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)[1] 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 presented 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 preferential pairing of homozygous Ia_α and Ia_β 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 examining the specificity of alloreactive or autoreactive T cells (8, 15, 16) and other studies examining the I-A mutant B6.C-H-2^{bm12} (17, 18), have provided evidence that multiple functional epitopes exist on a single Ia molecule.

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[1] *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.

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The study presented here is a systematic analysis of the ligand of the TcR. We have examined the individual contributions of the I-A^k_α chain, I-A^k_β 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^k-restricted, HEL-specific T cell hybridoma clones, (*b*) a panel of antigen-presenting B cell hybridomas that bear mutated I-A^k_α or I-A^k_β 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^k mutant APC lines has been previously described in detail (8, 9, 15, 19). Briefly, TA3 cells (an I-$A^{d/a}$/I-$E^{d/k}$-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 ($0.1–3 \times 10^4$) of the APC, either the wild-type TA3 or a mutant I-A^k line, were cocultured with 10^5 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, 5×10^{-5} 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 (4×10^3) 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 [^{3}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^k_β- and A^k_α-specific mAb were produced by Pierres and his coworkers (21).

Results

Class II I-A^k Mutant Cell Lines. The production and characterization of some of the A^k_β mutant cell lines has been described previously (8, 9, 15, 19). A more detailed characterization of the remainder of the A^k_β mutant cell lines and the A^k_α 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_1 spleen cells with the M12.4.1 BALB/c Ia^d-bearing B lymphoma (18). This was accomplished by cycles of negative and positive immunoselection with different mAb specific for A^k_β or A^k_α. 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^k_β variant cell lines and the three A^k_α variant cell lines, as defined by their reactivity to a large panel of A^k_β- and A^k_α-specific mAb, is shown in Tables I and II. Note that cell line 89 reacts with none of the anti-I-A^k mAb and thus expresses no I-A^k molecule. It does express an I-E^k 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^k, I-E^k, I-A^d, and I-E^d 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

TABLE I
Phenotypes of the A^k_β Variant Cell Lines

	Anti-A^k_β 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.

TABLE II
Phenotypes of the A^k_α Variant Cell Lines

	Anti-A^k_α 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^k_β mutant cell lines to present native HEL to the 10 HEL-specific I-A^k-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^k-restricted cell lines in addition to an I-E^k-restricted HEL-specific hybridoma, B14. The 89 cell line, as expected, stimulated only the I-E^k-restricted B14 cell line, confirming that the other 10 cell lines are I-A^k specific. Two of the A^k_β 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^k_β molecules on APC ability. On the other hand, the K5 mutant cell could stimulate none of the I-A^k-restricted hybrids: this was not due to a generalized loss of APC function, since it activated the B14, I-E^k-restricted hybrid perfectly well. The results obtained from an analysis of these 10 hybridomas with the remaining two A^k_β 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
A^k_β Variant APC Lines Divide the T Hybridomas into Several Groups

Group	HEL-specific T hybridoma	TA3	89	A19	LD3	JT1	K5	40LB3
					cpm			
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	116,634	584	520	14,532	111,206	947	108,750
II	3A9	46,164	441	82,336	572	145,468	406	42,254
III	A2.2B2	118,238	385	81,941	85,026	91,484	649	135,912
	18N.30	23,352	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	64,280	103,712	129,337	123,278	138,239

T hybridoma cells (10^5) 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 (4×10^3) were cultured for 36 h in the presence of 40% primary culture supernatant, and the degree of stimulation was measured by the incorporation of [^{3}H]thymidine into DNA. Varying numbers of stimulators of each type, from 1 to 30×10^3, were used. Only the maximum response obtained is shown. These were generally achieved with $1-10 \times 10^3$ 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^k-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 A^k_β variant APC lines, these HEL-specific T cells appear to recognize several distinct epitopes on the I-A^k molecule.

To further define determinants on the I-A^k molecule responsible for antigen presentation we examined the contribution of the A^k_α 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 A^k_α 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-A^k_β and I-A^k_α mutant cell lines to present antigen are combined, the panel of T cell hybridomas can be divided into six separate groups (Table VI). Groups I_a and I_b 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 A^k_α mutant APC lines. They are distinguished from each other by their differential reactivity with the JE50 cell. Groups I_c and I_d contain four T cells, C11,A2, 2A11, A4.A1, and 19.47, all of which have identical reactivity patterns to the I-A^k_β mutants. Two findings differentiate group I_c and I_d from I_a and I_b hybrids: (*a*) the ability of JE67 and 3J9 to present HEL

TABLE IV

A^k_α Variant APC Lines Further Subdivide the T Hybridomas

Group	HEL-specific T hybridoma	Experiment 1			Experiment 2	
		TA3	JE67	JE50	TA3	3J9.2
			cpm		*cpm*	
I_a	C10	59,201	1,360	18,579	112,010	787
I_b	C4.A1	47,286	619	943	83,305	3,957
I_c	C11.A2	65,730	118,770	575	101,561	98,187
	2A11	33,665	48,379	365	101,583	92,984
	A4.A1	45,102	107,751	531	49,873	108,880
I_d	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_c and I_d T cells. Groups I_c and I_d 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 Leu^{56} 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

TABLE V
Fine Antigen Specificity of HEL Hybridomas

HEL-specific T hybridoma	HEL	46-61	34-45
		cpm	
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 (10^5) were cocultured with 3×10^4 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.

TABLE VI

Summary of the Response Patterns to I-A^k_β Mutants, I-A^k_α Mutants, and Antigen Specificity

Group	Cell line	Wild-type TA3	I-A^k_β Mutants						I-A^k_α Mutants			Antigen Specificity		
			89	A19	LD3	JT1	K5	40LB3	JE67	3J9.2	JE50	46-61	34-45	??
I_a	C10	++	−	−	++	++	−	++	−	−	++	++	−	−
I_b	C4.A1	++	−	−	++	++	−	++	−	−	−	−	−	++
I_c	C11.A2	++	−	−	+	++	−	++	++	++	−	−	++	−
I_c	2A11	++	−	−	+	++	−	++	++	++	−	++	−	−
I_c	A4.A1	++	−	−	+	++	−	++	++	++	−	++	−	−
I_d	19.47	++	−	−	+	++	−	++	++	++	+	−	−	++
II	3A9	++	−	++	−	++	−	++	++	++	−	++	−	−
III	A6.A2	++	−	+	++	++	−	++	++	++	−	−	++	−
III	A2.2B2	++	−	++	++	++	−	++	++	++	−	++	−	−
III	18N.30	++	−	+	+	++	−	++	++	++	−	−	++	−
IV	B14	++	++	++	++	++	++	++	++	++	++	−	−	−

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

that 8 of the 10 I-A^k-restricted hybrids recognize, there are colinear but nonoverlapping sequences. Perhaps this is because the physiological processed fragment of HEL produced by the APC is a large fragment that contains both the HEL(34-45) and HEL(46-61) determinants. The remaining three cell lines, C4.A1, 19.47, and B14, did not respond to any of the tryptic fragments of HEL, including the HEL(34-45) and HEL(46-61) fragments. We therefore tested other proteolytic fragments of HEL for their ability to stimulate these three cell lines. One scheme for generating fragments was based on the assumption that trypsin was cleaving and thus inactivating the immunogenic determinant. We therefore tried a chymotryptic digest and a trypsin digest of HEL in which the Lys residues were blocked by citraconylation and only the arginyl bands were cleaved (23). We were still unable to identify a single peptide that could stimulate any of these three cell lines. Both the C4.A1 and 19.47 hybridomas did respond to the large CNBr fragment of HEL containing residues 13–105. Thus, for C4.A1 and 19.47 we can only localize the immunogenic determinant to the fragment HEL(13-105). Likewise, we have not been able to identify the HEL determinant recognized by the I-E^k-restricted B14 cell. The B14 cells do recognize some processed form of HEL since prefixed APC could not present native HEL to the B14 cells (not shown), but we have been unable to identify any fragment that can stimulate these cells. Thus, these experiments demonstrate that this panel of 11 H-2^k-restricted T hybridomas recognize, at a minimum, three distinct determinants on the HEL molecule.

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^k_β and A^k_α 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 I_a, I_c, II, and III. Similarly, those recognizing peptide 34-45 belong to groups I_c 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^k_β and A^k_α molecules are necessary to form the restriction element used, since the K5 A^k_β mutant cell line stimulated none of the T cells, while none of the T cells could be activated by all three A^k_α mutant cell lines. That the A_β and A_α 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^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^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^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^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^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 β chain genes from seven members of a BALB/c thymocyte library revealed remarkably little heterogeneity in the V_β 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_a determinant or peptide recognized and the variable, diversity, or joining (V, D, or J) TcR β and α 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_β 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_β 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_β 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_β 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_β chain to interact with, thus creating distinct determinants, or, alternatively, the peptide's interaction with A^k_β may be invariant and the differential reactivity of the 2A11 and 3A9 hybrids determined solely by the TcR–I-A^k_β 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 Ia 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 Ia 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_α chain, the I-A^k_β 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_α or I-A^k_β chains, and (*c*) proteolytic fragment of HEL and related synthetic peptides. The ability of the I-A^k_β and I-A^k_α 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^k 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$^k_\alpha$ chains, I-A$^k_\beta$ 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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