DIRECT EVIDENCE THAT A CLASS II MOLECULE AND A
SIMPLE GLOBULAR PROTEIN GENERATE MULTIPLE
DETERMINANTS

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It is widely accepted that helper T cells (Th) recognize antigen in the context
of Ia molecules on the surface of antigen-presenting cells (APC) (1, 2). Functional
and structural studies strongly suggest that, like B cells, T cells have a single
antigen receptor (TcR) (3–5). Unlike immunoglobulins, however, TcR appear
to recognize a bimolecular complex composed of a class II molecule and foreign
antigen (6, 7). Thus the ligand of the TcR is far more complex than that of the
B cell.

Little is currently known about the interaction of the TcR with its bimolecular
ligand. On the one hand, this three part interaction might limit the diversity of
T cell antigenic responses if Ia antigens can associate with only a limited number
of foreign antigenic determinants and if the TcR can recognize only a small
number of Ia epitopes. On the other hand, the diversity of the T cell response
would be greatly enlarged if many foreign antigenic determinants can be pre-
sented by Ia molecules and if numerous epitopes on these molecules can be
recognized by the TcR.

We have previously reported studies involving the Ia component of the TcR
ligand (8, 9). These class II Ia molecules are composed of two noncovalently
linked chains, α and β. It now appears that there are a limited number of class
II genes, with six to eight identified in the murine system (10, 11) and 10–12 in
the human system (12). Furthermore, it has been shown that there is a prefer-
ential pairing of homozygous Iaα and Iaβ chains and that not all possible hybrid
combinations are formed (13, 14). Thus, there does not seem to be a large
number of class II-encoded gene products. However, our recent studies exam-
íning the specificity of alloreactive or autoreactive T cells (8, 15, 16) and other
studies examining the I-A mutant B6.C-H-2$^{bmm12}$ (17, 18), have provided evidence
that multiple functional epitopes exist on a single Ia molecule.

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Abbreviations used in this paper: APC, antigen-presenting cells; HEL, hen egg-white lysozyme;
IL-2, interleukin 2; mAb, monoclonal antibody; PEG, polyethylene glycol; TcR, antigen-specific T
cell receptor; Th, helper T cell.
The study presented here is a systematic analysis of the ligand of the TcR. We have examined the individual contributions of the I-A<sub>k</sub> chain, I-A<sub>a</sub> chain, and different determinants on the hen egg-white lysozyme (HEL) molecule in the formation of the complex being recognized by the TcR. As functional probes we have used (a) a panel of 10 I-A<sub>k</sub>-restricted, HEL-specific T cell hybridoma clones, (b) a panel of antigen-presenting B cell hybridomas that bear mutated I-A<sub>k</sub> or I-A<sub>a</sub> chains, and (c) proteolytic fragments of HEL and related synthetic peptides. Our results provide evidence that a single class II molecule and a simple globular protein can generate multiple antigenic determinants. Thus the association of Ia molecules and foreign antigens can generate extraordinary diversity available for the Th cell immune response.

Materials and Methods

T Cell Hybridomas. T cell hybridomas specific for HEL were generated by following the established protocol of polyethylene glycol fusion of primed T cells with AKR thymoma BW5147 cells (3, 18). The resulting T cell hybridomas secreted interleukin 2 (IL-2) upon interaction with an Ia-bearing antigen-presenting cell in the presence of HEL. The T cell hybridomas 2A11, 3A9, 19.47, and 18N.30 were generated in two fusions from CBA/J mice; T hybrids A2.2B2, C10, C4.A1, A4.A1, A6.A2, and B14 were generated in two fusions from B10.A mice. All of the T hybrid lines were subcloned twice to insure the clonality of each line.

Antigen-presenting Cells (APC). The procedure for the production and characterization of the I-A<sup>k</sup> mutant APC lines has been previously described in detail (8, 9, 15, 19). Briefly, TA3 cells (an I-Ad<sup>−/−</sup>/I-E<sup>−/−</sup>-bearing B cell–B lymphoma hybridoma) were mutagenized and the mutant APC lines were isolated by cycles of negative and positive immunoselection.

Culture Conditions and IL-2 Assays. Varying numbers (0.1–3 x 10<sup>4</sup>) of the APC, either the wild-type TA3 or a mutant I-A<sup>k</sup> line, were cocultured with 10<sup>5</sup> T cell hybridoma cells in the continued presence of antigen for 24 h (18). The medium used in all of the experiments consisted of RPMI 1640 supplemented with 8% fetal calf serum, 3 mM L-glutamine, 5 x 10<sup>-5</sup> M 2-mercaptoethanol, penicillin, and Hepes. The level of T cell stimulation was determined by quantitating the amount of IL-2 present in the supernatant. HT-2 cells (4 x 10<sup>3</sup>) were cultured for 36 h in the presence of 40% primary culture supernatants, and the degree of T cell stimulation was measured by the incorporation of [<sup>3</sup>H]thymidine into DNA. We could quantitate the level of IL-2 by assaying a single concentration of primary culture supernatant, since we tested various numbers of APC for each group and used the maximal values that were on the linear portion of the standard curve. The values represent the mean counts per minute (cpm) of triplicate culture wells. The standard deviations never exceeded 20% of the mean.

HEL Peptides. The preparation and purification of the tryptic peptides of HEL have been previously described (20). For the HEL(46-61) tryptic fragment both the purified fragment and a corresponding synthetic peptide were used with identical results. The tryptic peptide HEL(34-45) was identified by determining the amino acid composition of the single high performance liquid chromatography (HPLC)-purified peak that stimulated the C11.A2, A6.A2, and 18N.30 T hybrids. The amino acid composition of this peak fit exactly with that predicted for the tryptic fragment HEL(34-45). The amino acid sequence of HEL(34-45) is Phe-Glu-Ser-Asn-Phe-Asn-Thr-Glu-Ala-Thr-Asn-Arg and the sequence for HEL(46-61) is Asn-Thr-Asp-Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Glu-Ile-Asn-Ser-Arg.

Monoclonal Antibodies (mAb). These have been described and referenced in previous reports (8, 9, 15, 19). The majority of the A<sup>k</sup>- and A<sup>a</sup>-specific mAb were produced by Pierres and his coworkers (21).
Results

Class II I-A<sup>a</sup> Mutant Cell Lines. The production and characterization of some of the A<sup>b</sup> mutant cell lines has been described previously (8, 9, 15, 19). A more detailed characterization of the remainder of the A<sup>b</sup> mutant cell lines and the A<sup>b</sup> variant cell lines will be presented elsewhere (L. Glimcher, D. McKean, in preparation). All of these lines were produced from the parent line, TA3, a B cell–B lymphoma hybridoma produced from the fusion of (BALB/c × A/J)F<sub>1</sub> spleen cells with the M12.4.1 BALB/c I<sup>a</sup>-bearing B lymphoma (18). This was accomplished by cycles of negative and positive immunoselection with different mAb specific for A<sup>b</sup> or A<sup>a</sup>. By varying the mAb selected for the negative and positive immunoselection steps, a panel of cell lines with distinct serologic phenotypes was obtained. The phenotypes of the six A<sup>b</sup> variant cell lines and the three A<sup>a</sup> variant cell lines, as defined by their reactivity to a large panel of A<sup>b</sup>- and A<sup>a</sup>-specific mAb, is shown in Tables I and II. Note that cell line 89 reacts with none of the anti-I-A<sup>b</sup> mAb and thus expresses no I-A<sup>b</sup> molecule. It does express an I-E<sup>b</sup> molecule (not shown) (9). The remainder of the cell lines lost reactivity with certain mAb but retained reactivity with others. Although several of the cell lines appear serologically similar (A19 and LD3; 3J9 and JE50) they have clear-cut functional differences, as shown below. These APC lines all (except 89) constitutively express I-A<sup>b</sup>, I-E<sup>b</sup>, I-A<sup>a</sup>, and I-E<sup>d</sup> molecules at comparable levels to the wild-type TA3 cell line.

Reactivity with Class II Mutant APC Divides the HEL Hybridomas into Multiple Groups. The panel of HEL-specific hybridomas arose from four separate fusions of primed lymph node cells from CBA/J mice (hybrids 2A11, 3A9, 19.47, and

| Anti-A<sup>b</sup> mAb | 10.2.16 | 40A | 40N | 39B | 39E | 40L | 40F | 40M |
|---------------------|--------|-----|-----|-----|-----|-----|-----|-----|
| TA3 (wild-type)     | ++     | ++  | ++  | ++  | ++  | ++  | ++  | ++  |
| A19                 | +/-    | -   | -   | +/- | -   | ++  | ++  |
| JT1                 | -      | ++  | ++  | ++  | ++  | ++  | ++  |
| 40LB3               | ++     | ++  | ++  | ++  | ++  | -   | NT  |
| K5                  | ++     | -   | -   | +/- | -   | ++  | ++  |
| LD3                 | +/-    | -   | -   | +/- | -   | ++  |
| 89                  | -      | -   | -   | -   | -   | -   | -   |
| ++, Wild-type; +/-, weak positive signal, occasionally negative; -, negative; NT, not tested.

| Anti-A<sup>a</sup> mAb | 39J | 39C | 40J | H116.32 | 1E9 | 2A11 |
|------------------------|-----|-----|-----|----------|-----|------|
| TA3 (wild-type)        | ++  | ++  | ++  | ++       | ++  |
| 3J9                    | -   | -   | -   | -        | ++  |
| JE67                   | -   | -   | -   | -        | ++  |
| JE50                   | -   | -   | -   | -        | ++  |
| ++, Wild-type; -, negative.
18N.30) or from B10.A mice (A2.2B2, C10, C4.A1, C4.A2, A4.A1, A6.A2, and B14) with the AKR thymoma BW5147. The basic experimental protocol involving coculturing these hybridomas with the class II mutant APC lines in the presence of native HEL and testing the supernatant for IL-2 activity 24 h later. The results of a typical experiment testing the ability of the panel of A\textdoublescript{b} mutant cell lines to present native HEL to the 10 HEL-specific I-A\textdoublescript{b}-restricted T cell hybridomas is shown in Table III. The wild-type TA3 cells were able to present HEL to all of the 10 I-A\textdoublescript{b}-restricted cell lines in addition to an I-E\textdoublescript{b}-restricted HEL-specific hybridoma, B14. The 89 cell line, as expected, stimulated only the I-E\textdoublescript{b}-restricted B14 cell line, confirming that the other 10 cell lines are I-A\textdoublescript{b} specific. Two of the A\textdoublescript{b} mutant lines, JT1 and 40LB3, presented HEL to all the hybridomas equally as well as the TA3 cell. Thus, in these lines, there was no discernible functional effect of the altered A\textdoublescript{b} molecules on APC ability. On the other hand, the K5 mutant cell could stimulate none of the I-A\textdoublescript{b}-restricted hybrids; this was not due to a generalized loss of APC function, since it activated the B14, I-E\textdoublescript{b}-restricted hybrid perfectly well. The results obtained from an analysis of these 10 hybridomas with the remaining two A\textdoublescript{b} mutant cell lines, A19 and LD3, however, subdivided the T hybridomas into three groups. The group I hybrids were not activated by the A19 cell line but were activated by the LD3 cell line, in a manner ranging from modest activation (19.47) to wild-

### Table III

| Group | HEL-specific T hybridoma | TA3   | 89     | A19   | LD3   | JT1   | K5     | 40LB3  |
|-------|---------------------------|-------|--------|-------|-------|-------|--------|--------|
| I     | C10                       | 117,183 | 695    | 425   | 105,600 | 108,690 | 1,218  | 123,858 |
|       | C4.A1                     | 24,839 | 571    | 265   | 21,118 | 107,989 | 395    | 16,599  |
|       |                            | 67,444 | 713    | 288   | 43,740 | 65,332  | 616    | 20,151  |
|       | C11.A2                    | 169,701 | 4,918   | 1,353 | 74,912 | 161,853 | 6,917  | 169,268 |
|       | 2A11                      | 101,583 | 1,126  | 939   | 29,320 | 92,551  | 1,049  | 111,223 |
|       | A4.A1                     | 71,966  | 829    | 638   | 27,115 | 68,037  | 486    | 55,738  |
|       | 19.47                     | 116,934 | 584    | 520   | 14,532 | 111,206 | 947    | 108,750 |
| II    | 3A9                       | 46,164 | 441    | 82,336 | 572 | 145,468 | 406    | 42,254  |
|       | A2.2B2                    | 118,238 | 385  | 81,941 | 85,026 | 91,484  | 649    | 135,912 |
|       | 18N.30                    | 25,352  | 262    | 5,861 | 7,632 | 56,160  | 375    | 21,487  |
|       | A6.A2                     | 137,081 | 585    | 14,078 | 74,036 | 123,870 | 408    | 115,98  |
| IV    | B14                       | 103,538 | 115,304 | 64,380 | 105,712 | 129,337 | 133,278 | 138,239 |

T hybridoma cells (10^6) were cocultured with the designated stimulator cells in 0.2 ml of complete medium in the presence of native HEL (100 μg/ml). After 24 h of culture, supernatants were collected and assayed for IL-2 content in a secondary culture of HT-2 cells. HT-2 cells (4 x 10^3) were cultured for 36 h in the presence of 40% primary culture supernatant, and the degree of stimulation was measured by the incorporation of [3H]thymidine into DNA. Varying numbers of stimulators of each type, from 1 to 30 x 10^3, were used. Only the maximum response obtained is shown. These were generally achieved with 1-10 x 10^3 stimulators per well. Positive responses are underlined. Responses were determined to be positive if they were twice the magnitude of the cpm obtained with the I-A\textdoublescript{b}-negative cell line, 89.
type (C10, C4.A1). The group II hybrid, 3A9, had a reciprocal pattern to the group I hybrids, since the A19 but not the LD3 cell could present antigen to this hybridoma. The group III hybrids showed yet another phenotype of activation, since both the A19 and LD3 cell lines could present HEL to these three hybrids. Thus, as probed with this panel of A8 variant APC lines, these HEL-specific T cells appear to recognize several distinct epitopes on the I-A8 molecule.

To further define determinants on the I-A8 molecule responsible for antigen presentation we examined the contribution of the A8 molecule to the formation of the restriction site used by each hybridoma. Table IV shows the results of a representative experiment testing the ability of the three A8 mutant lines to activate the panel of hybridomas. The 3J9 and JE67 lines are serologically and functionally identical while the JE50 cell line, although serologically similar, displayed very disparate functional properties. Specifically, the 3J9 and JE67 cell lines activated all the hybridomas except the two group I hybridomas, C10 and C4.A1, while the JE50 cell line activated only the group I hybridomas C10 and 19.47.

When the results of the ability of the I-A8 and I-A8 mutant cell lines to present antigen are combined, the panel of T cell hybridomas can be divided into six separate groups (Table VI). Groups Ia and Ib contain the T cells C10 and C4.A1, respectively. These two groups are distinguished from the other four by their failure to react to the JE67 and 3J9 A8 mutant APC lines. They are distinguished from each other by their differential reactivity with the JE50 cell. Groups I, and Id contain four T cells, C11,A2, 2A11, A4.A1, and 19.47, all of which have identical reactivity patterns to the I-A8 mutants. Two findings differentiate group Ic and Id from Ib hybridomas: (a) the ability of JE67 and 3J9 to present HEL

**Table IV**

| Group | HEL-specific T hybridoma | Experiment 1 | Experiment 2 |
|-------|--------------------------|--------------|--------------|
|       |                          | cpm          | cpm          | cpm          | cpm          |
|       |                          | TA3          | JE67         | JE50         | TA3          | 3J9.2        |
| Ia    | C10                      | 59,201       | 1,360        | 18,579       | 112,010      | 787          |
| Ib    | C4.A1                    | 47,286       | 619          | 943          | 83,305       | 3,957        |
| Ic    | C11,A2                   | 65,730       | 118,770      | 575          | 101,561      | 98,187       |
|       | 2A11                     | 33,665       | 48,379       | 365          | 101,583      | 99,984       |
|       | A4.A1                    | 45,102       | 107,751      | 531          | 49,873       | 108,880      |
| Id    | 19.47                    | 69,393       | 34,214       | 11,602       | 177,734      | 94,499       |
| II    | 3A9                      | 43,629       | 68,932       | 502          | 63,107       | 98,217       |
| III   | A2.2B2                   | 30,096       | 22,264       | 231          | 45,266       | 50,049       |
|       | 18N.30                   | 41,913       | 34,392       | 249          | 53,290       | 51,549       |
|       | A6.A2                    | 49,190       | 48,742       | 262          | 113,565      | 106,628      |
| IV    | B14                      | 61,970       | 52,506       | 67,585       | 109,671      | 107,730      |

Assay performed as detailed in the legend to Table III.
to the former, and (b) the diminished ability of LD3 to stimulate the group I, and I, T cells. Groups I, and I, are distinct since the JE50 cell can activate the 19.47 hybridoma but not the other hybrids. Group II consists of only one T cell hybrid, 3A9, whose unique response pattern is its activation by the A19 cell but not by the LD3 cell. Three T cell hybrids belong to group III, A2.2B2, 18N30, and A6.A2. Their distinguishing response pattern is that both the A19 and LD3 mutant cells can present HEL to them. Group IV contains the I-E-restricted T cell hybrid. This data is summarized schematically in Table VI.

**HEL Hybrids Recognize at Least Three Distinct Determinants on the HEL Molecule.** The second portion of this analysis involved determining the fine antigen specificity of the 11 HEL-specific T cell hybridomas. Two of the hybrids, 2A11 and 3A9, have already been extensively characterized and found to respond to the tryptic fragment of HEL containing residues 46–61 (20). It was subsequently shown (22) that the minimal stimulatory peptide for both 2A11 and 3A9 was HEL(52–61): furthermore, the immunogenicity of this peptide has been localized to the Leu56 residue.

We initially determined which if any of the other nine T cell hybrids were recognizing HEL(46–61). Using wild-type TA3 cells as the presenting cells we determined that three other T cell hybridomas, C10, A4.A1, and A2.2B2 responded to HEL(46–61). These three T cell lines responded to both the tryptic fragment HEL(46–61) and the synthesized HEL(46–61) and to no other HEL tryptic fragments (Table V). We next tested the ability of the other tryptic fragments of HEL to stimulate the remaining six hybridomas. Three of the hybridomas responded to a tryptic fragment containing the residues 34–45 (Table V). The C11.A2 and the A6.A2 cells responded strongly to HEL(34–45), while the 18.N30 cells' response was much weaker. These three T cell lines responded only to this HEL(34–45) tryptic fragment; thus, we have identified a second determinant(s) on the HEL molecule that three of the T cell hybrids recognize. It is interesting that, for the two determinants on the HEL molecule

| HEL-specific T hybridoma | HEL | 46-61 | 34-45 |
|--------------------------|-----|-------|-------|
| C10                      | 128,927 | 79,353 | 415   |
| C4.A1                    | 102,097 | 246   | 570   |
| C11.A2                   | 13,328  | 226   | 128,504 |
| A4.A1                    | 7,701   | 18,073 | 692   |
| A6.A2                    | 23,800  | 262   | 88,160 |
| 19.47                    | 123,783 | 221   | 740   |
| A2.2B2                   | 99,350  | 110,050 | 813   |
| 18N.30                   | 13,801  | 315   | 34,768 |
| B14                      | 109,671 | 2,987 | 2,656 |

T hybridoma cells (10⁶) were cocultured with 3 x 10⁴ TA3 cells along with either 7 μM HEL, 10 μM HEL(46-61), or 5 μM HEL(34-45). The level of T cell stimulation was determined as described in Table III. The positive responses are underlined.
MULTIPLE DETERMINANTS FORMED BY I-A^* MOLECULE AND LYSOZYME

**Table VI**

Summary of the Response Patterns to I-A^d Mutants, I-A^k Mutants, and Antigen Specificity

| Group | Cell line | Wild-type T cell line | I-A^d Mutants | I-A^k Mutants | Antigen Specificity |
|-------|-----------|-----------------------|---------------|---------------|---------------------|
| Ia    | G10       | ++                    | ++            | +++           | +                   |
| Ib    | C4.A1     | ++                    | ++            | +++           | ++                  |
| Ic    | C11.A2    | ++                    | ++            | +++           | ++                  |
| Id    | 2A11      | ++                    | ++            | +++           | ++                  |
| Ie    | A4.A1     | ++                    | ++            | +++           | ++                  |
| If    | 19.47     | ++                    | ++            | +++           | ++                  |
| IIa   | 5A9       | ++                    | ++            | +++           | ++                  |
| IIIB  | A6.A2     | ++                    | ++            | +++           | ++                  |
| IIIA  | A2.282    | ++                    | ++            | +++           | ++                  |
| IIIB  | 18N.30    | ++                    | ++            | +++           | ++                  |
| IVB   | B14       | ++                    | ++            | +++           | ++                  |

++, Wild-type response; +, positive but less than wild-type response; -, negative response; ??, antigen specificity was only localized to HEL(13-105).

Discussion

When the HEL antigen specificities recognized by the 10 I-A^k-restricted T cell hybridomas are combined with their response patterns to the A^b and A^b variant cell lines, a very complex pattern emerges. In fact, this analysis reveals that the 10 T cell hybrids can be divided into 8 distinct groups. There does not appear to be any correlation between the peptide recognized by the hybridomas and their response pattern to the panel of I-A variant APC lines. Thus, T cells that recognize the peptide 46-61 belong to the groups Ia, Ic, II, and III. Similarly, those recognizing peptide 34-45 belong to groups Ic and III. Of the 10 T cell
hybrids there are only two pairs that have the same reactivity pattern and antigen specificity: 2A11-A4.A1 and A6-A2-18N.30. The major conclusion from this analysis, therefore, is that many different determinants or TcR ligands can be generated from a single Ia molecule and a simple globular protein. Furthermore, in agreement with previous work (8, 19), it appears that both the A\textsubscript{b} and A\textsubscript{d} molecules are necessary to form the restriction element used, since the K5 A\textsubscript{b} mutant cell line stimulated none of the T cells, while none of the T cells could be activated by all three A\textsubscript{d} mutant cell lines. That the A\textsubscript{b} and A\textsubscript{d} molecules used must be homozygous is supported by the recent finding that very few hybrid molecules are present in these cells (24).

The T cell hybrids used in this study were generated from two different mouse strains, CBA/J and B10.A. From our analysis we found no correlation between the strain of origin of the hybrid and their reactivity pattern to the mutant I-A\textsuperscript{k} APC or their antigen specificity. In fact, the two pairs of T cell hybrids that we could not distinguish from each other, 2A11-A4.A1 and A6.A2-18N.30, were each composed of T hybrids from two different mouse strains. These experiments, therefore, identify an immunodominant determinant on the HEL molecule recognized by mice of the H-2\textsuperscript{k} haplotype. Clearly, the HEL(46-61) peptide contains the major determinant since 5 of the 10 T cell hybrids responded to this fragment. Three of the T cell hybrids recognized a second determinant located in the HEL(34-45) peptide. These two determinants appear to represent a reasonable portion of the T cell response in H-2\textsuperscript{k} mice, since 8 of the 10 hybrids responded to one or the other of them. We were unable to identify a proteolytic fragment of HEL other than the large CNBr fragment HEL(13-105), which the C4.A1 and the 19.47 cells recognize. Shastri et al. (25) have shown that a minor determinant recognized by H-2\textsuperscript{k} mice is located in the HEL(74-96) fragment. This fragment did not stimulate either C4.A1 or 19.47; however, the HEL(74-96) peptide could potentially contain part of an immunogenic determinant that was destroyed by the cleavage of HEL by trypsin to produce the fragment.

Obviously, this analysis is only as complete as is the definition of distinct regions on the I-A\textsuperscript{k} molecule, as well as the fine specificity of peptide recognition. Further efforts in these directions may result in an even more complex picture than is presented here. Nevertheless, this already striking heterogeneity must be reflected at the level of TcR proteins. In this regard it is interesting that the cloning and sequencing of TcR $\beta$ chain genes from seven members of a BALB/c thymocyte library revealed remarkably little heterogeneity in the $V_{\beta}$ genes used (26). It will be interesting to isolate and sequence both TcR beta and alpha chain genes from selected members of this panel of HEL-specific T hybridomas to determine whether a correlation exists between the I\textsubscript{a} determinant or peptide recognized and the variable, diversity, or joining (V, D, or J) TcR $\beta$ and $\alpha$ gene segments used.

The two T cell hybridomas whose fine specificity has been most extensively studied are the 2A11 and the 3A9 cell lines (21, 22). It has been shown that these two hybrids recognize very similar but distinct determinants composed of the same essential residues, 52-61 (22). In this study we have found a clear difference between these two T cell lines in their response patterns to the panel of mutant APC lines. The A19 cell line failed to present HEL to the 2A11 cells.
but did present to the 3A9 cells. Conversely, the LD3 cell line could present to the 2A11 cells, but not to the 3A9 cells. The site of the mutation of the A19 cell line has recently been determined to be a single base change resulting in a glutamine to a lysine substitution at position 67 in the I-A^k chain (M. Brown, L. Glimcher, E. Nielsen, W. E. Paul, and R. Germain, submitted for publication). The location of the mutation in the LD3 I-A^k chain is currently being determined.

Putting all of the patterns for the 2A11 and 3A9 T cells together allows us to analyze how the ligand recognized by these two TcR may be formed. Clearly, the same HEL peptide 46-61 can combine with I-A^k to differentially activate these two T cells. It appears that position 67 of the A^k chain is an essential residue in the formation of the determinant recognized by the 2A11 cells, but that it is not involved in the formation of the determinant recognized by the 3A9 cells. Conversely, the mutational site in the LD3 I-A^k chain is essential in the formation of the determinant recognized by the 3A9 cells but not by the 2A11 cells. Whether this is because these particular regions of the class II molecule interact with the HEL peptide 46-61, or whether it is the interaction between TcR proteins and these residues that determines the T cell specificity, cannot be determined from these data. In other words, the 52-61 peptide may use different residues of the A^k chain to interact with, thus creating distinct determinants, or, alternatively, the peptide's interaction with A^k may be invariant and the differential reactivity of the 2A11 and 3A9 hybrids determined solely by the TcR-I-A^k interaction. We think it is unlikely that the failure of the A19 or LD3 cells to activate certain T cells is secondary to an absolute inability of the peptide to associate productively with these mutant molecules, since the mutant cell lines can activate other T cells that also recognize this peptide. However, it is possible that the active peptide recognized by these T cells is, in fact, slightly different. The answer to some of these questions will require direct-binding studies of purified mutant La antigens to the relevant peptides as well as the crystallographic structure of the wild-type and mutant molecules. Since recent studies (27) have established that direct binding of the HEL peptide 46-61 to purified La antigens from the wild-type APC line, TA3, does occur, it should be possible to perform these same assays with purified mutant molecules.

Summary

We have examined the individual contributions of the I-A^k chain, the I-A^b chain, and the foreign antigen hen egg-white lysozyme (HEL) in the formation of the determinant being recognized by the T cell receptor. As functional probes we have used (a) a panel of 10 HEL-specific T cell hybridomas, (b) a panel of antigen-presenting cells (APC) possessing mutations in either the I-A^k or I-A^b chains, and (c) proteolytic fragment of HEL and related synthetic peptides. The ability of the I-A^k and I-A^b mutant cell lines to present antigen to the 10 T cell hybridomas divided these T cells into six distinct groups. These HEL-specific T cells therefore appear to recognize several distinct domains on the I-A^k molecule. The 10 T cell hybrids were then shown to recognize at least three distinct determinants on the HEL molecule, with 8 of the 10 hybrids recognizing one of two major determinants HEL(46-61) or HEL(34-45). Combining the response
patterns to the panel of I-A<sup>k</sup> mutant APC lines with the antigen specificity revealed that the 10 T cell hybrids recognized at least eight unique determinants formed by the I-A<sup>k</sup> chains, I-A<sup>b</sup> chains, and HEL peptides. This analysis provides direct evidence that a large number of different determinants or T cell receptor ligands can be generated from a single Ia molecule and a simple globular protein.

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