

MEMBRANE Ia EXPRESSION AND ANTIGEN-PRESENTING  
ACCESSORY CELL FUNCTION OF L CELLS  
TRANSFECTED WITH CLASS II MAJOR  
HISTOCOMPATIBILITY COMPLEX GENES

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Stimulation of inducer T lymphocytes to undergo clonal expansion and differentiation requires recognition of nominal antigen in the context of polymorphic determinants of class II major histocompatibility complex (MHC)<sup>1</sup> glycoproteins (Ia) displayed on specialized accessory cells (for review, see 1). Effective stimulation requires both an ill-defined "processing" of native antigen (2–6), perhaps corresponding to partial intracellular proteolysis and membrane reexpression of antigenic peptides, and the action of poorly characterized "lymphokines" (2, 4), possibly including, but not limited to, interleukin 1 (IL-1) (7). A more precise understanding of T cell antigen recognition thus requires greater knowledge of the structural features of the interacting components, i.e., the T cell receptor, Ia molecule, and immunogenic peptide, and more information about the additional membrane and soluble components involved in turning the recognition event into complete T cell activation.

Substantial progress has recently been made in each of these areas. Detailed analyses of the minimal stimulatory peptide(s) of protein antigens has been carried out in several laboratories (6, 8–10). A two-chain polypeptide structure for the antigen receptor of MHC-restricted T cells has been demonstrated (11–14) and a gene encoding one of these peptides has been cloned (15, 16). Finally, the molecular cloning of class II genes of mouse and man has provided a relatively complete picture of the primary structure of these glycoproteins and identified the major site of intraspecies polymorphism as the amino-terminal domain of both  $\alpha$  and  $\beta$  chains (reviewed in 17).

The availability of these cloned Ia genes has permitted the initiation of studies

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<sup>1</sup> *Abbreviations used in this paper:* APC, antigen-presenting cell; Con A, concanavalin A; cyto *c*, pigeon cytochrome *c*; DME-10, Dulbecco's modified Eagle's medium supplemented with 10% FCS; ER, 50:50 R medium and Eagle's high amino acid medium with 10% FCS; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GAT, L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup> random terpolymer; HAT, hypoxanthine, aminopterin, and thymidine; HEL, hen egg lysozyme; [<sup>3</sup>H]TdR, [<sup>3</sup>H]thymidine; IL-1 and -2, interleukin 1 and 2; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; MXH, mycophenolic acid, xanthine, and hypoxanthine; PBS, phosphate-buffered saline; PMA, phorbol myristic acetate; R medium, RPMI 1640 medium supplemented with 10% FCS, 2-mercaptoethanol, and Hepes; TK, herpes thymidine kinase gene.

using DNA-mediated gene transfer to dissect further the structure-function relationship of Ia molecules to T cell antigen recognition. Our initial work in this area (18) first demonstrated that such transfected Ia genes are functionally expressed by Ia<sup>+</sup> B cell lymphomas known to be capable of accessory cell activity, and revealed that the polymorphism between A<sub>β</sub> chains alone is sufficient to create a "restriction element" for at least some T cells. To derive a model system that (a) permitted complete control of the α and β chains available for Ia assembly, and (b) might permit dissecting the recognition of (antigen plus Ia) from subsequent activation steps, we have chosen to transfect Ia genes into a fibroblastoid cell line of murine origin. These Ia<sup>-</sup> L cells are not considered to be among the cell types typically capable of direct T cell stimulation. The present report demonstrates, in agreement with the work of others (19, 20), that such L cells express the products of the transfected Ia genes on their surface, and describes the accessory cell activity of these cells in stimulating responses of a variety of T lymphocytes.

### Materials and Methods

*Mice.* C3H/HN mice were obtained from the Small Animal Branch, NIH. C57BL/10, B10.A(4R), and B10.A mice were obtained from the barrier-sustained colony at NIH or Sprague-Dawley, respectively. All animals were maintained on food and water ad lib, and used at 8–20 wk of age.

*Antigens.* L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup> (GAT) was purchased from Vega Biochemicals, Tucson, AZ, and prepared as previously described (21). Pigeon cytochrome *c* (cyto *c*) cyanogen bromide cleavage fragment (1-65) was prepared as described (22). Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem-Behring Corp., La Jolla, CA and hen egg lysozyme (HEL) was purchased from Sigma Chemical Co., St. Louis, MO.

*Media.* Dulbecco's modified Eagle's medium with 4,500 mg/l glucose (Gibco Laboratories, Grand Island, NY) was supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS), and 20 μg/ml gentamycin (DME-10). RPMI 1640 (Biofluids, Inc., Rockville, MD) was supplemented with 10% FCS, 5 × 10<sup>-5</sup> M 2-mercaptoethanol, and 10 mM Hepes (R medium). A mixture of 50% RPMI 1640, 50% Eagle's high amino acid medium containing 10% FCS, 5 × 10<sup>-5</sup> 2-mercaptoethanol and 2 mM L-glutamine was termed ER. Interleukin 2 (IL-2)-containing medium was either the supernatant of the EL-4 thymoma stimulated with 10 ng/ml phorbol myristic acetate (PMA) for 24 h or the supernatant of a 5 × 10<sup>6</sup> cells/ml culture of rat splenocytes incubated with 5 μg/ml concanavalin A (Con A) for 24 h. Selective medium for transfection experiments consisted of DME-10 plus hypoxanthine (15 μg/ml), aminopterin (0.2 μg/ml), and thymidine (5 μg/ml) (HAT) (23), DME-10 plus mycophenolic acid (6 μg/ml), hypoxanthine (15 μg/ml), and xanthine (250 μg/ml) (MXH) (24), or a combination of the two (DME-10, HAT, MXH).

*Cells.* All cultured cell lines used in these experiments, their origin, and properties, are described in Table I. The DAP.3 line of Ltk<sup>-</sup> cells (23) was grown in DME-10 and passaged by 1:10 subculture two times per week after trypsin-EDTA treatment. DAP.3 and all L cell transfectants were prepared for use in experiments by trypsin-EDTA removal from the culture vessel, followed by washing and resuspension in FCS-containing medium. The M12.4.1 H-2<sup>d</sup> B lymphoma (25), the A20-2J H-2<sup>d</sup> B lymphoma (26), the TA3 (H-2<sup>a/d</sup> × H-2<sup>d</sup>) B cell hybridoma (25), the LK (H-2<sup>k</sup> × H-2<sup>d</sup>) B hybridoma (27), and the B cell transfectant T70.3.1 (H-2<sup>d</sup>, A<sub>β</sub><sup>k</sup>) (18) were all maintained by biweekly passage at 1:50 in R medium. T hybridomas were maintained in the same manner in DME-10, except for C10, which required ER medium for growth. The IL-2-dependent, GAT-specific T cell clone 11.4 (31), generously provided by Dr. J. Ashwell, NIH, was maintained in ER medium with 5% IL-2-containing supernatant. The 4R.6 pigeon cyto *c*-specific T cell line, kindly provided by Dr. G. Suzuki, NIH, was maintained by stimulating 2 × 10<sup>5</sup>

TABLE I  
Cell Lines Used in this Study

	Cell	Cell type	Ia			Reference
			Transfected	Endogenous	Expressed	
Group I	DAP.3	L (fibroblast)	None	None	None	23
	T43.6	L transfectant	A $\beta^k$	None	None	This paper
	T43.7	L transfectant	A $\beta^d$	None	None	This paper
	D3.4	L transfectant	A $\beta^k$ A $\alpha^k$	None	I-A $^k$	This paper
	T78.11.1	L transfectant	A $\beta^d$ A $\alpha^d$	None	I-A $^d$	This paper
	M12.4.1	B lymphoma	None	I-A $^d$ , I-E $^d$	I-A $^d$ , I-E $^d$	25
	T70.3.1	B transfectant	A $\beta^k$	I-A $^d$ , I-E $^d$	A $\beta^k$ , I-A $^d$ , I-E $^d$	18
	A20.2J	B lymphoma	None	I-A $^d$ , I-E $^d$	I-A $^d$ , I-E $^d$	26
	LK	B hybridoma	None	I-A $^{dk}$ , I-E $^{dk}$	I-A $^{dk}$ , I-E $^{dk}$	27
				Antigen specificity	Ia restriction	
Group II	4R.6B	T cell clone	Cytochrome <i>c</i> (1-65)	I-A $^k$	G. Suzuki, unpublished	
	11.4	IL-2 dependent T cell clone	GAT	I-A $^k$	31	
	CTL-L	IL-2 dependent T cell	—	—	29	
	HT-2	IL-2 dependent T cell	—	—	30	
	Group III	RF9.140	T hybridoma	GAT	I-A $^d$	28
SKK9.11		T hybridoma	KLH	I-A $^k$	J. Kappler & P. Marrack, unpublished	
SKK45.10		T hybridoma	KLH	I-A $^k$	J. Kappler & P. Marrack, unpublished	
C10		T hybridoma	HEL	I-A $^k$	R. Schwartz, unpublished	

cells with  $3 \times 10^6$  3,000 rad-irradiated B10.A spleen cells in the presence of 0.5  $\mu$ M pigeon cyto *c* cyanogen bromide fragment 1-65 for 7 d in 2 ml in the wells of 24-well cluster plates, then resting the cells on  $3 \times 10^6$  irradiated B10 spleen cells for 2-3 wk. Viable cells were recovered using Ficoll-Hypaque gradients and were washed before use. CTL-L and HT-2 cells for IL-2 assay were maintained in 5% rat Con A supernatant. DAP.3 cells cotransfected with Ia genes plus the herpes thymidine kinase gene (TK) were selected (see below) and grown in DME-10, HAT. DAP.3 cells sequentially cotransfected with A $\beta^d$  plus TK, then A $\alpha^d$  plus pSV2gpt (see below), were first selected and grown using DME-10, HAT, then DME-10, HAT, MXH.

*DNA Clones, Hybridization Probes, and Constructs.* Genomic clones of A $\beta^k$ , A $\beta^d$ , A $\alpha^d$ , and A $\alpha^k$  were isolated from  $\lambda$  phage vectors or cosmid libraries. The isolation and nucleotide sequence of the A $\beta^k$  and A $\beta^d$  genes has been previously described (26, 32), as has the construction in the plasmid vector pSV2gpt (24) of subclone pgI-A $\beta^k$ -gpt-1 containing an expressible A $\beta^k$  gene (18). To prepare a vector with A $\beta^d$ , the original  $\lambda$  phage clone of the A $\beta^d$  was digested with BamHI restriction endonuclease and the 5' 9.5 kilobase (kb) fragment and 3' 2.3 kb fragments subcloned into the BamHI site of pBR327. These BamHI fragments were recovered from the plasmid subclones and ligated together into the BamHI site of pSV2gpt. One such construct, containing the two fragments of the A $\beta^d$  gene in the proper orientation with respect to one another, was identified and termed pgI-A $\beta^d$ -gpt-49. The A $\alpha^d$  gene used in these studies was derived from the 5' BamHI/EcoRI fragment of the A $\alpha^d$  gene, which is missing the end of the 3' untranslated region containing the poly A addition signal (33), and the 3' EcoRI/BamHI fragment of A $\beta^k$ , which contains such a signal (32). These were ligated together in the BamHI site of pSV2gpt, generating pg5'I-A $\alpha^d$ 3'A $\beta^k$ -gpt-1. The BamHI/EcoRI fragment of A $\alpha^d$  was kindly provided by Dr. Mark Davis from a cosmid subclone constructed by Dr. M. Steinmetz (34). The A $\alpha^k$  gene was isolated from a subgenomic library prepared in the HindIII cloning site of the  $\lambda$  J1 phage vector using a 7-10 kb fraction of HindIII-digested (H-2 $^k \times$  H-2 $^a$ ) DNA. The library was screened with A $\alpha^d$ -coding region cDNA probe (35),

made available to us by Dr. M. Davis. One of several phage clones hybridizing to this probe was isolated and the 9 kb HindIII insert containing the  $A_\alpha^k$  gene subcloned into the HindIII site of pBR327 to generate pgI- $A_\alpha^k$ A21. The identity of this clone was confirmed by restriction map analysis of the insert DNA. Hybridization probe for Northern blot analysis of RNA consisted of a Cfo fragment containing the 3'  $A_\beta^d$  cDNA described in (26). All recombinant DNA manipulations were carried out essentially according to Maniatis et al. (36).

**Transfection.** Cloned class II MHC genes were introduced into DAP.3 cells using the calcium phosphate coprecipitation method, as previously described for class I genes (23). 48 h after transfection, stable transformants were selected by incubation in drug-containing medium. T43.7 represents a pool of cells from DAP.3 cotransfected with pBRTK plus  $\lambda$  phage DNA containing  $A_\beta^d$  and selected in DME-10, HAT. T43.7 cells were retransfected with DNA from pg5' I- $A_\alpha^d$ 3'  $A_\beta^k$ -gpt-1 and selected in DME-10, HAT, MXH. Clones growing in this medium were screened for surface I- $A^d$  expression using a modification of the polyester replica plating technique of Raetz et al. (37). Colonies were overlaid with polyester cloth and after 2 wk the filter was washed in phosphate-buffered saline (PBS), incubated with MKD6 (anti-I- $A^d$ ) antibody (38), washed, and incubated in  $^{125}\text{I}$ -goat anti-mouse IgG (New England Nuclear, Boston, MA). The clone (T78.11.1) giving the strongest autoradiographic signal was picked and expanded. D3.4 was derived as a pool of cells growing in DME-10, HAT, after cotransfection of DAP.3 with pgI- $A_\beta^k$ -gpt-1, pgI- $A_\alpha^k$ A21, and pBRTK. The M12.4.1 BALB/c B lymphoma transformant T70.3.1 expressing  $A_\beta^k$  was produced using spheroplast fusion as previously described (18).

**Analysis of Specific RNA Transcripts.** Cytoplasmic RNA was prepared according to Mushinski et al. (39). 10  $\mu\text{g}$  (for B cells) or 40  $\mu\text{g}$  (for transfectants) of this RNA were applied in 2.2 M formaldehyde to 1% agarose gels containing 0.22 M formaldehyde, and subjected to electrophoresis for 600 volt hours. The RNA was transferred to nitrocellulose filters according to the method of Southern (40), hybridized using  $^{32}\text{P}$  nick-translated probes, and autoradiographs were prepared as previously described (18).

**Monoclonal Antibodies.** The monoclonal antibodies used in these experiments were 10.2.16, anti-I- $A^k$  (Ia-m.27), specific for  $A_\beta^k$  (41); MKD6, anti-I- $A^d$  (38); M5/114, anti-I- $A^d$ , I-E $^{d/k}$  (42); and 11.4.1, anti-H-2K $^k$  (41). All monoclonal antibodies were in the form of cell-free sterile culture supernatants containing 5–50  $\mu\text{g}/\text{ml}$  specific antibody. They were added as indicated at culture initiation to a final concentration of 12.5–25% (vol/vol), or used as described below for fluorescent staining.

**Flow Cytometry.** Cell surface expression of Ia was assessed by microfluorimetric analysis on a FACS II (B-D FACS Systems, Sunnyvale, CA). Cells ( $5 \times 10^5$ ) in 50  $\mu\text{l}$  R medium were mixed with 50  $\mu\text{l}$  of the appropriate monoclonal antibody supernatant in the wells of a 96-well microtiter plate. After 30 min on ice, the cells were washed twice in PBS, resuspended in 50  $\mu\text{l}$  of R medium containing 1  $\mu\text{g}$  of fluorescein isothiocyanate (FITC)-goat F(ab) $'_2$  anti-mouse IgG (Cappel Laboratories, Cochranville, PA) and incubated for an additional 30 min on ice. Stained cells were washed and fixed in 1% paraformaldehyde in PBS. Profiles shown indicate log fluorescence (channel number) on the abscissa vs. percent of total cells on the ordinate. A total of 10,000 cells was analyzed for each profile.

**T Cell Assays.** Production of IL-2 by T cell hybridomas (38) was measured by culturing  $10^5$  T cell hybridoma cells with  $5 \times 10^4$  to  $10^5$  antigen-presenting cells (APC) and antigen for 24 h in 200  $\mu\text{l}$  ER medium in 96-well microtiter trays (Falcon 3040; Falcon Labware, Oxnard, CA). Individual culture supernatants from triplicate cultures were collected and tested for IL-2 content at a 1:4 dilution on  $4 \times 10^5$  cells of the IL-2-dependent cell line CTL-L or HT-2. 1  $\mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine ([ $^3\text{H}$ ]TdR) (6.7 Ci/mmol; New England Nuclear) was added at 24 h and the cultures harvested 16–20 h later using an automated multi-sample harvester. Data are shown as counts per minute plus or minus standard error.

Interaction of T cell clones with transfected cells was assayed by culturing  $2 \times 10^4$  rested viable 4R.6 T cells with either 12,000 rad-irradiated B cells or 18,000 rad-irradiated L cells, with or without 1  $\mu\text{M}$  pigeon cyto *c* fragment (1-65). Cells were cultured for 76 h, then labeled for 20 h with [ $^3\text{H}$ ]TdR and recovered for scintillation counting.

The GAT + I-A<sup>k</sup>-restricted, IL-2-dependent T cell clone, 11.4, was washed before use and similarly tested except that antigen consisted of 100  $\mu\text{g}/\text{ml}$  GAT (Vega Biochemicals) and the cultures were harvested at 72 h.

For mitogen responses, normal T cells were prepared from C3H/HN mice (NIH) by passing  $\sim 100 \times 10^6$  lymph node cells over a nylon wool column. These were then used after treating  $50 \times 10^6$  nylon-passed cells with anti-Ia antibody (10.2.16 [1:3], 1 ml; M5/114 [1:3]) and 1 ml rabbit complement (1:6) (Low-Tox; Accurate Chemical & Scientific Corp., Westbury, NY) for 30 min at 37°C. Ia<sup>-</sup> T cells prepared in this manner lacked direct Con A responsiveness. T cells were washed and cultured at  $2.5 \times 10^5$  per well with  $10^4$  irradiated stimulator cells for 96 h; [<sup>3</sup>H]TdR incorporation was assessed over the last 20 h of culture.

**Photography.** Cultures in flat-bottomed microtiter plates were photographed on Kodak technical pan film 2415 with a Leitz inverted microscope using phase optics.

## Results

**Transcription of I-A Genes In L Cells.** We have previously shown (18) that the product of the A $\beta^k$  gene introduced into an I-A<sup>d</sup>-bearing B cell tumor is expressed on the cell surface in normal amounts and functions as an MHC restriction element for T cell stimulation. To ascertain whether the Ia<sup>-</sup> murine L cell would possess accessory cell function in T cell activation assays if it bore Ia on its membrane, cloned I-A genes were introduced into L cells using the calcium phosphate precipitation technique. DNA from genomic clones of A $\beta^k$  or A $\beta^d$  in  $\lambda$  bacteriophage vectors, containing the entire gene plus several kilobases of 5' and 3' flanking DNA, was cotransformed with pBRTK DNA into DAP.3 TK<sup>-</sup> L cells. Stable transformants growing in HAT-selective medium were shown by Southern blot analysis to contain one to several copies of the cloned A $\beta$  genes (data not shown). To assess the transcriptional activity of these transfected genes, cytoplasmic RNA was prepared and analyzed by Northern blotting, using an A $\beta$  3' cDNA probe. Fig. 1 shows that no A $\beta$ -specific mRNA was detectable in the parental DAP.3 cells (lane 1), while T43.7 (lane 2), containing the transfected

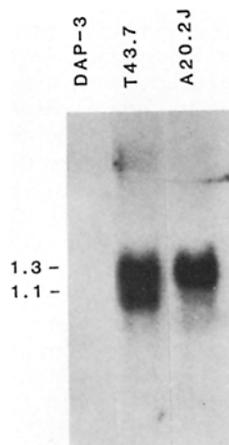


FIGURE 1. Northern blot analysis of A $\beta$ -transfected cells. Cytoplasmic RNA (40  $\mu\text{g}/\text{lane}$  for DAP.3 and T43.7, 10  $\mu\text{g}/\text{lane}$  for A20.2J) was electrophoresed on 1% agarose formaldehyde gels. The RNA was transferred to nitrocellulose and hybridized with a nick-translated A $\beta$  cDNA probe, and the washed blot was subjected to autoradiography.

$A_{\beta}^d$  gene, produced substantial amounts of  $A_{\beta}$  message. These autoradiograms show two major species of hybridizing transfectant RNA, at 1.3 and 1.1 kb, which are both present, although in strikingly different proportions, in B cell lymphoma RNA (lane 3). The amount of hybridizing RNA in transfected L cells vs. B lymphoma cells ranged from 30–50% of the level of  $A_{\beta}$  message in the M12.4.1 tumor line to 10–20% of the level in the A20-2J tumor line. It is important to note that the transcriptional activity detected in these studies did not require either cotransformation with or covalent linkage to DNAs containing viral enhancer elements.

Thus, L cells, which are constitutively  $Ia^{-}$  and cannot be induced to express  $Ia$  using  $\gamma$ -interferon-containing T cell supernatants (unpublished observations), nonetheless transcribe exogenously introduced  $A_{\beta}$  genes, including one ( $A_{\beta}^k$ ) identical to that already present in their genome.

*Surface Expression of I-A by L Cells Possessing Transfected  $A_{\alpha}$  and  $A_{\beta}$  Genes.* Analysis of the  $A_{\beta}$  L cell transfectants with monoclonal anti-I-A antibodies, including 10.2.16 (which is capable of binding isolated  $A_{\beta}^k$  polypeptides) revealed no surface  $Ia$  expression. This result is consistent with previous data on the requirement for  $\alpha/\beta$  dimers for  $Ia$  surface expression (43), including recent studies with gene transfectants (19, 20).

To derive cells potentially able to express surface  $Ia$ , the T43.7 cell was transfected with DNA from a recombinant clone (pg5' I- $A_{\alpha}^d$ 3'  $A_{\beta}^k$ -gpt-1) containing the entire coding region of  $A_{\alpha}^d$  spliced to the 3' untranslated region of  $A_{\beta}^k$ , which provides the poly A addition signal but no coding sequence. Selection in DME-10, HAT, MXH led to the growth of clones that were screened using a replica-plating method and cellular radioimmunoassay for surface  $I-A^d$  expression, as detected by the monoclonal antibody MKD6. One clone identified in this manner, T78.11.1, was chosen for further study. At the same time, DAP.3 cells were cotransfected with  $A_{\beta}^k$  (pgI- $A_{\beta}^k$ -gpt-1),  $A_{\alpha}^k$  (pgI- $A_{\alpha}^k$ A21), and pBRTK, and a pool of cells surviving selection in DME-10, HAT was designated D3.4. Transcription of the introduced  $A_{\alpha}$  genes was evaluated by Northern blotting. Substantial amounts of  $A_{\alpha}$  transcripts (10–20% the level observed in B cell tumors) were seen in T78.11.1 and D3.4 RNA, while such hybridizing species were absent from DAP.3 or the  $A_{\beta}$  alone transfectant T43.7. The  $A_{\alpha}$  transcripts in D3.4 transfected with  $A_{\alpha}^k$  co-migrated with B cell  $A_{\alpha}$  message at 1.4 kb. The  $A_{\alpha}$  transcripts derived from cells possessing the hybrid  $A_{\alpha}^d:A_{\beta}^k$  gene show the expected increase in size, due to an additional 3' untranslated sequence introduced from the  $A_{\beta}^k$  gene segment, as well as a larger species presumably representing the result of aberrant splicing secondary to creation of the hybrid gene (data not shown).

These  $A_{\alpha}:A_{\beta}$  transfectants were analyzed for surface expression of  $Ia$  by staining with monoclonal anti-I- $A^d$  (MKD6) or anti-I- $A^k$  (10.2.16) antibodies, followed by FITC-conjugated goat F(ab')<sub>2</sub> anti-mouse IgG (Fig. 2). Cells from  $A_{\alpha}^k:A_{\beta}^k$  transfectant pool D3.4 stained only with 10.2.16, not with MKD6, while T78.11.1 cells stained with MKD6 but not 10.2.16. T78.11.1 cells also stained with M5/114 (anti-I- $A^d$ ), and D3.4 cells stained with 39J (anti-I- $A_{\alpha}^k$ ) (data not shown). Thus, the transfected L cells not only transcribed both  $A_{\alpha}$  and  $A_{\beta}$ , but expressed the translated products of these mRNAs on the cell membrane. The

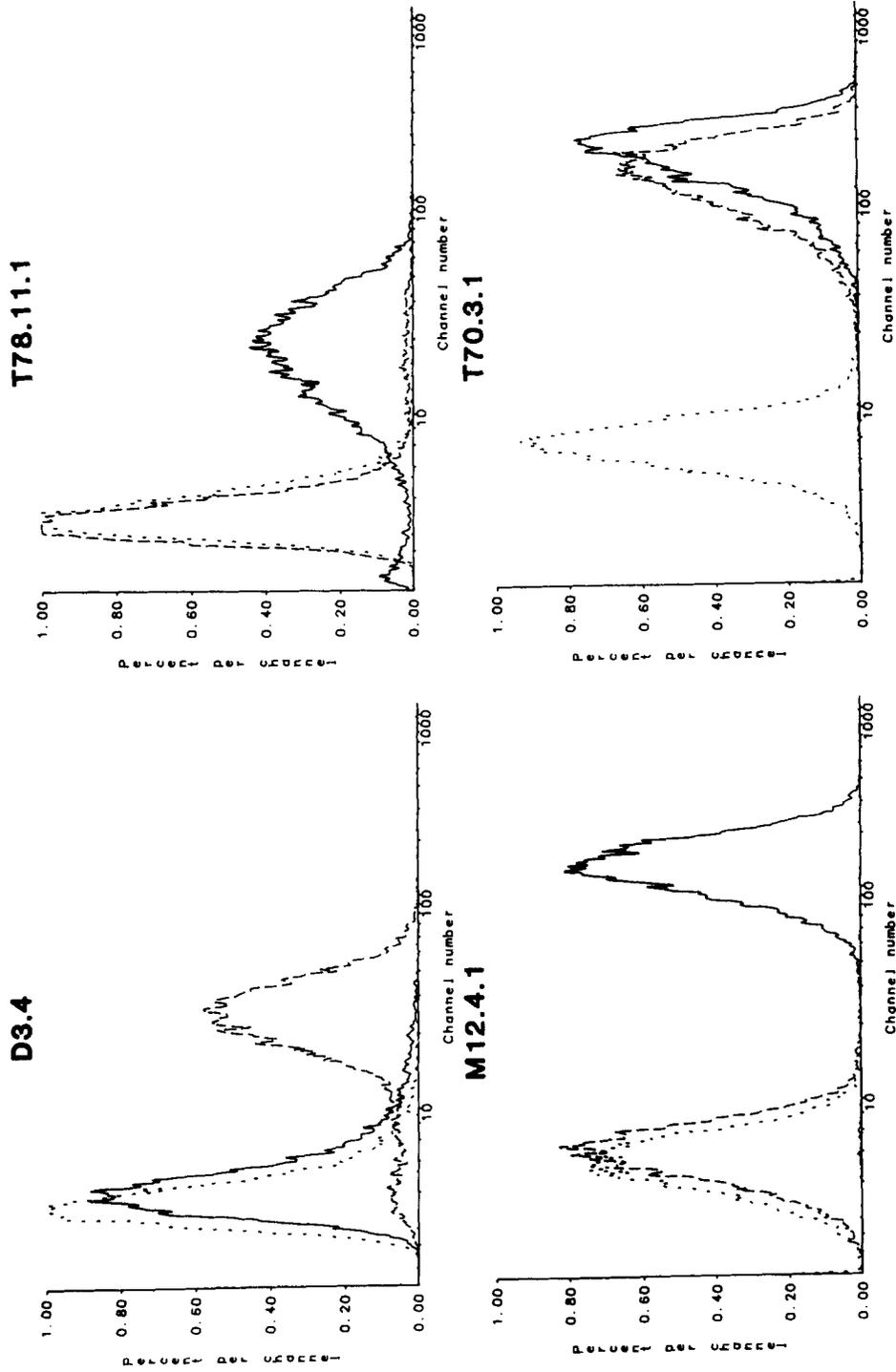


FIGURE 2. Surface Ia expression by transfected L cells. Cells were stained with monoclonal anti-I-A antibodies as outlined in Materials and Methods and analyzed on a FACS II flow cytometer. Profiles represent log fluorescent channel vs. the percent of total cells for 10,000 cells analyzed. (· · ·) FITC anti-Ig only; (- - -) 10.2.16 (anti-I-A $\beta$ ); (—) MKD6 (anti-I-A $\alpha$ ).

TABLE II  
Stimulation of T Cell Hybridomas by Ia-bearing L Cell Transfectants

APC	Cell type	Ia		Assay hybridomas*			
		Transfected	Endogenous	SKK9.11 (KLH:I-A <sup>k</sup> )	SKK45.10 (KLH:I-A <sup>k</sup> )	C10 (HEL:I-A <sup>b</sup> )	RF9.140 (GAT:I-A <sup>d</sup> )
Exp. 1							
LK	B hybridoma	None	I-A <sup>dk</sup> , I-E <sup>dk</sup>	19,300 ± 68	31,667 ± 401	35,957 ± 427	— <sup>‡</sup>
D3.4	L transfectant	A <sub>α</sub> <sup>k</sup> A <sub>β</sub> <sup>k</sup>	None	18,142 ± 125	2,768 ± 275	1,885 ± 125	—
T70.3.1	B transfectant	A <sub>β</sub> <sup>k</sup>	I-A <sup>d</sup> , I-E <sup>d</sup>	634 ± 76	716 ± 6	1,085 ± 255	—
Exp. 2							
LK	B hybridoma	None	I-A <sup>dk</sup> , I-E <sup>dk</sup>	—	—	—	88,394 ± 4,776
T43.7	L transfectant	A <sub>β</sub> <sup>d</sup>	None	—	—	—	2,557 ± 279
T78.11.1	L transfectant	A <sub>α</sub> <sup>d</sup> A <sub>β</sub> <sup>d</sup>	None	—	—	—	52,877 ± 4,194

\* 10<sup>5</sup> T hybridoma cells were cultured with 10<sup>5</sup> transfected L cells or 5 × 10<sup>4</sup> lymphoid APC and antigen for 24 h. Supernatants from these cultures were assayed at a 1:4 dilution on HT-2 cells. The data are given as total cpm ± SE for [<sup>3</sup>H]TdR incorporation for triplicate cultures. Antigen concentrations: KLH, 1 mg/ml; HEL, 200 μg/ml; and GAT, 500 μg/ml.

<sup>‡</sup> Not tested.

fluorescence profiles of both cell types are relatively homogeneous and indicate that they have 1/5 to 1/10 the total amount of surface I-A expressed by the B lymphoma transfectant T70.3.1.

*Antigen Presentation to T Cell Hybridomas by I-A-expressing L Cell Transfectants.* The transcription, translation, and surface expression of I-A by L cells containing transfected Ia genes permitted us to investigate whether such normally Ia<sup>-</sup> cells, without known accessory cell activity, could replace physiologic accessory cells in tests of T cell activation. I-A-expressing, transfected L cells were placed in culture with T hybridomas in the absence or presence of specific antigen and IL-2 release into the culture medium was measured 24 h later. The data presented in Table II show that both of the transfectants tested presented antigen to at least one T cell hybridoma. D3.4 cells (I-A<sup>k</sup> positive) stimulated SKK9.11, a KLH-reactive, I-A<sup>k</sup>-restricted T hybridoma (Exp. 1). A second KLH-specific hybridoma (SKK45.10) responded weakly, while an HEL-specific hybridoma assayed in this experiment did not respond to D3.4, but did respond to the control B cell hybrid LK. This differential ability of D3.4 to stimulate I-A<sup>k</sup>-restricted cells was seen in several similar experiments, and may reflect differences between the responding T cells in the quantitative level of Ia required for activation, variations in requirements for other elements lacking in the L cell transfectants, or differential requirements for antigen processing. These issues are currently under further investigation. SKK9.11, which responded to D3.4 cells expressing A<sub>α</sub><sup>k</sup>:A<sub>β</sub><sup>k</sup>, failed to be stimulated by the B lymphoma transfectant T70.3.1, which expresses A<sub>β</sub><sup>k</sup> without A<sub>α</sub><sup>k</sup>. This suggests that SKK9.11 requires the specific A<sub>α</sub><sup>k</sup> chain as part of its "restriction element", and that it may require the A<sub>β</sub><sup>k</sup>:A<sub>α</sub><sup>k</sup> dimer for this purpose. The I-A<sup>d</sup>-bearing T78.11.1 line could consistently stimulate the GAT-specific, I-A<sup>d</sup>-restricted hybridoma RF9.140, which was not triggered by the A<sub>β</sub><sup>d</sup>-only transfectant T43.7 (Table II, Exp. 2). The I-A<sup>k</sup>-bearing D3.4 cells did not stimulate RF9.140 in the presence of GAT, nor did the I-A<sup>d</sup>-bearing T78.11.1 cells activate the SKK9.11 cells in the presence of KLH (data not shown).

To substantiate further that the L cell transfectants were stimulating the T cell hybridomas by virtue of the Ia molecules expressed on their membranes,

monoclonal anti-Ia antibodies were used to inhibit the responses. Antigen presentation to these T hybridomas was specifically blocked by antibodies that bound to the surface-expressed products of the transfected Ia genes (Table III). Experiment 1 shows that antigen presentation to the GAT-specific hybridoma RF9.140 was inhibited by the anti-I-A<sup>d</sup> antibodies MKD6 and M5/114, whereas 10.2.16 (anti-I-A<sup>k</sup>), which does not bind to T78.11.1, or 11.4.1, which binds to the class I K<sup>k</sup> molecule, have no effect. On the other hand, accessory cell function of D3.4 cells was only blocked by 10.2.16. These data establish the specific involvement of I-A expressed by genes transfected into L cells in the antigen-presenting activity of these cells.

Since the L cell transfectants express 5–10-fold less I-A than antigen-presenting B cell tumors, it might be expected from previous work showing a relationship between level of Ia expression and T cell stimulation (44) that there would be quantitative differences in antigen-presentation by B lymphomas vs. transfected L cells. These expected differences were observed in both the cell and antigen dose response patterns of RF9.140 to B lymphomas vs. T78.11.1, as shown in Figs. 3 and 4. Fig. 3 illustrates the dose response to varying cell numbers in the presence of a fixed antigen concentration, and Fig. 4 shows the relationship between antigen concentration and response at a fixed cell number. In both titrations, the T78.11.1 cells were less efficient than B lymphoma cells in stimulating RF9.140, requiring more cells or antigen for comparable levels of IL-2 release.

*Antigen Presentation to T Cell Clones by I-A-expressing L Cell Transfectants.* The above results indicate that L cells bearing surface I-A molecules can effectively take up and present complex antigens to I-A-restricted T cell hybridomas. Experiments were next undertaken to explore whether these same transfectants could present antigen to, and activate, long-term T cell clones, which may have different physiologic requirements for stimulation compared with T hybridomas. The T cell clone 4R.6 is specific for pigeon cyto *c* fragment (1-65) in the context of I-A<sup>k</sup>, and it is maintained by repetitive stimulation and rest. As shown in Table IV, this T cell responds to D3.4 (I-A<sup>k</sup>) cells in the presence of antigen, but fails to proliferate in the presence of antigen together with either T78.11.1 (I-A<sup>d</sup>) or T70.3.1 (A<sub>β</sub><sup>k</sup>) transfectants. (The high background seen with L cell transfectants is due to the extreme radioresistance of these cells, which continue to incorporate [<sup>3</sup>H]TdR even after exposure to 18,000 rad.) The response to D3.4 could be specifically blocked with anti-I-A<sup>k</sup> monoclonal antibody. These data, together with the absence of a response by 4R.6 cells to L cells bearing the I-A of the wrong haplotype, even in the presence of antigen, and the failure of D3.4 cells to stimulate without antigen, indicate that the observed T cell responses required recognition of I-A on the L cells.

Tests of a second T cell clone gave different results. The clone 11.4 was maintained in the presence of Con A supernatant without any feeder cells. The effect of mixing this cloned T cell line with various transfectants and antigen is detailed in Table V, experiment 1. Although these T cells proliferated to antigen plus the A<sub>β</sub><sup>k</sup>-bearing B lymphoma transfectant T70.3.1, they failed to proliferate in response to the irradiated, I-A<sup>k</sup>-bearing D3.4 cells plus antigen. Rather, such experiments consistently showed a decrease in the background level

TABLE III  
Specific Inhibition of Antigen Presentation by Ia-bearing L Cell Transfectants Using Monoclonal Anti-Ia Antibody

APC	Ia		Assay culture with RF9.140 (GAT:I-A <sup>d</sup> ) hybridoma*					
	Transfected	Endogenous	No addition	GAT	GAT + 10.2.16 <sup>‡</sup>	GAT + M5/114 <sup>§</sup>	GAT + MKD6 <sup>¶</sup>	GAT + 11.4.1 <sup>¶</sup>
Exp. 1								
A20.2J	None	I-A <sup>d</sup> , I-E <sup>d</sup>	1,979 ± 313	63,283 ± 327	62,312 ± 5,713	1,373 ± 133	2,888 ± 402	57,567 ± 3,563
T78.11.1	A <sub>β</sub> <sup>d</sup> A <sub>α</sub> <sup>d</sup>	None	1,599 ± 140	21,091 ± 1,893	18,501 ± 224	2,063 ± 278	7,528 ± 758	19,055 ± 1,093
Exp. 2								
			Assay culture with SKK9.11 (KLH:I-A <sup>k</sup> ) hybridoma					
			No addition	KLH	KLH + 10.2.16	KLH + M5/114		
D3.4	A <sub>β</sub> <sup>k</sup> A <sub>α</sub> <sup>k</sup>	None	681 ± 169	13,899 ± 1,589	782 ± 128	25,331 ± 2,936		

\* Assay cultures were identical to those detailed in Table II except that monoclonal antibody supernatant was added at 25% final concentration to the hybridoma cultures. KLH was used at 1 mg/ml and GAT was used at 200 μg/ml.

‡ 10.2.16, anti-A<sub>β</sub><sup>k</sup>.

§ M5/114, anti-I-A<sup>d</sup>, I-E<sup>d</sup>.

¶ MKD6, anti-I-A<sup>d</sup>.

¶ 11.4.1, anti-K<sup>k</sup>.

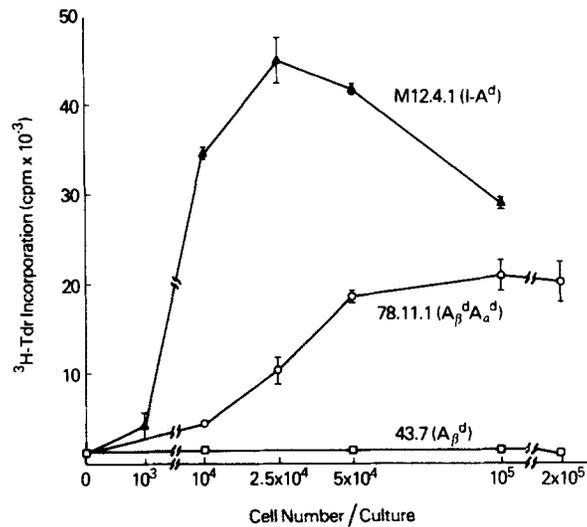


FIGURE 3. APC dose responses for the GAT-responsive T cell hybridoma, RF9.140.  $10^5$  RF9.140 were incubated with  $200 \mu\text{g/ml}$  of GAT and various numbers of the B lymphoma M12.4.1, the I-A<sup>d</sup> transfectant T78.11.1, or the A<sub>β</sub><sup>d</sup>-transfected L cell T43.7. Data are shown as [<sup>3</sup>H]TdR incorporation (cpm  $\pm$  SE) of CTL-L cells in response to a 1:4 dilution of culture supernatant from the stimulated T hybridoma.

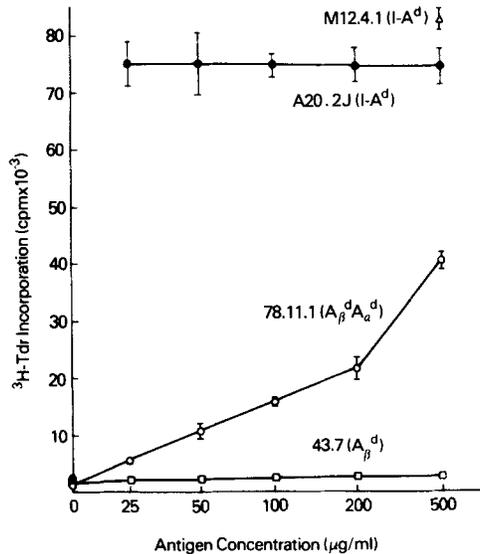


FIGURE 4. Antigen dose response of RF9.140 with various APC.  $10^5$  RF9.140 cells were cultured with either  $5 \times 10^4$  B lymphoma cells or  $10^5$  transfected L cells and various concentrations of GAT. Supernatants from 24-h cultures were assayed as in Fig. 3.

of [<sup>3</sup>H]TdR incorporated by the transfectants, which was most pronounced in the presence of antigen. This inhibitory effect on D3.4 was reversed by anti-I-A<sup>k</sup> but not anti-I-A<sup>d</sup> antibody. Experiment 2 was carried out using unirradiated transfected cells, and shows identical results on the I-A<sup>k</sup> L cells and a similar, though lesser, effect on the B lymphoma transfectant line.

TABLE IV  
Ia-transfected L Cells Present Antigen to Ia-restricted T Cell Clones

APC	Cell type	Ia				Culture additions*			
		Transfected	Endogenous	None	T cell	T + Ag†	T + Ag + M5/114‡	T + Ag + 10.2.16‡	T + Ag + M5/114‡
C3H spleen		None	I-A <sup>k</sup> , I-E <sup>k</sup>	712	5,231 ± 207	56,254 ± 3,536	685 ± 115	21,239 ± 1,117	
T70.3.1	B transfectant	A <sub>p</sub> <sup>k</sup>	I-A <sup>d</sup> , I-E <sup>d</sup>	1,422 ± 51	766 ± 141	806 ± 159	467 ± 66	493 ± 4	
T78.11.1	L transfectant	A <sub>p</sub> <sup>d</sup> A <sub>e</sub> <sup>d</sup>	None	12,037 ± 280	9,765 ± 368	8,363 ± 552	8,341 ± 18	6,581 ± 785	
D3.4	L transfectant	A <sub>p</sub> <sup>k</sup> A <sub>e</sub> <sup>k</sup>	None	8,282 ± 126	7,637 ± 424	40,228 ± 2,095	5,905 ± 336	37,618 ± 9,099	

\* 2 × 10<sup>4</sup> 4R.6, I-A<sup>k</sup>-restricted, cyto c (1-65)-specific T cells were cultured with either 5 × 10<sup>5</sup> irradiated C3H spleen cells, 5 × 10<sup>5</sup> B lymphoma cells, or 5 × 10<sup>5</sup> transfected L cells with and without 1 μM of pigeon cyto c (1-65). Data represent [<sup>3</sup>H]TdR incorporation (cpm × 10<sup>-3</sup> ± SE) over the last 20 h of a 96 h culture period.

† Ag, pigeon cyto c (1-65).

‡ Monoclonal antibody supernatants were added to 25% final concentration.

TABLE V  
Interaction of an IL-2-dependent I-A-restricted T Cell Clone With Ia-bearing L Cell Transfectants

APC	Ia		No addition	T cell	Culture additions			
	Transfected	Endogenous			T + GAT	T + GAT + 10.2.16	T + GAT + M5/114	T + GAT + 11.4.1
Exp. 1 Irradiated APC*								
DAP.3	None	None	1,386 ± 237	931 ± 405	1,105 ± 413	—	—	—
T70.3.1	A <sub>β</sub> <sup>k</sup>	I-A <sup>d</sup> , I-E <sup>d</sup>	1,319 ± 521	2,338 ± 380	39,228 ± 1,917	1,931 ± 403	20,413 ± 2,091	—
D3.4	A <sub>β</sub> A <sub>α</sub> <sup>k</sup>	None	15,193 ± 853	4,910 ± 622	1,208 ± 444	8,432 ± 1,185	1,479 ± 187	—
Exp. 2 Unirradiated APC†								
T70.3.1	A <sub>β</sub> <sup>k</sup>	I-A <sup>d</sup> , I-E <sup>d</sup>	25,593 ± 626	17,362 ± 1,808 (32)‡	8,734 ± 795 (65)	19,262 ± 2,391 (10)	4,863 ± 985 (68)	5,321 ± 1,048 (70)
T78.11.1	A <sub>β</sub> A <sub>α</sub> <sup>d</sup>	None	44,486 ± 3,640	36,030 ± 4,000 (19)	33,486 ± 2,156 (25)	41,219 ± 607 (30)	26,948 ± 3,755 (28)	43,470 ± 795 (11)
D3.4	A <sub>β</sub> A <sub>α</sub> <sup>k</sup>	None	28,712 ± 776	14,761 ± 437 (49)	1,324 ± 268 (95)	19,911 ± 4,017 (38)	999 ± 111 (96)	1,021 ± 197 (97)

\* 2 × 10<sup>4</sup> 11.4 cells were cultured with 10<sup>5</sup> irradiated T70.3.1 or 5 × 10<sup>5</sup> irradiated L cells with and without 100 μg/ml of GAT for 72 h. Monoclonal antibody was added in supernatant form to a final concentration of 25%. Data represent (total cpm ± SE) of incorporated [<sup>3</sup>H]TdR over the last 20 h of culture.

† APC added at 5 × 10<sup>5</sup> cells per well.

‡ Percent inhibition calculated as: [(cpm with no addition - cpm with addition)/(cpm with no addition)] × 100.

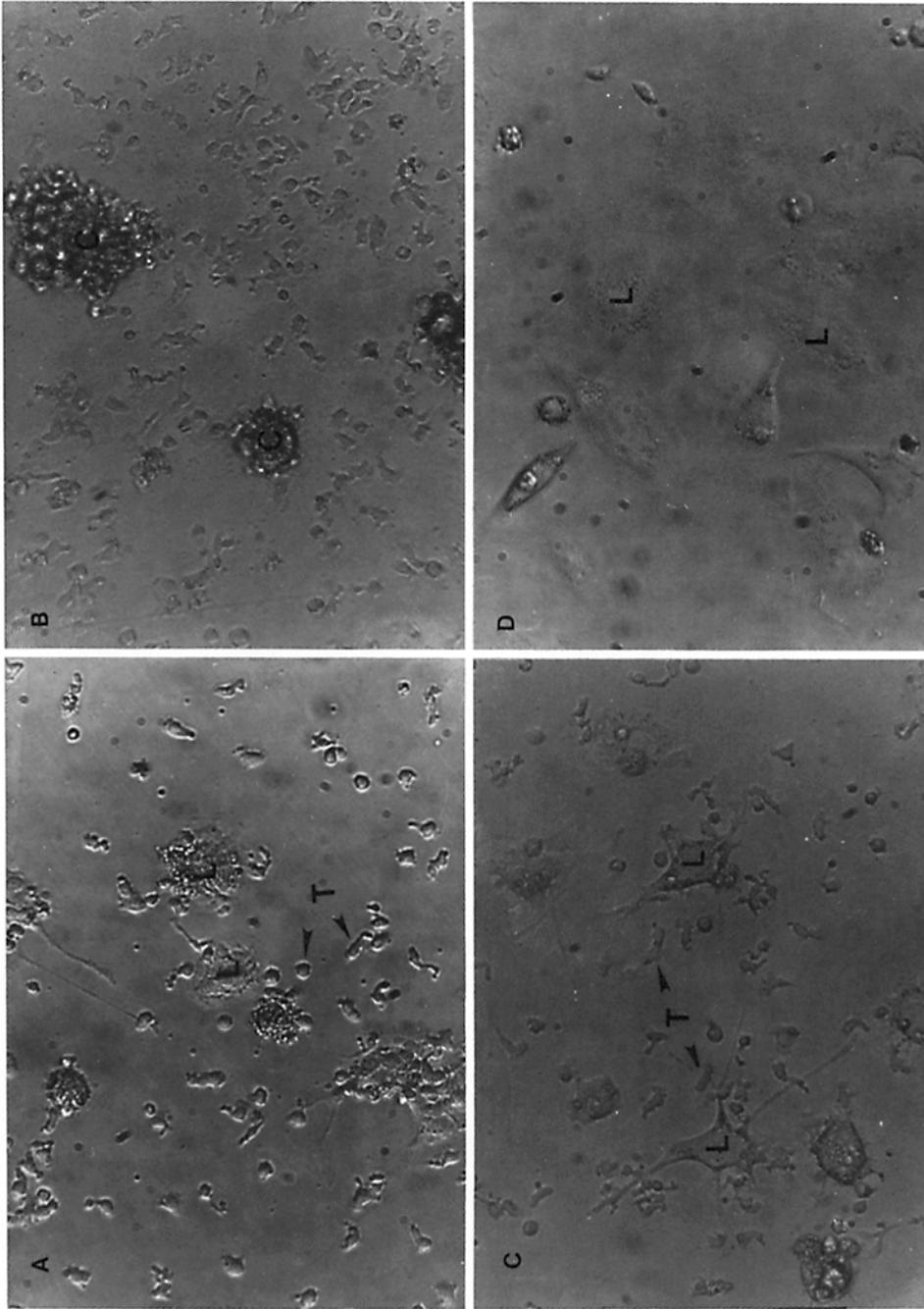


FIGURE 5. Photomicroscopy of I-A<sup>k</sup>-transfected D3.4 interacting with the T cell clone 11.4 T cells cultured with D3.4 without GAT (A), with GAT (B), and with GAT plus 10.2.16, anti-I-A<sup>k</sup> antibody (C). (D) D3.4 alone. Cells were photographed in microtiter plates with a Leitz Orthomat at 48 h of culture. T, T cell; L, transfactant; C, cluster.

TABLE VI  
*Accessory Cell Function of L Cells for T Cell Mitogen Responses*

Accessory cell*	Transfected Ia	No addition	T cell	Con A	Con A + 10.2.16	Con A + M5/114
None	—	ND <sup>‡</sup>	465 ± 27	1,596 ± 468	ND	ND
T43.6	A <sub>β</sub> <sup>k</sup>	28,613 ± 285	13,454 ± 1,403	188,511 ± 13,228	156,847 ± 3,477	178,924 ± 17,458
T78.11.1	A <sub>β</sub> <sup>d</sup> A <sub>α</sub> <sup>d</sup>	20,310 ± 605	13,644 ± 612	298,000 ± 8,014	161,994 ± 2,872	240,250 ± 13,294
D3.4	A <sub>β</sub> <sup>k</sup> A <sub>α</sub> <sup>k</sup>	15,422 ± 602	10,332 ± 74	200,111 ± 7,292	197,355 ± 19,270	179,155 ± 13,187
DAP.3	None		984 ± 92	176,754 ± 33,379		

\* Purified Ia-negative T cells ( $2.5 \times 10^5$ ) prepared as in Table V were cultured with  $10^4$  irradiated L cells with and without Con A (5  $\mu$ g/ml). Antibody supernatants were added to a final concentration of 25%. [<sup>3</sup>H]TdR incorporation was assayed over the last 20 h of a 96 h culture period. Data are presented as total cpm  $\pm$  SE.

<sup>‡</sup> Not done.

This impression of an antigen- and I-A-specific cytotoxic effect by 11.4 cells was confirmed by direct observation. As shown in Fig. 5, cultures containing L cell transfectants plus T cells, but without antigen, showed well-spread L cells interspersed randomly among T cells, a few of which are associated with the transfected cells (Fig. 5A). Upon the addition of antigen, large clusters of T cells formed on the I-A<sup>k</sup> (but not I-A<sup>d</sup>)-bearing cells (Fig. 5B). Transfected cells visible in such cultures had the appearance of damaged cells. Monoclonal antibody to I-A<sup>k</sup> reversed the clustering and the cytopathic effect (Fig. 5C). Such cytopathic changes were not seen in cultures of transfected cells and the 4R.6 clone. These experiments indicate that in the complete absence of feeder cell contamination, L cell transfectants bearing the appropriate I-A can, in the presence of antigen, cause specific changes in the activity of a cloned, nonmalignant T cell line. Taken together with the data on the 4R.6 clone, these results show that I-A-bearing L cells provide the accessory cell functions necessary for the restimulation of previously activated "memory" T cells.

*Effects of L Cells and L Cell Transfectants in Mitogen Responses.* One of the best characterized models of Ia-bearing accessory cell function is the mitogen response to lectins such as Con A. Experiments were therefore carried out to explore the ability of Ia-bearing L cell transfectants to replace normal Ia<sup>+</sup> accessory cells in this primary stimulation model. Lymph node cells from C3H mice were passed through nylon wool and treated with monoclonal anti-Ia plus complement to remove endogenous accessory cells. As shown in Table VI, such purified T cells failed to respond to Con A directly, as previously reported (45, 46). However, the addition of L cells, irrespective of their expression of Ia, equally reconstituted the response to this mitogen. These findings document the ability of L cells to function as accessory cells in a primary T cell response, but disagree with earlier results showing a requirement for Ia on accessory cells in this system. Ia per se is apparently not required for accessory cell activity in the Con A response.

## Discussion

The overall purpose of the experiments described above was to devise a model system capable of producing sets of cells, one member totally Ia negative, the others expressing only those Ia gene products experimentally introduced into the parent (Ia<sup>-</sup>) cell type. Such a system would permit examination of the regulation of Ia gene transcription and translation, as well as the assembly and

cell surface expression of these gene products. Cells expressing membrane Ia could be tested for their ability to stimulate various T lymphocytes, providing, via the use of in vitro generated Ia gene variants, a method for correlating Ia gene structure with function. Finally, it was possible that an uncoupling of T cell Ia recognition from subsequent activation steps might be observed, allowing a more precise analysis of the cofactors required in the postrecognition phase of T cell stimulation.

The results presented here demonstrate that most of these desired features can be achieved using DNA-mediated gene transfer to introduce full-length Ia genomic clones into mouse Ltk<sup>-</sup> cells. These cells are constitutively Ia<sup>-</sup> and do not express Ia after exposure to  $\gamma$ -interferon-containing culture supernatants (M. Norcross, unpublished observations). The introduction of A $\beta$ <sup>k</sup> or A $\beta$ <sup>d</sup> genes carried on  $\lambda$  phage vectors as a CaPO<sub>4</sub> coprecipitate with pBRTK DNA led to the production of cell lines that had stably incorporated these genes into chromosomal DNA. Further, such cells actively transcribed the transfected genes, producing mRNA of the same size as Ia-expressing B cell tumors. This transcription occurred even though in the case of A $\beta$ <sup>k</sup> the identical gene present in the Ltk<sup>-</sup> cells is inactive. These data indicate that the lack of A $\beta$  transcription in DAP.3 cells is not caused by a trans-acting repressor mechanism. Our findings are consistent with results recently published by Rabourdin-Combe and Mach (19), who demonstrated the transcription of genomic clones of human class II genes in mouse L cells after transfection. These observations differ from the results of Malissen et al. (20), who failed to see expression of A $\beta$ <sup>k</sup> and A $\alpha$ <sup>k</sup> after transfection, unless DNA containing strong viral enhancer/promoter elements was used for cotransformation. It is possible in these latter experiments that transcription did occur without the added enhancer elements, but generated very few clones with sufficient expression to register in their assay. Our own studies with L cells containing class II genes linked to strong viral promoter-enhancer elements are consistent with this interpretation, as we find 5–10-fold increases in Ia mRNA and protein levels in such cells (Germain, unpublished observations). Surface expression of Ia required the presence of both A $\beta$  and A $\alpha$  genes. Again, such results confirm both earlier studies on Ia expression by normal lymphoreticular cells (43), and recent gene transfection studies using human or murine genes (19, 20). The present studies do not address the role of invariant chain (47), which has been identified in L cells (48), in Ia membrane expression.

The Ia molecules expressed by the transfected L cells were recognized normally by the small panel of anti-Ia monoclonal antibodies used in this study. Additional antibodies will need to be tested to determine if all known serological specificities assigned to I-A<sup>k</sup> or I-A<sup>d</sup> are expressed by the transfectants. It is also important to determine if the heterogeneity of Ia molecules observed in immunoprecipitation experiments by some investigators using normal cells will be found also with these L cells (49).

The major focus of the present study was the functional activity of Ia-expressing L cell transfectants in T cell stimulation assays. Both I-A<sup>d</sup>-bearing and I-A<sup>k</sup>-bearing transfectants were able to cause IL-2 production by T cell hybridomas in an antigen-specific, MHC-restricted manner in experiments using both

polypeptide and complex protein antigens. These results confirm and extend the report of Malissen et al. (50) showing that an I-A<sup>k</sup>-bearing L cell could present the antigen KLH to several T hybridomas. The studies by Chestnut et al. (5), Shimonkevitz et al. (6), and Ziegler and Unanue (3) have demonstrated that antigen presentation of such molecules to T cells involves at least two distinct steps: (a) the processing of the native antigen, which is believed to involve partial lysosomal proteolysis, and (b) the display on the cell surface of processed antigen along with Ia. The present data suggest that L cells may be capable of processing antigen in a manner similar to normal accessory cells. However, direct testing of this presumption is needed to rule out the possibility that the L cells somehow circumvent the processing requirement, or that the particular hybridomas used respond to unprocessed antigen. Recent experiments indicate that I-A<sup>k</sup>-bearing L cells can present antigen to a hybridoma previously shown to require stimulation by processed antigen (R. Lechler and R. Germain, unpublished observation).

Additional tests of the accessory cell potential of the Ia transfectants involved nonmalignant T cells maintained in long-term cultures. These previously activated T cells also demonstrated antigen-dependent, I-A-restricted interactions with the transfected L cells. The 4R.6 cytochrome fragment (1-65)-specific clone proliferated in response to antigen plus the D3.4 (I-A<sup>k</sup>) cell line, but failed to respond to D3.4 alone, or to T78.11.1 (I-A<sup>d</sup>) with or without antigen. These findings essentially rule out the possibility that functional I-A<sup>k</sup>-bearing feeder cells were carried over into the assay culture, and, in the presence of nonspecific factors from the L cells, acted as APC. The findings do not eliminate the potential contribution of nonspecific factors by residual feeder cells that permit the L cells to fully trigger the T cell clone. Tests of the 11.4 clone, which is carried in IL-2-containing medium without feeder cells, revealed an antigen-specific, MHC-restricted cytotoxic effect. This activity establishes that such T cells can recognize antigen and Ia on the L cell surface. With respect to the observed cytotoxicity itself, several points can be made. First, L cells are the major cell type used over the years in assays of T cell-derived lymphotoxin (51). Although preliminary experiments have failed to demonstrate a soluble cytotoxin in culture supernatants of 11.4 cells stimulated with antigen and D3.4, this mechanism cannot be ruled out. Second, the activity does not appear to be due to 11.4 actually being an Ia-specific cytotoxic T lymphocyte, since, at moderate effector to target ratios, little chromium release is seen from labeled D3.4 cells after 18–24 h (M. Norcross, unpublished observations). Lastly, the finding that B cell tumors were also subject to a cytopathic effect under these conditions raises the possibility that we are observing a physiologic mechanism involved in homeostatic control of immunity. T cells may inactivate accessory cells and some B cells after interacting with them to reduce further immune stimulation.

There is an extensive literature on the cofactor requirements for full stimulation of T cell responses by accessory cells (reviewed in 52). In general, these studies have shown a requirement for IL-1 or other unspecified soluble mediators, in addition to the display of Ia. The ability of Ia-bearing L cells to serve as accessory cells in a variety of T cell responses needs to be assessed in this light. L cells have long been known to produce one or more growth factors, particularly colony-stimulating factor (CSF) (53), and L cell supernatants are a standard

source of conditioned medium for augmenting growth of other cell types. If the partially activated T cell lines require any cofactors, such mediators or adequate substitutes may be produced by the transfected L cells. The constitutive elaboration of such materials might also explain the ability of both Ia<sup>-</sup> and Ia<sup>+</sup> L cells to act as accessory cells in mitogen responses. The Ia<sup>+</sup> cell requirement for such function seen in earlier studies may relate to the possibility that only Ia<sup>+</sup> cells normally can produce the required cofactors (54), and that Ia recognition is not required by the T cell itself, if the requisite soluble mediators are present.

Taken together, these results indicate that transfection of Ia genes into L cells will be a powerful tool for dissection of the role of Ia in immunity. The experiments can control the precise mix of I-A or I-E subregion elements expressed by the cell, and introduce *in vitro* generated variant genes without needing to account for the effects of endogenous class II gene expression. The resultant cells function in a variety of T cell assays suitable for analysis of the regions of Ia that are recognized by the T cell receptor(s) and should provide a means for examining the relationship between immunogen and Ia structure underlying restricted antigen recognition.

### Summary

To study the relationship between the structure and function of Ia antigens, as well as the physiologic requirements for antigen presentation to major histocompatibility complex-restricted T cells, class II A<sub>α</sub> and A<sub>β</sub> genes from the k and d haplotypes were transfected into Ltk<sup>-</sup> fibroblasts using the calcium phosphate coprecipitation technique. Individually transfected genes were actively transcribed in the L cells without covalent linkage to, or cotransformation with, viral enhancer sequences. However, cell surface expression of detectable I-A required the presence of transfected A<sub>α</sub><sup>d</sup>A<sub>β</sub><sup>d</sup> or A<sub>α</sub><sup>k</sup>A<sub>β</sub><sup>k</sup> pairs in a single cell. The level of I-A expression under these conditions was 1/5–1/10 that of Ia<sup>+</sup> B lymphoma cells, or B lymphoma cells expressing transfected class II genes.

These I-A-expressing transfectants were tested for accessory cell function and shown to present polypeptide and complex protein antigens to T cell clones and hybridomas in the context of the transfected gene products. One T cell clone, restricted to I-A<sup>k</sup> plus GAT (L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup>), had a profound cytotoxic effect on I-A<sup>k</sup>- but not I-A<sup>d</sup>-expressing transfectants in the presence of specific antigen. Assays of unprimed T cells showed that both Ia<sup>+</sup> and Ia<sup>-</sup> L cells could serve as accessory cells for concanavalin A-induced proliferative responses. These data indicate that L cells can transcribe, translate, and express transfected class II genes and that such I-A-bearing L cells possess the necessary metabolic mechanisms for presenting these antigens to T lymphocytes in the context of their I-A molecules.

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