Presentation of Antigen by Mixed Isotype Class II Molecules in Normal H-2d Mice

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Summary

A panel of DBA/2 T cell hybridomas specific for the sperm whale myoglobin epitope 110-121 was found to recognize antigen presented by the mixed isotype class II molecule EcβAβ4. The response was blocked by monoclonal antibodies specific for Ecβ and Aβ3 chains; in addition, the hybridomas responded to antigen presented by L cells expressing EcβAβ3 molecules, and made no response with L cells expressing I-Aβ2 or I-Eβ2 molecules. Two more groups of hybridomas isolated from DBA/2 and B10.D2 mice immunized with myoglobin also recognized peptide 110-121 presented by EcβAβ4. Thus, although it is expressed at biochemically undetectable levels on spleen cells, the EcβAβ3 molecule is an important presenting element in normal H-2d mice making a conventional immune response to a protein antigen. These results suggest that high levels of class II expression are not a prerequisite for T cell activation.

Class II MHC-encoded molecules are heterodimeric membrane proteins that bind peptides and display them on the cell surface for recognition by T lymphocytes. The α and β chains of class II MHC molecules show very strong preferences for matched isotypic pairing (1, 2). High level expression of mixed isotype molecules can be achieved after transfection of certain combinations of α and β gene pairs into L cells (3–5). There is also biochemical, serological, and functional evidence for low-level expression on some B cell tumors (4, 6) and human EBV-transformed cells (7). EcβAβ molecules can be detected on the spleen cells of mouse strains with defective β expression (8), and also in mice expressing high levels of an Ec transgene (8–10,) where they can be recognized both as alloantigens (9) and as antigen-presenting molecules (10). Despite these latter results, it was thought that mixed isotype molecules were expressed at levels too low to play any physiological role in individuals with balanced class II chain expression. Here we show that the biochemically undetectable mixed isotype molecule EcβAβ3 constitutes a major presenting element in H-2d mice for a determinant on sperm whale myoglobin (SpW Mb).1 The quantitative aspects of this mixed isotype molecule suggest that the very low number of MHC molecules expressed is not limiting in T cell activation, and may have important implications for understanding certain autoimmune states.

Materials and Methods

Animals. DBA/2 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) or from the National Cancer Institute (Bethesda, MD). Males, 8–12 wk of age, were used for all experiments.

Antigens. SpW Mb was purchased from Serva (Heidelberg, Germany). SpW Mb peptides and 110-121 SpW Mb long chain biotinylated (LCB) peptide at the NH2 terminus were synthesized using standard solid phase methods as previously described (11).

Antigen-presenting Cells. The L cell transfectants RT 7.7H7 14.3 (EcβAβ3) (5), RT 10.3 C1 (Ecββ3) (3), RT 2.3.3H D6 (AαAβ3) (12), and CA36.2.1.Ii (EcβAβ3 and invariant chain) (13), and the A20 B cell lymphoma line (14) have been previously described. APC were exposed to 3,000 rad from a cesium source before coculture with T cells.

T Cell Hybridomas. The T cell hybridomas in Table 1, A and D were obtained by fusion of SpW Mb–specific T cell clones (15, 16) with the BW5147 TCR-α/β cell line (17) as previously described (18). Briefly, 3 d before fusion, the T cell clones were restimulated as previously described (16) in the presence of 5 U/ml of human rIL-2 (Cetus Corp., Emeryville, CA). After removal of dead cells on a Ficoll-Paque gradient, 2–10 × 10⁶ cells were fused with 40 × 10⁶ BW5147 cells as described (18). The antigen fine specificity of the T cell hybridomas was indistinguishable from that

1 Abbreviations used in this paper: LCB, long chain biotinylated; SpW Mb, sperm whale myoglobin.
of the original T cell clones. The T cell hybridomas in Table 1, B and C were produced by fusion of SpW Mb-immune lymph node cells stimulated once in vitro and will be reported elsewhere (K. Sellins et al., manuscript in preparation).

**IL2 Assays.** 0.1–1 × 10⁵ hybridoma cells and 0.5–1 × 10⁵ irradiated APC were cultured with serial dilutions of antigen in flat-bottomed microtiter wells. After a 24-h incubation at 37°C, 100 µL supernatant was harvested from each well, frozen, thawed, and added to 0.5–1.0 × 10⁵ HT-2 cells in 100 µL medium to measure IL-2 production as previously described (18). Plates were pulsed with [3H]Tdr (1 µCi/well) at 24 h, and harvested at 24 h. Results are given as the mean of triplicate wells ± SD.

**Antibody Blocking.** A20 APC (10⁵/well) were incubated at room temperature for 30 min with serial twofold dilutions of mAb. 10⁴ hybridoma cells/well and antigen were then added, and the plates incubated for 24 h at 37°C. 100 µL supernatant was harvested from each well and assayed for IL-2 as described above.

**Peptide Binding Assays.** Binding assays with SpW Mb 110–121 LCB peptide were performed as previously described (11, 19). Briefly, class II MHC-negative cells; DAP3, EoEβ¹, AαAθβ, and EoAβ were transfected with the L cell transfectants (3 × 10⁴) were incubated in Dulbecco's PBS containing biotinylated peptide at different concentrations at 37°C for 4 h in 96-well round-bottomed plates. Subsequently, the cells were stained with successive layers of fluoresceinated avidin D (10 µg/ml in PBS/0.1% BSA; Vector Laboratories, Burlingame, CA), biotinylated anti-avidin D (10 µg/ml in PBS/0.1% BSA; Vector Laboratories), and again fluoresceinated avidin D, each for 30 min at 4°C. The mean fluorescence of 5,000 stained cells was determined by flow cytometry on a FACScan® analyzer (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The background, measured in the absence of peptide, as well as nonspecific binding signal to a class II MHC-negative fibroblast cell line, DAP3, was subtracted from each signal.

Results and Discussion

Analysis of a panel of DBA/2 (H-2d) SpW Mb–specific T cell clones (20) originally suggested that six independent clones recognized the determinant 110-121 when presented by the EoEβ¹ class II molecule. First, the response could be blocked by the EoE-specific mAb 14.4.4S, and was apparently unaffected by the Aβ¹-specific antibody MKD6. Second, there was no response to antigen presented by D2.GD spleen cells; the D2.GD strain has functional Aβ¹, Act, and Eβ¹ genes, but carries a defective EoE chain (21), and is therefore Aβ¹Aβ—(I-A)⁺, and EoEβ¹(I-E)⁺.

Many of these clones were subsequently fused with the TCR-αββ⁻ BW5147 variant fusion partner (17) to generate T cell hybridomas, which were assayed on class II–expressing B cells and L cell transfectants. The EoEβ¹Eβ⁺ L cell line CA 36.2.14Li, transfected with the EoEβ¹ and invariant chain genes (13), presented antigen very efficiently to the hybridoma 13.26.8-H6, specific for an epitope within residues 132–147, confirming the EoEβ¹ presentation of antigen to T cells specific for this epitope (22). However, CA 36.2.14Li cells were unable to present myoglobin or peptide to the 110-121–specific hybridoma 12.2.9-H7. Both hybridomas made excellent control responses to antigen presented by the BALB/c (H-2b) B lymphoma cell line A20 (data not shown). Similar results were obtained using several other 110-121–specific hybridomas and EoEβ¹-transfected L cell lines (G. Ruberti, unpublished results).

These experiments suggested that the 110-121–specific hybridomas did not recognize 110-121 presented by EoEβ¹, even though the EoE chain clearly formed part of the presenting molecule. EoE chains expressed in H-2d cells associated preferentially with Eβ¹ chains, but co-transfection experiments have shown that EoE can pair with Aβ¹ to be expressed as an isotype-mismatched class II molecule (2-4). Since all our data were consistent with EoEβ¹-associated recognition of the 110-121 epitope, the antibody inhibition experiments were repeated using higher concentrations of MKD6 and 14.4.4S. The specificity of the antibody preparations is shown in Fig. 1, A and B. MKD6 blocked the response of an Aβ¹Aβ¹/106-118–specific hybridoma (11.12.8b-H5.1), but had no effect on the response of the EoEβ¹/132-147–specific hybridoma 13.26.8-H6.1. Conversely, 14.4.4S

![Figure 1](image-url)
Table 1. Response of DBA/2 and B10.D2 T Cell Hybridomas to Peptide 110-121 Presented by EoA0 is, EoE9 is, and A0A0 is L Cell Transfectants

| Hybridomas          | EoA0 is | EoE9 is | A0A0 is |
|---------------------|---------|---------|---------|
| A                   |         |         |         |
| 11.3.7e-H2          | 152,354 | 274     | 529     |
| 8.2.1d-H1a          | 129,437 | 440     | 927     |
| 12.2.9-H7           | 97,202  | 923     | 228     |
| 9.4.23-H1b          | 141,817 | 2,456   | 493     |
| 14.12.21-H10        | 119,136 | 2,410   | 2,425   |
| 14.16.12-H7b        | 131,807 | 1,791   | 2,683   |
| B                   |         |         |         |
| DB10 10.7-H7        | 115,140 | 2,924   | 1,590   |
| DB7 2.E2            | 133,117 | 1,591   | 1,339   |
| DB10 9.C6           | 84,063  | 2,751   | 1,227   |
| C                   |         |         |         |
| B17 9.B4            | 129,299 | 2,410   | 2,324   |
| B17 9.C9            | 140,681 | 1,378   | 1,234   |
| B17 9.A10           | 195,889 | 722     | 1,226   |
| B13 5.G5            | 133,383 | 1,910   | 2,312   |
| B13 5.D10           | 82,717  | 780     | 210     |
| B13 5.C11           | 123,662 | 1,386   | 2,624   |
| B13 5.B8            | 141,378 | 440     | 927     |
| B19 4.E8            | 139,560 | 960     | 1,110   |
| D                   |         |         |         |
| 13.26.8-H6          | 2,090   | 101,170 | 2,241   |
| 11.12.8b-H5         | 1,378   | 1,110   | 108,768 |

(A) 110-121-specific DBA/2 hybridomas derived by fusion of T cell clones isolated from long-term SpW Mb-specific T cell lines, as previously described (15, 16); (B) 110-121-specific DBA/2 hybridomas produced by fusion of SpW Mb-immune lymph node cells stimulated once in vitro; (C) 110-121-specific B10.D2 hybridomas produced by fusion of SpW Mb-immune lymph node cells stimulated once in vitro; (D) control responses of the DBA/2 hybridomas 13.26.8-H6 and 11.12.8b-H5 (15). All the hybridomas in B and C appear to be independent, as determined by TCR V8 sequence or antigen fine specificity. (A, B, and C) hybridomas were stimulated with peptide 110-121 (50 µM); (D) 13.26.8-H6 and 11.12.8b-H5 were stimulated with peptides 132-147 (50 µM) and 102-120 (50 µM), respectively. Peptide was presented by the L cell transfectants RT 7.7H7 14.3 (EoA0 is) (3), RT 10.3 C1 (EoE9 is) (3), and RT 2.3.3H D6 (A0A0 is) (12). Representative results of four experiments are shown. Results are expressed as the mean of triplicate wells in counts per minute. The SDs were within 10% of the mean values.

completely blocked the response of 13.26.8-H6.1, but did not inhibit the response of 11.12.8b-H5.1. Both antibodies blocked the response of four independent 110-121-specific hybridomas, 8.2.1d-H1a.5, 9.4.23-H1b.2, 11.3.7e-H2.2.3, and 12.2.9-H7.2 (Fig. 1, C–F). It was essential to assay antibody blocking at limiting antigen concentrations; at higher antigen concentrations, 14.4.4S still blocked the response almost completely, whereas MKD6 gave little if any inhibition. It has been shown that MKD6 binds poorly to EoA0 is, compared with A0A0 is (3, 4); this could explain why we previously saw no inhibition of the 110-121 response with MKD6, under conditions where conventional A0A0 is-controlled responses were blocked quite efficiently.

To test directly whether EoA0 is could present the 110-121 determinant, the hybridomas were assayed on L cells transfected with A0A0 is (RT 2.3.3H D6) (12), EoE9 is (RT 10.3 C1) (3), or EoA0 is (RT 7.7H7 14.3) (3). Table 1A shows that six independent 110-121-specific hybridomas responded to peptide 110-121 presented by the EoA0 is-expressing L cells, but were not stimulated by this peptide on A0A0 is or EoE9 is transfectant cells. The control A0A0 is (11.12.8b-H5)- and EoE9 is (13.26.8-H6)-restricted hybridomas were stimulated by peptides 102-120 and 132-147 on A0A0 is+ and EoE9 is+ L cells, respectively, but were unable to recognize peptide presented by EoA0 is transfectants (Table 1D).

These six DBA/2 110-121-specific hybridomas were made from clones isolated from T cell lines stimulated several times in vitro before cloning. A second set of hybridomas was made from myoglobin-immune DBA/2 lymph node cells stimulated just once in vitro before fusion (K. S. Sellins et al., manuscript in preparation). Those hybridomas that made no response to antigen presented by D2.GD spleen cells were also

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**Figure 2.** Comparison of antigen presentation by spleen cells, A20 cells, and class II MHC-transfected L cells. T cell hybridomas were cultured with serial fivefold dilutions of peptides 110-121 (A and B), 132-147 (C), or 102-120 (D), presented by D2.GD spleen cells (O), A20 (@) or L cells (I) expressing EoA0 is (A and B), EoE9 is (C), or A0A0 is (D); details of these L cell lines are given in the legend to Table 1. Hybridomas were: (A) 9.4.23-H1b; (B) 11.3.7e-H2; (C) 13.26.8-H6; and (D) 11.12.8b-H5. Representative results are shown from three experiments. Results are represented as counts per minute. Standard deviations were within 10% of the mean values. Similar results were obtained with the 110-121-specific hybridomas 8.2.1d-H1a and 14.16.12-H7b (data not shown).
found to recognize antigen presented by EoAβ^d, with no
crossreactive recognition of peptide presented by Acαβ^d or
EoEβ^d (Table 1 B). Finally, 110-121-specific hybridomas
from the H-2^d strain, B10.D2, also made after a single in
vitro restimulation and unresponsive to antigen presented
by D2.GD spleen cells (K. S. Sellins, et al., manuscript in prepa-
ration), were again found to recognize antigen presented by
EoAβ^d (Table 1 C). Thus, presentation of peptide 110-121
by the mixed isotype molecule EoAβ^d was a reproducible
phenomenon that could be observed in two different H-2^d
mouse strains.

In normal H-2^d cells, with balanced expression of the four
class II chains, it has been estimated that EoAβ^d molecules
account for <5% of the class II molecules (2). To test this,
L cells (Acαβ^d, EoEβ^d, or EoAβ^d) expressing MHC mol-
cules at levels similar to that on A20 and H-2^d spleen cells
were compared for their ability to present myoglobin pep-
tides to the various hybridomas. For Acαβ^d (Fig. 2 D)
and EoEβ^d (Fig. 2 C) restricted responses, the L cells gave
dose response curves very similar to those seen with A20 and
H-2^d spleen cells. In contrast, the EoAβ^d-transfected L cells
allowed half-maximal responses at peptide concentrations three
to five orders of magnitude lower than with A20 or H-2^d
spleen cells (Fig. 2, A and B). These results were consistent
with very low expression of EoAβ^d relative to Acαβ^d and
EoEβ^d on H-2^d cells, and demonstrated the efficiency of T
cell recognition of peptide 110-121 presented by EoAβ^d
molecules.

Given the very low level of EoAβ^d expression on H-2^d
cells, the repeated isolation of 110-121-specific T cells that
recognized the antigen presented by this molecule was rather
surprising. One possibility was that peptide 110-121 bound
very efficiently to EoAβ^d, so that the density of 110-
121/EoAβ^d complexes on the antigen-presenting cell sur-
face was high enough to trigger T cell activation. Thus, al-
though EoAβ^d expression was low relative to Acαβ^d or
EoEβ^d, the number of 110-121/EoAβ^d complexes could have
achieved that of peptides 102-120 and 132-147 bound to
Acαβ^d and EoAβ^d, respectively. This possibility was
tested by measuring the mean fluorescent signals generated by
the binding of LCB 110-121 peptide to EoEβ^d, Acαβ^d,
and EoAβ^d L cell transfectants. As demonstrated by data
presented in Fig. 3, this peptide did not bind with detectably
higher affinity to the hybrid MHC molecule than to the
Acαβ^d or EoEβ^d molecules. Another possibility was that
those T cells specific for 110-121/EoAβ^d expressed TCRs
with high affinity for this ligand. Among the 17 cell lines
described here, all but one expressed VB8.2 of these, 14/15
VB8.2^+ chains sequenced were VB8.2/Dβ2.1/Jβ2.6, while
the remaining clone expressed VB8.2/Dβ2.1/Jβ2.5 (15, 16,
and K. S. Sellins et al., manuscript in preparation). The
VB8.2/Dβ2.1/Jβ2.6 chain can pair with at least four different
TCR-α chains to form TCR-α/β heterodimers specific for
110-121/EoAβ^d (16), and it is tempting to speculate that
this TCR-β chain confers particularly high TCR affinity for
peptide 110-121 presented by EoAβ^d.

There has been considerable debate about any function in
vivo for mixed isotype class II molecules (2, 23). The crucial
issue concerns the level of cell surface expression, rather than
any special properties of mismatched α/β pairs. Activation
of class II-specific T cells in the periphery depends critically
on class II density on the APC (24–26). Mixed isotype class
II molecules, when expressed at sufficiently high levels, ap-
ppear to have properties indistinguishable from those of isotype-
mismatched molecules. They can be recognized as alloantigens
(9), present conventional protein antigens (4, 6, 10), and can
also cause partial deletion of T cell subsets in Mls-positive
mice (8). However, the association of class II chains is in-
fluenced by strong isotype and haplotype preferences in o~/B
pairs (2–4, 27–29), so that cells with balanced Ac, AcB,
Eo, and EoB chain synthesis express low or undetectable
amounts of mixed isotype molecules. In previous reports,
mixed isotype molecules have been recognized by T cells only
when their expression is favored by co-transfection of partic-
ular class II α or β chains (4), by unbalanced synthesis of
the isotypically matched α or β chain (6–8), or by the in-
duction of Eor transgenes expressed at high levels (8–10).

The present study shows that normal mice with balanced
class II α and β chain synthesis can use isotypically mismatched
molecules in a conventional immune response, even though
these molecules are expressed at such low levels, relative to
the isotypically matched class II molecules, that they can be
biochemically undetectable on spleen cells (2, and R. Ger-
main, unpublished results). These results suggest that high
levels of class II expression are not a prerequisite for T cell
activation. It is not possible to say from these experiments
whether the low level of class II expression required for acti-
vation in the periphery reflects equally low thresholds for positive or negative selection in the thymus; it has been shown by others that levels of class II expression too low to stimulate antigen-specific or allogeneic responses nevertheless induce tolerance to much higher levels of the same class II molecule (9, 10, 30). These data raise questions about the role that mismatched MHC molecules might play in a physiological immune or autoimmune response without the necessity of invoking any unbalanced expression of isotype-matched chains (2, 23). Antigen presentation by mixed isotype molecules may well depend both on the particular antigen and on the available TCR repertoire. Presentation by mixed isotype molecules in vivo would greatly increase the repertoire of class II-restricted T cell responses, especially in humans, where individuals heterozygous for DR, DQ, and DP alleles potentially express a very large number of different class II molecules. Certain autoimmune diseases correlate with extended HLA-D haplotypes, rather than with particular DR, DQ, or DP alleles (31–33). Our results support the possibility that these diseases are induced or maintained by responses involving mixed isotype molecules (23).

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References

1. Kaufman, J.F., C. Auffray, A.J. Korman, D.A. Shackelford, and J. Strominger. 1984. The class II molecules of the human and murine major histocompatibility complex. Cell. 36:1.
2. Sant, A.J., and R.N. Germain. 1989. Intracellular competition for component chains determines class II MHC cell surface phenotype. Cell. 57:797.
3. Germain, R.N., and H. Quill. 1986. Unexpected expression of a unique mixed-isotype class II MHC molecule by transfected L-cells. Nature (Lond.). 320:72.
4. Malissen, B., N. Shastrí, M. Pierres, and L. Hood. 1986. Cotransfer of the Eoα and Eoβ genes into L-cells results in the surface expression of a functional mixed-isotype Ia molecule. Proc. Natl. Acad. Sci. USA. 83:3958.
5. Lechler, R.I., A.J. Sant, N.S. Braunstein, R. Sekaly, E. Long, and R.N. Germain. 1990. Cell surface expression of hybrid murine/human MHC class II Eoα dimers. J. Immunol. 144:329.
6. Spencer, J.S., and R.T. Kubo. 1989. Mixed isotype class II antigen expression. J. Exp. Med. 169:625.
7. Lotteau, V., L. Tetton, D. Burroughs, and D. Charron. 1987. A novel HLA class II molecule (DRαα-DQββ) created by mismatched isotype pairing. Nature (Lond.). 329:339.
8. Anderson, G.D., and C.S. David. 1989. In vivo expression and function of hybrid Ia dimers (Eoαβ) in recombinant and transgenic mice. J. Exp. Med. 170:1003.
9. Kimoto, M., K. Seki, M. Matsunaga, and T. Mineta. 1989. Unique mixed lymphocyte-stimulating determinants in Eoα gene-introduced C57BL/6 transgenic mice. Immunology. 67:154.
10. Matsunaga, M., K. Seki, T. Mineta, and M. Kimoto. 1990. Antigen-reactive T cell clones restricted by mixed isotype Aββ/Eoα class II molecules. J. Exp. Med. 171:577.
11. Rothbard, J.B., R. Bush, K. Howland, V. Bal, C. Fenton, W.R. Taylor, and J.L. Lamb. 1989. Structural analysis of a peptide-HLA class II complex: identification of critical interactions for its formation and recognition by a T cell receptor. Int. Immunol. 1:479.
12. 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 complex molecule. Proc. Natl. Acad. Sci. USA. 82:2940.
13. Stockinger, B., U. Pessara, R.H. Lin, J. Habicht, M. Grez, and N. Koch. 1989. A role for Ia-associated invariant chains in antigen processing and presentation. Cell. 56:683.
14. Kim, K.J., C. Kanellopoulos-Langevin, R. Merwin, D. Sachs, and R. Asofsky. 1979. Establishment and characterization of BALB/c lymphoma lines with B cell properties. J. Immunol. 128:2164.
15. Morel, P.A., A.M. Livingstone, and C.G. Fathman. 1987. Correlation of T cell receptor VB gene family with MHC restriction. J. Exp. Med. 166:583.
16. Danska, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. J. Exp. Med. 172:27.

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17. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D.P. Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. *J. Immunol.* 143:1822.

18. 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.

19. Hill, C.M., J.D. Hayball, A.A. Allison, and J.B. Rothbard. 1991. Conformational and structural characteristics of peptides binding to HLA-DR molecules. *J. Immunol.* 147:189.

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

21. Mathis, D.J., C. Benoist, V.E. William II, M. Kanter, and H.O. McDevitt. 1983. Several mechanisms can account for defective Eα gene expression in different mouse haplotypes. *Proc Natl. Acad. Sci. USA.* 80:273.

22. Berkower, I., H. Kawamura, L.A. Matis, and J.A. Berzofsky. 1985. T cell clones to two major T cell epitopes of myoglobin: Effect of Iα/Iβ restriction on epitope dominance. *J. Immunol.* 135:2628.

23. Lechler, R.I. 1988. MHC class II molecular structure – permitted pairs? *Immunol. Today.* 9:76.

24. McNicholas, J.M., D.B. Murphy, L.A. Matis, R.H. Schwartz, E.A. Lerner, C.A. Janeway, and P.P. Jones. 1982. Immune response gene function correlates with the expression of an Iα antigen. *J. Exp. Med.* 155:490.

25. Matis, L.A., P.P. Jones, D.B. Murphy, S.M. Hedrick, E.A. Lerner, C.A. Janeway, J.M. McNicholas, and R.H. Schwartz. 1982. Immune response gene function correlates with the expression of an Iα antigen. *J. Exp. Med.* 155:508.

26. Matis, L.A., L.H. Glimcher, W.E. Paul, and R.H. Schwartz. 1983. Magnitude of response of histocompatibility-restricted T cell clones is a function of the product of the concentrations of antigen and Iα molecules. *Proc. Natl. Acad. Sci. USA.* 80:6019.

27. Germain, R.N., D.W. Bentley, and H. Quill. 1985. Influence of allelic polymorphism on the assembly and surface expression of class II MHC (Ia) molecules. *Cell.* 43:233.

28. Braunstein, N.S., and R.N. Germain. 1987. Allele-specific control of Ia molecule surface expression and conformation: implications for a general model of Ia structure-function relationship. *Proc. Natl. Acad. Sci. USA.* 84:2921.

29. Sant, A.J., N.S. Braunstein, and R.N. Germain. 1987. Predominant role of amino-terminal sequences in dictating efficiency of class II major histocompatibility complex αβ dimer expression. *Proc. Natl. Acad. Sci. USA.* 84:8065.

30. T. Mizuochi, L. Tentori, S.O. Sharrow, A.M. Kruisbeek, and A. Singer. 1988. Differentiation of Ia-reactive CD8+ murine T cells does not require Ia engagement. Implications for the role of CD4 and CD8 accessory molecules in T cell differentiation. *J. Exp. Med.* 168:437.

31. B. Nepom, D. Schwartz, J. Palmer, and G.T. Nepom. 1987. Transcomplementation of HLA genes in IDDM. HLA-DQ alpha- and beta-chains produce hybrid molecules in DR 3/4 heterozygotes. *Diabetes.* 36:114.

32. Sheehy, M.J., S.J. Scharf, J.R. Rowe, M.H. Neme de Gimenez, L.M. Meske, H.A. Erlich, and B.S. Nepom. 1988. A diabetes susceptible HLA haplotype is best defined by a combination of HLA-DR and DQ alleles. *J. Clin. Invest.* 83:830.

33. Todd, J.A., H. Acha-Orbea, J.I. Bell, N. Chao, Z. Pronek, C. Jacob, M. McDermott, A. Sinha, L. Timmerman, L. Steinman, and H.O. McDevitt. 1988. A molecular basis for MHC class II associated autoimmunity. *Science (Wash. DC).* 240:1003.