

# Predominant role of amino-terminal sequences in dictating efficiency of class II major histocompatibility complex $\alpha\beta$ dimer expression

(Ia antigens/gene transfection)

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**ABSTRACT** Cell surface expression of class II major histocompatibility complex-encoded (Ia) molecules depends on association of the component  $\alpha$  and  $\beta$  chains into a stable heterodimer. In the mouse, two isotypes of class II molecules have been identified,  $A_\beta A_\alpha$  and  $E_\beta E_\alpha$ . However, experiments from this laboratory have shown that, following DNA-mediated gene transfer into murine L cells, an  $A_\beta E_\alpha$ -mixed-isotype molecule can be assembled and expressed at the cell surface. In the present study, we have investigated the structural features of the  $\beta$  chain that control the extent of association and level of membrane expression of  $A_\beta E_\alpha$  interisotypic pairs. The use of intact allelic  $A_\beta$  genes demonstrated that only  $A_\beta^d$  chains, but not  $A_\beta^b$  or  $A_\beta^k$  chains, can be coexpressed on the surface membrane with  $E_\alpha$  chains. Transfection of recombinant  $A_\beta$  genes that encode all or half of the  $\beta_1$  domain from one allele and the rest of the chain from another allele revealed that the 5–7 polymorphic residues in the amino-terminal 50 residues of the  $A_\beta$  chain completely controlled this variation in expression with  $E_\alpha$ . Isotypically mixed  $\beta$  genes encoding the  $A_{\beta 1}$  domain of either  $A_\beta^d$  or  $A_\beta^k$  chains and the  $\beta_2$ , transmembrane, and intracytoplasmic portions of  $E_\beta$  chains were used to assess the role of isotypically conserved structures in  $\alpha\beta$  pairing and expression. In marked contrast to the major alterations in expression accompanying changes in the amino-terminal polymorphic residues, exchange of these carboxyl-terminal isotypic segments had no detectable influence on the efficiency of expression with either  $A_\alpha$  or  $E_\alpha$  chains. These results argue strongly that variations in the efficiency with which distinct Ia  $\alpha\beta$  dimers assemble and are transported to the membrane is determined almost exclusively by a critical chain interaction involving the amino-terminal domains of the molecules.

The major histocompatibility complex-encoded class II (Ia) antigens are transmembrane glycoproteins whose cell surface expression is dependent on the stable association of a 33- to 35-kDa  $\alpha$  chain and a 27- to 29-kDa  $\beta$  chain. Both  $\alpha$  and  $\beta$  chains consist of an amino-terminal domain containing virtually all the sites of the extensive intraspecies polymorphism characteristic of histocompatibility antigens, and a series of carboxyl-terminal segments showing conservation of sequence among alleles at a given locus, but extensive divergence among products of distinct loci (1, 2). The locus-specific structure of the carboxyl termini of class II  $\alpha$  and  $\beta$  chains suggested that interactions of these regions with each other could explain the immunochemical observation of selective dimer formation restricted to  $\alpha$  and  $\beta$  chains derived from closely linked genes [for example,  $A_\beta A_\alpha$  or  $DR_\beta DR_\alpha$  (isotype-specific pairing)] (3–6). However, our initial attempts to test this hypothesis led to the unexpected observation that mixed-isotype  $A_\beta E_\alpha$  dimers can in fact form and

be expressed at the cell surface and that allelic variation in the  $\beta$  chain affects the extent of expression of such molecules (7). In the present study, we have analyzed in detail the roles of the polymorphic amino-terminal domain and of the isotype-conserved carboxyl-terminal region in controlling expression of class II  $\alpha\beta$  dimers. Our results led to the surprising conclusion that the 5–7 allelically variable residues in the amino-terminal 50 amino acids of the  $\beta$  chain are of critical importance in determining the efficiency of mixed-isotype Ia expression, whereas the 40 or more locus-specific residues in the highly conserved carboxyl terminus of this chain have no detectable role in selective  $\alpha\beta$  pairing or transport.

## MATERIALS AND METHODS

**Genes.**  $A_\beta$  wild-type and recombinant genes were genomic clones, created and subcloned into pSV2gpt as described (8).  $E_\alpha^{a/k}$ ,  $A_\alpha^b$ , and  $A_\alpha^k$  genomic clones were subcloned into pUC8, pcEXV, and pBR327, respectively (7, 9, 10). Recombinant genes containing portions of both  $A_\beta$  and  $E_\beta$  were created by a sequential procedure in which a 3.6-kilobase (kb) *Bam*HI–*Kpn*I  $E_\beta$  genomic fragment (containing sequences coding for  $\beta_2$ , transmembrane, and intracytoplasmic regions) was first subcloned into pSV2gpt (11) to yield pSV2gpt- $E_\beta$ . The 7.0-kb *Bgl*II–*Bam*HI genomic fragment of  $A_\beta$  (containing the coding sequences for the leader and  $\beta_1$  domains of either  $A_\beta^b$  or  $A_\beta^k$ ) was then cloned into the *Bam*HI site of pSV2gpt- $E_\beta$  to yield pSV2gpt- $A_\beta 1$ - $E_\beta$ . The  $E_\alpha$  and the  $A_\alpha^d$  cDNA constructs were full-length cDNA subcloned into pcEXV-3 expression vector as described (refs. 12 and 13, respectively).

**Cell Maintenance and Transfection.** The thymidine kinase (TK)-negative L cell subline Dap.3 was maintained in culture, transfected with  $\alpha$  and  $\beta$  gene-containing plasmids, and selected for DNA incorporation and expression essentially as described (10, 14), except that no carrier DNA was used.

**Immunofluorescent Staining and Flow Cytometry.** Transfected cell lines were stained with the mixture of Ia  $\alpha$ - and  $\beta$ -chain-specific antibodies as indicated. The chain assignments of the monoclonal antibodies 14-4-4S and 40B (15); MKD6, 10-2.16, M5/114, and 3JP (8); K24-199 (16); 25-9.17 and H116-32 (17) have been reported. Indirect immunofluorescence staining was performed as described (8). Stained cells were analyzed on either an EPICS-V flow microfluorimeter (Coulter) or a fluorescence-activated cell sorter (FACS) analyzer (Becton Dickinson).

**Cell Surface Labeling, Immunoprecipitation, and NaDod-SO<sub>4</sub> Gel Analysis.** L-cell transfectants were labeled with <sup>125</sup>I using lactoperoxidase-catalyzed iodination as described (18), and the labeled cells were solubilized with a solution of 0.5% Nonidet P-40, 0.05 M Tris-HCl (pH 7.6), 0.15 M NaCl, 5 mM EDTA, and the protease inhibitors phenylmethylsulfonyl fluoride (100  $\mu$ g/ml) and aprotinin (5  $\mu$ g/ml) (Sigma). The

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Abbreviation: FACS, fluorescence-activated cell sorter.

post-nuclear supernatants of the cell lysates were preincubated with normal rabbit serum and protein A-Sepharose for 4 hr at 4°C, and aliquots were then incubated with the appropriate antibodies overnight at 4°C. Antigen-antibody complexes were isolated with protein A-Sepharose, and immunoprecipitated material was analyzed by 10% NaDodSO<sub>4</sub>/PAGE (19). The rabbit antisera used for immunoprecipitation were raised against the following antigens: (i) monoclonal antibody affinity column purified I-A<sup>k</sup> (20), (ii) a synthetic peptide corresponding to the 15-amino acid carboxyl-terminal segment of A<sub>β</sub><sup>d</sup>, and (iii) a synthetic peptide corresponding to the 16-amino acid carboxyl-terminal segment of E<sub>α</sub>. The latter two antisera were produced and kindly provided by Lee Maloy (Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases).

**RNA Gel Blot Analysis of Transfected L Cells.** Cytoplasmic RNA was prepared according to Mushinski *et al.* (21) and fractionated on formaldehyde/agarose gels as described (22, 23). RNA was transferred to nitrocellulose and probed with DNA probes labeled with <sup>32</sup>P by the random hexamer primer method (24). The E<sub>α</sub> probe used corresponds to a 0.7-kb *Pst* I fragment isolated from an E<sub>α</sub> cDNA clone. The A<sub>β</sub> probe was derived by partial *Pst* I digestion of an A<sub>β</sub><sup>d</sup> cDNA clone and corresponds to the 3' half of the A<sub>β</sub> transcript (25).

## RESULTS

**A<sub>β</sub>-chain Polymorphism Determines the Efficiency of Cell Surface Coexpression with E<sub>α</sub> Chains.** The experimental system used to evaluate the influence of β-chain polymorphism on cell surface expression of Ia dimers was an L-cell transfection model. Genes encoding α and β chains as well as a selectable marker were cotransfected into L cells by DNA-mediated gene transfer. After growth in selective medium, surviving colonies were pooled and examined for

cell surface expression of αβ heterodimers by FACS analysis using a mixture of monoclonal antibodies.

In the first experiment, three allelic forms of A<sub>β</sub> chains were compared for their ability to pair and be coexpressed with E<sub>α</sub> chains. FACS profiles of these transfectants are shown in Fig. 1a. As had been shown by our laboratory (7), A<sub>β</sub><sup>d</sup> can pair and be coexpressed with E<sub>α</sub> on the surface of L cells. In contrast to A<sub>β</sub><sup>d</sup>, no cell surface expression was detected when A<sub>β</sub><sup>b</sup> or A<sub>β</sub><sup>k</sup> was used as the partner chain for E<sub>α</sub>. Fig. 1d shows that the proteins encoded by the A<sub>β</sub><sup>b</sup> and A<sub>β</sub><sup>k</sup> genes can achieve high levels of cell surface expression when they are paired with an isotype- and haplotype-matched α chain (A<sub>α</sub><sup>b</sup> and A<sub>α</sub><sup>k</sup>, respectively). These results demonstrate that (i) α and β glycoproteins of different isotypic origin are not precluded from pairing and being coexpressed at the cell surface and (ii) allelic polymorphism of A<sub>β</sub> chains dramatically influences their ability to be coexpressed with E<sub>α</sub>.

**Polymorphism in the Amino-Terminal Half of the A<sub>β</sub>1 Domain Determines the Extent of Cell Surface Expression with E<sub>α</sub>.** As shown in Fig. 2, polymorphic residues among alleles of A<sub>β</sub> genes are most prevalent in the amino-terminal domain, where they occur in clusters or hypervariable regions, and they occur with less frequency in the second external domain and the transmembrane and intracytoplasmic regions. It was possible that any or all sites of polymorphism among A<sub>β</sub><sup>d</sup>, A<sub>β</sub><sup>b</sup>, and A<sub>β</sub><sup>k</sup> were responsible for the differences observed in dimer expression with E<sub>α</sub>. To evaluate the contributions of different regions of allelic variation to the selective αβ dimer expression observed in the preceding experiment, various recombinant A<sub>β</sub> chains were tested for expression with E<sub>α</sub>.

The first type of recombinant β chain tested was created by exon shuffling, in which the exons encoding the amino-terminal domain of A<sub>β</sub><sup>b</sup>, A<sub>β</sub><sup>d</sup>, or A<sub>β</sub><sup>k</sup> were exchanged. By this technique, four types of recombinant genomic clones were prepared, A<sub>β</sub><sup>ddb</sup>, A<sub>β</sub><sup>dkd</sup>, A<sub>β</sub><sup>bbd</sup>, and A<sub>β</sub><sup>kkd</sup>, where the first two

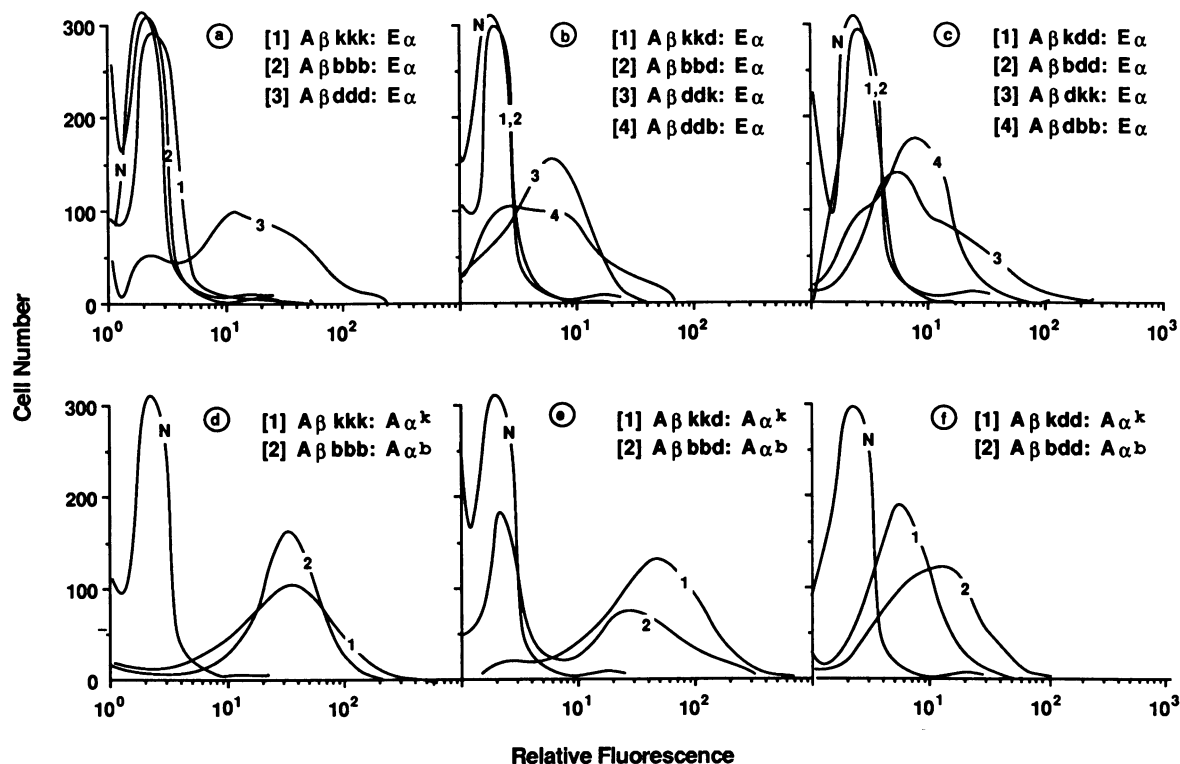


FIG. 1. Allelic influences on interisotypic Ia dimer expression. L cells were transfected with Ia α and β genes as indicated, and pools of 20–50 colonies were stained with a mixture of monoclonal antibodies. The number on each FACS profile corresponds to the combination of α and β genes used for transfection, as indicated in the upper right corner of each panel. The following antibodies were used for staining. Profiles: a1, b1, c3, 10.2-16 (A<sub>β</sub>) and 14.4-4S (E<sub>α</sub>); a2, a3, b2, b3, b4, c1, c2, and c4, MKD6 (A<sub>β</sub>, M5/114) (A<sub>β</sub><sup>d</sup>), and 14.4.4S; d1 and e1, H116 (A<sub>α</sub><sup>k</sup>) and 10.2-16; d2, e2, and f2, 3JP (A<sub>α</sub><sup>b</sup>) and M5/114; f1, H116 and M5/114. Profile N, fluorescent second antibody only (background).

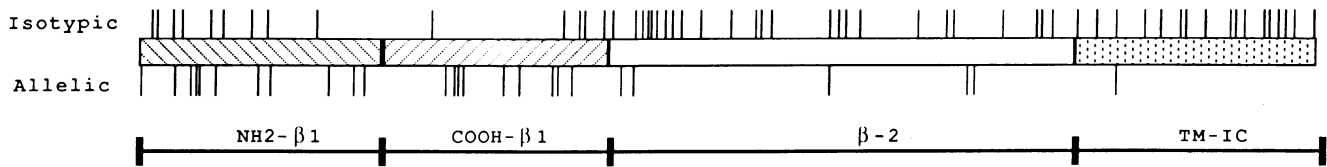


FIG. 2. Isotypic and allelic residues in Ia  $\beta$  chains. The amino acid sequences (26) of three alleles of  $A_\beta$  (b, d, k) and three alleles of  $E_\beta$  (b, d, k) were aligned. Two types of residues were noted and are depicted. Isotypic (locus-specific) residues (bars above line) are those that are common to the  $A_\beta$  alleles or common to the  $E_\beta$  alleles but that differ between  $A_\beta$  and  $E_\beta$ . Allelic residues (bars below line) are those in which variability was detected among three alleles of  $A_\beta$  compared. NH<sub>2</sub>- $\beta$ 1 and COOH- $\beta$ 1,  $\beta$ -2, and TM-IC refer to the amino- and carboxyl-terminal halves of the first external ( $\beta$ <sub>1</sub>) domain, the second external ( $\beta$ <sub>2</sub>) domain, and the transmembrane and intracytoplasmic regions of the  $\beta$  chain, respectively.

letters of the superscript denote the amino-terminal or  $\beta$ <sub>1</sub> domain and the third letter denotes the remaining carboxyl-terminal region of the molecule, including the second external domain and the transmembrane and intracytoplasmic regions. Each of these recombinant  $A_\beta$  genes was cotransfected together with an  $E_\alpha$  gene into L cells. As before, each of these  $\beta$  genes was also cotransfected with the appropriate haplotype- and isotype-matched  $A_\alpha$  gene. Fig. 1b demonstrates that molecules containing the amino-terminal domain of  $A_\beta^d$  ( $A_{\beta 1}^d$ ) were efficiently expressed with  $E_\alpha$ , whereas those containing  $A_{\beta 1}^k$  or  $A_{\beta 1}^b$  could not be detected at the cell surface. As shown in Fig. 1e, the latter two recombinant  $A_\beta$  chains were well expressed with their isotype-matched  $A_\alpha$  partner. Thus, the differences in the ability of allelic  $A_\beta$  chains to show interisotypic pairing and expression are determined by sequence variation in the  $\beta$ <sub>1</sub> domain.

Further dissection of the contribution of polymorphic subregions to the control of  $A_\beta E_\alpha$  expression was achieved by creating "hemi-exon" shuffled genes. The proteins encoded by these genes have a hybrid  $\beta$ <sub>1</sub> domain in which the amino-terminal half is derived from one allele and the carboxyl-terminal half from another. The remainder of the molecule is of the same allele as the carboxyl-terminal half of the  $\beta$ <sub>1</sub> domain. The following four types of recombinant  $\beta$  chains were tested for heterodimer expression with  $E_\alpha$ : amino-terminal  $A_\beta^d$  with the remainder of  $A_\beta^k$  or  $A_{\beta 1}^d$  and amino-terminal  $A_\beta^k$  or  $A_\beta^b$  with the remainder of  $A_{\beta 1}^d$ . Flow cytometric analysis of these transfectants (Fig. 1c) showed that coexpression with  $E_\alpha$  was observed only when the amino-terminal half of the  $\beta$ <sub>1</sub> domain was from the *d* allele.

Two control experiments were performed to determine if the negative expression data obtained with  $A_\beta^k E_\alpha$  and  $A_\beta^b E_\alpha$  pairs were due to serological or transfection artifacts. To verify that FACS analysis using monoclonal antibodies accurately reflects membrane Ia expression, cell membrane Ia levels were also assayed by surface iodination and immunoprecipitation using rabbit antisera raised against either denatured mouse Ia glycoproteins or peptides corresponding to the carboxyl-terminal segments of  $A_\beta$  or  $E_\alpha$ . Each of these antisera react with denatured, as well as native Ia, and most likely recognize determinants not sensitive to the conformation of the amino-terminal domains of the Ia dimer. Fig. 3A shows that, consistent with the monoclonal antibody staining results, cell membrane-associated Ia can only be detected on transfectants containing  $E_\alpha$  and an  $A_\beta$  chain whose amino terminus is derived from the *d* allele. In addition, RNA gel blot experiments (Fig. 3B) established that transfected cell lines lacking detectable membrane Ia expression nonetheless have levels of  $A_\beta$  and  $E_\alpha$  mRNA equivalent to those transfectants expressing Ia on their membranes. The results in Fig. 3 confirm the conclusion that variation in the ability of  $A_\beta$  chains to be coexpressed with  $E_\alpha$  is determined solely by the small number (5–7 residues) of allelically polymorphic residues in the amino-terminal half of the  $\beta$ <sub>1</sub> domain.

**Isotypically Distinct Carboxyl-Terminal  $\beta$ -Chain Segments Do Not Influence the Relative Efficiency of  $\alpha\beta$  Dimer Expression.** Although clearly demonstrating an important role for allelic polymorphism in the control of interisotypic Ia molecule expression, the preceding experiments did not reveal a structural basis for the apparent isotype preference in  $\alpha\beta$ -

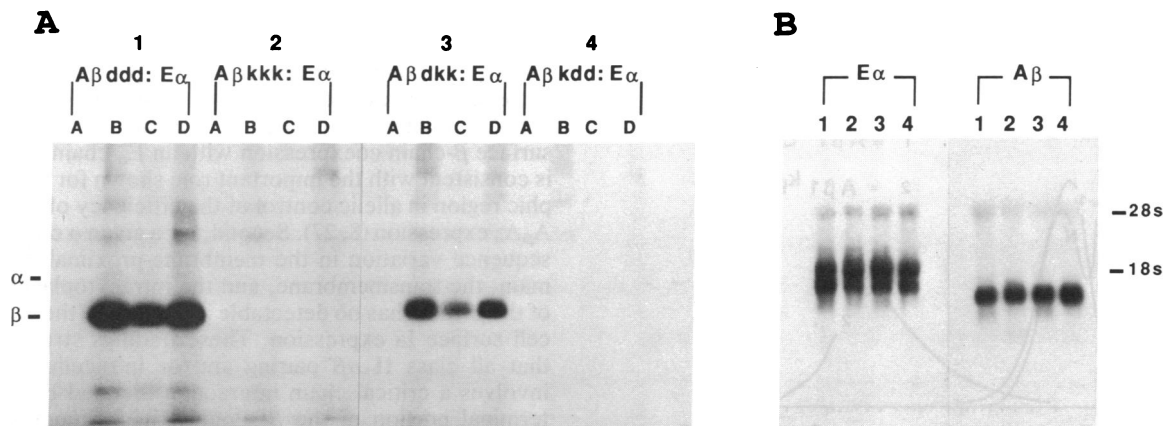


FIG. 3. (A) NaDodSO<sub>4</sub>/PAGE analysis of anti-Ia immunoprecipitates prepared from L-cell transfectants. L cells shown in Fig. 1, transfected with the  $A_\beta$  and  $E_\alpha$  genes indicated above each lane, were cell surface labeled with <sup>125</sup>I. Detergent lysates of the labeled cells were immunoprecipitated with rabbit antisera raised against the following antigens: isolated  $A_\beta A_\alpha$  glycoproteins (lanes B), synthetic peptides corresponding to the carboxyl-terminal segment of  $E_\alpha$  (lanes C), the carboxyl-terminal segment of  $A_\beta$  (lanes D), or human syncytial virus, the negative control (lanes A). (B) RNA gel blot analysis of transfected L cells. Cytoplasmic RNA from the transfectants shown in A was isolated and fractionated on formaldehyde/agarose gels. RNA was transferred to nitrocellulose and probed with the <sup>32</sup>P-labeled DNA probes specific for the genes indicated above the lanes. The  $E_\alpha$  probe used corresponds to a 0.7-kb *Pst* I fragment isolated from a  $E_\alpha$  cDNA clone, and the  $A_\beta$  probe is a 0.45-kb fragment isolated after a partial *Pst* I digest of an  $A_\beta^d$  cDNA clone that codes for the 3' half of  $A_\beta$  (25).

chain pairing and expression seen in immunoprecipitation studies. As shown in Fig. 2, isotopic (locus-specific) residues in class II  $\beta$  chains are most prevalent in the  $\beta_2$ , the transmembrane, and the intracytoplasmic regions. To directly evaluate the importance of this carboxyl-terminal half of the  $\beta$  chain in determining generalized isotopic preference in dimer expression, an Ia  $\beta$  chain gene was constructed containing the  $\beta_1$  domain of either  $A_{\beta}^k$  or  $A_{\beta}^d$  and the  $\beta_2$ , transmembrane, and intracytoplasmic segments of  $E_{\beta}^k$ .

By using the  $A_{\beta_1}^k E_{\beta}$  construct in a cotransfection with  $E_{\alpha}$ , it was possible to determine whether an isotopic match in the carboxyl-terminal segments of the  $\alpha$  and  $\beta$  chains could override the negative effects of  $A_{\beta}^k$  in the first domain, thus allowing cell surface expression with  $E_{\alpha}$ . Similarly, by using  $A_{\alpha}^k$  as the test partner chain for the  $A_{\beta_1}^k E_{\beta}$  hybrid, we could evaluate whether an isotopic "mismatch" in the second domain, transmembrane, and intracytoplasmic regions would prevent cell surface expression of the heterodimer. L cells were transfected with  $A_{\beta_1}^k E_{\beta}$  genes and with either  $E_{\alpha}^{a/k}$  or  $A_{\alpha}^k$  genes. Surviving colonies were pooled and examined for cell surface expression of Ia (Fig. 4).  $A_{\beta_1}^k E_{\beta}$  was not coexpressed with  $E_{\alpha}^{a/k}$ , although this recombinant  $\beta$  chain was able to pair and be coexpressed with  $A_{\alpha}^k$ . Thus, replacement of the carboxyl-terminal isotopic portions of  $A_{\beta}$  with those of  $E_{\beta}$  could not overcome the negative influence of  $A_{\beta_1}^k$  polymorphic residues on coexpression with  $E_{\alpha}$  nor did this change prevent coexpression with the appropriate  $A_{\alpha}$  chain.

A similar set of transfectants with the hybrid  $A_{\beta_1}^d E_{\beta}$  gene permitted us to look for more subtle effects of isotype matching on heterodimer formation and expression. Because the native  $A_{\beta}^d$  chain can be coexpressed with  $E_{\alpha}$ , we anticipated that the hybrid  $\beta$  chain would be able to also. A quantitative analysis could thus be performed by comparing the hybrid or native  $A_{\beta}^d$  chain for cell surface coexpression with  $A_{\alpha}^d$  or  $E_{\alpha}^{a/k}$  chains. Fig. 5 shows a comparison of transfectants containing either the native  $A_{\beta}^d$  gene or the hybrid  $A_{\beta_1}^d E_{\beta}$  gene plus either the  $E_{\alpha}^{a/k}$  or the  $A_{\alpha}^d$  gene. For each  $\alpha$  chain, the same level of cell surface expression was detected regardless of whether the carboxyl-terminal portion of the  $\beta$  chain was of the same or a different isotopic origin, that is, neither positive nor negative influences of this region of the molecule on the efficiency of Ia molecule expression could be discerned. This result was observed with  $\alpha$ -chain-specific antibodies, such as 14-4-4S and K24-199,  $\beta$ -chain-specific antibodies, such as MKD6 and 25-9-17 (data not shown), or an antibody, 40B, that reacts with  $A_{\beta} A_{\alpha}$  and  $E_{\beta} E_{\alpha}$ .

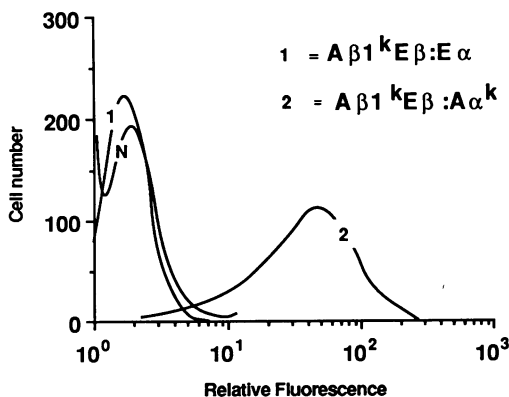


FIG. 4. Influence of isotopic residues on Ia heterodimer expression. L cells were transfected with  $A_{\beta_1}^k E_{\beta}$  and  $E_{\alpha}^{a/k}$  genes or  $A_{\beta_1}^k E_{\beta}$  and  $A_{\alpha}^k$  genes. Surviving colonies were pooled and stained with a mixture of 10.2-16 and 14.4-4S antibodies or of 10.2-16 and H116-32 antibodies and then analyzed by flow microfluorimetry. Profile N, fluorescent second antibody only (background).

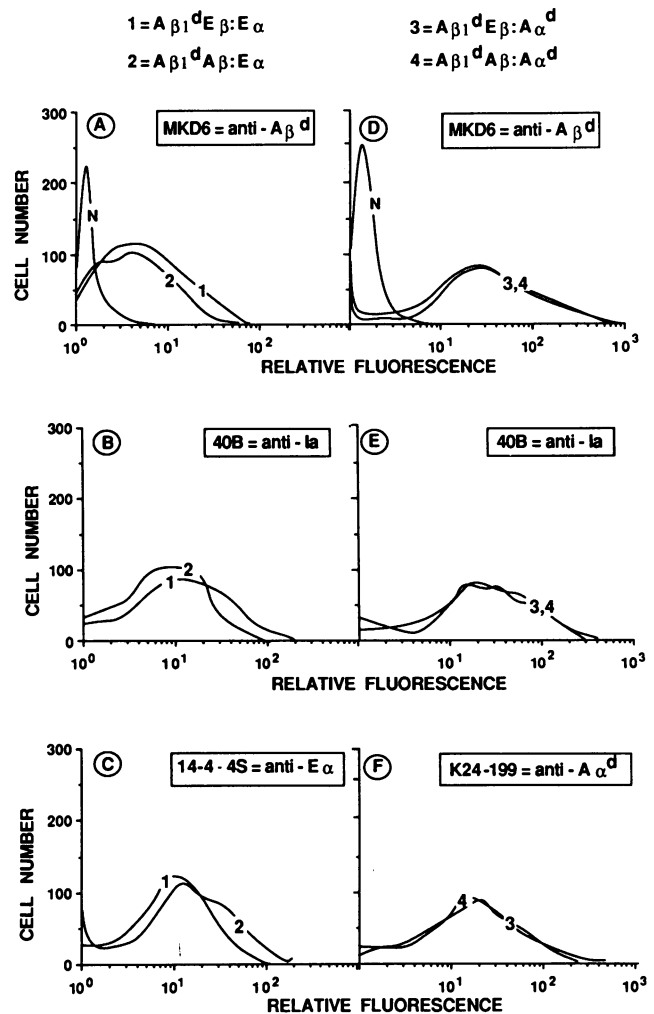


FIG. 5. Effect of isotype matching of the  $\beta_2$ , transmembrane, and intracytoplasmic regions of Ia on the efficiency of dimer formation and expression. L cells were transfected with  $A_{\beta_1}^d E_{\beta}$  and  $E_{\alpha}^{a/k}$  sequences,  $A_{\beta_1}^d E_{\beta}$  and  $E_{\alpha}^{a/k}$  sequences,  $A_{\beta_1}^d A_{\beta}$  and  $A_{\alpha}^d$  sequences, or  $A_{\beta_1}^d E_{\beta}$  and  $A_{\alpha}^d$  sequences. Pools of colonies surviving drug selection were stained with the antibodies indicated and analyzed by flow microfluorimetry.

## DISCUSSION

Two main points emerge from the present study. First, allele-specific residues in the amino-terminal 50 residues of the  $A_{\beta_1}$  domain dramatically influence interisotypic cell surface  $\beta$ -chain coexpression with an  $E_{\alpha}$  chain. This finding is consistent with the important role shown for this polymorphic region in allelic control of the efficiency of intraspecific  $A_{\beta} A_{\alpha}$  expression (8, 27). Second, for a given  $\alpha$  chain, isotopic sequence variation in the membrane-proximal external domain, the transmembrane, and the intracytoplasmic regions of the  $\beta$  chain has no detectable influence on the efficiency of cell surface Ia expression. These findings strongly suggest that all class II  $\alpha\beta$  pairing and/or intracellular transport involves a critical chain interaction dictated by the amino-terminal portion of the  $\beta_1$  domain in conjunction with an unmapped region of the  $\alpha_1$  domain.

This interaction has significant effects on the immunologically relevant structure of the Ia heterodimer (8) and may play a role in determining the binding properties of Ia for certain antigenic peptides (28, 29). The precise consequences of variability in this interaction on Ia structure are not known at this time, but, clearly in the extreme cases of  $A_{\beta}^k E_{\alpha}$ ,  $A_{\beta}^k E_{\alpha}$ , and  $A_{\beta}^d A_{\alpha}^k$  (8, 27), an inappropriate interaction leads to a total

lack of detectable membrane expression. We do not yet know if this absence of membrane expression reflects a primary defect in  $\alpha\beta$  association or whether dimerization occurs but results in a conformation that prevents an obligate post-translational processing or intracellular transport event.

Isotype-specific residues make up  $\approx 35\%$  of the amino acids in the second external domain, the transmembrane, and the intracytoplasmic portions of the  $\beta$  chain. It was, therefore, surprising that changing the isotypic origin of these regions had no detectable influence in determining the efficiency of expression with either  $A_\alpha$  or  $E_\alpha$  chains. At least two explanations can be offered for this observation. The first is that there are few, if any, molecular interactions between  $\alpha$  and  $\beta$  chains in these regions. This would allow substantial variability in sequence without affecting dimer formation and expression. Alternatively, these domains may interact significantly and the apparent lack of an effect of isotypic residues may reflect a highly conserved secondary structure between these two types of  $\beta$  chains. Only substitutions preserving the proper structure may thus have been permitted during evolutionary divergence of the  $\beta$  chains. The actual contact residues between the  $\alpha$  and  $\beta$  chains might involve those residues conserved between  $A_\beta$  and  $E_\beta$ , which to a large extent are those common to members of the immunoglobulin gene superfamily (1). A highly conserved secondary and tertiary structure in the second external domain and the transmembrane and intracytoplasmic regions may be required for other effector functions of Ia, such as interactions with other membrane molecules in the same cell (for example, invariant chain) or on other cells (for example, L3T4) or as a binding site for molecules directing intracellular trafficking of Ia.

We have not yet investigated the importance of isotypic residues in the first external domain of the  $\beta$  chain in Ia dimer formation and expression. These residues are rare in the carboxyl-terminal half of the  $\beta_1$  domain but are prevalent in the amino-terminal half, which is the region we believe to be the most critical in selective dimer expression. If this region of the  $\beta$  chain directly forms contacts with the  $\alpha$  chain, then isotypic residues as well as the allelic polymorphic sites could potentially influence the efficiency of this interaction and contribute to selective dimer expression.

Lastly, although we have demonstrated that in L cells, with no other partners available,  $A_\beta^d$  and  $E_\alpha$  are coexpressed at the cell surface, the prevalence and importance of interisotypic Ia dimers *in vivo* is not yet known. In normal lymphoid cells synthesizing multiple allelic and isotypic  $\alpha$  and  $\beta$  chains, asymmetries in the rate of synthesis of each chain and allele-dependent variation in the efficiency of formation and transport of particular pairs could lead to the apparent selective expression of Ia molecules that has been reported (3–6). It will be important to examine the effects of intracellular competition among multiple  $\alpha$  or  $\beta$  chains on the nature of the Ia molecules expressed by transfected L cells, as well as reexamining the spectrum of Ia heterodimers expressed by resting and lymphokine-induced  $Ia^+$  hematopoietic cells. These studies should aid in understanding how the class II surface phenotype of cells may vary during distinct differentiation states of a given cell, contributing to both normal and pathologic immune responses.

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