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Immunology

Cotransfer of the E^d_α and A^d_β genes into L cells results in the surface expression of a functional mixed-isotype Ia molecule

(gene transfer/Ia molecule/α/β chain gene pairing/T-cell recognition/immune response genes)

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ABSTRACT **Ia molecules play a key role in antigen recognition by T lymphocytes. To analyze the structural features of the individual α and β chains relevant to the assembly of intact Ia molecules, mouse fibroblasts were cotransfected with various combinations of haplotype- and isotype-mismatched *Ia* α/β gene pairs. Two important points emerged. First, the level of surface expression of a given haplotype-mismatched $A_\alpha A_\beta$ pair appears to depend upon the α and β chain alleles involved. Second, transfection with some isotype-mismatched combinations such as $E^d_\alpha A^d_\beta$ results in a significant level of surface expression of a stable mixed-isotype dimer, which also appears to be normally expressed at a low level by an Ia^d-positive B lymphoma. Moreover, a T-cell hybridoma specific for human gamma globulin and restricted by the E^d molecule was found to be efficiently stimulated by the $E^d_\alpha A^d_\beta$-positive transfectant in the presence of antigen. The stimulation was specifically inhibited by monoclonal antibodies directed to either the Ia or the L3T4 molecule. These findings suggest that the estimates of the potential number of Ia molecules available in an animal for restricting T-lymphocyte recognition of antigens must be revised.**

The *I* region of the mouse major histocompatibility complex encodes four polypeptide chains which are denoted A_α, A_β, E_α, and E_β and are involved in the control of immune responsiveness (1). Both α and β chains may be divided into two extracellular regions of approximately 90 residues each, a transmembrane region of about 30 residues, and a cytoplasmic tail of about 15 residues. The A_α and A_β chains associate noncovalently inside the cell as one isotypic heterodimer, the I-A molecule, which is then transported to the cell surface. Similarly, the E_α chain combines noncovalently with the E_β chain to give rise to a second cell-surface isotype, the I-E molecule (2). Association between the α and β chains is apparently required for the expression of either at the cell surface (3). The expression of a homodimer such as $E_\alpha E_\alpha$ or of mixed-isotype dimers (e.g., $A_\alpha E_\beta$ or $E_\alpha A_\beta$) have not been described to date. This suggests that the assembly of an isotype-matched α/β chain dimer is controlled by amino acid residues that are conserved in each chain within an isotype (4). In accordance with this hypothesis, analysis of *I*-region-heterozygous animals has revealed the existence of "hybrid" (transcomplementing) Ia molecules, resulting from the combinatorial association between isotype-matched α and β chains (5, 6). For instance, in ($H\text{-}2^b \times H\text{-}2^k$) F_1 mice, comparable amounts of $A^b_\alpha A^k_\beta$ and $A^k_\alpha A^b_\beta$ dimers are expressed on the cell surface in addition to the parental $A^b_\alpha A^b_\beta$ and $A^k_\alpha A^k_\beta$ molecules. However, reports of widely differing concentrations of different $A_\alpha A_\beta$ or $E_\alpha E_\beta$ complexes in cells from some *I*-region heterozygotes (7–9) suggest that polymorphic residues present on the first domain of α/β chains may also contribute to dimer formation.

Recent studies have shown that L cells transfected with isotype- and haplotype-matched pairs of α/β chain genes can express the corresponding Ia (*I* region-associated) molecules on the cell surface (reviewed in ref. 10). The ability to express unique combinations of *Ia* genes in isolation after transfer into mouse L cells offers the opportunity to analyze the precise rules controlling the pairing of α/β polypeptide chains. In this report, we examine this question directly by transfecting L cells with haplotype-mismatched and isotype-mismatched pairs of genes. Although L cells expressing $A^d_\alpha A^k_\beta$ molecules were readily detected, surface expression of the reciprocal combination $A^k_\alpha A^d_\beta$ was not observed. Unexpectedly, transfection of L cells with the isotype-mismatched $E^d_\alpha A^d_\beta$ gene pair resulted in the cell surface expression of a mixed-isotype Ia dimer. That this molecule was nevertheless functional was seen by the ability of this $E^d_\alpha A^d_\beta$-expressing L-cell transfectant to present the antigen human gamma globulin (HGG) to an HGG-specific and I-E^d-restricted T-lymphocyte hybridoma. The earlier estimates generally assumed the existence only of intraisotypic α/β combinatorial association. Our results suggest that the potential number of Ia-restricting elements available in an animal may be larger than these estimates. This may be beneficial to the animal, as it has been suggested that combinatorial association expands the immunologic response capacities of *I* region-heterozygous animals over those of homozygous animals (11–13). Thus, our data suggest that the Ia-molecule repertoire size may be even larger and more accurately estimated, at least for some haplotype combinations (e.g., $H\text{-}2^d \times H\text{-}2^k$), as the total number of compatible intra and interisotype α/β chain associations.

MATERIALS AND METHODS

Mice. All strains, the origin of which have been described (14), were maintained in our animal facilities.

Cell Lines. The AODH7.1 T-lymphocyte hybridoma was kindly provided by P. Marrack (15). It originates from DBA/2 mice and produces interleukin 2 (IL-2) when stimulated with the antigen HGG in the presence of I-E^d-positive antigen-presenting cells. The IL-2-dependent cytotoxic T lymphocyte L cell line was maintained as described (16). M12.4.1 and A20-2J AG^R are two Ia^d-positive B-cell lymphomas (17, 48).

Transfected Fibroblast Cell Lines. The transfectants were generated by cotransfer of the genes of Ia subunits (6 μg each) and thymidine kinase (100 ng) into the mouse Ltk^- cell line (18). The selection of clones CA14.11.14 ($A^k_\alpha A^k_\beta$), CA36.3.2 ($E^d_\alpha E^d_\beta$), and CA36.1.3 ($E^k_\alpha E^{d/k}_\beta$) has been described (18, 19). The CA42.13.10 L-cell transfectant clone expresses the A^d_β chain in combination with the E^d_α chain. Plasmid pCA52

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Abbreviations: HGG: human gamma globulin; IL-2: interleukin 2.

containing the entire A^d_β gene from BALB/c liver DNA was constructed by inserting into pSV2gpt a 9-kilobase *Eco*RI–*Bam*HI fragment obtained by partial digestion of cosmid *H-2*^{d} 34.2 (20). Plasmid pCA66 containing the complete E^d_α gene was constructed by inserting into pUC9 a 6.5-kilobase *Hin*dIII fragment from cosmid *H-2*^{d} 17.2 (20). The A^d_α gene used in construction of CA29.5.2 and CA34.9.5 L-cell transfectants was derived as described by Norcross *et al.* (21). The CA45.6.7 and CA45.8.9 L-cell transfectants express hybrid E_β chain in combination with E^k_α (unpublished data).

Monoclonal Antibodies. Fig. 1 *Left* summarizes the main characteristics of the different anti-Ia monoclonal antibodies used in this study (14, 22, 23). Antibodies MKD6 (24) and 17/227 (25) were kindly provided by P. Marrack and G. Hämmerling, respectively. Antibodies 34.5.3S (26), 14.4.4S (27), and 10.2.16 (28) were obtained from the American Type Culture Collection Distribution Center. H129-326 is a rat anti-T200 monoclonal antibody and H129-19 is a rat anti-L3T4 monoclonal antibody (16).

Radioimmunoassay. Two-step radioimmunoassay of cell-surface Ia molecules were performed as described (14). The presence of a stable $E^d_\alpha A^d_\beta$ dimer in the CA42.13.10 L-cell transfectants was assessed by using a two-site "sandwich" immunoassay (29). For this purpose, anti-Ia monoclonal antibodies were diluted in 10 mM phosphate-buffered saline (pH 7.4) to a concentration of 40 μg/ml and were used to coat the wells of polyvinyl microtiter plates by passive adsorption. Unbound monoclonal antibodies were washed out, and the remaining solid-phase binding sites were saturated with bovine serum albumin. Cells to be analyzed were lysed with 0.5% Nonidet P-40/1% bovine serum albumin/0.1 mM phenylmethylsulphonyl fluoride/0.02% sodium azide in phosphate-buffered saline. After 15 min at 0°C and centrifugation at 100,000 × *g* for 1 hr, 50 μl of the cell lysate was applied to each antibody-coated well for 1 hr. After the unbound antigens were washed out, the wells were incubated at 4°C overnight with the ^{125}I-labeled 81C monoclonal antibody (5 × 10^5 cpm per well). Finally, unbound radiolabeled probe was washed off, and the radioactivity associated with the solid phase was measured.

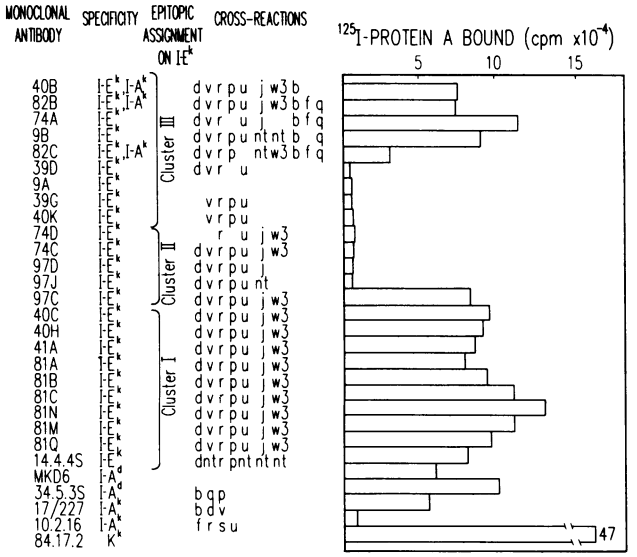

FIG. 1. Serological analysis of $E^d_\alpha A^d_\beta$ molecules present on the surface of the L-cell transfectant CA42.13.10 with 3.3 μg of each of the purified monoclonal antibodies per ml. Cell-bound antibodies were detected with ^{125}I-labeled protein A (^{125}I-protein A). The values obtained with monoclonal antibodies 10.2.16, 9A, 39G, 40K, and 74D represent background binding. The K^k-specific monoclonal antibody was used as a positive control.

Antigen-Presentation Assay. Microcultures in 96-well tissue culture plates were prepared containing 1 × 10^5 AODH7.1 hybridoma T cells, 1 × 10^5 antigen-presenting cells, and 250 μg of HGG (Sigma) per ml in a total volume of 250 μl. Stimulation of AODH7.1 was assessed by estimating the amount of IL-2 present in a 24-hr supernatant as described (24).

RESULTS

Cotransfer of the E^d_α and A^d_β Genes Results in the Surface Expression of a Mixed-Isotype Ia Molecule. Mouse L-cell fibroblasts were transfected with various combinations of α/β chain gene pairs. Surface expression of the resulting Ia molecules was monitored by using 10.2.16, an A^k_β chain-specific monoclonal antibody (30); MKD6, an anti-I-A^d monoclonal antibody; and 81C, an anti-I-E^k monoclonal antibody recognizing an epitope analogous to the E_α chain-determined specificity Ia.7. The transfectants CA14.11.14, CA29.5.2, and CA36.2.1, which received isotype- and haplotype-matched α/β chain genes, expressed the corresponding Ia molecules at a high level (18, 19, 21) (Fig. 2). Note that sequence comparisons have revealed that the E^d_α and E^k_α genes are identical; therefore, E^d_α and E^k_α are synonymous alleles. CA34.9.5 cells express a low but detectable level of the haplotype-mismatched $A^d_\alpha A^k_\beta$ dimer. However, we were unable to detect an $A^k_\alpha A^d_\beta$ complex at the surface of CA34.11.1 cells despite the presence of normal levels of A^k_α and A^d_β mRNA transcripts (data not shown). This lack of expression of the $A^k_\alpha A^d_\beta$ molecule was further confirmed by our inability to detect the chain-determined specificities with several monoclonal antibodies (31–33). Similarly, cotransfer of the E^d_α and A^k_β genes did not result in the expression of a detectable Ia molecule (clone CA42.12.5). However, cotransfection of the E^d_α and A^d_β genes led to the unexpected expression of an 81C- and MKD6-positive Ia molecule on the surface of the CA42.13.10 transfectant. It should be noted that L cells transfected with the E^d_α or the A^d_β chain gene alone showed no staining above background with antibodies 81C or MKD6, respectively (data not shown). These results allow us to draw two major conclusions. First, intraisotypic α/β chain free assembly is not a universal property of Ia molecules; its

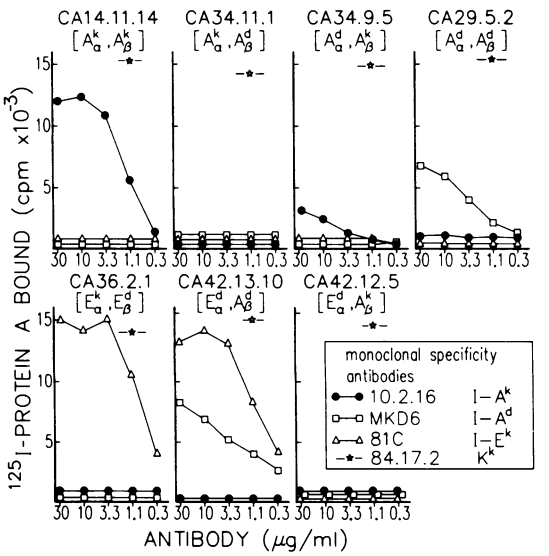

FIG. 2. Analysis of the Ia molecules present at the surface of L-cell transfectant cloned lines. Cells (5 × 10^5) were incubated with 1:3 serial dilutions of the indicated monoclonal antibodies. Cell-bound antibodies were detected by using ^{125}I-labeled protein A (^{125}I-protein A). Results represent the mean of three experiments. The K^k-specific monoclonal antibody was used as a positive control.

occurrence depends both on the haplotype and on the α/β chain pair under consideration. Second, at least one combination of isotype-mismatched chains can result in the surface expression of an interisotypic Ia molecule, the properties of which were analyzed in detail in the following experiments.

Serological Analysis of the $E^d_\alpha A^d_\beta$ Molecule-Expressing L Cells. Three monoclonal antibodies raised against (MKD6 and 34.5.3S) or cross-reactive with (17/227) the I-A^d molecule demonstrated significant binding to the CA42.13.10 transfectant. However, analysis of the full titration curves of these antibodies with CA42.13.10 cells (Fig. 2; data not shown) indicates that they gave a reproducibly weaker binding relative to the antibodies reacting with the epitopes designated as "cluster I" of the I-E molecule (see below). Thus, the A^d_β chain-determined epitopes recognized by these antibodies seem to be partially affected by the nature of the α-chain isotype. Twenty monoclonal antibodies raised against the I-E^k molecule and cross-reactive with the I-E^d molecule were subsequently analyzed for their ability to bind the expressed $E^d_\alpha A^d_\beta$ molecule. As summarized in Fig. 1, these antibodies define three clusters of topologically distinct epitopes on the I-E molecule (12, 14). "Cluster I" antibodies reacted strongly with $E^d_\alpha A^d_\beta$-expressing CA42.13.10 cells. These data formally demonstrate that the specificity of cluster I antibodies is primarily determined by the nonpolymorphic E_α chain irrespective of the isotype of the associated β chain. None of the I-E^d cross-reactive "cluster II" antibodies (74C, 97D, 97J) reacted with CA42.13.10 cells. Therefore, it appears that the epitopes recognized by this set of antibodies are dependent upon the interaction between isotype-matched chains. Finally, analysis of the I-E^d cross-reactive "cluster III" antibodies with CA42.13.10 cells divides them into a negative group (39D, 9A, 39G, 40K) and a positive group (40B, 82B, 74A, 9B, 82C). Interestingly, most of the antibodies pertaining to this latter group cross-react with strains that do not express the I-E molecule (C57BL/10, B10.GD, B10.M, or D2.GD) and therefore recognize determinants also present on the I-A^b, I-A^q, I-A^f, and I-A^d molecules (Fig. 1). Subsequent analysis of one of these antibodies (40B) indicates that it recognizes the I-A and I-E isotypes in both the *k* and *d* haplotypes equally well (23). The present results extend the distribution of this highly conserved epitope to the mixed isotype $E^d_\alpha A^d_\beta$ molecule.

The above studies do not, however, prove the existence of stable $E_\alpha A_\beta$ dimers in CA42.13.10 cells. To formally demonstrate this point, we have used a two-site sandwich immunoassay (29) that specifically detects the $E^d_\alpha A^d_\beta$ dimers present both at the cell surface and in the cytoplasm. A crude cell lysate was prepared, and the Ia molecules containing the A^d_β chain were specifically immobilized with monoclonal antibodies such as MKD6, 34.5.3S, or 17/227 (see above). A second ^{125}I-labeled monoclonal antibody (81C) directed against an E_α chain-determined epitope was then used to detect putative $E^d_\alpha A^d_\beta$ dimers. Such an analysis of the CA42.13.10 L-cell transfectants unequivocally demonstrated the presence of stable $E_\alpha A_\beta$ dimers (Table 1). Analysis of an $E^k_\alpha E^d_\beta$-positive transfectant (CA36.2.1) confirmed the specificity of this assay and prompted us to examine the Ia^d-positive B lymphoma A20.2J AG^R for the presence of $E^d_\alpha A^d_\beta$ dimers. A20.2J AG^R cells appear to express a low but significant level of mixed-isotype dimer. However, in such cells competition between A^d_β and E^d_β for E^d_α may favor intraisotype pairing (Table 1).

A T-Lymphocyte Hybridoma Uses the $E^d_\alpha A^d_\beta$ Molecule as a Restriction Element. The functional relevance of the $E^d_\alpha A^d_\beta$ dimer was analyzed by using a panel of 11 T-cell hybridomas specific for the copolymer $(Glu\text{-}Ala\text{-}Tyr)_n$ and restricted by the I-A^d molecule. None of these hybridomas was able to use the $E^d_\alpha A^d_\beta$ dimer as a restricting element (date not shown). However, another T-cell hybridoma, AODH7.1, specific for HGG and restricted by the I-E^d molecule, was found to be efficiently stimulated by the $E^d_\alpha A^d_\beta$-positive transfectant in the presence of HGG (Table 2). Analysis of the Ia specificity of this hybridoma on a panel of L cells transfected with "exon-shuffled" E_β chain genes assigns its reactivity to the highly polymorphic first domain of the E_β chain (Table 2). Therefore, regardless of the exact constitution of the Ia restriction element recognized by the AODH7.1 cells, at least a portion must be formed by sequence shared between the first polymorphic domains of the E^d_β and A^d_β chains. The generality of this observation remains to be determined.

Further evidence for the direct involvement of the $E^d_\alpha A^d_\beta$ dimer in the antigen presentation function was obtained by using monoclonal anti-Ia antibodies to inhibit this function. Activation of the AODH7.1 T-cell hybridoma, induced by HGG, in the presence of either the CA42.13.10 transfectant or an Ia^d-positive B lymphoma, could be inhibited by antibodies directed toward the mixed-isotype dimer (e.g., 81M and 81C in Table 3). Parenthetically, the antibodies MKD6 and 34.5.3S, which are directed to the A^d molecule and cross-react with the $E^d_\alpha A^d_\beta$ dimer, failed to inhibit the activation of AODH7.1 when the CA42.13.10 cells were used as APC (Table 3). This may be due to their relatively weak binding to CA42.13.10 cells (Fig. 2) compared to the E^d_α-reactive antibodies.

Finally, the anti-L3T4 monoclonal antibody H129-19 was a potent inhibitor of antigen-induced activation of AODH7.1 in the presence of $E^d_\alpha A^d_\beta$-positive L cells (Table 3). This suggests that if L3T4 is an Ia-binding molecule (34, 35), its binding site is still present on the mixed-isotype dimer.

Table 1. A two-site sandwich radioimmunoassay provides direct evidence for the existence of the $E^d_\alpha A^d_\beta$ dimer in transfectant CA42.13.10, transfectant CA36.2.1, and Ia^d-positive B-cell lymphoma

Monoclonal antibody fixed on wells	Binding of ^{125}I-labeled 81C antibody, cpm			
	CA42.13.10 ($E^d_\alpha A^d_\beta$)	CA36.2.1 ($E^d_\alpha E^d_\beta$)	A20.2J AG^R (A^d, E^d)	
	1×10^6 cells	1×10^6 cells	3×10^6 cells	1×10^6 cells
81C (E^k, cluster I)	995	1,313	1,910	888
74A (E^k, cluster III)	34,335	68,335	57,654	32,310
MKD6 (A^d)	11,033	1,024	4,706	1,956
34.5.3S (A^d)	22,450	1,506	6,813	2,560
17/227 (A^k)	5,064	1,324	5,328	3,114
74C (E^k, cluster II)	1,064	22,666	27,543	150,683
97J (E^k, cluster II)	1,635	ND	17,812	7,007

Cell lysates obtained from CA42.13.10 ($E^d_\alpha A^d_\beta$ L-cell transfectants), CA36.2.1 ($E^d_\alpha E^d_\beta$ L-cell transfectants), or A20.2J AG^R (Ia^d-expressing B-cell lymphoma) were analyzed by using the two-site sandwich radioimmunoassay as described. Each well was incubated with 50 μl of cell lysate corresponding to the equivalent of either 10^6 or 3×10^6 cells. Results are expressed in cpm and represent the mean of three experiments. The specificity of the monoclonal antibodies has been described in Fig. 2.

Table 2. Antigen-induced IL-2 response of hybrid AODH.7.1 in the presence of different antigen-presenting cells (APC)

APC	Ia expression: Transfected E_α	Transfected E_β: L	$\beta 1$	$\beta 2,TM,CY$	Endogenous	IL-2 production by AODH7.1 in presence of: Medium	HGG
M12.4*			None		A^d,E^d	1916	31,900
BALB/c†			None		A^d,E^d	2204	14,700
DBA/2†			None		A^d,E^d	2516	29,754
D2.GD†			None		A^d	2132	2,124
CA36.1.3‡	k	d	k	k	None	2104	2,140
CA36.2.1‡	k	d	d	d	None	1988	24,012
CA45.6.7‡	k	d	d	k	None	2132	18,936
CA45.8.9‡	k	d	k	d	None	2656	2,488
CA42.13.10‡		$E^d_\alpha A^d_\beta$			None	1852	16,710

AODH7.1, an HGG-specific I-E^d-restricted T-cell hybridoma, was cultured with various APC in the presence or absence of HGG. After 24 hr of culture, supernatants were assayed for the presence of IL-2 at a 1:6 dilution by the incorporation of [^{3}H]thymidine by the IL-2-dependent cytotoxic T-cell L-cell line. The origin (domain) of the exons constituting the hybrid E_β genes used for transfection is indicated: *L*, leader; *TM*, transmembrane; *CY*, cytoplasmic. The values represent the mean cpm of triplicate culture wells.
*B-cell lymphoma line.
†Spleen cells.
‡L-cell transfectants.

DISCUSSION

The generation of L-cell transfectants expressing various combinations of haplotype- and isotype-mismatched Ia polypeptide chains leads to conclusions that contrast with those previously derived from immunoprecipitation and two-dimensional gel analysis. First, the level of surface expression of a given haplotype-mismatched $A_\alpha A_\beta$ pair appears to depend on the α and β chain alleles involved. For instance, $A^b_\alpha A^k_\beta$, $A^k_\alpha A^b_\beta$ (12), and $A^d_\alpha A^k_\beta$ molecules appear to constitute permissive combinations, whereas $A^k_\alpha A^d_\beta$ molecule has in fact not been detected (this paper; refs. 9 and 36). Second, transfection with some isotype-mismatched combinations such as $E^d_\alpha A^d_\beta$ results in significant surface expression of a stable mixed-isotype dimer. The absence of surface expression of the $E^d_\alpha A^k_\beta$ combination supports the importance of allelic polymorphism in the first domains of the β subunits in control of α/β chain pairing. Furthermore, we have demonstrated that the assembly of an $E^d_\alpha A^d_\beta$ dimer also occurs normally in an Ia^d B-cell lymphoma. However, in such cells, isotypically conserved sequences appear to drive α/β chain assembly predominantly toward intraisotypic pairing. These observations of interisotypic pairing are consistent with the recent results of Maloy *et al.* (37), who reported the existence of mixed-isotype Ia molecules in spleen cells of $H\text{-}2^d$ haplotype mice by using a combination of sequential immunoprecipitation and radiochemical amino acid sequencing. In addition, they support the view that the genes encoding α and β chains of the I-A and I-E isotypes have each evolved from a common ancestral gene by duplication and diversification, while their products still keep the potential (at least in one combination) to assemble together. Our present results on surface expression of $E_\alpha A_\beta$ dimers also suggest an alternative explanation to the observations made with the *I*-region recombinant strain A.TFR5. A.TFR5 cells do not synthesized an E_β chain but react weakly with anti-Ia.7 (E_α) antiserum. These findings have been interpreted in favor of the existence of free E_α polypeptides at the cell surface (38, 39). However, in analogy to the existence of the $E^d_\alpha A^d_\beta$ molecule, it is possible that the $E^k_\alpha A^f_\beta$ dimer is expressed at a low level at the cell surface in this strain. The important point is that combinatorial association between allelic products of the same isotype (e.g., $A^b_\alpha A^k_\beta$) and between allelic products of different isotypes (e.g., $E^d_\alpha A^d_\beta$) may considerably expand the repertoire of Ia molecules available both to homozygous and heterozygous mice. This conclusion may have important implications for the phenomena of immune responsiveness.

Table 3. Ia and L3T4 molecules are involved in the recognition of antigen by AODH7.1 T-cell hybridoma in presence of antigen-presenting CA42.13.10 or M12.4.1 cells

mAB	Specificity	[^{3}H]Thymidine incorporation per culture, Δ cpm: CA42.13.10 ($E^d_\alpha A^d_\beta$)	M12.4.1 (A^d,E^d)
H129-326	T200	17,549	42,080
H129-19	L3T4	3,219	8,612
MKD6	A^d	17,053	12,069
34.5.3S	A^d	16,533	41,200
81M	I-E^k, cluster I	2,312	3,750
82C	I-E^k, cluster III	1,908	8,541

Anti-Ia and anti-L3T4 monoclonal antibodies (mAbs) were analyzed for their ability to block antigen presentation by CA42.13.10 or M12.4.1 cells. Culture conditions and assay for IL-2 production were identical to those described in Table 2. Monoclonal antibodies were included in the cultures at 20 μg/ml. The nonblocking antibody HI29-326 was used as a negative control.

It is interesting to consider these observations with regard to the existence of a putative "hot spot" for recombination within the *I* region (40). As a result of its localization in the intron between the $\beta 1$ and $\beta 2$ exons of the E_β gene (ref. 41; and J. Kobori, E. Strauss, K. Minard, and L.H., unpublished data), the polymorphic A_β and A_α genes appear to be in linkage disequilibrium. Such a unique structural organization may in fact be evolutionarily advantageous in preventing the occurrence of recombinant haplotypes with nonpermissive A_α and A_β alleles (e.g., $A^k_\alpha A^d_\beta$) and the subsequent generation of homozygotes lacking a functional I-A molecule. Conversely, the nonpolymorphic E_α chain appears to recombine more or less independently of the polymorphic $E_{\beta 1}$ region.

It should be emphasized that the occurrence of interisotypic pairing and of nonpermissive intraisotypic combinations may have important implications for the understanding of the associations existing between human Ia molecules

(HLA-DP, -DQ, and -DR) and the susceptibility to certain diseases. In this regard, the formation of *trans*-complementing HLA-DQ molecules has been proposed recently as a plausible explanation for the increased susceptibility of *HLA-DR3/4* heterozygotes to insulin-dependent diabetes mellitus (42).

"Exon-shuffling" and haplotype-mismatched α/β chain gene pairing studies support the view that class I $\alpha 1$ and $\alpha 2$ and Ia $\alpha 1$ and $\beta 1$ polymorphic domains interact tightly to form unique quaternary structures recognized by T-cell receptor molecules (10). For instance, T-cell clones restricted by hybrid Ia molecules are not stimulated by the parental Ia molecules and vice versa. In agreement with the above observations, we have shown that the $E^d_\alpha A^d_\beta$-positive L cells were unable to activate antigen-specific T-cell hybridomas restricted by the I-A^d molecule. However, AODH7.1 T-cell hybridoma constitutes an exception to this rule, since AODH7.1 is able to use both $E^d_\alpha E^d_\beta$ and $E^d_\alpha A^d_\beta$ molecules as a restricting element. Note that the AODH7.1 normal T-cell parent originated from a DBA/2 (*H-2^d*) animal, which leaves open the possibility that it could have been originally activated by HGG in the context of either the $E^d_\alpha E^d_\beta$ or $E^d_\alpha A^d_\beta$ molecules. Our findings parallel the recent observations of an alloreactive T-cell clone that recognizes a determinant shared by $E^k_\alpha E^b_\beta$ and $A^b_\alpha A^{bm12}_\beta$ (43) and of bulk T-cell populations able to recognize sheep insulin in the context of the $A^b_\alpha A^{bm12}_\beta$, $E^k_\alpha E^k_\beta$, or $E^k_\alpha E^b_\beta$ molecules (44). A more precise estimate of the frequency of these T-cell clones directed to "public" *Ia* determinants broadly shared by different alleles and genetic subregions (45–47) must await limiting dilution analyses. Finally, the functional studies of "hybrid" F_1 determinants (12) have suggested that the $E^d_\alpha A^d_\beta$ dimer may also encode unique "interisotypic" determinants recognized by allo- or self-restricted T cells.

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