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Immunology

Allele-specific control of Ia molecule surface expression and conformation: Implications for a general model of Ia structure–function relationships

(class II genes/major histocompatibility complex/monoclonal antibodies/gene transfection/recombinant DNA)

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ABSTRACT **Sequence polymorphism of class II major histocompatibility complex-encoded molecules (Ia) not only accounts for the allelic variability in Ia structure relevant to T-lymphocyte responses but also seems to result in differential quantitative expression of particular Ia heterodimers. The contributions of different allelically variable regions of Ia molecules to both of these processes were analyzed by transfection of L cells with various A_β and A_α gene pairs. The results show that, with regard to quantitative and qualitative aspects of Ia expression, the polymorphisms in the A_β chain segregate into two groups. Those in the NH_2-terminal half of $A_{\beta 1}$ have a consistent role in controlling β–α chain interactions, efficiency of dimer expression, and Ia conformation and probably are in the interior of the Ia molecule at the site of β–α domain interaction. Polymorphisms in the COOH-terminal half of $A_{\beta 1}$ contribute to those structures that directly interact with antibodies, antigen, and/or T-cell receptors, consistent with their presence on the surface of the Ia heterodimer. This analysis provides a model for understanding both overall class II molecular structure and the relationship between this structure and immune recognition. It also suggests an explanation for the evolution of certain features of class II genes.**

Class II major histocompatibility complex-encoded molecules (Ia) are heterodimeric ($\beta\alpha$) cell-surface glycoproteins that show extensive intraspecies polymorphism (1). The only well-recognized functions of these molecules are to participate in intrathymic repertoire selection of T-cell precursors and in corecognition of antigen by mature T lymphocytes (2). As might be expected, sequence variation in the α and β chains has been shown to contribute to qualitative aspects of Ia structure related to T-cell corecognition of antigen and class II molecules (3, 4) and to repertoire selection (2). Somewhat surprisingly, it is now clear that class II allelic sequence variation may also have an effect on the quantitative expression of Ia heterodimers (5–8).

The suggestion that intraspecies allelic polymorphism may affect both Ia-restricted antigen recognition by T lymphocytes and the efficiency of membrane Ia expression has significant implications for understanding the relationship between Ia structure and function, as well as the evolution of class II genes. Therefore, we have examined in detail the effects of allelic polymorphism of A_β and A_α chains on quantitative and qualitative aspects of Ia expression using a gene transfection system. The results of these experiments indicate that intimate interactions of the polymorphic regions of the β and α chains determine structural features of Ia essential for membrane expression and immunological recognition. Our findings also suggest how selective pressures may have influenced the coevolution of the tightly linked A_β and A_α genes.

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MATERIALS AND METHODS

Wild-Type Genes and the Construction of Recombinant Genes. The generation of expressible genomic clones of wild-type A^b_β, A^d_β, A^k_β, A^b_α, and A^k_α, and of an A^d_α cDNA inserted into the cDNA expression vector pcEXV-1 has been described (9–12). A set of recombinant A_β genes was constructed by first converting the *Bgl* II site on the 5′ side of the A^d_β exon I to a *Kpn* I site using *Kpn* I linkers (see Fig. 1*a*). The common *Kpn* I–*Xho* I restriction fragments from A^b_β, A^d_β, and A^k_β were then subcloned into the vector pcEXV-2 (13) to facilitate the exchange of gene segments. Using these pcEXV-2-A_β subclones, first the *Z* regions from A^b_β or A^k_β (*Bam*HI–*Xho* I fragment, see Fig. 1*a*) and A^d_β (*Hin*dIII–*Xho* I fragment) were isolated and independently inserted into A^b_β or A^k_β from which the *Z* region (*Bam*HI–*Xho* I fragment) had been excised. Second, the *Y* region from A^d_β (*Bst*EII–*Hin*dIII fragment) was isolated and inserted into A^b_β or A^k_β from which the corresponding fragment (*Bst*EII–*Bam*HI) had been removed. Third, *YZ* regions (*Bst*EII–*Xho* I fragment) from the three wild-type genes and from the recombinants thus far created were inserted into *Bst*EII/*Xho* I-digested vectors containing only the *X* region of A^b_β, A^d_β, or A^k_β. The 27 different *XYZ* combinations were cut with *Kpn* I, made blunt-ended using T4 polymerase, and digested with *Xho* I. The excised *Kpn* I (blunt)–*Xho* I fragments were subcloned into *Sma* I/*Xho* I-digested pgIA^d_βgpt-49 (10) (the A^d_β gene inserted into the vector pSV2gpt, Fig. 1*b*). This provided the missing 3′ end to all the recombinant gene fragments and also covalently linked the A_β genes to the *Escherichia coli xgpt* gene. The recombinant genes and their protein products are named according to the allelic origin of their *X*, *Y*, and *Z* segments, respectively (e.g., A^{bdd}_β or A^{kbb}_β). In addition, an independently derived recombinant A^{kkd}_β gene (11) was used to generate some of the transfectants used in the analysis shown in Fig. 5.

Gene Transfection and Cell Maintenance. DAP-3, a tk^- subclone of mouse L cells, was transfected with a total of 10 μg of DNA in any given experiment using a standard calcium phosphate precipitation technique, except that no carrier DNA was used (14). For all the transfectants shown in Figs. 2–4 and most shown in Fig. 5, L cells were transfected with either a 3:1 molar ratio of A_α/A_β or with equimolar amounts of A_β and A_α genes plus 250 μg of a plasmid containing the herpes simplex virus *tk* gene. In the former case, cells were selected in medium containing mycophenolic acid (6 μg/ml), xanthine (250 μg/ml), and hypoxanthine (15 μg/ml) (MXH). In the latter case, selection was with MXHAT medium [MXH plus aminopterin (0.2 μg/ml) and thymidine (5 μg/ml)] (4). Critical comparisons for quantitative level of expression were performed in parallel with the same gene ratios and selection

procedure. Some of the transfectants used in the qualitative serologic analysis shown in Fig. 5 were independently generated in this laboratory using various molar combinations of A_β, A_α, and *tk* genes (4, 7, 10–12). Transfectant lines were maintained in culture as described (4).

Monoclonal Antibody Staining and Flow Cytometry. The monoclonal antibodies 10-2.16 (15), M5/114 (16), 3JP (called Y3P in the original reference) (17), 39H, 39J, 40B, 40F, 82C (18, 19), 25-9-3, 28-16-8, 34-5-3 (20, 21), H116-32 (22), and K24-199 (23) were used as culture supernatants. Protein A-purified 11-5.2 (15) and MK-D6 (24) were generously provided by A. Kruisbeek (National Institutes of Health). Staining for flow cytometry was done as described (4), except that, when appropriate, fluorescein-conjugated goat anti-mouse IgM (a gift of J. Mizuguchi, National Institutes of Health) was used. Cells were analyzed fresh or were washed in PBS (Biofluids) and fixed overnight at 4°C in PBS containing 1% paraformaldehyde. Cells were analyzed on either an EPICS-V or FACS Analyzer flow microfluorimeter.

RESULTS

Control of Surface Membrane Ia Heterodimer Expression Maps to the NH_2-Terminal Half of the β_1 Domain. After class II gene transfection into mouse L cells, there are marked differences in the apparent efficiency of Ia surface expression of certain $A_\beta A_\alpha$ pairs (7). This same experimental system was utilized to determine if allelic variation similarly affected the expression of other $A_\beta A_\alpha$ pairs and to map the portion of the A_β gene controlling this phenomenon. Recombinant A_β genes (see Fig. 1) or wild-type A_β genes were transfected into L cells along with various A_α genes. The drug-resistant colonies (representing 20–100 independent clones) derived from a particular transfection were pooled and analyzed by antibody staining and flow microfluorimetry. The results of this analysis are shown in Fig. 2 and are summarized in Fig. 3.

The transfection of haplotype-matched A_β and A_α genes always resulted in the maximum observed cell-surface Ia expression. This high level of expression was comparable for all three haplotype-matched $\beta\alpha$ dimers (i.e., $A^k_\beta A^k_\alpha$, $A^b_\beta A^b_\alpha$, and $A^d_\beta A^d_\alpha$). In comparison, transfection of A^k_α with A^b_β resulted in poor membrane expression, while transfectants prepared with A^k_α and A^d_β expressed no detectable Ia on their surface. Using the recombinant β genes, it can be seen that the presence of A^k_β-derived sequence encoding the NH_2-terminal half of $A_{\beta 1}$ (the X region, Fig. 1*a*) was both necessary and sufficient for achieving high-level expression of A^k_α-containing heterodimers, similar to that attained by the wild-type $A^k_\beta A^k_\alpha$ haplotype-matched dimer. Recombinant A_β genes containing A^b_β or A^d_β sequence in this region, however, yielded the same low level of expression as obtained when wild-type A^b_β or A^d_β genes, respectively, were cotransfected with A^k_α. These latter transfectants demonstrated that the presence of A^k_β sequence in either the second half of $A_{\beta 1}$ or in more COOH-terminal parts of the A_β chain did not overcome the negative effect of a mismatch between the allelic origins of the A_α chain and the NH_2-terminal half of $A_{\beta 1}$. For A^d_α, a pattern similar to that seen with A^k_α was obtained. That is, the d-haplotype ("matched") sequence in the NH_2-terminal half of $A_{\beta 1}$ was both necessary and sufficient for high-level heterodimer expression with A^d_α, and d sequences in other portions of the A_β chain could not overcome the negative effects of a haplotype mismatch between A^d_α and the NH_2-terminal half of $A_{\beta 1}$. For A^b_α, high levels of surface expression were obtained when this chain was paired with A^b_β, A^k_β, any of the chains encoded by recombinants between A^b_β and A^k_β, or with A^{db-}_β (where db- indicates that the allelic origin of the Z region had no effect). Thus, high-level class II heterodimer expression requires an appropriate combination of polymorphic sequences in the NH_2-terminal half of $A_{\beta 1}$ and A_α. For A^k_α and A^d_α, there is a stringent requirement for haplotype-matched $A_{\beta 1}$ sequences, while for A^b_α, NH_2-terminal $A_{\beta 1}$ sequences from any of the three alleles tested give high-level Ia expression.

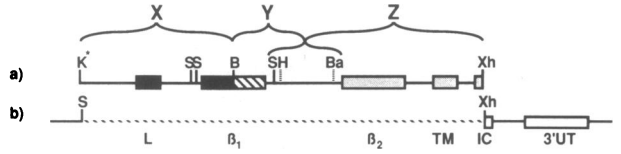

FIG. 1. Partial restriction map of A^b_β, A^d_β, and A^k_β genes. (*a*) Portion of A_β genes subcloned into pcEXV-2. (*b*) The 3′ segment of pgIA$^d_\beta$gpt-49. Dotted line represents part of pgIA$^d_\beta$gpt-49 removed by *Sma* I/*Xho* I digestion. Boxes represent the following exons: L (leader), β_1, β_2, TM (transmembrane), IC (cytoplasmic), and 3′UT (3′-untranslated portion of mRNA) according to the different portions of the A_β polypeptide (or mRNA) encoded. B, *Bst*EII; Ba, *Bam*HI ($A^{b \text{ and } k}_\beta$ only); H, *Hin*dIII (A^d_β only); K, *Kpn* I (*, *Bgl* II site in A^d_β was converted to a *Kpn* I site); S, *Sma* I; Xh, *Xho* I. The *Bst*EII and *Xho* I sites are in the middle of the β_1 and intracytoplasmic exons, respectively. X, Y, and Z indicate the three exchangeable A_β segments *X*, *Y*, and *Z*.

In addition to showing that allele-specific residues in the NH_2-terminal half of $A_{\beta 1}$ have a predominant effect in controlling Ia heterodimer expression, these experiments also demonstrated that A^d_β gene-derived residues in the second half of $A_{\beta 1}$ could have a separate, negative effect on dimer expression. However, this negative effect of A^d_β sequence in the second half of $A_{\beta 1}$ was not observed when there was haplotype matching of the NH_2-terminal half of $A_{\beta 1}$ with A^d_α or A^k_α. Thus the allele-specific effect of COOH-terminal $A_{\beta 1}$ residues on expression is subordinate to that of NH_2-terminal $A_{\beta 1}$ residues.

Quantitative Analysis of mRNA Expression and of Antibody Reactivity Following Gene Transfection. The preceding results could have reflected either real differences in the efficiency of expression of particular $A_\beta A_\alpha$ pairs or experimental artifacts due to either inadequate gene transcription following transfection of certain gene pairs and/or altered antibody affinity for the Ia molecules composed of haplotype-mismatched $A_\beta A_\alpha$ pairs. Several types of experiments were performed to address these possibilities.

(*i*) RNA gel blot analysis indicated that similar levels of normal size A_β and A_α mRNA were present in primary transfectant pools regardless of the wide variation detected in levels of surface Ia (data not shown). Additionally, as all of the recombinant β genes gave rise to cell-surface Ia molecules when expressed with at least one α gene, no mutations were introduced during the construction of the recombinants that affected the ability of their mRNA to be properly translated.

(*ii*) To rule out selective extinction of Ia epitopes on those pairs not detected by M5/114 ($A^{dd-}_\beta A^k_\alpha$ and $A^{bd-}_\beta A^d_\alpha$), these transfectants were analyzed with additional monoclonal antibodies (34-5-3, MK-D6, 39J, 28-16-8, H116-32, 11-5.2, K24-199, 39H, 82C, and 40B) and polyvalent rabbit anti-Ia antiserum (25) with distinct specificities. In no case was staining observed.

(*iii*) To evaluate whether the observed staining of the various transfectants resulted from different levels of Ia expression or altered antibody reactivity for certain Ia pairs, the relative affinities of the anti-Ia antibodies for the Ia molecules expressed by the transfectants was assessed by staining cloned transfectant lines with various dilutions of certain antibodies. In such an analysis, if two lines stain differently in conditions of antibody excess because of altered affinity of the antibody for its ligand, then this

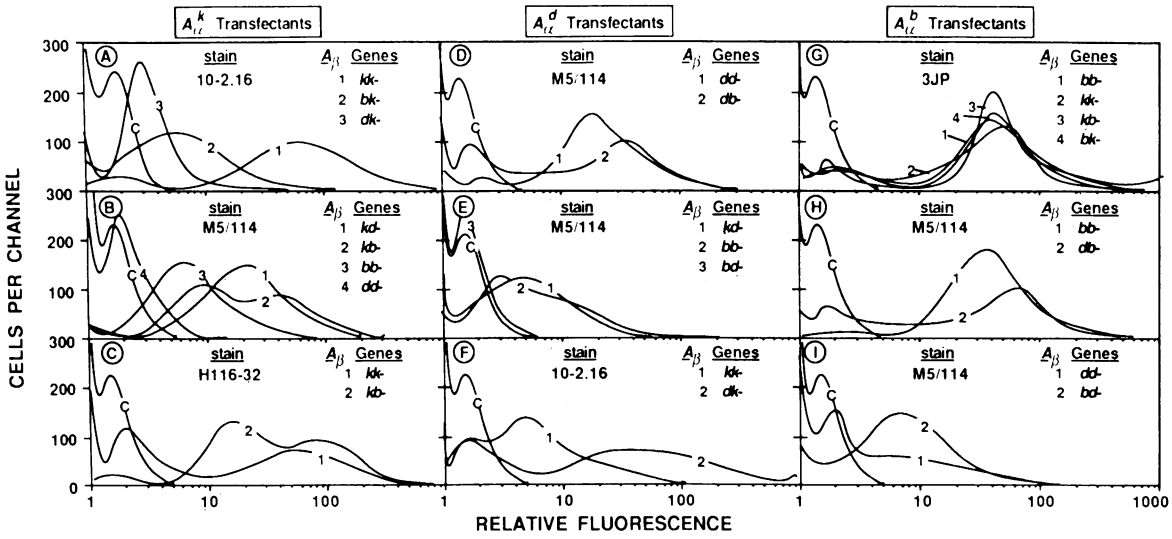

FIG. 2. Monoclonal antibody staining of primary transfectants. Drug-resistant colonies of L cells transfected with the indicated A_β and A_α gene pairs were pooled in a given experiment and analyzed by flow cytometry using the antibodies shown. The allelic origins of the *X* and *Y* regions of the A_β gene are indicated. The dashes indicate that all the different *Z* regions tested gave identical profiles. Curves labeled C represent background staining of L-cell transfectants incubated only with the fluorescein-conjugated second antibody. 10-2.16 reacts with cells expressing A_β^k gene sequences in the *Y* region of A_β. M5/114 reactivity similarly depends on A_β^b or A_β^d sequence in the *Y* region. H116-32 and 3JP react with A_α^k and A_α^b, respectively.

differential staining should be preserved when antibody is limiting. Should the antibody's affinity for the Ia pairs being analyzed be similar enough so that it quantitatively stains the various transfectants, then when antibody is limiting, staining intensity should be dependent only on the amount of antibody available. Thus, the cell lines should stain equally. By including transfectants expressing high and low levels of wild-type molecules, one can distinguish affinity differences from avidity effects on antibody binding due to variation in surface Ia density among the transfectants.

An example of this type of analysis is shown in Fig. 4. M5/114 stains all Ia molecules containing sequences in the COOH-terminal half (Y region) of $A_{\beta 1}$ encoded by the *b* or *d* genes. When this antibody is limiting, the cell lines shown stain equivalently. This indicates that M5/114 has a similar affinity for its A_β^d chain-dependent epitope regardless of the A_α chain partner. A similar result was obtained with M5/114 when tested on cells expressing the appropriate A_β^b epitope, and with 10-2.16 for A_β^{k-}-containing molecules. Therefore, differences in transfectant staining seen with saturating amounts of these antibodies reflect differences in the quantity of cell surface Ia molecules.

This quantitative staining was not seen with all the antibodies. For example, MK-D6 staining requires sequences in the COOH-terminal half of $A_{\beta 1}$ encoded by the *d* gene. However, it can be seen in Fig. 4 that when MK-D6 is limiting, $A_\beta^{kd-}A_\alpha^d$-expressing cells are stained less well than cells expressing $A_\beta^{kd-}A_\alpha^k$ or other MK-D6 reactive Ia molecules, and the curve for $A_\beta^{kd-}A_\alpha^d$ staining with MK-D6 diverges from the others. This indicates that, in contrast to M5/114, MK-D6 has a lower affinity for this Ia heterodimer than for the others.

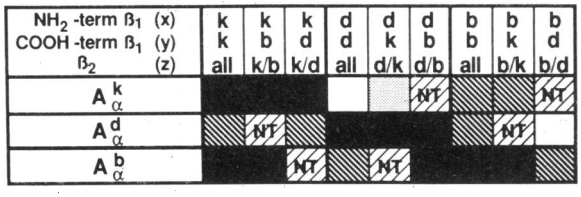

FIG. 3. Relative level of surface expression of individual $A_\beta A_\alpha$ pairs. Solid boxes, maximal expression. Hatched boxes, <20% maximal expression. Stippled boxes, <5% maximal expression. White boxes, no detectable expression. NT, not tested. Level of expression is based on the results shown in Fig. 2.

Qualitative Serologic Analysis of Ia Transfectants. The results of staining all the transfectants with different antibodies (shown in Fig. 5) were also analyzed in terms of how sequence polymorphisms affect Ia structure. This analysis allowed the identification of two different types of serologic epitopes: segment specific and conformational.

Segment-specific epitopes depend on the polymorphism of a particular region of the A_β or A_α chain and are unaffected by polymorphic variation elsewhere in the molecule. This does not necessarily imply that the antibodies bind to these particular segments, as nonpolymorphic portions of the same chain or the partner chain may contribute to the binding site. Nonetheless, for these epitopes the presence of only one discrete polymorphic region, in the context of an $A_\beta A_\alpha$ heterodimer, is both necessary and sufficient for antibody binding. Examples include the epitopes reactive with 10-2.16 and 40F (anti-A_β^{k-}), and M5/114 and 34-5-3 (anti-A_β^{b-} or A_β^{d-}).

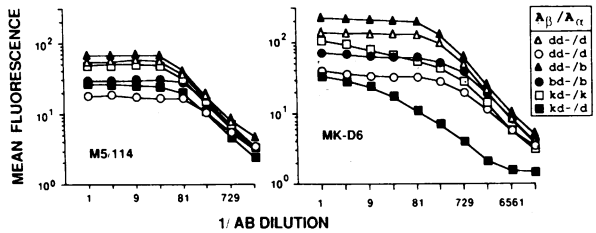

FIG. 4. Analysis of antibody affinity for different $A_\beta A_\alpha$ pairs. For each point, 2.5×10^5 transfected L cells from a given clone or sorted cell line expressing the $A_\beta A_\alpha$ pair indicated were stained with the appropriate dilution of the antibody shown. Cells (10^4 cells) were analyzed by flow cytometry, and the arithmetic mean of the relative fluorescence was calculated. Dilution 1 represents either 100 μl of undiluted antibody-containing culture supernatant (M5/114) or 10 μg of protein A-purified antibody (MK-D6) in 100 μl of PBS containing 2.5% (vol/vol) fetal calf serum. The A_β Z regions are represented by a dash as substitution of different allelic Z segments had no effect on antibody reactivity. Two different clones expressing high (△) or low (○) levels of the $A_\beta^{dd-}A_\alpha^d$ molecule were used to control for avidity effects related to surface Ia density.

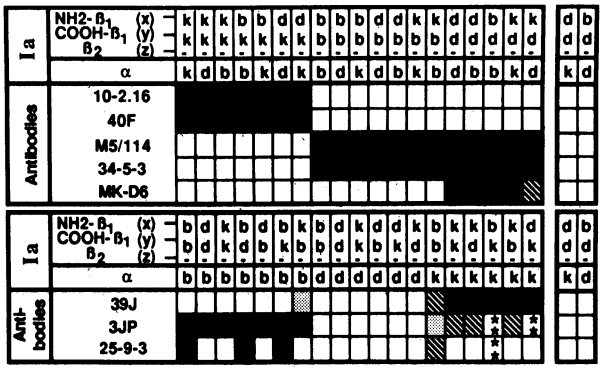

FIG. 5. Analysis of Ia epitopes defined by monoclonal antibodies. (*Upper*) Antibodies dependent primarily on A_β sequence polymorphisms. (*Lower*) Antibodies dependent primarily on A_α sequence polymorphisms or on combined A_β and A_α polymorphisms. The A_β Z regions are represented by a dash as substitution of different allelic *Z* segments had no effect on antibody reactivity. Solid boxes, full reactivity. Hatched boxes, 20–50% reactivity. Stippled boxes, <20% reactivity. Open boxes, no reactivity. ★, no reactivity; however, because of the low level of Ia expression on these cells, <30% reactivity cannot be excluded. Percent reactivity is based in part on a dilutional analysis as shown in Fig. 4 (10-2.16, M5/114, 34-5-3, MK-D6, and 39J) and on maximal staining obtained with saturating amounts of antibody. All analyses were performed on cloned or repetitively sorted transfectant lines.

Conformational epitopes require an A_β or A_α chain or segment expressed in a particular $A_\beta A_\alpha$ dimer context. The anti-Ia.19 antibody 39J recognizes such an epitope. It has a normal, high level of reactivity for cells expressing A^k_α paired with $A^{kk\text{-}}_\beta$, $A^{kb\text{-}}_\beta$, $A^{kd\text{-}}_\beta$, $A^{bk\text{-}}_\beta$, or $A^{dk\text{-}}_\beta$. However, when this α chain is paired with $A^{bb\text{-}}_\beta$, reactivity is diminished. In addition, the molecule $A^{kb\text{-}}_\beta A^b_\alpha$ can be stained by this antibody, albeit relatively inefficiently. These results suggest that the association of the NH_2-terminal A^k_β region with A^b_α either permits or induces the expression of the Ia.19 epitope by the A^b_α chain while haplotype mismatching of A^b_β with A^k_α alters the Ia.19 epitope on the α chain. The epitope recognized by the antibody 3JP might also be considered in this category. While this antibody has a similar reactivity for all A^b_α-expressing cells, its cross-reactivity on A^k_α-bearing cells appears to depend on the presence of A^k_β sequence in the NH_2-terminal half of $A_{\beta 1}$. The antibody 25-9-3 reacts with an epitope dependent on A^b_β sequence in the NH_2-terminal half of $A_{\beta 1}$ expressed with A^b_α (high affinity) or A^k_α (low affinity), but does not react if A^d_α is present.

A final example of a conformational determinant is that recognized by MK-D6 (Figs. 4 and 5). Staining with this antibody requires the presence of allele-specific residues from A^d_β in the COOH-terminal half of $A_{\beta 1}$. However, as shown above, the molecule $A^{kdk}_\beta A^d_\alpha$ is poorly stained with this antibody, even though it contains the appropriate A^d_β chain segment expressed with the parental A^d_α chain. On the other hand, MK-D6 has high affinity for the molecule $A^{kdk}_\beta A^k_\alpha$ that contains the appropriate $A^{d\text{-}}_\beta$ segment in a molecule that contains mostly nonparental A^k_β and A^k_α sequences. MK-D6, therefore, defines an epitope dependent on COOH-terminal $A^d_{\beta 1}$ allele-specific residues whose conformation or access to antibody is affected by the relationship of the NH_2-terminal $A_{\beta 1}$ and the A_α sequences present in the dimer.

DISCUSSION

In this study, it was shown that, first, polymorphic residues in the NH_2-terminal half of $A_{\beta 1}$ have a predominant effect in determining with which allelic A_α chains a particular A_β chain can achieve efficient cell-surface expression.* In particular, haplotype matching of the NH_2-terminal half of $A_{\beta 1}$ with A_α is necessary and sufficient for efficient expression of A^d_α- and A^k_α-containing heterodimers. Second, it was demonstrated that NH_2-terminal $A_{\beta 1}$ and A_α sequences together control the expression of the epitopes recognized by the antibodies 25-9-3 and 3JP. Third, analysis of the MK-D6 and 39J epitopes showed that the combination of polymorphic residues in these same two regions affected the conformation of distantly encoded Ia epitopes. These three results strongly suggest that structures dependent on the allelically variable residues in the NH_2-terminal half of $A_{\beta 1}$ and in A_α become intimately associated when the two chains pair. This interaction controls overall Ia conformation and determines the efficiency of Ia expression, either directly through effects on dimer formation or indirectly through the effects of altered Ia conformation on intracellular Ia transport or processing.

The experiments reported here reveal that with respect to their involvement in determining quantitative and qualitative aspects of Ia expression, different segments of the $A_{\beta 1}$ domain have distinct roles regardless of the allele studied. Thus, the NH_2-terminal half of β_1 is particularly important in controlling β–α chain interactions affecting molecular conformation and efficiency of membrane expression. Allele-specific residues in this region appear to contribute strongly to formation of a "normal" Ia molecule that preserves the appropriate spatial relationships among the remainder of the Ia variable domain segments. Distortions in these latter relationships can account for the observed effects of $\beta\alpha$ chain "mispairing" on serologic epitopes, as detailed here, and for our previous demonstration that haplotype mismatching of the NH_2-terminal portion of A_β with A_α generates Ia molecules incapable of effective antigen presentation to or recognition by the majority of T cells analyzed (4). Conversely, polymorphisms in the COOH-terminal half of $A_{\beta 1}$ have a detectable but weak effect on the efficiency of Ia heterodimer expression but direct the formation of many Ia epitopes seen by monoclonal antibodies (this report; refs. 26–28). Further, analysis of T-cell recognition of Ia molecules involving A_β chains subjected to site-directed mutagenesis indicates a special importance of the region around the COOH-terminal $A_{\beta 1}$ residues 65–75 (12, 27–29).

The existence of highly conserved regions within the variable domains of Ia, together with this pattern of fixed function mediated by chain segments having variable sequences, strongly suggests that Ia molecules have a common and consistent pattern of secondary chain folding and relatively conserved tertiary and quarternary configurations that are formed regardless of the specific polmorphic residues present in the two chains. Based on this analysis, we suggest a model of Ia in which polymorphic variation can affect qualitative immune recognition of Ia in two distinct ways (Fig. 6). First, the local structural features exposed on the external surface of the β and α chains (e.g., βHV3) control the effects of allelic polymorphism on T-cell receptor and/or antigen binding through direct contacts with these components of the ternary recognition complex (Fig. 6). Second, changes in the domain-pairing region within the interior of the dimer (e.g., NH_2-terminal region of β_1) will alter either the relationships between the residues of the exposed α and β polymorphic segments or the orientation of such residues, thus indirectly disturbing the recognition process. This "in-

*In the construction of the recombinants, the exon encoding the leader peptide was always carried with the 5′ half of the β_1 exon. Other work in this laboratory using an A_β gene that recombines the leader and β_1 exons of two alleles has shown that sequence differences in the leader peptide do not contribute to the allele-specific control of Ia expression described here (A. Sant, N.S.B., and R.N.G., unpublished results).

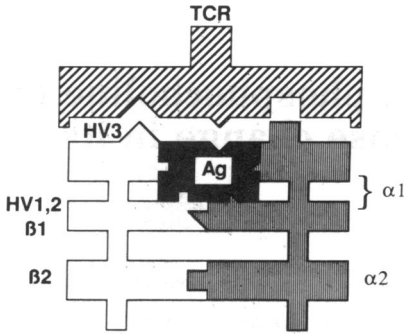

FIG. 6. Model of Ia structure. TCR, antigen-specific, Ia-restricted T-cell receptor. HV1,2; HV3, hypervariable regions 1 (residues 2–17), 2 (residues 26–47), and 3 (residues 63–78) of the Ia-β_1 domain. β_1, β_2, α_1, α_2, extracellular domains of Ia β and α chains. Ag, antigen. The NH_2-terminal half of the β1 domain (HV1,2) is depicted below the COOH-terminal half (HV3) to indicate postulated internal position within the Ia heterodimer.

terior" domain-interaction region may also contribute directly to the binding of small peptides. Our model is most consistent with there being only a single or small number of available major histocompatibility complex restriction sites and antigen-binding sites on an Ia molecule, as suggested by Guillet *et al.* (30).

The results presented in this report also bear on the issue of evolution of Ia genes. Residues with critical roles in the structural interaction of two polypeptide chains tend to be highly conserved within and even between species. The striking effects of allelic polymorphism on membrane Ia levels indicate that such conservation is not always the case for class II molecules. This suggests that there may be an overriding selective pressure that compensates for any negative effects of a mutation on quantitative aspects of Ia expression. Based on the structure–function model of Ia proposed above, such a force might be provided by conformational effects induced by the same residues controlling dimer expression, leading to new Ir phenotypes. As polymorphic residues in β and α chains control Ia conformation and efficiency of expression, selection can only operate on the $\beta\alpha$ heterodimeric unit. Thus mutations in the critical portions of A_β or A_α controlling these phenomena will only be preserved in the species if the two genes are in sufficiently strong linkage disequilibrium to allow selection to operate on the pair in the absence of frequent recombination (cis selection) (31).

Class I molecules can be considered to have carried the process of linkage disequilibrium to the extreme by forming a single gene encoding the two polymorphic domains carried on separate transcription units for class II molecules. Given the structural similarities of class I and class II molecules (32) and the observation that class I- and class II-restricted T cells use the same set of α and β receptor genes (33), the structure–function correlations ascribed in this paper to particular portions of $A_{\beta 1}$ most likely apply to corresponding segments of the homologous domain (α_2 or C1) of class I molecules as well (34, 35).

The generation of effective immune responses requires appropriate helper T-lymphocyte recognition of antigen and Ia. The segmental relationship of Ia polymorphism to structure described here provides new insight into the molecular basis of Ia function in T-cell activation.

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