*Proc. Natl. Acad. Sci. USA*
Vol. 82, pp. 2940–2944, May 1985
Immunology

# "Exon-shuffling" maps control of antibody- and T-cell-recognition sites to the $NH_2$-terminal domain of the class II major histocompatibility polypeptide $A_\beta$

**(gene transfer/histocompatibility antigens/immune response/antigen presentation)**

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*Communicated by William E. Paul, December 31, 1984*

ABSTRACT **To investigate the role of the highly polymorphic amino-terminal ($\beta_1$) domain of the class II major histocompatibility polypeptide $A_\beta$ during recognition by T cells and antibodies, "exon-shuffling" was carried out between genomic recombinant DNA clones of $A^k_\beta$ and $A^d_\beta$ to generate a hybrid gene containing $A^k_\beta$ exons for the amino-terminal domain followed by the $A^d_\beta$ exons for the remainder of the molecule. L-cell gene transfectants expressing this hybrid $A_\beta$ gene in combination with $A^k_\alpha$ were compared to L cells expressing wild-type $A^k_\beta A^k_\alpha$ dimers in tests of antigen-presentation to T-cell clones and hybridomas and for staining by a panel of anti-I-$A^k$-specific monoclonal antibodies. These antibodies were also tested for their reactivity with a B-lymphoma transfectant expressing $A^k_\beta$ in the absence of $A^k_\alpha$. The results showed no qualitative differences in either T-cell or antibody-mediated recognition of I-$A^k$ molecules containing either the exon-shuffled or wild-type $A^k_\beta$. Together with the data involving the B cell transfectant expressing only $A^k_\beta$, these results map control of the $A_\beta$ contribution to the immunologically relevant determinants of I-$A^k$ to the highly polymorphic amino-terminal domain and indicate little, if any, contribution to allele-specific recognition by amino acid sequence variations in the remaining portions of the $A_\beta$ polypeptide.**

Self/nonself discrimination by and communication within the immune system are dependent on T-lymphocyte recognition of cell surface major histocompatibility complex (MHC) gene products (reviewed in refs. 1 and 2). The recognition of Ia (class II) cell surface MHC molecules by alloantibodies, by allospecific T lymphocytes, and by Ia-restricted, antigen-specific T cells in particular involves determinants controlled by sites of intraspecies polymorphism (1). A major interest among cellular immunologists studying this recognition process is the molecular distribution of the relevant polymorphic sites on Ia molecules and the correlation of this distribution with the phenomena of restricted antigen recognition and immune-response gene function.

A first step in this analysis has been the molecular cloning and nucleotide sequencing of several allelic forms of $A_\alpha$ (3), $A_\beta$ (4–6), $E_\alpha$ (7, 8) and $E_\beta$ (9–11) class II MHC genes. This work has revealed that $A_\alpha$, $A_\beta$, and $E_\beta$ show the expected extensive amino acid polymorphism between alleles and that the majority of such substitutions are localized in "hypervariable" regions within the $NH_2$-terminal ($\alpha_1$ or $\beta_1$) domain of each polypeptide. Similar results have also been obtained for the human equivalents of these murine genes (12). These findings suggest that these amino-terminal domains may play critical roles in T-cell and antibody recognition of MHC molecules. However, data showing that only a few amino acid substitutions in a critical region can affect the immunologic function of MHC molecules has been accumulated through study of existing class I and class II gene mutants (13, 14). It is therefore important to examine directly the hypothesis that the highly polymorphic $NH_2$-terminal regions are of special significance for humoral and cell-mediated recognition of Ia. To explore this question, we have employed the technique of DNA-mediated gene transfer, which has proved extremely valuable in analyzing the structure–function relationships of class I MHC gene products (15–21). In the present experiments, "exon-shuffling" (17) has been carried out between genomic subclones of $A^k_\beta$ and $A^d_\beta$, generating a recombinant gene encoding the $A^k_{\beta_1}$ (amino-terminal) domain, with the remainder of $A^d_\beta$. L cells were transfected with this "exon-shuffled" $A_\beta$ gene product, together with $A^k_\alpha$, and such transfected cells expressing the new class II molecule on their surface were compared with transfectants expressing wild-type I-$A^k$ molecules in tests involving recognition by a panel of monoclonal anti-I-$A^k$ antibodies, antigen-specific I-$A^k$-restricted T-cell clones, or T-cell hybridomas.

## MATERIALS AND METHODS

**DNA Clones and Constructs.** Genomic clones of $A^k_\beta$, $A^d_\beta$, and $A^k_\alpha$ were isolated from λ genomic libraries by hybridization to previously described cDNA probes (4, 22). For use in transfection experiments, DNA fragments from these phage clones, containing complete class II genes plus variable amounts of 5′ and 3′ flanking DNA, were subcloned in plasmid vectors. The construction of pgI-$A^k_\beta$-1, pgI-$A^k_\beta$-gpt-1, pgI-$A^d_\beta$-gpt-49, and pgI-$A^k_\alpha$A21 has been reported in detail elsewhere (22, 23). pgI-$A^k_\beta$(TA3)-gpt-1 was prepared (unpublished work) from a λ Charon 4A clone derived from DNA of the *H-2*$^{a/k}$ B-cell hybridoma TA3 (24).

The construction of pgI-$A_\beta$ ($\beta^k_1$;$\beta_2$,*TM*,*IC*$^d$)-gpt-1 was carried out as follows: pgI-$A^k_\beta$-1 was digested to completion with *Bam*HI and *Bgl* II restriction endonucleases, and the 5.3-kilobase (kb) *Bgl* II–*Bam*HI fragment containing the first (5′ untranslated and leader) and second ($\beta_1$) exons was isolated. The ends of this fragment were made flush by using the Klenow fragment of DNA polymerase. pgI-$A^d_\beta$-gpt-49 (22) was digested to completion with *Sma* I restriction endonuclease, and the 12-kb fragment containing the pSV2gpt vector (25) plus $\beta_2$, transmembrane (*TM*), intracytoplasmic (*IC*$_1$ and *IC*$_2$), and 3′ untranslated regions of $A^d_\beta$ was isolated and purified. This DNA fragment was treated with calf intestinal phosphatase and ligated to the blunt-ended $A^k_\beta$ fragment. After transformation of competent *E. coli* K-12 strain MC1061 with the ligated DNA, colonies were picked by hybridization to a 0.55-kb *Sma* I fragment containing the $\beta^k_1$ exon from

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s).

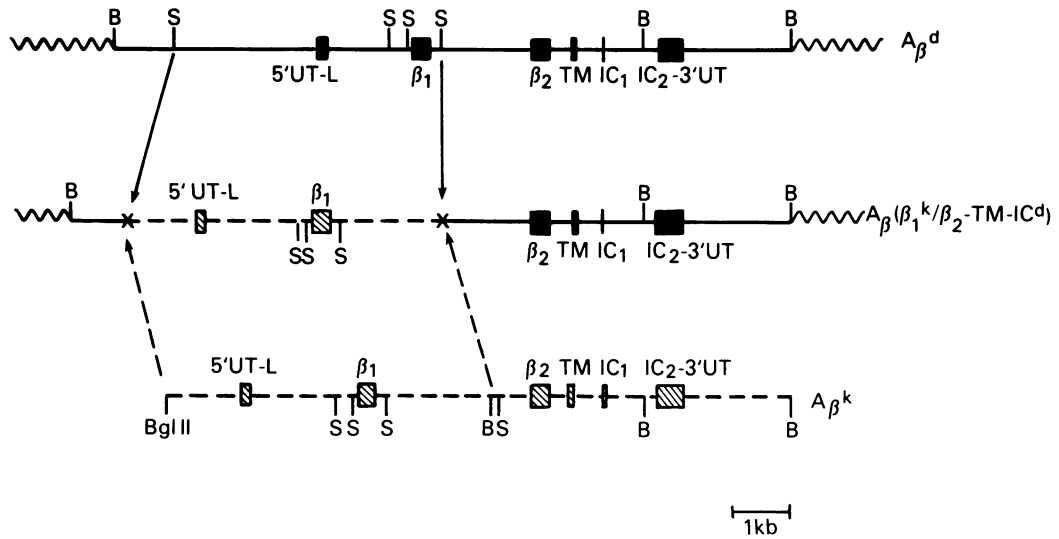


FIG. 1. Strategy for construction of exon-shuffled $A_\beta$ recombinant DNA clone. See *Materials and Methods* for details. Exons are represented by boxes. B, *Bam*HI; S, *Sma* I; wavy line, vector DNA; solid line, $A_\beta^d$ DNA; dashed line, $A_\beta^k$ DNA; 5'UT, 5' untranslated region; L, leader (signal) peptide exon; TM, transmembrane region exon; IC, intracytoplasmic region exons; 3'UT, 3' untranslated region.

pgI-$A_\beta^k$-1. Positive colonies were used to prepare DNA and restriction-mapped to identify a recombinant clone with a single $\beta_1^k$ exon in the correct orientation.

A cDNA clone containing the entire coding region of $A_\alpha^d$ (26) was kindly provided by M. Davis. A *Bst*EII–*Pvu* II fragment of this clone was made blunt-ended and inserted into the similarly treated *Eco*RI cloning site of the cDNA expression vector pcEXV-1, derived from Okayama and Berg's (27) pcDV and pL1 plasmids (unpublished work). This construct [pcEXV($A_\alpha^d$)] is capable of directing the expression of a complete $A_\alpha^d$ polypeptide in various eukaryotic cells.

**Southern Blot Analysis.** Ten micrograms of high molecular weight DNA and 10 ng of the cloned plasmid DNAs pgI-$A_\beta^k$-gpt-1 and pgI-$A_\beta(\beta_1^k;\beta_2,TM,IC^d)$-gpt-1, were individually digested to completion with *Bam*HI restriction endonuclease and used for Southern blot analyses as described (23). The hybridization probe consisted of the 464-base-pair $A_\beta^d$ cDNA described by Robinson *et al.* (28), labeled by nick-translation to a specific activity of $\geq 4 \times 10^8$ cpm/$\mu$g of DNA.

**DNA-Mediated Gene Transfer.** The thymidine kinase negative, L-cell subline DAP.3 was transfected with various DNAs using the calcium phosphate precipitation technique, as detailed previously (15, 22). Sublines or clones expressing the desired level of membrane Ia were isolated by preparative cell-sorting to produce (*a*) D3.11H3, containing pgI-$A_\beta^k$-gpt-1 and pgI-$A_\alpha^k$A21 ($A_\beta^kA_\alpha^k$); (*b*) RT7.3H3.B, containing pgI-$A_\beta^k$(TA3)-gpt-1 and pgI-$A_\alpha^k$A21 ($A_\beta^kA_\alpha^k$); (*c*) RT4.15HP, containing pgI-$A_\beta(\beta_1^k;\ \beta_2,\ TM,\ IC^d)$-gpt-1 and pgI-$A_\alpha^k$A21 [$A_\beta(\beta_1^k;\ \beta_2\ TM,\ IC^d)\ A_\alpha^k$]; and (d) RT2.3.3H, containing pgI-$A_\beta^d$-gpt-49 and pcEXV($A_\alpha^d$) [$A_\beta^dA_\alpha^d$]. T70.3.1 is a transfectant produced by introduction of pgI-$A_\beta^k$-gpt-1 into the $Ia^d$-expressing B-cell lymphoma M12.4.1 (23).

**Monoclonal Antibodies.** The following monoclonal antibodies were used, either as culture supernatants from growing hybridomas or as protein A-purified antibodies (0.5–1 $\mu$g/50 $\mu$l): 10.2.16 (29), MKD6 (30), 16.1.2 (31), 39B, 39E, 40A, 40F, 40L, 40M, 40N, 39F, 39J, 40J, 39A, 8B and 8C (32, 33).

**Flow Cytometry.** L-cell transfectants were grown in bacteriologic Petri dishes, and B cells, in suspension cultures. All cells were harvested without trypsinization. Harvested cells ($\approx 10^6$) were washed in phosphate-buffered saline/10% fetal calf serum; incubated for 30 min on ice with 50–100 $\mu$l of the indicated monoclonal antibody-containing culture supernatant, diluted purified antibody, or control culture supernatant; washed; incubated for 30 min on ice with fluorescein-labeled F(ab')$_2$ fragment of goat anti-mouse immunoglobulin antibody; washed; and analyzed with an EPICS flow microfluorimeter. All analyses and the preparative cell-sorting described in the text were kindly carried out by A. Palini and M. Waxdal in the National Institute of Allergy and Infectious Diseases Flow Microfluorimetry Facility. For the panel analysis with multiple cells and antibodies, the data from a single run on the EPICS V were analyzed to yield mean channel fluorescence for each antibody.

**Cell Lines, Clones, Hybridomas, and Functional Assays.** Other than the above-described L-cell transfectants, all the cells used in these experiments and their maintenance, origin, properties, and use for *in vitro* assays of responses to antigen and accessory cells have been described in detail (22, 23). Precise conditions are given in the tables.

## RESULTS AND DISCUSSION

$A_\beta^k$ differs from $A_\beta^d$ by 14 amino acids (including 2 deletions) in the $\beta_1$ domain and by 4 amino acids in the $\beta_2$ domain (4). The strategy employed for replacing the exon encoding the $\beta_1$ domain of $A_\beta^d$ with the equivalent $\beta_1^k$ exon is shown in Fig. 1. This construction keeps the leader-containing exon of $A_\beta^k$ paired with the $\beta_1^k$ exon so that the first 5 amino acids of the mature $A_\beta$ polypeptide, which are encoded in the leader exon (4, 5), will also be "k." DNA from this recombinant $A_\beta$ gene [pgI-$A_\beta(\beta_1^k;\ \beta_2,\ TM,\ IC^d)$gpt-1], together with DNA from a genomic subclone of $A_\alpha^k$ and DNA from the herpes thymidine kinase gene, was used to cotransfect mouse L cells by the calcium phosphate precipitation method (15, 22). The primary transfectant pool (RT4.15) arising after selection in hypoxanthine/aminopterin/thymidine medium (15) was analyzed for I-A surface-membrane expression by staining separately with two different monoclonal anti-I-A antibodies: 10.2.16 (anti-Ia.m27, ref. 29), an $A_\beta^k$-specific reagent (34) that nonetheless might fail to react with these transfectants due to the exon-shuffle of $A_\beta$, and H116.32 (anti-Ia.m19, ref. 35), a putative anti-$A_\alpha^k$ antibody (36) that should react with the expressed I-A molecule of these transfectants via the wild-type $A_\alpha^k$ polypeptide. In fact, both antibodies stained a substantial number of primary transfectant cells (data not shown), and cells from the brightest 1% fraction stained with 10.2.16 plus fluorescein-labeled goat anti-mouse

Table 1. Reactivity of class II gene transfectants with monoclonal anti-Ia antibodies

| | | | | Fluorescence intensity, mean channel number* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | B cells | | | L cells | |
| | | | | M12.4.1 | T70.3.1 | TA3 | RT7.3H3-B | RT4.15HP |
| | Antibody | Nominal assignment | *Ia expression Transfected: Endogenous:* | None $I\text{-}A^d,I\text{-}E^d$ | $A^k_\beta$ $I\text{-}A^d,I\text{-}E^d$ | None $I\text{-}A^{d,k},I\text{-}E^{d,k}$ | $A^k_\beta A^k_\alpha$ None | $A_\beta(\beta^k_1;\beta_2, TM, IC^d)A^k_\alpha$ None |
| | None | — | | 15 | 30 | 20 | 30 | 30 |
| Group 1 | 10.2.16 | $A^k_\beta$ | | 15 | 150 | 145 | 120 | 185 |
| | 39B | $A^k_\beta$ | | 20 | 125 | 140 | 110 | 170 |
| | 39E | $A^k_\beta$ | | 20 | 125 | 140 | 110 | 170 |
| | 40A | $A^k_\beta$ | | 15 | 125 | 140 | 100 | 170 |
| | 40F | $A^k_\beta$ | | 20 | 145 | 150 | 110 | 180 |
| | 40L | $A^k_\beta$ | | 20 | 145 | 150 | 110 | 180 |
| | 40M | $A^k_\beta$ | | 20 | 145 | 140 | 110 | 180 |
| | 40N | $A^k_\beta$ | | 20 | 95 | 115 | 100 | 130 |
| Group 2 | 39F | $A^k_\alpha$ | | 25 | 20 | 115 | 80 | 130 |
| | 39J | $A^k_\alpha$ | | 15 | 20 | 140 | 100 | 170 |
| | 40J | $A^k_\alpha$ | | 15 | 20 | 115 | 80 | 110 |
| Group 3 | 39A | ? | | 30 | 80 | 120 | 60 | 110 |
| Group 4 | 8B | $I\text{-}A^k$ | | 20 | 20 | 70 | 50 | 65 |
| | 8C | $I\text{-}A^k$ | | 20 | 30 | 70 | 50 | 70 |

*EPICS channels are linearly distributed across a 3-decade logarithmic scale; full scale = 1–255.

IgG were cloned by preparative sorting on an EPICS flow microfluorimeter, generating RT4.15H and RT4.15HP.

To verify that RT4.15 HP had in fact received the exon-shuffled $A_\beta$ construct and not wild-type $A^k_\beta$, high molecular weight DNA was prepared, digested with *Bam*HI restriction endonuclease, and used for Southern blot analysis. Hybridization to a 464-base-pair 3′ $A_\beta$ cDNA probe (28) gave the results shown in Fig. 2. The shuffling procedure has deleted a *Bam*HI site in the intron between the $\beta_1$ and $\beta_2$ exons of $A^k_\beta$ (see Fig. 1). This converts the 2.0-kb *Bam*HI fragment containing the $\beta_2$, *TM*, and *IC-1*$^k$ exons into a 9.5-kb fragment containing all of the recombinant $A_\beta$ gene upstream of the 3′ untranslated region but leaves intact the 2.4-kb 3′ *Bam*HI fragment seen in both $A^d_\beta$ and $A^k_\beta$. Consistent with this, DNA from RT4.15HP shows only a 2.4-kb band in the short-exposure autoradiogram (lane 3) and an intense 2.4-kb band (representing multiple integrated gene copies) plus a faint 2.0-kb band (representing the single-copy endogenous L cell $A^k_\beta$ gene) in the autoradiogram exposed with an intensifying screen ("long" exposure) (lane 8). The 2.4-kb band comigrates with the equivalent *Bam*HI fragment from pgI-$A_\beta(\beta^k_1;\beta_2,TM,IC^d)$gpt-1 (lanes 4 and 9). Both the 2.0- and 2.4-kb bands are intense in the pgI-$A^k_\beta$-gpt-1 and wild type-transfectant (D3.11H3) lanes (lanes 1 and 2, short exposure; lanes 6 and 7, long exposure). These data indicate that RT4.15HP has received the shuffled $A_\beta$ gene.

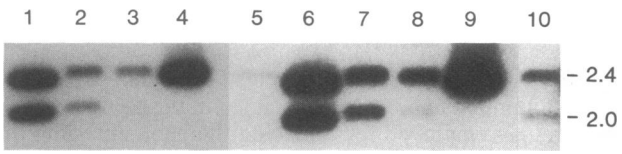


FIG. 2. Southern blot analysis of *Ia*-transfected L cells. Exposure for lanes 1–4 was 20 hr with no screens; for lanes 5–9, 20 hr with a Cronex Lightning-plus intensifying screen; and for lane 10, 60 hr with a screen. Lanes 1 and 6; pgI-$A^k_\beta$-gpt-1. Lanes 2 and 7: D3.11H3. Lanes 3 and 8: RT4.15HP. Lanes 4 and 9: pgI-$A_\beta(\beta^k_1;\beta_2, TM, IC^d)$-gpt-1. Lanes 5 and 10: 2B4 (an $H\text{-}2^a/H\text{-}2^k$ T-cell hybridoma). Sizes (in kb) of major hybridizing fragments are at right.

The ability to stain and preparatively sort the original transfectant pool with the 10.2.16 antibody had established that the $\beta^k_1$ domain controlled the Ia.m27 epitope seen by this reagent. Table 1 summarizes the results of similar flow microfluorimetric analysis of RT4.15HP stained with a panel of anti-$I\text{-}A^k$ antibodies from the various epitope groups defined by Pierres *et al.* (32, 33). These same antibodies are also evaluated for TA3, an $I\text{-}A^{k,d}$-expressing B-cell hybrid (24); RT7.3H3-B, a wild-type $I\text{-}A^k$-expressing L-cell transfectant (unpublished observations); T70.3.1, an $H\text{-}2^d$ B-lymphoma cell line transfected with $A^k_\beta$ and expressing this gene's product in the absence of $A^k_\alpha$ (23); and M12.4.1, the parent $I\text{-}A^d$ B-lymphoma line used to prepare T70.3.1 (37). Several findings emerge from this analysis. First, as previously suggested, the epitope(s) recognized by the antibodies in group 1 correlate exactly with $A^k_\beta$ expression by T70.3.1, and members of this group are not influenced in their binding by the *d/k* allelic polymorphism of the $A_\alpha$ chain. Most other antibodies require $A^k_\alpha$ for binding and are either $A^k_\alpha$-specific or $I\text{-}A^k$-specific. Recent data on transfectants expressing $A^b_\beta$ togeth-

Table 2. Proliferative response of the 4R.6 T-cell clone

| | | | [$^3$H]Thymidine incorporation, cpm | | | |
|---|---|---|---|---|---|---|
| | Ia expression | | No | | Antigen | Antigen |
| APC | Endogenous | Transfected | addition | Antigen | + 10.2.16 (anti-$A^k_\beta$) | + 16.1.2 (anti-$D^k,K^k$) |
| D3.11H | None | $A^k_\beta A^k_\alpha$ | 5521 | 89,175 | 6121 | 73,411 |
| RT4.15H | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | 7694 | 91,210 | 6869 | 130,050 |
| TA3 | $I\text{-}A^{d,k},I\text{-}E^{d,k}$ | None | 2341 | 48,190 | 1723 | 43,884 |
| RT2.3H | None | $A^d_\beta A^d_\alpha$ | 8859 | 7,392 | 8203 | 10,676 |

Cells ($10^4$) of the $I\text{-}A^k$-restricted T-cell clone 4R.6 specific for pigeon cytochrome *c* fragment(1–65), prepared and maintained as described (39), were assayed for proliferative response in the presence of the indicated combinations of antigen (1 $\mu$M) and antigen-presenting cells (APC; $10^5$). The indicated antibodies were added as sterile culture supernatants, to 25% (vol/vol) final concentration. Proliferation was measured as [$^3$H]thymidine incorporation from 68 to 84 hr of culture.

Table 3. Cytopathic effect of 11.4 T-cell clone

| APC | Ia expression | | % control response | | | |
|---|---|---|---|---|---|---|
| | Endogenous | Transfected | Antigen | Antigen + 10.2.16 | Antigen + MKD6 (Anti-I-$A^d$) | Antigen + Anti-$D^k$,$K^k$ |
| D3.11H | None | $A^k_\beta A^k_\beta$ | 30 | 99 | 39 | 42 |
| RT4.15H | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | 9 | 106 | 15 | 12 |
| RT2.3H | None | $A^d_\beta A^d_\alpha$ | 97 | 109 | 96 | 110 |

Cells ($3 \times 10^4$) of the I-$A^k$-restricted T-cell clone 11.4 specific for poly($Glu^{60}Ala^{30}Tyr^{10}$) were assayed for cytopathic effect on L-cell transfectants as previously described (22). Antibodies were used as described in the legend to Table 2. Data are given as percent of control-response [$^3$H]thymidine incorporation, calculated as follows: (cpm for culture with given additions/cpm for culture without additions) $\times$ 100%. Control responses: D3.11H, 6201 cpm; RT4.15H, 19,970 cpm; RT2.3H, 27,710 cpm. APC, antigen-presenting cells.

er with $A^k_\alpha$ suggest that the 39J group (group 2) is $A^k_\alpha$-specific (unpublished observations). The proportionately lower binding of 39A on T70.3.1, compared to that of the group 1 antibodies, may indicate that this reagent is predominantly anti-$A^k_\beta$ but influenced in its binding by $A_\alpha$ polymorphism. Second, the pattern of reactivity of all anti-I-$A^k$ antibodies tested is the same for TA3, RT7.3H3-B, and RT4.15HP. Thus, all direct and indirect involvement of $A_\beta$ in creating the epitopes seen by these antibodies maps to the $\beta_1$ domain, and expression of these class II gene products by L cells, as compared to B cells and macrophages, does not appear to grossly influence the serology of I-$A^k$.

To evaluate changes in the ability of the variant I-$A^k$ molecule expressed by RT4.15HP to be recognized by I-$A^k$-restricted, antigen-specific T lymphocytes, these transfectants were used as accessory cells in assays of T-cell stimulation. As previously reported by Malissen *et al.* (38) and Norcross *et al.* (22), I-A-expressing L cells present antigen to long-term T-cell clones and T hybridomas. This presentation can be measured as T-cell proliferation and/or interleukin 2 release and, in the case of certain T-cell clones, by a cytopathic effect on the transfectants. As summarized in Tables 2–4, RT4.15H or RT4.15HP were qualitatively identical in functional tests to the D3.11H or D3.11H3 transfectants expressing wild-type I-$A^k$. This was true even for clone 11.4, which has previously been shown to require the $A^k_\beta$ chain, irrespective of the $A_\alpha$ (d or k) present in the I-A molecule of the accessory cell (22, 23). This essentially rules out the possibility that all the T cells tested require only $A^k_\alpha$ recognition and ignore $A_\beta$ polymorphism.

Although additional shuffled genes need to be tested to establish the generality of the above findings, they do indicate that the polymorphic sites within the $NH_2$-terminal domain of the $A^k_\beta$ polypeptide play a predominant, if not exclusive, role in the contribution of this chain to epitopes recognized on intact I-A molecules by the humoral and cell-mediated components of the immune system. This is consistent with the striking effect of the *bm12* mutation (which affects amino acids 68, 71, and 72 in $A^b_{\beta_1}$; see ref. 40) on T-cell recognition of Ia molecules containing this $A_\beta$ polypeptide (41, 42). These data contrast with the results obtained with "exon-shuffled" class I MHC molecules. In the latter case, most (20, 21), though not all (19), recombinant molecules mixing N (amino-terminal) and $C_1$ (penultimate) domains fail to be recognized by T cells restricted to parental MHC molecules. Such results are consistent with the view that the N and $C_1$ domains of class I molecules are the functional homologues of the $\alpha_1$ and $\beta_1$ domains of class II molecules and have a similar spatial relationship with respect to each other, to antigen, and to the T-cell receptor during the recognition process. This view also implies that $N/C_1$ shuffles are equivalent to "hybrid" or transcomplementing $A_\alpha$:$A_\beta$ pairs, which bring new $\alpha_1$ and $\beta_1$ domains together, and are not analogous to intramolecular $\alpha_1$:$\alpha_2$ or $\beta_1$:$\beta_2$ recombinants. This model is consistent with the documented occurrence of unique, nonparental "$F_1$" determinants on transcomplementing $\alpha$:$\beta$ pairs, as assessed using T-cell clones (43).

Finally, the results described above are not entirely unexpected, since naturally occurring intra-I-region recombination has been found to occur primarily in the intron between the exons encoding the $\beta_1$ and $\beta_2$ domains of $E_\beta$ (44, 45) and since the resultant naturally exon-shuffled genes give rise to $E_\beta$ polypeptides that are functionally identical to the $E_\beta$ of the $\beta_1$-domain donor (1). Nonetheless, the present observations are significant with regard to unraveling Ia structure–

Table 4. Interleukin-2 production by T-cell hybridomas

| Hybridoma | Specificity | | | Ia expression | | [$^3$H]Thymidine incorporation, cpm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ia | Antigen | APC | Endogenous | Transfected | No addition | Antigen | Antigen + 10.2.16 | Antigen + MKD6 | Antigen + 16.1.2 |
| SKK9.11 | I-$A^k$ | KLH | None | — | — | 2124 | 2,261 | — | — | — |
| | | | RT2.3.3H | None | $A^d_\beta A^d_\alpha$ | — | 3,763 | — | — | — |
| | | | D3.11H3 | None | $A^k_\beta A^k_\alpha$ | — | 34,870 | 3,130 | 48,564 | 45,381 |
| | | | RT4.15HP | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | — | 103,124 | 2,400 | 116,641 | 111,095 |
| | | | LK | I-$A^{d,k}$;I-$E^{d,k}$ | None | — | 165,103 | 2,866 | 183,566 | 169,855 |
| SKK45.10 | I-$A^k$ | KLH | RT1.1.12 | None | None (TK* only) | — | 1,092 | — | — | — |
| | | | RT4.15HP | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | — | 23,158 | 863 | — | — |
| 3A9 | I-$A^k$ | HEL | RT1.1.12 | None | None (TK only) | — | 2,971 | — | — | — |
| | | | RT4.15HP | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | — | 18,934 | 1,184 | — | — |
| RF9.140 | I-$A^d$ | GAT | RT2.3.3H | None | $A^d_\beta A^d_\alpha$ | — | 22,090 | 20,165 | 9,139 | — |
| | | | RT4.15HP | None | $A_\beta(\beta^k_1;\beta_2,TM,IC^d)A^k_\alpha$ | — | 1,687 | 1,641 | 1,326 | — |

Cells ($10^5$) of the indicated T-cell hybridomas were cultured in 200 $\mu$l of medium for 24 hr with the specified additions, and the supernatants were assayed for IL-2 content as previously described, using CTLL indicator cells (22). Antibodies were used as described in the legend to Table 2. Data are reported as [$^3$H]thymidine incorporation (total cpm) for each condition. KLH, keyhole limpet hemocyanin; HEL, hen egg lysozyme; GAT, poly($Glu^{60}Ala^{30}Tyr^{10}$); APC, antigen-presenting cells. SKK9.11 and SKK45.10 were provided by P. Marrack and J. Kappler; 3A9 was the gift of P. Allen; RF9.140 was provided by K. Rock.
*Thymidine kinase.

function relationships; in an *in vitro* model system, they directly identify the portion of a class II $\beta$ gene that needs to be further analyzed to determine the precise sites critical in immune-response gene function and MHC-restricted antigen presentation.

We wish to thank Dr. Ronald Schwartz for his critical review of this manuscript, Shirley Starnes for her excellent editorial assistance, and Drs. D. McKean and R. Hodes for providing monoclonal anti-Ia antibodies. R.I.L. was supported by a fellowship from the Wellcome Trust. G.S. is a Fogarty Visiting Fellow.

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