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Altered I-J phenotype in E_α transgenic mice

(*H-2* locus/major histocompatibility complex/class II gene/Ia molecules/suppressor T cells)

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ABSTRACT **One of the more intriguing puzzles in immunology is the genetic basis for control of murine T-cell I-J determinants. Molecules bearing I-J determinants (I-J molecules) play a role in information trafficking among immunocompetent cells, probably serving as self-recognition molecules that channel regulatory factors to their appropriate target cells. Although it is clear that I-J polymorphism is influenced by the major histocompatibility complex (MHC), molecular genetic studies provide evidence that an MHC gene does not encode I-J molecules. A possible explanation for this paradox is that I-J molecules are a set of non-MHC-encoded T cell receptors that are directly or indirectly selected for by self-MHC products. One key to resolving the genetic and molecular basis for control of I-J determinants is the identification of the MHC gene(s) involved. Herein, data are presented which show that E_α transgenic mice express an altered I-J phenotype, providing clear evidence that I region class II genes influence I-J polymorphism. Although further study is required to resolve how class II genes mediate this effect, this is a major piece to the I-J puzzle.**

The I region of the murine major histocompatibility complex (MHC) or *H-2* gene complex on chromosome 17 contains polymorphic class II genes that encode glycoprotein molecules found on B cells and macrophages (1, 2). These molecules, M_r 28,000–35,000, are also referred to as I-region-associated or Ia molecules. At present, four class II genes have been well characterized: A_α and A_β mapping in the I-A subregion, E_α mapping in the I-E subregion, and E_β spanning both subregions (linear order A_β, A_α, E_β, E_α). Products of these genes are expressed as noncovalently associated cell surface dimers, designated $A_\alpha A_\beta$ and $E_\alpha E_\beta$ complexes, which are readily detected by antibodies produced in I-region-incompatible strains. Class II molecules are intimately involved in the presentation of foreign antigen to the immune system—i.e., helper T-cell activation and interaction is dependent on recognition of foreign antigen plus self class II molecules on macrophage and B-cell surfaces. Class II molecules are thus important self-recognition elements for helper T-cell function (3). Recent studies with transgenic mice have formally proven that class II molecules are the products of immune response or *Ir* genes, which determine high or low responsiveness to numerous foreign protein antigens (4–6).

Evidence for additional genes mapping in the I region came from studies which showed that some antibodies produced in I-region-incompatible strains reacted with suppressor T cells and T-cell-derived suppressor factors but *not* with B cells or helper T cells (refs. 7–9, reviewed in refs. 10–12). These antibodies clearly recognized a determinant controlled by the MHC or by a closely linked gene on chromosome 17, as judged by analysis of numerous MHC congenic and recombinant mouse strains. On the basis of differential reactivity of these antibodies with intra-I region recombinant strains, we postulated that a distinct I region gene, *Ia-4*, mapping between the I-A and I-E subregions, controlled determinants expressed on suppressor T cells, and that crossovers in the recombinant strains defined a previously unknown subregion, designated I-J (7, 9, 10).[¶] We now refer to these determinants collectively as "I-J determinants," and we refer to the molecules that bear these determinants as "I-J molecules."

Subsequent studies have shown that I-J determinants are expressed on several T-cell subsets and factors involved in the generation of suppressor activity (11–15), a T-cell subset that augments helper T-cell activity (12), T-cell subsets and factors involved in the generation of contrasuppressor activity (11), and macrophages involved in the generation of helper and suppressor activity (11, 13, 16). Different I-J determinants are expressed on several of these cell types and factors (11, 12, 17), raising the possibility that distinct loci encode the determinants. I-J molecules in several systems determine the self *Igh-V*-region-restricted and/or MHC-restricted activity of suppressor factors (*Igh-V*, variable region of immunoglobulin heavy chain gene complex), providing evidence that these molecules play a role in information trafficking among immunoregulatory cells (11, 13, 15, 18). Studies with chimeras, showing that the self-restricted activity of suppressor factors or I-J molecules is determined by the environment in which stem cells mature, are compatible with the concept that I-J molecules are a set of T-cell receptors that channel regulatory signals to their appropriate target (11, 12, 15). Under this view, I-J molecules on macrophages are probably passively acquired. Several studies suggest that I-J molecules are polypeptides, M_r 25,000 (12, 15, 19).

Problems with the original interpretation of the genetic basis for control of I-J determinants arose during molecular genetic analysis of the I region (reviewed in ref. 10). Crossovers in the strains utilized to map the *Ia-4* gene between the I-A and I-E subregions and define the I-J subregion were localized to a recombination hot spot within the E_β gene (20,

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Abbreviations: MHC, major histocompatibility complex; Ia, I-region-associated; *Igh-V*, variable region of immunoglobulin heavy chain gene complex; kb, kilobase(s); PFC, plaque-forming cells; SRBC, sheep erythrocytes; TsiF, T suppressor-inducer factor; ABM_{si}, antigen-binding molecule from TsiF; I-J_{si}, I-J molecule from TsiF.

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[¶]Key recombinant strains in the mapping of the *Ia-4* locus and the definition of the I-J subregion include B10.A(3R), which types $Ia\text{-}4^b$ and was judged to be I-A^b I-J^b/I-E^k, and B10.A(5R), which types $Ia\text{-}4^k$, and was judged to be I-A^b/I-J^k I-E^k. Both strains appear to carry the same alleles at class II loci, and both express $A^b_\alpha A^b_\beta$ and $E^k_\alpha E^b_\beta$ complexes. Similar results were obtained with two additional intra-I region recombinant strains, B10.HTT ($Ia\text{-}4^s$ and I-A^s I-J^s/I-E^k) and B10.S(9R) ($Ia\text{-}4^k$ and I-A^s/I-J^k I-E^k). See refs. 7, 9, and 10 for assumptions and detail.

21). In addition, no mRNA was detected in I-J$^+$ T-cell hybridomas with DNA probes that overlap the E_β gene and span most of the I region (22). Thus, although it is still clear that an I-region gene influences polymorphism in I-J molecules, it would appear that a non-I-region gene encodes I-J molecules. For example, I-J molecules may be non-MHC-encoded T-cell receptors that are directly or indirectly selected for by I-region products during ontogeny (10–13, 15, 20, 23, 24). Tentative support for this receptor–ligand model comes from chimeras which show that I-J phenotype is influenced by the environment in which T cells mature (25, 26). Further complicating the I-J story is the observation that a gene (*Jt*) mapping on chromosome 4 influences either cell surface expression of I-J molecules or the relative number of I-J$^+$ T cells in the periphery (24, 27). There is no evidence that the *Jt* gene influences the qualitative nature of or encodes I-J molecules (28, 29).

One key to understanding the genetic basis for control of I-J determinants is identification of the I-region gene(s) involved. We (10, 11) and others (12–15, 20, 23, 24) have speculated that class II genes influence I-J polymorphism. A direct test of this hypothesis was achieved by analyzing E_α transgenic mice for their I-J phenotype. As the data will show, these mice display an altered I-J phenotype, providing clear evidence that class II genes play a role in determining I-J polymorphism. This is an important advance in our understanding of the genetics and biology of I-J molecules, and it sets the stage for solving the I-J mystery.

MATERIALS AND METHODS

Mice. C57BL/6 (abbreviated B6), SJL, (C57BL/10 × A)F_1 [abbreviated (B10 × A)F_1], and (B6 × SJL)F_1 mice utilized for suppressor factor production were bred and maintained at the Laboratoire de Génétique Moléculaire des Eucaryotes, Strasbourg, France. B10.A(3R) mice were bred and maintained at Yale University School of Medicine, while B10.A(5R) mice and B6/J mice utilized in the suppressor assay were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Construction and Analysis of E^k_α Transgenic Mice. Production of the E_α16 line of transgenic mice has been described (4). Briefly, an 8.2-kilobase (kb) *Bgl* I fragment containing the E^k_α gene was injected into (B6 × SJL)F_2 embryos. The injected DNA derives from the genome of A/J mice and contains all of the E^k_α exons plus 2 kb of DNA 5′ to the cap site and 1.3 kb 3′ to the polyadenylylation site. The founder E_α16 male mouse was backcrossed to B6 and one male offspring, E_α16-6 (figure 6 of ref. 4), was determined positive for E^k_α expression by Southern blotting of tail DNA. This mouse was backcrossed to several B6 female mice, and the progeny of this cross were used in these experiments. The presence of the E^k_α transgene was assessed by preparing liver DNA from the animals and analyzing on Southern blots *Bam*HI and *Eco*RV double digests, using as a probe the appropriate *Bam*HI–*Eco*RV fragment. This probe allows distinction of the E^k_α transgene from its endogenous counterpart, which is smaller due to a deletion in the E^b_α and E^s_α genes (figure 1 of ref. 4). Detection of cell surface expression of the E^k_α gene product was accomplished by cytofluorometric analysis of spleen cells stained with fluorescein isothiocyanate-labeled monoclonal antibody 14.4.4 (4). The MHC haplotype of test mice was determined on the basis of a *Pst* I restriction fragment length polymorphism in the A_α gene, using as a probe the A_α cDNA clone pAAC6 (30).

Antibodies and Antigens. Monoclonal anti-Lyt2.2 serum was generously supplied by F. W. Shen (Memorial Sloan–Kettering Cancer Center, New York). Monoclonal anti-I-J^k antibodies (WF8.C12.8) and anti-I-J^b antibodies (WF9.40.5) were generated as previously described (31) and were a generous gift of Carl Waltenbaugh (Northwestern University Medical School, Chicago). Monoclonal anti-E_α antibody (14.4.4) was a generous gift of H. O. McDevitt (Stanford University, Palo Alto, CA) (32). Sheep erythrocytes (SRBC) were obtained from Colorado Serum Co. (Denver, CO).

Preparation of T-Cell-Derived Suppressor-Inducer Factor (TsiF). Preparation of SRBC-specific TsiF has been previously described (18). Briefly, a suspension of spleen cells from mice hyperimmunized with SRBC was treated with anti-Lyt2.2 monoclonal antibody and complement and subsequently cultivated *in vitro* for 48 hr in RPMI 1640 medium plus 10% fetal calf serum at 10^7 cells per ml. After 48 hr, supernatant fluids were harvested and passed through Millipore filters before use. Depletion of cells bearing the Lyt-2 marker was achieved by incubating 1×10^7 cells per ml of antibody appropriately diluted in balanced salt solution for 45 min at room temperature, washing, and incubating with complement for 45 min at 37°C. Complement used in these experiments was serum from rabbits selected for low natural cytotoxicity to mouse spleen cells.

Suppression Assay. B6 spleen cells were washed in balanced salt solution and were suspended in RPMI 1640 medium supplemented with glutamine, antibiotics, mycostatin, 20 μM 2-mercaptoethanol, and 10% fetal calf serum. All cells were suspended at 10^7 spleen cells in 1 ml and were cultured with 0.05 ml of a 1% (vol/vol) suspension of SRBC in Falcon 3008 plates (Falcon Labware, Becton Dickinson) in a 5% CO_2/95% air incubator at 37°C for 5 days. Suppressive activity of TsiF or separated molecules was determined by adding these materials to cultures of unprimed spleen cells at a final dilution of 10% on day 0 of culture. The number of plaque-forming cells (PFC) in control and test cultures on day 5 was determined by using the Cunningham modification (44) of the Jerne–Nordin plaque assay. Results are given as the mean of three independent calculations from each culture condition (33).

Analysis of I-J Molecules Associated with TsiF (I-J$_{si}$). TsiF is composed of two molecules: an I-J$^+$ antigen-nonbinding molecule (I-J$_{si}$ molecule) and an I-J$^-$ antigen-binding molecule (ABM$_{si}$). Both molecules are required to interact with an Lyt-1$^+$,2$^+$ transducer T cell to induce suppressive activity (33). The phenotype of I-J$_{si}$ molecules associated with TsiF was determined by absorption-elution analysis with anti-I-J immunoabsorbent columns (33). Briefly, TsiF was passed over immunosorbent columns made by coupling anti-I-J^b or anti-I-J^k antibody to Sepharose 4B (Pharmacia). We have previously shown that TsiF passed over anti-I-J columns separates the I-J$^-$ ABM$_{si}$ molecule (which passes through the column in the unbound filtrate) from the I-J$_{si}$ molecule (which is retained by the anti-I-J column) (18). After extensive washing, the columns were eluted with 0.2 M sodium carbonate, pH 11.0, to obtain any bound I-J$_{si}$ molecules, and immediately neutralized with 0.3 M sodium borate buffer, pH 8.3. The column eluates were then concentrated back to their original volume and dialyzed overnight, first against phosphate-buffered saline, then against RPMI 1640 medium. Eluates from these columns were then tested for the presence of I-J$_{si}$ molecules by their ability to restore suppressor activity in cultures containing ABM$_{si}$ and spleen cells. Filtrates obtained by passing TsiF over an anti-I-J column served as a source of ABM$_{si}$. TsiF from individual E_α16-6-derived backcross progeny and pooled control mice were typed for both I-J^k and I-J^b determinants. Suppressor factors prepared from control strains B10.A(3R) (I-J^b) and B10.A(5R) (I-J^k) were tested before *and* after testing experimental TsiF to ensure the I-J specificity of the typing immunosorbent (see Table 2). This general method of I-J typing is routinely used in several laboratories (12, 13, 15, 34–37). E_α16-6 backcross progeny were typed blind for E^k_α expression and I-J phenotype.

RESULTS

To determine the influence of class II genes on I-J phenotype, we tested transgenic mice that were constructed by injecting the E_{α}^{k} gene into (B6 × SJL)F_2 (H-2^b × H-2^s) fertilized eggs (4). One of the successful transgenic mice, $E_{\alpha}16$, was shown to express E_{α}^{k} with a tissue and cell specificity similar to that of normal mice. This transgenic mouse was backcrossed to strain B6, and 7 of 16 progeny were shown to carry the E_{α}^{k} gene, including male $E_{\alpha}16$-6 (4). In the present series of experiments, we tested 10 additional B6 backcross mice, all derived from male $E_{\alpha}16$-6, which was heterozygous for both the MHC and the injected E_{α}^{k} gene. The rationale for testing these particular transgenic mice for I-J phenotype is as follows. Strains carrying the H-2^b or H-2^s haplotype carry defective E_{α} alleles, and do not synthesize E_{α} chains (38, 39). In these strains, E_{β}^{b} and E_{β}^{s} chains are synthesized but are found only in the cytoplasm. Such strains routinely type I-J^k-negative (7). In contrast, some closely related strains, which synthesize similar or identical E_{β} chains and which also synthesize E_{α}^{k} chains and express $E_{\alpha}E_{\beta}$ complexes on the cell surface, type I-J^k-positive [e.g., strain B10.A(5R), $E_{\alpha}^{k}E_{\beta}^{b}$, and strain B10.S(9R), $E_{\alpha}^{k}E_{\beta}^{s}$]. These observations raise the possibility that cell surface expression of $E_{\alpha}E_{\beta}$ complexes can influence I-J phenotype. One might then expect H-2^b/H-2^b or H-2^b/H-2^s E_{α}^{k}-positive transgenic mice to type I-J^k-positive (due to cell surface expression of $E_{\alpha}^{k}E_{\beta}^{b}$ and/or $E_{\alpha}^{k}E_{\beta}^{s}$ complexes). Control littermates that do not carry E_{α}^{k} should type I-J^k-negative. Since all progeny were derived by backcrossing to the I-J^b-positive strain B6, typing for I-J^b was included as a control. In the experiments below, E_{α} and I-J typing were done independently with coded samples.

Southern blots of liver DNA detect the presence of the E_{α}^{k} transgene in 6 of the 10 animals (Table 1). In no case was DNA rearrangement evident (data not shown). The MHC haplotypes were also determined by Southern blotting of genomic DNA (Table 1). Most important, we confirmed that E_{α}^{k} is expressed on spleen cells in mice that carry the gene by fluorescent staining with the E_{α}-chain-specific monoclonal antibody 14-4-4 followed by cytofluorometric analysis (Table 1). Finally, the I-J phenotype of the mice was determined by serological analysis of I-J molecules associated with TsiF. Suppressor factor produced in each mouse was passed over an anti-I-J^k (experimental) and an anti-I-J^b (control) column, the columns were eluted at high pH, and the eluate was tested for functional activity. As can be seen in Table 2, negative control (B6 × SJL)F_1 and 5 backcross progeny (numbers 1, 5, 6, 20, and 25) type I-J^{b+}, I-J^{k-}. In contrast, positive control (B10 × A)F_1 and 5 other backcross progeny (numbers 10, 11, 12, 21, and 22) type I-J^{b+}, I-J^{k+}. Specificity of the anti-I-J columns was confirmed before (pre) *and* after (post) testing factors from these mice [see data with B10.A(3R) and B10.A(5R), Table 2]. When the I-J phenotype is compared with E_{α}^{k} expression in the backcross progeny, it can be seen that 5 out of 6 mice (numbers 10, 11, 12, 21, and 22) that carry and express E_{α}^{k} type I-J^{k+}, while all 4 mice (numbers 1, 5, 6, and 25) that do not carry or express E_{α}^{k} type I-J^{k-} (Table 1).

DISCUSSION

Data above clearly show that the MHC class II E_{α}^{k} gene influences I-J^k expression in the transgenic mice. Failure of one E_{α}^{k}-positive transgenic mouse (number 20) to express I-J^k determinants could be due to experimental variability among individual mice in producing TsiF, the influence of another segregating gene on I-J^k expression, or possibly variability in E_{α}^{k} expression in different tissues, which may be important in selecting for I-J^k expression. Although we have established that E_{α}^{k} is expressed normally in the thymus in $E_{\alpha}16$ progeny (unpublished observation), we did not test mouse 20. We

Table 1. E_{α}^{k} and I-J^k phenotype in transgenic mice

Progeny number*	E_{α}^{k} presence†	% spleen cells expressing E_{α}^{k}‡	MHC haplotype§	% suppression with eluate from antibody column¶: Anti-I-J^b	Anti-I-J^k	I-J^k phenotype
1	−	2.8	*bb*	72 ± 3	10 ± 13	−
5	−	2.4	*bs*	76 ± 8	0 ± 9	−
6	−	2.9	*bb*	68 ± 8	9 ± 13	−
10	+	27.9	*bs*	74 ± 12	66 ± 6	+
11	+	29.9	*bb*	69 ± 5	70 ± 10	+
12	+	28.3	*bb*	76 ± 11	72 ± 6	+
20	+	33.9	*bb*	78 ± 8	8 ± 17	−
21	+	35.7	*bb*	69 ± 5	57 ± 12	+
22	+	41.2	*bs*	61 ± 7	66 ± 10	+
25	−	1.2	*bs*	75 ± 9	3 ± 10	−
(B6 × SJL)F_1	−	1.8	*bs*	77 ± 8	12 ± 2	−
(B10 × A)F_1	+	19.4	*ba*	68 ± 7	71 ± 3	+

*Progeny derived by backcrossing the $E_{\alpha}16$–6 transgenic male, which is heterozygous for both H-2 (H-2^b/H-2^s) and the injected E_{α}^{k} gene (E_{α}^{k}/−), to inbred strain B6 females (homozygous H-2^b, do not carry E_{α}^{k}). The (B6 × SJL)F_1 control is included to show that H-2^b/H-2^s heterozygotes type E_{α}^{k}-negative and I-J^k-negative. The (B10 × A)F_1 control is included to show that E_{α}^{k} heterozygous mice express I-J^k determinants. Note that these latter mice also express I-J^b determinants (Table 2), providing further evidence for codominant expression of I-J genes.

†Presence of the E_{α}^{k} transgene was assessed by Southern blotting of liver DNA utilizing a cloned E_{α}^{k} DNA probe that does not hybridize to DNA from H-2^b or H-2^s mice.

‡Expression of the E_{α}^{k} gene was tested by cytofluorometric analysis of lipopolysaccharide-stimulated spleen cells utilizing the E_{α}-chain specific monoclonal antibody, 14-4-4.

§MHC haplotype determined by restriction fragment length polymorphism analysis of the A_{α} gene.

¶I-J phenotype was determined by eluting I-J molecules involved in the induction of suppressor activity (I-J_{si}) from appropriate anti-I-J immunoabsorbent columns and testing these eluted I-J_{si} molecules for activity in Mishell–Dutton cultures (see Table 2 for details). Percent suppression represents the mean ± SEM of four independent experiments and was calculated according to the formula (PFC response in cultures containing I-J_{si} + ABM_{si})/(PFC response in cultures containing ABM_{si}).

Table 2. I-J phenotype of E_{α}^{k} transgenic mice

Source of I-J_{si} molecule*	Eluate from anti-I-J column†	ABM_{si}‡	Anti-SRBC PFC per culture§				I-J phenotype
			Exp. I		Exp. II		
			A	B	C	D	
—	—	−	6840 ± 560	7760 + 720	5000 ± 480	2960 ± 240	
—	—	+	6520 ± 760	8080 + 760	4760 ± 280	2760 ± 240	
B10.A(3R)-pre	I-J^b	+	**1080 ± 280**	**3120 ± 240**	ND	ND	I-J^b
B10.A(3R)-pre	I-J^k	+	6680 ± 520	7360 ± 360	ND	ND	
B10.A(5R)-pre	I-J^b	+	5680 ± 320	6400 ± 360	ND	ND	I-J^k
B10.A(5R)-pre	I-J^k	+	**2080 ± 320**	**2600 ± 360**	ND	ND	
(B6 × SJL)F_1	I-J^b	+	**1280 ± 240**	**1240 ± 80**	**1600 ± 320**	ND	I-J^b
(B6 × SJL)F_1	I-J^k	+	5880 ± 240	7240 ± 560	4080 ± 320	ND	
(B10 × A)F_1	I-J^b	+	**2400 ± 680**	**3040 ± 280**	**1040 ± 160**	ND	I-J^b and I-J^k
(B10 × A)F_1	I-J^k	+	**2120 ± 680**	**2480 ± 240**	**1180 ± 320**	ND	
1	I-J^b	+	**2080 ± 360**	**2320 ± 600**	**1080 ± 320**	**840 ± 160**	I-J^b
1	I-J^k	+	4840 ± 200	7240 ± 600	4080 ± 440	3080 ± 280	
5	I-J^b	+	**1120 ± 280**	**1240 ± 200**	**1520 ± 120**	**920 ± 200**	I-J^b
5	I-J^k	+	5840 ± 520	7440 ± 520	5080 ± 280	3080 ± 280	
6	I-J^b	+	**2680 ± 600**	**3080 ± 80**	**960 ± 240**	**800 ± 240**	I-J^b
6	I-J^k	+	5960 ± 680	6520 ± 240	3720 ± 200	3120 ± 320	
10	I-J^b	+	**2880 ± 480**	**2360 ± 140**	**920 ± 240**	**320 ± 120**	I-J^b and I-J^k
10	I-J^k	+	**2272 ± 480**	**3440 ± 220**	**1440 ± 320**	**800 ± 200**	
11	I-J^b	+	**1640 ± 360**	**3040 ± 480**	**1320 ± 200**	**920 ± 240**	I-J^b and I-J^k
11	I-J^k	+	**2560 ± 440**	**3280 ± 80**	**800 ± 160**	**680 ± 200**	
12	I-J^b	+	**720 ± 240**	**1240 ± 200**	**1360 ± 200**	**1080 ± 280**	I-J^b and I-J^k
12	I-J^k	+	**1640 ± 520**	**2080 ± 120**	**1040 ± 200**	**1080 ± 440**	
20	I-J^b	+	**1200 ± 200**	**2640 ± 400**	**1120 ± 400**	**320 ± 200**	I-J^b
20	I-J^k	+	6320 ± 600	5840 ± 160	3840 ± 240	3240 ± 440	
21	I-J^b	+	**2160 ± 320**	**2320 ± 320**	**1160 ± 360**	**1080 ± 320**	I-J^b and I-J^k
21	I-J^k	+	4880 ± 520	**3560 ± 400**	**960 ± 280**	**960 ± 280**	
22	I-J^b	+	**3120 ± 240**	**2960 ± 160**	**1320 ± 200**	**1200 ± 320**	I-J^b and I-J^k
22	I-J^k	+	**3040 ± 400**	**2120 ± 200**	**1040 ± 200**	**1160 ± 280**	
25	I-J^b	+	**1720 ± 520**	**3120 ± 200**	**600 ± 200**	**640 ± 120**	I-J^b
25	I-J^k	+	6120 ± 640	6880 ± 560	4440 ± 520	3160 ± 560	
B10.A(3R)-post	I-J^b	+	**3280 ± 320**	ND	**1280 ± 320**	ND	I-J^b
B10.A(3R)-post	I-J^k	+	6520 ± 520	ND	4800 ± 240	ND	
B10.A(5R)-post	I-J^b	+	6160 ± 480	ND	4960 ± 440	ND	I-J^k
B10.A(5R)-post	I-J^k	+	**1080 ± 280**	ND	**1260 ± 320**	ND	

*Suppression of primary SRBC PFC responses in Mishell–Dutton cultures is induced by TsiF secreted by Ly-1^+,2^- T cells primed with SRBC *in vivo*. TsiF is composed of two molecules: an I-J^+ antigen-nonbinding molecule (I-J_{si} molecule) and an I-J^- antigen-binding molecule (ABM_{si}). *Both* molecules are required to activate the feedback suppression system. TsiF prepared from control strains B10.A(3R) (*I-J^b*) and B10.A(5R) (*I-J^k*) were tested before (pre) *and* after (post) testing TsiF prepared from other control mice and the E_{α}16-6 backcross progeny.

†I-J_{si} were prepared by passing TsiF over a monoclonal anti-I-J^b (WF.9.40.5) or anti-I-J^k (WF.8.C12.8) immunoabsorbent column and eluting with carbonate buffer. Eluates were concentrated back to their original volume and added to cultures at a final concentration of 10%.

‡ABM_{si} were prepared by passing B6 (*H-2^b*, *I-J^b*) TsiF over an anti-I-J^b (WF.9.40.5) column and harvesting the passed volume. The ABM_{si} preparation was added at a final concentration of 10%.

§*In vitro* primary anti-SRBC PFC response by B6 spleen cells on day 5. Boldfaced values indicate significant suppression. Experiments I and II represent independent passages of TsiF over anti-I-J immunoabsorbents. I-J_{si} molecules prepared in experiment I (columns A and B) and experiment II (columns C and D) were tested twice. ND, not done.

infer from these results that class II genes also influence I-J phenotype in other strains carrying the same MHC haplotypes found in the transgenic mice and in strains carrying different MHC haplotypes.

These findings provide important information regarding the genetic basis for control of I-J determinants. In particular, there is now no need to postulate that there is a novel I region or MHC gene involved in controlling I-J phenotype. Study can instead focus on how class II molecules influence I-J expression. The data also suggest a more involved role for class II molecules in immunoregulation than previously thought. In addition to being self-recognition elements for helper T lymphocytes, class II molecules may directly or indirectly select for the self-recognition capability of I-J (and other suppressor?) molecules during ontogeny (e.g., some suppressor factors are MHC restricted in their activity (11–15, 40). An understanding of this process is critical for elucidating the molecular basis for cellular interaction and regulation in suppressor systems.

Despite this advance, several basic questions concerning the I-J puzzle remain. First, how does the E_{α}^{k} gene influence I-J phenotype in the transgenic mice? It is extremely unlikely that I-J determinants reside on modified or altered E_{α} chains or on cell surface $E_{\alpha}E_{\beta}$ (or $A_{\alpha}A_{\beta}$) complexes (14, 41), since no hybridization has been observed with class II probes and RNA from I-J^+ somatic T-cell hybrids (22), and since class II alloantibodies generally fail to react with I-J molecules (7, 9). In addition, the anti-I-J^k antibody used to detect I-J^k determinants in the E_{α}^{k} transgenic mice was made by immunizing an E_{α}^{k}-positive mouse—i.e., [DBA/2 × B10.A(3R)]F_1 anti-B10.A(5R) (31). Rather, the E_{α}^{k} gene appears to directly or indirectly influence expression of a second gene that encodes I-J molecules. As discussed in detail elsewhere (10–13, 15, 20, 23, 24), I-J molecules may be non-MHC-encoded T-cell receptors that recognize self class II molecules and/or T-cell receptors that recognize (or are recognized by) self class II receptors. These putative receptors appear to be distinct from those expressed by helper T cells, since most I-J^+ T-cell

hybrids fail to rearrange T-cell receptor β genes (42). Selection for these receptors by class II molecules would occur during ontogeny, presumably in the thymus. Under this view, the E_{α}^{k} gene of mouse $E_{\alpha}16$ allows cell surface expression of $E_{\alpha}^{k}E_{\beta}^{b}$ and/or $E_{\alpha}^{k}E_{\beta}^{s}$ complexes, which, in turn, select for I-J^{k} expression.

Second, we have tested for I-J^{k} expression with the monoclonal antibody WF8.C12.8, which displays the appropriate specificity with strains B10.A(3R) (I-J^{b}) and B10.A(5R) (I-J^{k}) (Table 2). Although it is clear that the determinant detected by this monoclonal antibody on TsiF is influenced by E_{α}^{k} in the transgenic mice, it remains to be resolved whether this will hold for other monoclonal I-J^{k} antibodies that detect different determinants on I-J molecules associated with other functional T cells and T-cell-derived regulatory factors (11, 12, 17).

Third, expression of E_{α} and/or $E_{\alpha}E_{\beta}$ complexes on the cell surface cannot account for the I-J phenotype in strains that do not synthesize E_{α} chains—e.g., strains B10 or B6, which are I-J^{b}, and strain SJL, which is I-J^{s}. We assume that $A_{\alpha}A_{\beta}$ complexes [or possibly $A_{\alpha}E_{\beta}$ complexes (43) or free E_{β} chains?] influence the I-J phenotype in these strains. If the former proves to be the case, then it is still not clear why antibodies reactive with I-J^{b} can be made in strain B10.A(5R) that react with strain B10 when both strains express the same $A_{\alpha}A_{\beta}$ complexes. At the very least, data with (B10 × A)F_1 mice and E_{α} transgenic progeny (10–12, 21, 22) show that expression of I-J^{k} and/or E_{α}^{k} determinants does not preclude expression of I-J^{b} determinants in heterozygotes (Table 2).

Finally, the difference between recombinant strains utilized to map genes controlling I-J determinants remains a mystery—i.e., strains B10.A(3R) and B10.A(5R), both of which have functional E_{α}^{k} genes, type I-J^{b} and I-J^{k}, respectively. Since it is now clear that class II genes influence I-J polymorphism, and it appears that these two strains carry the same class II genes (21), the strains may differ at the locus that encodes I-J molecules (see ref. 10 for discussion).

In conclusion, data presented herein formally prove that class II genes influence I-J phenotype. These data are most compatible with I-J molecules being T-cell receptors that interact directly or indirectly with class II molecules. Molecular characterization of I-J molecules and genes may well reveal an additional family of receptors critical for immune regulation. Clearly, assembly of the I-J puzzle is far from complete. However, this study adds an important piece to a gradually emerging picture.

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