing (three times) 10^4 anti-DR1 T cells were cultured with different doses of irradiated DR1-expressing B-LCL.

can be processed by T cells. In contrast, MHC class II expression by T cells could have precisely the opposite effect. As shown in the experiments described in this study, in the absence of accessory cells, presentation of antigen by T cells leads to anergy. It is not clear whether at sites of T cell proliferation in vivo a T cell can interact exclusively with another T cell, with no receipt of signals from local accessory cells. One speculation is that this could occur during the clonal expansion of T cells, that such T cells would be likely to express class II molecules, and that this may provide a mechanism to make an immune response self limiting. The members

of a locally proliferating T cell clone may present antigen to each other, leading to arrest of further proliferation. The fact that Jenkins and Schwartz (20) were able to induce nonresponsiveness in vivo after the injection of mice with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-treated, antigen-bearing spleen cells suggests that exclusive interactions of the kind that are required to induce anergy can occur in vivo. Whether these observations can be applied to the induction of nonresponsiveness in the context of transplantation remains to be investigated.

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References

1. Thorsby, E., E. Berle, and H. Nousiainen. 1982. HLA-D region molecules restrict proliferative T cell responses to antigens. *Immunol. Rev.* 66:39.
2. Unanue, E.R., and P.M. Allen. 1987. The basis for the immunoregulatory role of macrophages and other accessory cells. *Science (Wash. DC)*. 236:551.
3. Hirschberg, H., Q.-J. Bergh, and E. Thorsby. 1980. Antigen-presenting properties of human vascular endothelial cells. *J. Exp. Med.* 152:249.
4. Geppert, T.D., and P.E. Lipsky. 1985. Antigen presentation by γ -interferon treated endothelial cells and fibroblasts: differential ability to function as antigen presenting cells despite comparable Ia expression. *J. Immunol.* 135:3750.
5. Gaspari, A.A., M.K. Jenkins, and S.I. Katz. 1988. Class II MHC-bearing keratinocytes induce antigen-specific unresponsiveness in hapten-specific Th1 clones. *J. Immunol.* 141:2216.
6. Bal, V., A. McIndoe, G. Denton, D. Hudson, G. Lombardi, J. Lamb, and R. Lechler. 1990. Antigen presentation by keratinocytes induces tolerance in human T cells. *Eur. J. Immunol.* 20:1893.
7. Markman, J., D. Lo, A. Naji, R.D. Palmiter, R.L. Brinster, and E. Heber-Katz. 1988. Antigen-presenting function of class II MHC-expressing pancreatic beta cells. *Nature (Lond.)*. 336:476.
8. Yu, D.T.Y., R.J. Winchester, S.M. Fu, A. Gibofsky, H.S. Ko, and H.G. Kunkel. 1980. Peripheral blood Ia positive T cells increased in certain diseases and after immunization. *J. Exp. Med.* 151:91.
9. Ko, H.S., S.M. Fu, R.J. Winchester, D.T.Y. Yu, and H.G. Kunkel. 1979. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen and antigen activated T cells. *J. Exp. Med.* 150:246.
10. Tribel, F., S. De Roquefeuil, C. Blanc, D. Charron, and P. Debre. 1986. Expression of MHC class II and Tac antigens on IL-2-activated human T cell clones that can stimulate in MLR, AMLR, PLT and can present antigen. *Hum. Immunol.* 15:302.
11. Russo, C., F. Indivieri, V. Quaranta, G.A. Molinaro, M.A. Pellegrino, and S. Ferrone. 1981. Stimulation of human T lymphocytes: analysis of the role of Ia-like antigens with monoclonal antibodies. *Immunogenetics*. 12:267.
12. Damle, N.K., and S. Gupta. 1982. Autologous mixed lymphocyte reaction in man. V. Functionally and phenotypically distinct human T-cell subpopulations respond to non-T and activated T cells in AMLR. *Scand. J. Immunol.* 16:59.
13. Pawelec, G., M. Blaurock, M.E. Schneider, S. Shaw, and P. Wernet. 1982. Alloactivated long-term cultured human T lymphocytes express both HLA-DR and SB antigens but lack lymphocyte stimulation capacity. *Eur. J. Immunol.* 12:967.
14. Hewitt, C.R.A., and M. Feldmann. 1989. Human T cell clones present antigen. *J. Immunol.* 142:1429.
15. Gerrard, T.L., D.J. Volkman, C.H. Jurgensen, and A.S. Fauci. 1985. Activated human T cells can present alloantigens but cannot present soluble antigens. *Cell. Immunol.* 95:65.
16. LaSalle, J., K. Ota, and D.A. Hafler. 1991. Presentation of autoantigen by human T cells. *J. Immunol.* 147:774.
17. Lanzavecchia, A., E. Roosnek, T. Gregory, P. Berman, and S. Abrignani. 1988. T cells can present antigens such as HIV gp120 targeted to their own surface molecules. *Nature (Lond.)*. 334:530.
18. Lamb, J.R., B.J. Skidmore, N. Green, J.M. Chiller, and M. Feldman. 1983. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. *J. Exp. Med.* 157:1434.
19. Quill, H., and R.H. Schwartz. 1987. Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative non-responsiveness. *J. Immunol.* 141:2216.
20. Jenkins, M.K., and R.H. Schwartz. 1987. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J. Exp. Med.* 165:3704.
21. Jenkins, M.K., M.D. Pardoll, J. Mizuguchi, H. Quill, and R.H.

- Schwartz. 1987. T-cell unresponsiveness *in vivo* and *in vitro*: fine specificity of induction and molecular characterization of the unresponsiveness state. *Immunol. Rev.* 95:113.
22. Dimuthu, R. De Silva, K.B. Urdahl, and M.K. Jenkins. 1991. Clonal anergy is induced *in vitro* by T cell receptor occupancy in the absence of proliferation. *J. Immunol.* 147:3261.
23. Lampson, L.A., and R. Levy. 1980. Two populations of Ia-like molecules on a human B cell line. *J. Immunol.* 125:293.
24. Lechler, R.I., V. Bal, J.B. Rothbard, R.N. Germain, R. Sekaly, E.O. Long, and J.R. Lamb. 1988. Structural and functional studies of HLA-DR-restricted antigen recognition by human helper T lymphocyte clones using transfected murine L-cell lines. *J. Immunol.* 141:3003.
25. Lombardi, G., S. Sidhu, J.R. Lamb, J.R. Batchelor, and R.I. Lechler. 1989. Co-recognition of endogenous antigens with HLA-DR1 by alloreactive human T cell clones. *J. Immunol.* 142:753.