T CELL SURFACE I-J GLYCOPROTEIN

Concerted Action of Chromosome-4 and -17 Genes Forms an Epitope Dependent on α-D-Mannosyl Residues

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The murine major histocompatibility complex *I-J* subregion has recently been the subject of much controversy. The *Ia-4* locus within *I-J* encodes a suppressor T lymphocyte surface marker (1), as well as a suppressor T cell factor determinant (2). Using recombinant strain distribution patterns, independent investigators mapped *I-J* between *I-B* and *I-B* and *I-E*, in the chromosomal segment bounded by recombination points in strains B10.A(5R) and B10.S(9R) on the left and B10.A(3R) and B10.HTT on the right (1, 2). However, when Steinmetz and coworkers (3) mapped restriction site polymorphisms in *I* region DNA, B10.A(3R) and B10.A(5R) DNA were identical. The interval between *I-A* and *I-E* was confined to 3.4 kb, including at least 2 kb of the E_β gene (3, 4). Despite considerable evidence supporting placement of *I-J* within the *I* region (1, 2, 5, 6), these results suggested *I-J* had been incorrectly mapped.

We recently reconciled these conflicting results (7). I-J expression is controlled by two unlinked genes; one (*Jt*) maps to chromosome 4 near the *b* and *Fv-1* loci and a second is located within the I region, possibly *I-E*. Strains B10.A(3R) and B10.A(5R) each provide the requisite $H\text{-}2^k$ gene for I-J^k expression, but the former apparently lacks the appropriate I-J^k locus on chromosome 4 (reference 7; summarized in Table I).

This report describes experiments to probe the *Jt* contribution to the I-J^k molecule. Complementation tests suggest that strain B10.HTT, like B10.A(3R), is probably not congenic with C57BL/10 and differs from B10.S(9R) outside rather than inside *H-2* (the murine major histocompatibility complex)[1]. Binding of I-J^k-specific monoclonal antibodies to cell surface I-J^k determinants depends on α-D-mannosyl residues associated with protein. The *Jt* gene product may glycosylate an *I*-region gene product; alternatively the *Jt* gene product may itself be an I-J–bearing glycoprotein expressed only in $H\text{-}2^k$ strains.

Materials and Methods

Animals. B10.BR and A/J mice were produced in our colony from breeding pairs supplied by The Jackson Laboratory, Bar Harbor, ME. B10.A, A.TH, C57BL/10, B10.A(5R), B10.S(9R), and B10.A(4R) breeding pairs were generously provided by Dr.

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[1] *Abbreviations used in this paper:* C, guinea pig serum complement; FCS, fetal calf serum; *H-2*, murine major histocompatibility complex; MuLV, Murine Leukemia virus.

TABLE I

Concerted Action of Chromosome-4 and -17 genes Is Required for the Expression of I-J^k Determinants (7)

Genes		I-J^k expression	Strains
H-2	*Jt*		
k	+	+	B10.BR, B6-H-2^k, CBA/J, C3H/HeJ, C58/J, MA/MyJ, MRL/MpJ$^+$, RF/J, AKR-Fv-1^b
k	−	−	AKR/J, CE/J
b	+	−	C57L, C57BL/6, C57BL/10
b	−	−	AKR-H-2^b
b/k	+/−	+	(C57L × AKR)F$_1$, (C57BL/6 × AKR)F$_1$, (C57BL/10 × AKR)F$_1$
i3/b	−/+	+	(B10.A(3R) × C57BL/10)F$_1$
i3/k	−/−	−	(B10.A(3R) × AKR)F$_1$
k	+	+	AKXL strains 13, 14, 28, 38
k	−	−	AKXL strains 6, 8, 21

F. H. Bach (University of Minnesota, Minneapolis, MN). Dr. J. H. Stimpfling (McLaughlin Research Institute, Great Falls, MT) kindly donated B10.A(3R) breeding pairs. B6-H-2^k mice were the gift of Dr. E. A. Boyse (Memorial Sloan-Kettering Cancer Center, New York, NY). Guinea pigs were from Research Animals Resource Center, University of Wisconsin, Madison, WI.

Media. All media and supplements were purchased from Grand Island Biological Co., Grand Island, NY. Fetal calf serum (FCS) was from Flow Laboratories, Rockville, MD.

Antibodies. Anti–Thy-1.2 serum was made according to Reif and Allen (8). Goat anti-mouse immunoglobulin was produced in our laboratory (9). Culture supernatant containing anti–I-J^k monoclonal antibody WF8.C12.8 was kindly provided by Dr. C. Waltenbaugh (Northwestern University Medical School, Chicago, IL).

Hybridoma Production. B10.A(5R)-immune B10.A(3R) splenocytes were fused with P3NS1/1-Ag4-1 (10) or Sp2/0-Ag14 (11) exactly as described (12). Fused cells were dispensed into 10 96-well plates giving clonal growth in medium with hypoxanthine, aminopterin, and thymidine (12). An enzyme-linked immunosorbent assay (12) or a microcytotoxicity assay (13) identified clones secreting I-J^k–specific antibodies. Assays used B10.A(3R) and B10.A(5R) T cells. Clones were subcloned by limiting dilution (12). Ouchterlony gel diffusion on agar-coated microscope slides determined monoclonal antibody heavy and light chain isotype (14). Heavy and light chain-specific rabbit anti-mouse immunoglobulin reagents were from Litton Bionetics, Kensington, MD.

Microcytotoxicity Assay. A two-stage, dye-exclusion microcytotoxicity assay was performed as described (13). With WF8.C12.8 IgG$_1$ monoclonal antibodies, a 15-min incubation of cells with rabbit anti-mouse IgG$_1$ (0.5% in medium 199 with 5% FCS; Litton Bionetics, Kensington, MD) followed by a wash step–preceded guinea pig complement addition. Percentage-specific lysis was corrected for background complement lysis (15). Complement backgrounds were <15% on untreated T cells, and <30% on neuraminidase-treated T cells.

T Cell Separation. Lymph node cells devoid of plastic-adherent cells were passed through a goat anti-mouse immunoglobulin-Sepharose column as described (16). Effluent cells were usually 80–90% Thy-1 positive.

Enzymes. Alpha-mannosidase (from jack bean), α-galactosidase (from *Aspergillus niger*), β-*N*-acetylglucosaminidase (from bovine epididymus), and α-L-fucosidase (from bovine epididymus) were from Sigma Chemical Co., St. Louis, MO. *Vibrea cholerae* neuraminidase was from Calbiochem-Behring, La Jolla, CA; trypsin, chymotrypsin, and β-galactosidase (from *Escherichia coli*) were from Millipore Corp., Freehold, NJ. Glycosidases were purified by high pressure liquid chromatography on a Varian TSK G3000 SW column to obtain fractions with specific glycosidase activity but without contaminating glycosidase or pro-

tease activities. Glycosidase activity was measured by incubating 100 μl enzyme with 100 μl *p*-nitrophenylglycoside (Sigma), 1.0 mM in 0.1 M acetate buffer, pH 4.5, for 1 h, 37°C. 1 ml glycine buffer, 0.133 M, with 0.06 M NaCl and 0.083 M Na_2CO_3, pH 10.7, was added and absorbance at 400 nm measured. 1 U of glycosidase will hydrolyze 1 μmol *p*-nitrophenylglycoside per hour at 37°C, pH 4.5. Specific activities calculated for purified glycosidases were: α-mannosidase, 8.37 U/mg; α-galactosidase, 4.15 U/mg; β-*N*-acetylglucosaminidase, 4.76 U/mg; α-L-fucosidase, 7.22 U/mg; β-galactosidase, 11.67 U/mg. Protease activity was assayed using casein, 1% in 0.05 *M* Tris-phosphate buffer, as a substrate and trypsin and chymotrypsin as standards. Enzyme (0.02 ml) was incubated with 1.0 ml casein 10 min, 37°C. 1 ml 10% trichloroacetic acid was added, the mixture was filtered, and absorbance of the filtrate at 280 nm measured.

Enzyme Digestion of T Cells. Purified T cells (4×10^6/ml) were incubated in Hank's balanced salt solution with 0.1% sodium azide at pH 6.5 (neuraminidase; 0.02 IU/ml), 7.0 (other glycosidases), or 7.4 (trypsin) containing 0.005–0.1 IU glycosidase/ml or 0.1 mg trypsin/ml for 30 min, 37°C in 7.5% CO_2. Adding excess cold Hank's balanced salt solution with 10% FCS terminated the digestion. Hypotonic lysis removed nonviable cells. Cell recoveries ranged from 19–65%; cells were 76–96% viable by trypan blue exclusion.

Cell Culture. Culturing trypsin-treated T cells at 1×10^6/ml in Mishell-Dutton medium (17) supplemented with 1×10^{-5} M 2-mercaptoethanol and 5% FCS for 30–60 min, 37°C, 7.5% CO_2, removed sodium azide. Growth at 5×10^6/ml in the same medium for 18 h, 37°C, 7.5% CO_2 allowed protein resynthesis. To some cultures cycloheximide (15 μg/ml; Sigma Chemical Co., St. Louis, MO), tunicamycin (4 μg/ml; Sigma) or monensin (1.0 μM; Calbiochem-Behring) were added. After 18 h cells were harvested and nonviable cells removed by hypotonic lysis.

Results

Neuraminidase Digestion Alters T Cells I-J^k Expression. To detect T cell surface I-J^k determinants, I-J^k–specific monoclonal antibody WF8.C12.8 (5) and five others produced in our laboratory (Jk.4, Jk.5, Jk.11, Jk.14, and Jk.18) were tested on peripheral T cells from several recombinant inbred strains. The monoclonal antibodies lysed B10.A, B10.A(5R), and B10.S(9R), but not C57BL/10, B10.A(3R), B10.HTT, or A.TH T cells (Table II and reference 7), charac-

TABLE II

Neuraminidase Digestion Enhances T Cell I-J^k Determinant Expression without Altering the Strain Distribution Pattern

T cell donor strain	Thy-1.2 antibody		% Cells lysed:* WF8.C12.8		Jk.18	
	Untreated	NA‡	Untreated	NA	Untreated	NA
B6-H-2^k	87 ± 1	84 ± 6	13 ± 3	42 ± 6	12 ± 2	42 ± 1
C57Bl/10	84 ± 5	87 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0
B10.A(3R)	82 ± 3	88 ± 2	0 ± 1	1 ± 1	0 ± 0	0 ± 0
B10.A(4R)	82 ± 2	84 ± 0	0 ± 0	2 ± 2	1 ± 1	2 ± 2
B10.A(5R)	81 ± 1	87 ± 3	13 ± 2	49 ± 10	10 ± 1	46 ± 9
B10.HTT	80 ± 1	85 ± 5	0 ± 0	0 ± 0	0 ± 0	1 ± 0
B10.S(9R)	85 ± 4	98 ± 2	16 ± 2	51 ± 9	14 ± 2	51 ± 11
A.TH	83 ± 6	87 ± 4	1 ± 1	0 ± 0	1 ± 1	2 ± 1
A/J	84 ± 4	82 ± 1	13 ± 1	32 ± 1	14 ± 2	32 ± 3
AKR/J	ND§	ND	1 ± 1	1 ± 1	0 ± 0	1 ± 1

* Mean ± SEM; two to four experiments.

‡ Neuraminidase treated.

§ *ND*, not determined.

teristic of the I-J^k strain distribution (18). B10.A(5R)-immune B10.A(3R) splenocytes, fused with P3NSI/1-Ag4-1 myeloma cells, produced clones Jk.4, Jk.11, and Jk.18; an Sp2/0-Ag14 myeloma cell fusion yielded clones Jk.5 and Jk.14. Jk.11 and Jk.18 are IgM antibodies with kappa light chains. None of the I-J^k–specific antibodies lysed B cells (not shown).

One difficulty in detecting T cell surface I-J molecules by antibody-mediated cytotoxicity is the low I-J determinant frequency in normal T cell populations (18). Kanno et al. (6) and others (19) routinely treat cells with neuraminidase before I-J detection by cytotoxicity (6), fluorescence-activated cell sorter analysis (6, 19), and enzyme immuno-electron microscopy (19). We found that neuraminidase specifically increased T cell I-J^k expression without altering the normal I-J^k strain distribution pattern (Table II). WF8.C12.8 and Jk.18 lysis of B10.A(5R) and B10.S(9R) T cells increased three- to fourfold, while B10.A(3R) and B10.HTT T cells remained unreactive. Jk.4, Jk.5, Jk.11, and Jk.14 gave similar results (not shown). B10.A(3R) anti-B10.A(5R) I-J^k–specific serum lysed up to 90% of neuraminidase-treated B10.S(9R) peripheral T cells (not shown). AKR/J (Table II) and CE/J (not shown) T cells remained I-J^k negative (7) after neuraminidase treatment.

I-J^k Expression on F$_1$ Hybrid T Cells. We previously reported (7) that a nonexpressor *Jt*$^-$ allele possibly accounts for B10.A(3R)'s failure to express I-J^k structures. Like B10.A(3R), B10.HTT does not express I-J^k structures, although it has *k* haplotype genes in *I-E*. We made F$_1$ hybrids between strains B10.HTT and AKR or C57BL/10 (Table III). Without neuraminidase treatment, I-J^k expression by F$_1$ hybrid T cells was equivocal (not shown). After neuraminidase digestion, antibodies to I-J^k lysed (C57BL/10 × AKR/J)F$_1$ and (B10.HTT × C57BL/10)F$_1$ T cells (Table III). Without prior neuraminidase digestion, I-J^k molecules on (3R × C57BL/10)F$_1$, (C57BL/10 × AKR/J), and (B10.HTT × C57BL/10)F$_1$ T cells are probably too sparse to detect in our cytotoxic assay system. That (C57BL/10 × AKR)F$_1$ hybrid T cells express I-J^k determinants indicates successful gene complementation between two heterozygous loci. The somewhat surprising result that B10.HTT complements with C57BL/10 but not

TABLE III

Two Complementing Genes Control I-J^k Expression in F$_1$ Hybrid T Cells

T cells‡ donor strain	Genes		% Cells lysed*		
	I-E	*Jt*	Anti-Thy-1.2	WF8.C12.8	Jk.18
(C57BL/10 × AKR)F$_1$	b/k	+/−	88 ± 2	17 ± 3	25 ± 4
(B10.HTT × AKR)F$_1$	k/k	−/−	91 ± 1	5 ± 2	2 ± 6
(B10.HTT × C57BL/10)F$_1$	k/b	−/+	92 ± 3	17 ± 6	19 ± 6
(5R × AKR-H-2^b)F$_1$	k/b	+/−	90 ± 2	27 ± 2	31 ± 7
(AKR × B6-H-2^k)F$_1$	k/k	−/+	91 ± 1	21 ± 6	22 ± 0
(B6 × B6-H-2^k)F$_1$	b/k	+/+	85 ± 2	48 ± 6	50 ± 2
(B10.A(4R) × AKR)F$_1$	b/k	+/−	84 ± 3	18 ± 2	19 ± 5
(B10.A(4R) × B10.A(3R))F$_1$	b/k	+/−	87 ± 4	20 ± 11	22 ± 6

* Mean ± SEM; two to five experiments.

‡ Neuraminidase treated as in Materials and Methods.

with AKR/J to yield I-J^k expression suggests that B10.HTT provides the requisite *H-2*^{k} gene, but may lack the *Jt*$^+$ allele. B10.HTT must be equivalent to B10.S(9R) in *H-2*, but dissimilar elsewhere.

F_1 T cell I-J^k expression is lower than I-J^k expression by B10.A(5R) or C57BL/6-H-2^k (Table II). We tested possible explanations for this observation (Table III). The hybrid (5R × AKR-H-2^b)F_1 is heterozygous at both genes, but a single parent (B10.A(5R)) donated both positive alleles; I-J^k expression on (5R × AKR-H-2^b)F_1 T cells was not significantly greater than on (C57BL/10 × AKR)F_1 T cells, where each of the two positive alleles came from a different parent. The hybrid (AKR × B6-H-2^k)F_1 is homozygous *H-2*^{k} but heterozygous *Jt*$^{+/-}$. I-J^k expression on these T cells was also diminished, compared with B10.A(5R) or C57BL/6-H-2^k. In contrast, (B6 × B6-H-2^k)F_1 mice, which are homozygous *Jt*$^+$ but heterozygous *H-2*$^{b/k}$, exhibited T cell I-J^k expression comparable to B10.A(5R) and B6-H-2^k. Thus, heterozygosity at *Jt* but not *H-2* appeared to decrease I-J^k expression.

Mapping the H-2^k I-J–Controlling Gene. We investigated the *H-2*^{k} gene contributing to I-J^k expression using recombinant strain B10.A(4R). B10.A(4R) T cells were I-J^k negative even after neuraminidase treatment (Table II). Crossing B10.A(4R) with B10.A(3R) and AKR (7) yielded I-J^k positive F_1 T cells (Table III); B10.A(4R) thus has a functional *Jt*$^+$ gene but lacks the *H-2*^{k} gene required for I-J^k expression. B10.A(4R) is A_β^k, A_α^k, E_β^k, E_α^b; the $A_\beta A_\alpha$ polypeptide complex is expressed on the cell surface in B10.A(4R) mice, whereas the $E_\beta E_\alpha$ complex is not. These results rule out the possibility that A_α and A_β genes contribute to the I-J^k determinant.

Inhibiting Protein Synthesis or Glycosylation Affects T Cell I-J^k Reexpression. Enhanced I-J^k expression after neuraminidase treatment (Table II) suggested that carbohydrate structures might be involved in the I-J^k molecule. Previous studies (16) demonstrated that the I-J^k determinant was associated with protein. We therefore attempted to block I-J^k reexpression by preventing protein synthesis or glycosylation. Trypsin treatment removed T cell surface I-J^k determinants (16). Digested cells were cultured 18 h to allow cell protein resynthesis. Trypsin-treated T cells reexpressed I-J^k determinants after 18 h in culture (Fig. 1). Either inhibiting protein synthesis with cycloheximide (20), or blocking protein glycosylation with tunicamycin (21) or monensin (22) prevented I-J^k reexpression (Fig. 1). Four other I-J^k–specific monoclonal antibodies with different clonal origins, Jk.4, Jk.5, Jk.11, and Jk.14, yielded similar results (not shown). Tunicamycin allowed cell surface expression of the unglycosylated protein moiety of HLA (23) and Ia (24) proteins. Similarly, unglycosylated membrane-bound IgM was detected in the presence of monensin (22). However, blocking glycosylation prevents cell surface expression of other membrane glycoproteins such as Thy-1 antigen (25). Apparently, a glycoprotein carried the I-J^k determinant. Either the I-J^k epitope involves carbohydrate chains or unglycosylated I-J protein does not reach the cell surface.

Glycosidase Treatment Affects I-J^k Expression. To determine whether the I-J^k epitope involves carbohydrate residues, we measured binding of I-J^k antibodies to T cells before and after glycosidase digestion. T cells were treated with neuraminidase and then with varying concentrations of different purified gly-

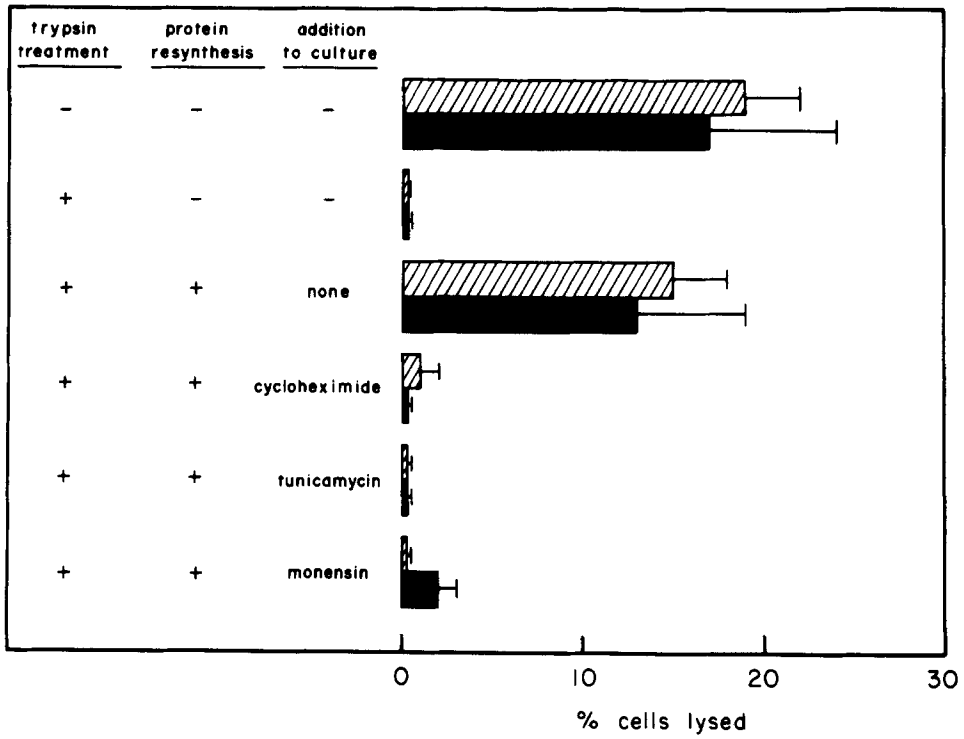

FIGURE 1. Blocking protein synthesis or glycoslyation prevents T cell I-J^k reexpression after its proteolytic removal. B10.A T cells were treated twice with 0.1 mg trypsin/ml for 30 min, 37°C. Trypsin-treated T cells were cultured for 18 h with or without cycloheximide (15 μg/ml), tunicamycin (4 μg/ml), or monensin (1.0 μM), I-J^k expression was measured by a microcytotoxicity assay with Jk.18 (■) and WF8.C12.8 (▨). Mean ± SEM; two to three experiments.

cosidases. Alpha-mannosidase completely abrogated I-J^k antibody-dependent lysis of neuraminidase-treated T cells in a concentration-dependent manner; heat-inactivated α-mannosidase had no effect (Fig. 2). Alpha-galactosidase, β-galactosidase, α-L-fucosidase, and β-*N*-acetylglucosaminidase also had no effect, either at concentrations comparable to effective α-mannosidase concentrations (Table IV), or at 2–20-fold higher concentrations (not shown). Similar results were obtained with Jk.4, Jk.5, Jk.11, and Jk.14 antibodies (not shown). Adding the competitive α-mannosidase inhibitor *p*-nitrophenyl-α-D-mannoside blocked I-J^k determinant removal from neuraminidase-treated T cells (Fig. 3). Thus decreased I-J^k is due to α-mannosidase activity and not to a contaminating enzyme activity. Alpha-mannosidase treatment also destroyed the I-J^k epitope on T cells without prior neuraminidase treatment (not shown). Thus α-D-mannosyl residues appear to influence the I-J^k epitope recognized by the monoclonal antibodies; if other sugar residues are involved, they are not in terminal positions where they would be removed by a single glycosidase (26).

Discussion

Considerable debate has recently focused on the placement of the enigmatic I-J suppressor determinant controlling gene (4, 27, 28). Previous experiments

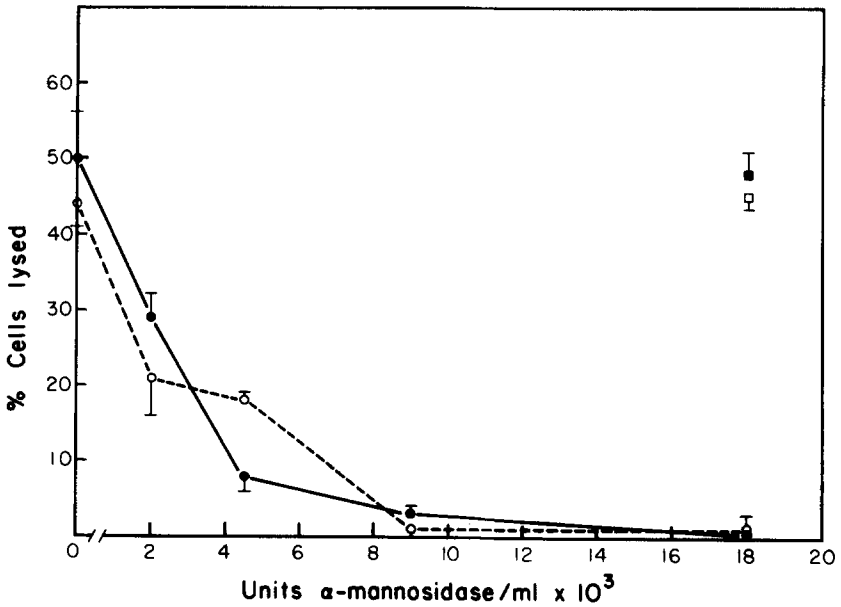

FIGURE 2. Alpha-mannosidase removes I-J^k from the T cell surface. B10.BR T cells were treated with 0.02 IU neuraminidase/ml for 30 min, 37°C as in Materials and Methods. Neuraminidase-digested T cells were then treated with increasing concentrations of α-mannosidase for 30 min. I-J^k was measured in a microcytotoxicity assay with Jk.18 (●) and WF8.C12.8 (○). Mean ± SD; one representative experiment of 3. Squares indicate T cells treated with 0.018 IU heat-inactivated α-mannosidase/ml and tested with Jk.18 (■) or WF8.C12.8 (□).

TABLE IV

*Alpha-Mannosidase Removes I-J^k Determinants from Neuraminidase-treated T Cells**

Enzyme treatment§	% Cells lysed‡	
	WF8.C12.8	Jk.18
None	13 ± 3	13 ± 6
NA¶	44 ± 9	35 ± 9
NA + α-galactosidase	47 ± 2	46 ± 6
NA + β-galactosidase	51 ± 5	37 ± 10
NA + α-L-fucosidase	47 ± 11	37 ± 16
NA + α-mannosidase	2 ± 3	0 ± 0
NA + β-*N*-acetylglucosaminidase	44 ± 4	31 ± 3

* B10.BR T cells, neuraminidase treated as in Materials and Methods.
‡ Mean ± SEM; two to six experiments.
§ Enzyme digestion conditions: 0.035 IU α-galactosidase/ml, 0.035 IU β-galactosidase/ml, 0.035 IU α-L-fucosidase/ml, 0.018 IU α-mannosidase/ml, 0.035 IU β-*N*-acetylglucosaminidase/ml.
¶ Neuraminidase.

provided evidence that the action of an *H-2*^{k} gene together with a non–*H-2* gene is required for I-J^k expression (7). A non–*H-2* autosomal dominant locus, termed *Jt* and linked to the *b* coat color gene and *Fv-1* on chromosome 4, controls I-J^k expression. The failure of strain B10.HTT, like B10.A(3R) (7), to express I-J^k specificities can be attributed to its background genes, rather than to an inappro-

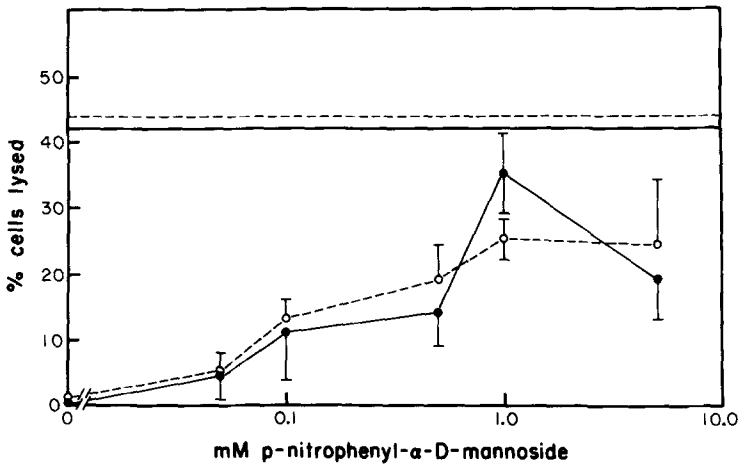

FIGURE 3. Addition of *p*-nitrophenyl-α-D-mannose blocks I-J^k removal by α-mannosidase. Neuraminidase-treated B10.BR T cells were incubated with 0.018 IU α-mannosidase/ml and increasing concentrations of *p*-nitrophenyl-α-D-mannoside for 30 min, 37°C. I-J^k was measured by cytotoxicity with Jk.18 (●) or WF8.C12.8 (○). Horizontal lines indicate anti-I-J^k lysis after neuraminidase treatment alone with Jk.18 (——) or WF8.C12.8 (- - -). Mean ± SEM; two experiments.

priate *H-2*^{k} gene. Hybrid (B10.HTT × C57BL/10)F$_1$ expressed I-J^k determinants, whereas (B10.HTT × AKR)F$_1$ did not. In the I-J^k–expressing hybrids, B10.HTT contributed the needed *H-2*^{k} gene (lacking in C57BL/10), while C57BL/10 provided the necessary non–*H-2* gene. In the non–I-J^k-expressing hybrid, neither B10.HTT nor AKR could contribute the requisite non–*H-2* gene. We therefore suggest that B10.HTT is not fully *H-2*–congenic with C57BL/10; it lacks a locus or loci (present in C57BL/10) which permits I-J^k expression by B10.A(5R) and B10.S(9R). Moreover, the B10.A(3R) – B10.A(5R) (7) and B10.HTT – B10.S(9R) pairs are apparently identical at the *H-2*^{k} locus required for I-J^k expression.

The original recombinant (*H-2*^{a1}) in B10.HTT's ancestry occurred among progeny of a C3H × DBA-T cross (29). This animal, crossed with A.SW, produced a second recombinant (*H-2*^{t1}) (30). The second recombinant, crossed with B10.S, produced a third recombinant, HTT (30). HTT was backcrossed an unknown number of times to C57BL/10 producing the B10.HTT line (29). One of these parent strains (most likely A.SW) evidently lacks the *Jt*$^+$ gene required for I-J^k expression, and donated its *Jt*$^-$ genotype to B10.HTT.

Genes in the I-E subregion probably contribute to the I-J^k molecule. Hood et al. (4) located only four structural genes, A_β, A_α, E_β, E_α, and one pseudogene, $E_{\beta 2}$, in the *I* region. The I-J^k positive strains B10.A(5R) and B10.S(9R) are *k* at E_α, but *b* and s, respectively, at the other three loci. B10.A(3R) and B10.HTT, which have the appropriate *H-2*^{k} gene, are both *k* at E_α but not at the other loci. B10.A(4R) is A_β^k, A_α^k, E_β^k but E_α^b and is I-J^k negative although it has a functional *Jt*$^+$ gene (Table III). The B10.A(4R) results alone do not rule out E_β^k as the I-J^k–associated *H-2*^{k} gene, since the E_β chain is not expressed on the cell surface

without intact E_α chain (lacking in B10.A(4R); references 31, 32). Taken together, however, these results imply that the $E_\alpha{}^k$ gene is required for I-J^k expression. Alternatively, in differentiated T lymphocytes the $E_{\beta 2}$ pseudogene product may contribute to I-J^k molecules.

Our data suggest that the integrity of the I-J^k epitope recognized by monoclonal antibodies depends on terminal α-D-mannosyl residues associated with protein. T cells do not reexpress I-J^k structures when protein synthesis or glycosylation is blocked. Alpha-mannosidase cleaves terminal mannosyl residues (26); this enzyme destroyed I-J^k epitopes. Wieder et al. (33) drew a different conclusion; they found I-J^k determinants on in vitro translated proteins presumably devoid of carbohydrate. Terminal mannosyl residues occur on simple, or high-mannose types of oligosaccharides (34). Neuraminidase treatment increased T cell I-J^k expression; this probably does not imply that sialic acid residues are attached to the nonreducing ends of some high-mannose, I-J^k–bearing carbohydrate chains. Only complex type carbohydrate chains have to date borne terminal sialic acid residues (34). Furthermore, it seems unlikely that mannosyl groups themselves form the I-J^k epitope, since these residues are so prevalent on mammalian cells. Mannosyl residues might instead be important in maintaining the structure of the I-J^k protein or carbohydrate chains.

Mannosyl residues may be implicit in functional suppressor T cell interactions. Interestingly, the T cell mitogenic lectin concanavalin A, whose ligand is α-D-mannose, bound specifically to I-J positive T cells initiating T cell proliferation (35) and increased nonspecific suppressor cell activity (36). Moreover, methyl-α-D-mannoside inhibited suppressor T cell induction in an allogeneic response (37). Finally, α-D-mannose reversed infectious mononucleosis-associated suppressor T cell activity in vitro (38).

A number of models may account for expression of the I-J^k molecule. It is unlikely that the I-J^k epitope is formed by association of two polypeptide chains, since I-J specificities occur on single polypeptide chains (39, 40). Likewise, DNA rearrangements occurring in T lymphocytes to juxtapose the *H-2* and *Jt* genes such that a single transcript might encode a polypeptide with two domains are unlikely because the two genes are unlinked. Finally, intra-*I*-region rearrangements have not been found in T cells (27).

We consider two models most likely. First, one gene product might modify (cleave, glycosylate, phosphorylate, or acylate) the other structural gene product (4). Since B cells synthesize I-region polypeptides but do not express I-J determinants (4, 15, 17, 18), the *Jt* gene product might be enzymatically active only in differentiated T lymphocytes. I-J^k molecules might be formed from E_α or E_β polypeptides processed differently in *Jt*-expressing T cells than in B cells or macrophages. Ikezawa et al. (41) concluded that I-J^k determinants resulted from $E_\beta{}^k$ chain modification. However, the Y17 monoclonal antibody used in experiments from which these conclusions were drawn does not bind E_α or E_β chains alone (42), but recognizes a combinatorial $E_\alpha E_\beta$ determinant (42). Furthermore, if the I-J^k–bearing structure were a receptor binding to E_α or E_β chains (see below) both I-E and I-J determinants could be closely associated, as these studies indicate, but not part of the same polypeptide; conditions that would dissociate noncovalently bound polypeptides were not used. T suppressor cell lines did not

yield *I*-region hybridizing mRNA (28). However, I-J^k expression is cell cycle dependent (19); nonsynchronous cells were used and I-J^k molecule synthesis was not demonstrated at the time of mRNA extraction (28). Finally, E_α or E_β polypeptide molecular weights do not match reported I-J^k polypeptide weights (40, 43–46).

A second hypothesis postulates that I-J synthesis is regulated by a second gene. Thus, *Jt* may be a structural gene, its transcription and/or translation controlled by E_α or E_β gene expression on other cells. For example, the T cell receptor molecule specific for self $E_\alpha E_\beta$ complexes on antigen-presenting cells or B cells might bear I-J^k epitopes. The receptor may be induced only when appropriate H-2 molecule(s) (e.g. I-E) are expressed during maturation. Genetic control of the receptor, and therefore of I-J^k specificities, would then apparently map to the *H-2* gene. Some evidence supports this model. I-J–bearing, antigen-specific suppressor T cells are restricted by, and probably bind, *I-E*; Baxevanis and coworkers (47–49) demonstrated that monoclonal anti–I-E antibodies blocked antigen-specific suppressor T cell induction. Furthermore, antibodies to I-J^k blocked binding of primed T cells to antigen-pulsed macrophages (K. Klyczek, preliminary results). I-J alleles might represent different receptor molecules coselected with the I-E gene product or selected by adaptive modification of the *Jt* gene product.

Genetic control of the Murine Leukemia virus (MuLV) antigen G_{IX} shows striking similarities to that of I-J^k. G_{IX} and I-J^k are not expressed in all mouse strains or tissues. Both show hemizygous expression in heterozygotes and Mendelian segregation of loci (50). G_{IX} is expressed as a thymocyte carbohydrate differentiation antigen carried on the viral glycoprotein gp70 (51, 52). The same genes that govern G_{IX} viral antigen expression could potentially also affect I-J expression. *Gv-1* governs G_{IX} cell surface density (53). The exact location of *Gv-1* is unclear. Segregation data suggested genetic linkage of *Gv-1* to both *Gpd-1* (chromosome 4) and *H-2* (chromosome 17) (54). Stockert et al. (54) attributed this "quasi-linkage" to the fact that both *Fv-1* (very near *Gpd-1*) (55) and an *H-2D*–linked gene (56) appear to influence MuLV replication, and thus simulate genetic linkage. The apparent chromosomal location of the I-J–controlling genes on chromosomes 4 and 17 might reflect a similar mechanism. The possibility that *I-J* genes may be derived from or associated with genes influencing virus expression is intriguing. Others have speculated that integrated viral genomes are not simply intruders, but are themselves elements of the cellular genome with vital physiological functions (57).

Summary

Two genes acting in concert control murine T cell I-J^k expession. We determined I-J^k expression with I-J^k–specific monoclonal antibodies WF8.C12.8 and five others produced in our laboratory in a cytotoxicity assay. Previous experiments established that an *H-2*^{k} gene and a chromosome 4 gene, *Jt*, regulate I-J^k expression (7). We show here that B10.HTT and B10.S(9R) do not differ at the *H-2*^{k} locus required for I-J^k expression. Rather B10.HTT, like B10.A(3R), lacks some important non–*H-2* gene (possibly *Jt*). The intra–*H-2*^{k} I-J–controlling locus maps to the right of the I-A subregion. The I-J^k determinant involves a carbo-

hydrate structure associated with protein; inhibiting either protein synthesis or glycosylation prevents T cell I-J^k reexpression after proteolytic removal. Treatment with α-mannosidase destroys I-J^k determinants, implicating terminal α-D-mannosyl residues in the I-J^k epitope. Models for *H-2* and *Jt* control of I-J expression are discussed.

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