EPITOPES ASSOCIATED WITH MHC RESTRICTION SITE OF T CELLS

III. I-J Epitope on MHC-restricted T Helper Cells

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I-J is a cell surface molecule having unique genetic polymorphism linked to the murine MHC (1, 2). This has been supported by a number of studies using T cell hybridomas and clones with continuous I-J expression detected by anti-I-J mAb (3–10). The expression of I-J is generally associated with a known T cell function, suggesting that I-J itself is involved in a site essential for the T cell function. Although these notions compelled us to conclude that I-J is a genetic marker controlled by a locus mapped within the I region of MHC, recent molecular analyses of MHC have excluded the presence of the I-J structural gene within the MHC chromosomal segment (11–14).

Because I-J had solely been defined by the specificity of alloantibodies, we reexamined the effect of anti-I-J mAb on T cell functions to find that anti-I-J^k mAb block syngeneic and allogeneic mixed lymphocyte reactions (MLR) of H-2^k by reacting with the responder but not with stimulator cells (15). This target specificity was distinct from the effect of conventional anti–class II antibodies, and confirmed the notion that I-J is on T cells but not on macrophages and B cells. It also suggested that I-J epitopes are in association with a recognition structure for self- and allo-class II antigens.

In previous reports (16, 17), we demonstrated that another I region–controlled determinant selectively expressed on T cells (Iat) is not the direct product of I region genes but is associated with the T cell receptor that sees the self Ia. The epitopes were found to undergo systematic adaptive alterations according to the acquisition of a new MHC restriction specificity in radiation bone marrow chimeras (17). Therefore, we hypothesized that I-J is not a mere genetic marker, but is associated with the T cells' recognition site for a self MHC. In the present study, the nature of I-J epitope on the MHC-restricted Th cells was analyzed by using radiation bone marrow chimeras constructed by the combination of intra-H-2 recombinant mice. The contradictory expression of I-J epitopes on Th cells will be described, and its biological significance will be discussed.

Materials and Methods

Animals. C57BL/6 (B6), C57BL/10 (B10), B10.BR, B10.A, C3H, (B6 × A/J)F_1 (B6AF_1), (B6 × C3H)F_1 (B6C3F_1), BALB/c *nu/nu* mice were purchased from the Shizuoka

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Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. B10.A(3R) (3R), B10.A(4R) (4R), B10.A(5R) (5R), (B10 × B10.BR)F_1, (B10 × B10.A)F_1, and (4R × 5R)F_1 mice were bred in our animal facility.

Chimeras. Chimeras used in this study were prepared as previously described (18), and were designated as bone marrow donor → irradiated recipient. Chimeras were used no earlier than 8 wk after irradiation and reconstitution. Spleen cells of chimeric mice were examined to ascertain their donor origin by the cytotoxicity assay with anti-H-2 reagents where <5% of cells were found to be of host origin.

Antigens. Keyhole limpet hemocyanin (KLH)[1] (Calbiochem-Behring, San Diego, CA) was conjugated with 2,4,6-trinitrobenzene sulfonic acid (Pierce Chemical Co., Rockford, IL) (TNP-KLH) as previously described (19). The substitution rate was 20 TNP residues per 100 kD KLH.

Antibodies. Anti-I-J^k mAb (1G8, 4B11, and KN34) are those described in detail in previous papers (15). They are the products of hybridomas derived from spleen cells of 3R immune to 5R lymphoid cells and have been selected by their reactivity with suppressor T cell (Ts) hybridomas and clones expressing I-J^k determinant(s). JK10-23 is a newly established monoclonal anti-I-J^k produced through a similar manner. Ascites containing mAb were produced in BALB/c *nu/nu* mice. This antibody was able to absorb T suppressor factor (TsF) from KLH-primed T cells of H-2^k mice but not of other strains. Anti-H-2^b and anti-H-2^k antisera were prepared as previously described (17). Anti-H-$2K^k$ mAb (11-4.1) (20) was obtained from the Salk Institute Cell Distribution Center, San Diego, CA. Anti-H-$2K^b$ mAb (28-13-3s) (21) was a gift from Dr. D. H. Sachs, National Institutes of Health, Bethesda, MD. Anti-H-$2D^b$ mAb (H141-30) was made by Dr. G. Hämmerling (German Cancer Center, Heidelberg, Federal Republic of Germany). Anti-L3T4a mAb (GK1.5) (22) was made by Dr. F. W. Fitch, University of Chicago, Chicago, IL. Cytotoxic treatment of T cells was carried out at 10^7 cells/ml with the predetermined dilution of the antibody and selected rabbit complement (C).

Immunization. Mice were immunized with 100 μg of KLH or TNP-KLH in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) intraperitoneally. Fully allogeneic chimeras were immunized with KLH-pulsed irradiated host type accessory (Acc) cells as described previously (23). Briefly, unprimed spleen cells were depleted of T cells by the treatment with a T cell–specific rabbit anti-mouse brain serum (RAMB) + C followed by 3,000 rad irradiation. The cells were pulsed with antigen by incubating with 100 μg/ml of KLH in RPMI 1640 medium containing 10% FCS.

Preparation of Cells. B cells were prepared by treating the TNP-KLH-primed spleen cells with RAMB + C (19). T cells were separated by panning the KLH-primed spleen cells on anti-mouse immunoglobulin–coated plastic dishes (19). Nonadherent cells consisting of >95% Thy-1^+ cells were used as Th cells.

Culture Conditions for the In Vitro Antibody Responses. The in vitro secondary antibody response was induced as previously described (19). Briefly, the mixture of 3×10^6 TNP-primed B cells and 10^6 KLH-primed Th cells was incubated with 1 ng/ml TNP-KLH in a volume of 2 ml per 16-mm-diam flat-bottomed well (3524; Costar, Cambridge, MA) for 5 d at 37°C in 5% CO_2 humidified air atmosphere. Medium used was RPMI 1640 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, 5×10^{-5} M 2-ME, and 10% FCS. Harvested cells were assayed for plaque-forming cells (PFC) on TNP-conjugated sheep erythrocyte (19). All points shown in each experiment represent the arithmetic mean IgG PFC responses of identical triplicate cultures.

Limiting-dilution Analysis. The frequency of Th cells was estimated by the limiting-dilution analysis (24). Graded doses of KLH-primed F_1 Th cells were cultured with 5×10^5 TNP-primed parental B cells in the presence of 1 ng/ml TNP-KLH. The cultures were performed in 48-replicate wells at each Th cell dose in microculture plates (3072; Falcon Labware, Oxnard, CA). The amount of anti-TNP antibody in the culture super-

[1] *Abbreviations used in this paper:* Acc, accessory; KLH, keyhole limpet hemocyanin; IJIM, I-J-interacting molecule; PFC, plaque-forming cells; RAMB, rabbit anti-mouse brain serum; TsF, suppressor T cell factor.

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TABLE I

I-J^k Epitopes Are Expressed on H-2^k-restricted but not H-2^b-restricted F_1 Th Cell Population

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture			
	B10 B cells		B10.BR B cells	
	B10 Th	F_1 Th	B10.BR Th	F_1 Th
Control	1,445 ± 101	1,637 ± 222	977 ± 152	1,368 ± 51
Anti-I-J^k: 1G8	1,551 ± 145	1,837 ± 226	205 ± 74	504 ± 118
4B11	1,453 ± 152	1,824 ± 122	940 ± 68	1,272 ± 78
KN34	1,464 ± 257	1,881 ± 117	177 ± 86	658 ± 134
JK10-23	1,560 ± 139	1,761 ± 102	238 ± 45	304 ± 62
Anti-L3T4: GK1.5	54 ± 7	19 ± 11	52 ± 12	71 ± 52

10^6 KLH-primed B10, B10.BR, and B6C3F_1 Th cells were treated with indicated antibody + C. The residual cells were cocultured with 3 × 10^6 TNP-primed B10 or B10.BR B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC.

natant was estimated by the enzyme-linked immunosorbent assay as previously described (25). Responses that exceed the mean plus three SD of the control were scored as positive.

Results

Association of I-J^k Epitopes with the H-2^k Restriction Element of Th Cells in H-$2^{k \times b}F_1$. KLH-primed T cells from B10, B10.BR, and B6C3F_1 mice were treated with anti-I-J^k and C, and the residual cells were cultured with TNP-primed B10 or B10.BR B cells to induce the in vitro secondary anti-TNP antibody response. The helper function measured in this experimental condition has been known to be due only to the cognate-type Th cells (19). Table I shows that the treatment of Th cells of B10.BR but not of B10 with three out of four anti-I-J^k mAb (1G8, KN34, and JK10-23) abrogated the anti-TNP antibody formation by syngeneic B cells. This indicates that I-J epitopes are present on the cognate-type Th cells.

F_1 Th cells were found to help B cells of B10 and B10.BR equally well to induce a comparable secondary anti-TNP antibody response (Table I). The helper activity restricted to B10.BR was greatly reduced by the cytotoxic treatment of F_1 T cells with the anti-I-J^k mAb. The same treatment of F_1 Th cells did not alter the response mounted by B10 B cells, indicating that I-J^k epitopes are expressed only on I-A^k-restricted but not on I-A^b-restricted Th cells in the F_1 T cell population. These mAb were also able to block the I-A^k-restricted helper activity when they were simply added to the culture of F_1 Th and B10.BR B cells (data not shown).

Assignment of I-J^k Epitopes on Th Cells in the MHC. To determine whether I-J epitopes on Th cells are controlled by the I-J locus assigned with Ts and TsF (16, 17), the cells from various congeneic and recombinant mice were tested for the expression of I-J^k. KLH-primed T cells of B10, 3R, 4R, 5R, and B10.A were treated with anti-I-J^k mAb and C, and the residual cells were cultured with TNP-primed syngeneic B cells in the presence of TNP-KLH. As shown in Table II, the helper function of T cells of B10.A and 5R was abrogated by the treatment with the same three of the anti-I-J^k mAb (1G8, KN34, and JK10-23), while that of B10, 3R, and 4R T cells was not. The results indicate that the I-J epitopes detected by our anti-I-J^k mAb on Th cells are controlled by the locus mapped at the previously prescribed position in the MHC.

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TABLE II

I-J Epitopes on Th Cells Map Between the I-A and I-E Subregions

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture in TNP-primed B cells and KLH-primed Th cells from:				
	B10	3R	4R	5R	B10.A
Control	1,782 ± 4	738 ± 53	1,143 ± 57	745 ± 66	1,876 ± 23
Anti-I-J^k: 1G8	1,832 ± 83	569 ± 59	1,174 ± 15	83 ± 21	338 ± 47
4B11	1,900 ± 98	730 ± 94	1,042 ± 27	889 ± 98	1,825 ± 141
KN34	2,017 ± 141	730 ± 14	1,104 ± 28	90 ± 43	389 ± 57
JK10-23	1,750 ± 141	743 ± 101	1,177 ± 66	151 ± 104	406 ± 65
Anti-L3T4: GK1.5	67 ± 8	15 ± 15	17 ± 5	25 ± 25	16 ± 9

10^6 KLH-primed T cells were treated with indicated antibody + C. The residual cells were cocultured with 3×10^6 TNP-primed syngeneic B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC.

TABLE III

Expression of I-J^k on Th Cells of 5R but Not of 3R

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture in B10 B cells		
	B10 Th	3R Th	5R Th
Control	882 ± 162	781 ± 104	982 ± 145
Anti-I-J^k: 1G8	670 ± 58	889 ± 46	209 ± 84
4B11	889 ± 88	922 ± 108	914 ± 36
KN34	871 ± 150	846 ± 166	378 ± 20
JK10-23	939 ± 166	813 ± 65	315 ± 91
Anti-L3T4: GK1.5	57 ± 15	50 ± 29	32 ± 21

10^6 KLH-primed B10, 3R, or 5R Th cells were treated with indicated antibody + C. The residual cells were cocultured with 3×10^6 TNP-primed B10 B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC.

Expression of I-J^k Epitopes on I-A^b-restricted Th Cells in 5R but not in 3R. 5R but not 3R T cells express I-J^k (16, 17). Th cells in these recombinant strains are, however, both I-A^b-restricted. An experiment was, therefore, carried out to assess whether their I-A^b-restricted Th cells carry I-J^k using KLH-primed T cells with TNP-primed B10 (H-2^b) B cells. Under this condition, the expression of I-J^k only on Th cells restricted to I-A^b is detected. The results shown in Table III show that both 3R and 5R Th cells can provide comparable degrees of help to B10 B cells. The helper function of 5R T cells was abrogated by the cytotoxic treatment with anti-I-J^k, whereas that of 3R Th cells was unaffected. The results indicate that Th cells of 5R but not 3R, although both are restricted to I-A^b, express I-J^k epitopes.

Both 3R and 5R Bone Marrow Stem Cells Have Potential to Express I-J^k Epitopes on Th Cells. The potential ability to express I-J epitopes on Th cells of 3R and 5R bone marrow stem cells can be determined by analyzing T cells developed under the condition of radiation bone marrow chimera. 3R → B10, 5R → B10, 3R → B10.A, and 5R → B10.A chimeras were primed with KLH that had been pulsed to the host type Acc cells. The Th cells of these chimeras were treated with anti-I-J^k and C and were cultured with appropriate TNP-primed B cells to

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TABLE IV
Expression of I-J^k Epitopes on Th Cells of 3R and 5R Bone Marrow Cells Matured in B10.A but not B10 Chimera Host

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture			
	B10 B cells		B10.A B cells	
	3R → B10 Th	5R → B10 Th	3R → B10.A Th	5R → B10.A Th
Control	613 ± 48	547 ± 64	647 ± 29	780 ± 140
Anti-I-J^k: 1G8	520 ± 46	673 ± 37	153 ± 153	347 ± 18
JK10-23	600 ± 20	540 ± 60	47 ± 24	147 ± 24
Anti-D^b: H141-30	613 ± 13	687 ± 24	ND	ND
Anti-K^b: 28-13-3S	ND	ND	13 ± 13	27 ± 27

10^6 KLH-pulsed host-type Acc-primed T cells of the chimeras were treated with indicated antibody + C. The residual cells were cocultured with 3×10^6 TNP-primed B10 or B10.A B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC.

which major MHC restriction was directed (Table IV). Th cells of 3R → B10 and 5R → B10 chimeras were able to help B10 B cells equally well. None of the anti-I-J^k mAb was able to kill these chimeric Th cells regardless of the origin of bone marrow cells. In contrast, the helper activity of Th cells of both 3R → B10.A and 5R → B10.A chimeras, which collaborate with B10.A B cells, was eliminated by the cytotoxic treatment with anti-I-J^k. The bone marrow origin of these chimeric Th cells was confirmed by the cytotoxic test with appropriate anti-H-2 mAb. The results indicate that I-J^k epitopes are expressed on Th cells differentiated in B10.A but not in B10, regardless of whether they are derived from 3R or 5R bone marrow stem cells.

I-J Haplotype of the Host Determines the Phenotype of I-J on Th Cells. To investigate what determines the expression of I-J^k on Th cells, chimeras were constructed by injecting B6AF$_1$ or (B10 × B10.A)F$_1$ bone marrow cells into irradiated B10, 3R, 4R, 5R, and B10.A mice. They were primed in situ with KLH, as all the Acc cells are of the same F$_1$ marrow origin. Th cells of these chimeras were restricted to help B cells carrying the host-type I-A. The Th cells obtained from these chimeras were treated with anti-I-J^k + C before culturing with B10 or B10.A B cells for the in vitro secondary antibody response. The H-2 restriction specificity of these chimeric Th cells was directed to the host-type I-A. As shown in Table V, three anti-I-J^k mAb were able to remove Th activity of F$_1$ → 5R and F$_1$ → B10.A chimeric T cells even though F$_1$ → 5R Th cells were able to help B10 B cells. On the other hand, Th cells of F$_1$ → B10 and F$_1$ → 3R chimeras were not affected by anti-I-J^k. Furthermore, Th cells of F$_1$ → 4R chimeras were also insensitive to the cytotoxic treatment with anti-I-J^k, regardless of their I-A^k restriction specificity. The origin of the functioning Th cells were confirmed to be the same F$_1$ bone marrow donor origin, as determined by the cytotoxic treatment with anti-H-2K^k (11-4.1) and anti-H-2K^b (28-13-3S). These results indicate that the phenotypic expression of I-J^k on Th cells is primarily determined by the I-J haplotype of the chimeric host.

I-J Phenotype of Th Is Determined by the Environment for Maturation but not for Priming T Cells. To exclude the possibility that the I-J phenotype is determined by the environment of antigen-priming, we examined the expression of I-J on

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TABLE V

I-J Genotype of Chimera Host Determines the Phenotype of I-J on Th Cells

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture				
	B10 B cells			B10.A B cells	
	$F_1 \rightarrow$ B10 Th	$F_1 \rightarrow$ 3R Th	$F_1 \rightarrow$ 5R Th	$F_1 \rightarrow$ 4R Th	$F_1 \rightarrow$ B10.A Th
Control	2,116 ± 50	1,428 ± 95	1,554 ± 17	2,285 ± 113	1,764 ± 22
Anti-I-J^k: 1G8	2,250 ± 63	1,159 ± 29	302 ± 14	1,958 ± 9	739 ± 73
4B11	2,469 ± 150	1,411 ± 44	1,494 ± 61	2,184 ± 51	1,855 ± 122
KN34	2,149 ± 144	1,512 ± 109	370 ± 77	2,084 ± 53	646 ± 134
JK10-23	2,318 ± 169	1,260 ± 104	446 ± 103	2,066 ± 116	646 ± 49
Anti-K^k: 11-4.1	42 ± 17	32 ± 4	50 ± 8	ND	ND
Anti-K^b: 28-13-3S	ND	ND	ND	42 ± 12	21 ± 7

10^6 KLH-primed B6A$F_1 \rightarrow$ B10, B6A$F_1 \rightarrow$ B10.A, B6A$F_1 \rightarrow$ 3R, B6A$F_1 \rightarrow$ 4R, and B6A$F_1 \rightarrow$ 5R chimera Th cells were treated with anti-I-J^k + C. The residual cells were cocultured with 3×10^6 TNP-primed B10 or B10.A B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC. All Th cells used were restricted to help host- but not nonhost-type B cells.

TABLE VI

I-J Phenotype of a Th Cell Is Determined by the Maturation but not by the Priming Environment

Cytotoxic treatment of Th cells with:	IgG anti-TNP PFC/culture				
	B10 B cells			B10.A B cells	
	F_1 (B10) Th	F_1 (3R) Th	F_1 (5R) Th	F_1 (4R) Th	F_1 (B10.A) Th
Control	633 ± 29	687 ± 77	667 ± 114	493 ± 64	400 ± 64
Anti-I-J^k: 1G8	680 ± 12	660 ± 70	593 ± 74	27 ± 13	33 ± 33
JK10-23	600 ± 46	560 ± 50	547 ± 77	27 ± 27	20 ± 20
Anti-L3T4: GK1.5	0	7 ± 7	0	0	0

10^6 KLH-pulsed (indicated in parentheses) Acc-primed B6AF_1 T cells were treated with indicated antibody + C. The residual cells were cocultured with 3×10^6 TNP-primed B10 or B10.A B cells in the presence of 1 ng/ml TNP-KLH. Th cells did not provide help to B cells of the I-A haplotype, which were not used for priming. B cells alone gave no PFC.

Th cells of (B10 × B10.A)F_1 mice that had been primed with KLH-pulsed Acc cells of different haplotype origin. Acc cells from B10, 3R, 4R, 5R, and B10.A were pulsed in vitro with KLH and then injected into F_1 mice. Under this condition of priming, no allogeneic effects are expected. Th cells thus obtained were treated with anti-I-J and examined for ability to help TNP-primed B10 or B10.A B cells. As shown in Table VI, the F_1 Th cells primed with KLH-pulsed Acc cells of B10, 3R, and 5R were able to help B10 but not B10.A B cells. On the other hand, F_1 Th cells primed on 4R and B10.A Acc cells could help only B10.A but not B10 B cells, indicating that the priming with KLH in context of the given I-A molecule on Acc cells determines the H-2 restriction of functioning Th cells (Table VI). When these Th cells were treated with anti-I-J^k and C, those restricted only to I-A^k were sensitive to the treatment regardless of the absence or presence of I-J^k on Acc cells used for priming. Also, phenotype of I-J subregion of priming Acc cells did not determine the I-J^k expression on I-A^b-restricted F_1 Th cells. The observed pattern of I-J^k expression on F_1 Th was identical to that

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TABLE VII

An MHC-restriction Specificity and an I-J Phenotype Are Expressed on a Th Cell in a Cis but Not a Trans Fashion

Origin of F_1 Th cells	Cytotoxic treatment of Th cells with:		IgG anti-TNP PFC/culture in F_1 Th cells cocultured with:			
			B10 B cells	5R B cells	4R B cells	B10.A B cells
(B10 × B10A)F_1	Control		2,889 ± 131	904 ± 93	907 ± 43	1,008 ± 58
	Anti-I-J^k:	1G8	2,310 ± 163	1,016 ± 220	210 ± 66	134 ± 73
		JK10-23	3,024 ± 227	915 ± 132	159 ± 55	159 ± 16
	Anti-L3T4:	GK1.5	117 ± 60	42 ± 22	0	0
(4R × 5R)F_1	Control		1,310 ± 81	705 ± 52	1,621 ± 84	1,394 ± 111
	Anti-I-J^k:	1G8	344 ± 60	50 ± 14	1,528 ± 198	1,276 ± 137
		JK10-23	268 ± 117	67 ± 22	1,646 ± 74	1,327 ± 226
	Anti-L3T4:	GK1.5	25 ± 14	0	0	50 ± 50

10^6 KLH-primed (B10 × B10.A)F_1 or (4R × 5R)F_1 Th cells were treated with indicated antibody + C. The residual cells were cocultured with 3 × 10^6 TNP-primed B10, 5R, 4R, and B10.A B cells in the presence of 1 ng/ml TNP-KLH. B cells alone gave no PFC.

of F_1 Th primed in situ, regardless of the haplotype origin of Acc cells used for priming. The results indicate that I-J epitopes expressed on Th cells are determined primarily by the maturation environment before the priming.

MHC Restriction Specificity and I-J Phenotype on Th Cells Show Only Cis Expression. The relationship between an MHC restriction specificity and I-J phenotype was studied in (B10 × B10.A)F_1 and (4R × 5R)F_1 Th cells. KLH-primed Th cells from these mice were treated with anti-I-J^k + C followed by coculture with TNP-primed B10, 4R, 5R, and B10.A B cells. The results are summarized in Table VII. Th cells from (B10 × B10.A)F_1 were able to help B cells of all these strains with different H-2-restriction specificity; I-A^b-restriction to help B10 and 5R B cells, and I-A^k-restriction to help 4R and B10.A B cells. The treatment of F_1 Th cells with anti-I-J^k eliminated with I-A^k restricted Th function leaving the I-A^b-restricted function intact. When Th cells of (4R × 5R)F_1 were treated by the same anti-I-J^k mAb, only the I-A^b-restricted Th function was affected. The same anti-I-J^k mAb did not alter the response induced by the I-A^k-restricted Th of (4R × 5R)F_1. The results indicate that only the combinations of I-A-restriction and I-J expression represented in parental haplotypes are expressed among F_1 Th populations.

This was further confirmed by the limiting-dilution analysis using (B10 × B10.A)F_1 Th cells. Graded numbers of (B10 × B10.A)F_1 Th cells were cultured with TNP-primed B10 or 4R B cells to enumerate I-A^b- and I-A^k-restricted Th cells, respectively. The frequency of Th cells with I-J^k epitopes on a functioning site was estimated in the presence of anti-I-J^k in limiting-dilution cultures. Fig. 1 shows the frequency analysis of I-A^b- and I-A^k-restricted Th cells in the presence and absence of anti-I-J^k or anti-L3T4. The frequency of KLH-specific, I-A^b-restricted Th cells, which cooperated with B10 B cells, was 1 in 6.2 × 10^4. This frequency was not altered in the presence of anti-I-J^k (1 in 6.1 × 10^4). The frequency of KLH-specific, I-A^k-restricted Th cells estimated with 4R B cells was 1 in 5.3 × 10^4, the frequency of which was reduced to 1 in 4.6 × 10^5 when anti-

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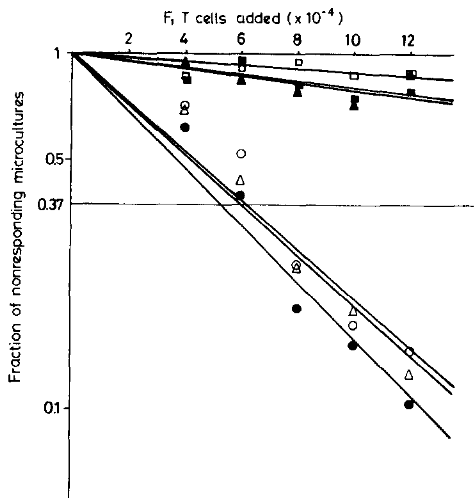

FIGURE 1. The limiting-dilution analysis of I-J^k-positive cells in (B10 × B10.A)F_1 Th cell subpopulations. Graded numbers of KLH-primed (B10 × B10.A)F_1 Th cells were cultured with 5×10^5 TNP-primed B10 (○, △, □) or 4R (●, ▲, ■) B cells in the presence of 1 ng/ml TNP-KLH. The parallel cultures were carried out in the absence of antibody (○, ●) or in the presence of 10% culture supernatant of anti-I-J^k (JK10-23) (△, ▲) or 10% culture supernatant of anti-L3T4 (□, ■) in 48 replicate wells at each Th cell dose in microculture plates. The amount of anti-TNP antibody in the culture supernatant was estimated by the enzyme-linked immunosorbent assay. Responses that exceed the mean plus three SD of the control were scored as positive.

I-J^k was present in the culture. This reduction of the frequency in the functional Th was comparable with that induced by anti-L3T4. An additional experiment with (B10 × B10.BR)F_1 Th cells gave essentially the similar pattern (data not shown). These results indicate that there is no detectable number of Th cells bearing I-J^k in I-A^b-restricted Th cell population in (B10 × B10.A)F_1 and (B10 × B10.BR)F_1.

Discussion

Previous studies in this series (16, 17) indicated that some of the anti-Ia mAb raised by the combination of I region–incompatible strains of mice did not bind to class II antigens but reacted with the site on T cells involved in the MHC-restricted cell interaction. These antibodies were reactive only with T cells but not with B cells and macrophages, and thus were designated as anti-Iat antibodies (26, 27). They were able to block class II–restricted macrophage–T cell and T–B cell interactions, resulting in the inhibition of the in vitro secondary antibody response (16, 17). The target site of these antibodies are detected on Th cells of F_1 animals restricted to only one parental strain of mice but not to the other, and can be adaptively acquired by Th cells of different genotype origin when developed in radiation bone marrow chimeras (17). These results indicated that Iat antigens are not the direct products of class II genes but are associated with a structure on T cells that sees the MHC polymorphism.

I-J is in many respects analogous to Iat, as it is expressed only on functional T cells (5). Although I-J was first demonstrated on Ts and TsF (1, 2), it has been detected on a variety of T cell subsets including Th, Ts-inducer, and MLR responders (15, 28–31). It has recently been demonstrated that I-J can be adaptively expressed by T cells of different genotype that have developed in the radiation bone marrow chimera and in transgenic mice (29–32). The present experiments were undertaken to elucidate the nature of I-J in the light of adaptive changes of restriction specificity in bone marrow chimeras. A newly established anti-I-J mAb JK10-23 was used to study the expression of I-J on Th, as this mAb was able to absorb TsF and to kill Th of H-2^k origin.

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Four essential findings were obtained: first, I-J^k epitopes are expressed on Th cells of H-$2^{k\times b}F_1$ mice restricted to help H-2^k B cells, but not on those restricted to H-2^b B cells. This was demonstrated by both the elimination of H-2^k-restricted Th by the anti-I-J^k + C and the blocking of H-2^k-restricted Th activity without C. The same treatments of F_1 Th cells with anti-I-J^k did not alter the helper activity for H-2^b B cells, indicating that I-J^k is exclusively expressed on H-2^k-restricted Th of H-$2^{k\times b}F_1$ spleen cells. The limiting-dilution analysis of I-J^k-positive Th cells in (B10 × B10.A)F_1 capable of helping 4R B cells indicated that virtually all I-A^k-restricted Th cells carry the I-J^k epitope. This suggested that I-J^k epitopes are associated with the H-2^k restriction site of Th cells.

However, there was no absolute association between the I-J phenotype and the MHC restriction specificity. I-J^k was found on Th of B10.BR and B10.A having the H-2^k restriction specificity, whereas Th from 4R having the same H-2^k restriction specificity was not killed by the anti-I-J^k. Furthermore, Th cells of 5R were shown to be positive for I-J^k regardless of the fact that they are H-2^b-restricted. The same H-2^b-restricted Th of both 3R and of B10 were absolutely negative for I-J^k. The results bring us back to the original mapping of I-J in between I-A and I-E based on the genetic studies (1, 2).

Third, all above findings were verified in radiation bone marrow chimeras, where these recombinant mice were used as recipients of bone marrow stem cells of different genotypes. Without exception, the restriction specificity of Th cells followed the host type, and the I-J expression on Th was exactly the same as that expected for the host haplotype. By examining the I-J expression of Th cells of $F_1 \rightarrow$ recombinant chimeras, where Th cells were primed with the identical F_1-type antigen-presenting cells (APC), the I-J expression of at least Th cells is solely determined by the selection in the thymic environment, rather than by priming with APC.

The final most striking finding is the linked expression of the parental H-2 restriction specificity and the I-J phenotype in the F_1 Th cell population. This was best exemplified in Th cells in (4R × 5R)F_1, where Th cells were either H-2^k- or H-2^b-restricted. I-J^k was found only on H-2^b-restricted but not on H-2^k-restricted Th cells. As H-2^b restriction specificity was a trait possessed by 5R, the strain with I-J^k, there was an absolute linkage between the restriction specificity and the I-J phenotype in F_1 Th cells. If we refer to the original mapping of I-J, the results obtained here indicate that there is only a *cis* expression of the restriction specificity and I-J phenotype in the F_1 (e.g., 5R has the linked expression of I-A^b and I-J^k, and 4R, I-A^k and I-J^b). The experiments with chimeras also support the notion that there is no case where expression of I-J^k is associated with MHC restriction other than that originally possessed by the host.

All these results indicate that I-J is an isomorphic structure T cells acquire in conjunction with their H-2 restriction during early differentiation in the thymus. I-J is probably directly involved in the MHC-restricted cell interaction, as anti-I-J mAb can block the response. The simplest deduction is that I-J is an inducible T cell receptor for the self class II polymorphism, which is involved in the T cells' H-2-restricted functions.

However, the present results indicate that the I-J isomorphism does not always correlate with the specificity of the MHC restriction. The Th of 5R restricted to

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I-A^b expressed I-J^k, whereas the H-2^k-restricted 4R Th lacked I-J^k. The Th from 3R and 5R differed in I-J phenotype regardless of their identical I-A^b restriction specificity. Therefore, I-J^k does not always reflect the H-2^k restriction specificity.

On the other hand, the parental restriction specificity and the I-J phenotype were always coexpressed in F_1 T cells. If the expression of I-J is merely due to an educational process in T cells according to the class II expression of the thymic environment, there should be F_1 T cells with I-J expression associated with the both parental H-2 restriction specificities. The absence of I-J^k-positive T cells having the opposite I-A^b restriction specificity in the (B10 × B10.A)F_1 suggests that the I-J isomorphism is physically associated with, or even identical to, the MHC restriction site of T cells. However, I-J isomorphic structure does not correspond to the given restriction specificity.

Although we do not want to reiterate the debates on the nature of I-J molecule (reviewed in 33 and 34), we have to examine here a few critical points. Is I-J indeed associated with the known T cell receptor (TcR)? There are very little biochemical data available to verify or disprove the identity of I-J determinants as a substructure of TcR (35, 36). The discrepancy between the I-J phenotype and the restriction specificity in recombinant mice, as well as their exclusive *cis* expression in F_1 suggests that I-J is not merely the epitope on the anti-MHC portion of TcR. Although it is related to the acquirable restriction specificity of T cells, it does not directly correlate with the specificity per se.

Second, what does the I-J represent? The prevalence of the I-J among class II–restricted population of T cells indicates that I-J is an isomorphic structure shared by a large population of T cells having class II MHC–restricted functions involved either in help or suppression. Our more recent results indicated that the generation of both suppressor and augmenting regulatory T cells was inhibitable by the administration of the anti-I-J mAb into the in vitro culture of primed lymphocytes (37). It has been known that I-J is predominantly expressed on Ts cells (1–10, 33, 34), while it has become clear that other regulatory T cells, including Th, also carry I-J epitopes. Detailed information about the identity of I-J on Th and Ts is still lacking. In addition, the mode of acquisition of MHC restriction by Th and Ts seems to be different. However, since these regulatory T cells are selectively interacting with specified target cells, the isomorphism of I-J should be an ideal device to direct the cell interactions. Thus, the I-J appears to be involved in the construction of MHC-restricted regulatory circuits.

Finally, the origin of I-J polymorphism is still unknown. The present results indicated that both class II and TcR genes by themselves do not explain the I-J specificity (see above discussion). Recently, Kawasaki and colleagues (38) demonstrated that the antiidiotypic reagent against a monoclonal anti-I-J inhibited the induction of I-J^+ Ts by reacting with APC and B cells. The target of antiidiotype of I-J was not class II antigen, and hence was designated as I-J-interacting molecule (IJIM). As we cannot find the direct correlation between H-2 restriction specificity and I-J, a possibility should be considered that MHC polymorphism generates a second-order polymorphic structure, such as IJIM on APC and thymic cells, that dictates the I-J epitope. The linked inheritance of I-J restriction specificity in F_1 suggests that such a putative IJIM should be influenced by the class II antigen.

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Although the gene transfer experiments demonstrated that the conventional TcR α/β heterodimer can explain both the MHC restriction and antigen-specificity (39, 40), the presence of additional polymorphic structure having a broad MHC restriction specificity has not been excluded. We may refer to some studies that proposed the two-receptor model for MHC plus antigen and MHC alone (41). The present results with anti-I-J also encourage further study of the specialized MHC restriction site associated with or in addition to the known TcR molecules.

The I-J enigma is one of the first-rated paradoxes in current cellular immunology and immunogenetics. Not much progress has been made in the molecular genetics of membrane biochemistry, despite numerous efforts. Nevertheless, the I-J issue provides important clues to study the mechanism whereby T cells recognize the right partner cells with which to interact for the construction of regulatory circuits.

Summary

I-J epitopes were found to be associated with the functional site of the class II MHC–restricted helper T (Th) cells: Virtually all of the H-2^k-restricted Th cell function of H-$2^{k \times b}F_1$ T cells was inhibited by the anti-I-J^k mAb, leaving the H-2^b-restricted function unaffected. The I-J^k epitope was inducible in Th cells of different genotype origin according to the environmental class II antigens present in the early ontogeny of T cells. Although above results suggested that I-J is the structure reflecting the inducible MHC restriction specificity, further studies revealed some interesting controversies: First, the I-J phenotype did not always correlate with the class II restriction specificity, e.g., I-A^b-restricted Th from 5R was I-J^k-positive, whereas I-A^k-restricted Th of 4R was not. Second, there was no *trans* expression of parental I-J phenotypes and restriction specificities in F_1 Th, e.g., the I-J phenotype was detected only on I-A^b-restricted Th of (4R × 5R)F_1, whereas it was absent on I-A^k-restricted Th. This strict linkage between the restriction specificity and I-J phenotype was also found on Th cells developed in bone marrow chimera constructed with intra-H-2-recombinant mice. The expression of I-J^k was always associated with the restriction specificity of the relevant host. Thus, the restriction specificity of Th cells followed the host type, and the I-J expression on Th was exactly the same as that expressed by the host haplotype. These results indicate that I-J is an isomorphic structure adaptively expressed on Th cells that is involved in the unidirectional regulatory cell interactions, and that the polymorphism cannot be explained merely by the restriction specificity of the conventional T cell receptor heterodimer.

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