DIRECT VISUALIZATION OF T LYMPHOCYTES BEARING Ia ANTIGENS CONTROLLED BY THE *I-J* SUBREGION*

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The immune responses to a number of antigens are known to be under the control of immune response (*Ir*) genes which are linked to the *H-2* complex (1–3). These *Ir* genes are located in a chromosomal segment between the *K* and *S* regions of the *H-2* complex and this segment of chromosome 17 is designated the *I* region. Crossovers within the *I* region have permitted its division into three subregions, designated *I-A*, *I-B*, and *I-C*.

By cross-immunizing between selected mouse strains, several laboratories have raised antibodies against *I*-region-associated (Ia) antigens (2–5). Whether these Ia antigens actually represent the *Ir*-gene product is still a matter of controversy. However, it was possible to detect Ia antigens controlled by loci (*Ia-1* and *Ia-3*) which map in the *I-A* and *I-C* subregions (2–5).

Recently, on the basis of Ia antigen studies, it has been possible to subdivide the *I* region into two additional subregions, namely *I-J* (6, 7) and *I-E* (8), which are marked by the *Ia-4* and *Ia-5* loci. The *I-J* subregion has stimulated great interest as it appears that the Ia antigens coded for by this subregion are expressed on suppressor T cells (6) and concanavalin A-reactive T cells (9), whereas the Ia antigens controlled by the other subregions appear predominantly on B lymphocytes (2–5). Furthermore, it has been shown that the *I-J* subregion also codes for determinants on soluble factors which suppress antibody responses (7).

In previously published studies the Ia antigens controlled by the *I-J* subregion were detected by indirect methods (6, 7). Direct visualization of lymphocytes which bear *I-J*-controlled determinants was difficult as only a small proportion of lymphocytes carry these antigens (6). In this report we describe a sensitive rosetting procedure which enabled us to directly estimate the proportion of T lymphocytes expressing *I-J*-defined antigens.

Materials and Methods

Mice. The mouse strains used in the experiments are listed in Table I, together with the haplotype origin of the *H-2* regions they carry.

Antisera. Two anti-Ia sera were used in these studies: A.TH anti-A.TL (anti-I^k, S^k, G^k, Tla^c)

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TABLE I
Haplotype Origin of H-2 Regions Carried by Mouse Strains Used in This Study

Strain	Haplotype	Region*									Allele‡
		K	I					*S*	*G*	*D*	*Tla*
			A	*B*	*J*	*E*	*C*				
C57BL/6	b	b	b	b	b	b	b	b	b	b	b
BALB/c	d	d	d	d	d	d	d	d	d	d	c
CBA/H	k	k	k	k	k	k	k	k	k	k	b
DBA/1	q	q	q	q	q	q	q	q	q	q	b
A/Tb	a	k	k	k	k	k	d	d	d	d	a
B10.A(4R)	h4	k	k	b	b	b	b	b	b	b	b
B10.A(5R)	i5	b	b	b	k	k	d	d	d	d	a
A.TL	t1	s	k	k	k	k	k	k	k	d	c
A.TH	t2	s	s	s	s	s	s	s	s	d	a
B10.HTT	t3	s	s	s	s	k	k	k	k	d	c
B10.S(9R)	t4	s	s	?	k	k	d	d	d	d	a
AQR	y1	q	k	k	k	k	d	d	d	d	a

* Haplotype origin of regions according to Shreffler and David (2), Klein et al. (10), David et al. (11), Murphy et al. (6), and Shreffler et al. (8).
‡ Tla allele according to Frelinger et al. (13).

and A.TL anti-A.TH (anti-I^s, S^s, G^s, Tla^a). The immunization regimens have been described previously (12). The A.TH anti-A.TL serum is unlikely to contain any known anti-Tla antibody, although A.TL anti-A.TH could contain anti-Tla-1,3 (13). This serum could also contain anti-Qa-1, but not anti-Qa-2 (14, 15). The preparation and characteristics of the anti-Thy-1.2, anti-Ly-1.1, and anti-Ly-2.1 sera have been reported earlier (12, 16). In preparing anti-I-J sera, 1.0 ml of a 1/10 dilution of anti-Ia serum was absorbed with 3×10^8 Ig^+ splenic lymphocytes (either CBA/H or A.TH) for 30 min on ice.

Preparation of Ig^- and Ig^+ Lymphocytes. Single cell suspensions of spleen, lymph node, thymus, bone marrow, and peritoneal cells were prepared as previously reported (17). Cells bearing surface Ig were removed from lymphoid cell suspensions by forming Ig rosettes and separating the rosetting and nonrosetting cells by centrifugation on Isopaque/Ficoll (18). Briefly, sheep erythrocytes (SRBC) were coated, via $CrCl_3$, with sheep Ig specific for mouse IgG and were mixed with lymphoid cell suspensions. The mixture of SRBC and spleen cells was pelleted by centrifugation, the pellet resuspended in its supernate, and the rosette-forming cells then sedimented on Isopaque/Ficoll. The Ig^- cells were harvested from the Isopaque/Ficoll-medium interface, and Ig^+ cells were recovered from the erythrocyte pellet.

Lymphocytotoxicity Tests. A two-stage dye exclusion microcytotoxic test was performed as reported previously (18). Guinea pig complement (C) was used for the anti-Thy-1.2 treatments and rabbit C for the anti-Ly and anti-Ia sera. In some cases, after treatment with antiserum, dead cells were removed by centrifugation on Isopaque/Ficoll (19).

Rosetting for Ia Antigens on Lymphocytes. The binding of mouse anti-Ia antibodies to mouse Ig^- lymphocytes was detected by a one-stage rosetting procedure. To 0.05 ml of various dilutions of mouse antiserum in phosphate-buffered saline (PBS)[1]/10% fetal calf serum (FCS) was added 0.05 ml of Ig^- lymphocytes (4×10^7 cells/ml in PBS/10% FCS). After reacting for 30 min on ice the cells were sedimented by centrifugation at 4°C and resuspended in 0.25 ml of PBS/10% FCS. Then 0.25 ml of a 2% suspension of SRBC (4×10^8 SRBC/ml in PBS/10% FCS) coated, via $CrCl_3$, with sheep Ig [40% $(NH_4)_2SO_4$ cut from immune serum] specific for mouse Ig was added. The method of coating

[1] *Abbreviations used in this paper:* FCS, fetal calf serum; HRBC, horse erythrocytes; PBS, phosphate-buffered saline; PFC, plaque-forming cells.

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SRBC with Ig preparations has been previously published (20). The erythrocyte-lymphocyte mixtures were then centrifuged for 5 min at 300 *g* and 4°C to encourage rosette formation. The cell pellets were gently resuspended in their supernates and 0.1-ml samples taken, mixed with 0.1 ml of crystal violet-staining solution (17), and examined for rosette content. Any lymphocyte which bound four or more SRBC was scored as a rosette and in each sample approximately 500–1,000 lymphocytes were scanned.

Induction of Suppressor T Cells. Antibody tolerance to horse erythrocytes (HRBC) was induced by a combination of antigen and cyclophosphamide (21). Mice were injected with 0.5 ml packed HRBC intraperitoneally (i.p.) and 24 h later with cyclophosphamide (100 mg/kg) given subcutaneously. 7 days later a further i.p. injection of 0.2 ml of 30% HRBC was given and mice were used 10–14 days after the first injection of HRBC. These animals are specifically tolerant to HRBC and contain HRBC-specific suppressor T cells (21).

Irradiations and Cell Transfers. Mice were placed in cylindrical containers on rotating stages 15 cm from a ^{60}Co rod source and given 850 rads whole body irradiation. Cell suspensions (0.5 ml) were injected, intravenously (i.v.), into mice 24 h after irradiation. In suppressor T-cell assays, mixtures of lymphoid cells were incubated for 2 h at 37°C before transfer (21).

Enumeration of Plaque-Forming Cells (PFC). The numbers of HRBC-specific PFC were assessed by the method of Cunningham and Szenberg (22).

Results

A sensitive rosetting system was developed for detecting Ia antigens on the surface of T lymphocytes. The system measures the ability of T (Ig$^-$) lymphocytes, which have been reacted with mouse anti-Ia sera, to bind SRBC coated with sheep anti-mouse Ig. Thus, the system represents a direct binding assay and therefore has the potential of detecting subpopulations of lymphocytes which react with the anti-Ia sera but which are not lysed by C. Furthermore, unlike the C lysis techniques, this assay has a very low background (<1% in most cases). The system can, of course, only be applied to lymphoid populations which have been depleted of B (Ig$^+$) cells.

Characterization of Anti-I-J Sera. Two anti-Ia sera were used in these studies, A.TH anti-A.TL and A.TL anti-A.TH. Both of these sera have the potential of reacting with a range of intra-*H-2* gene products as well as Qa and Tl antigens. Initially the two antisera were absorbed with B (Ig$^+$) lymphocytes to eliminate antibodies directed against Ia antigens carried by B lymphocytes, i.e., Ia antigens controlled by the *I-A*, *I-C*, and *I-E* subregions (2, 4–9). It was found that this absorption virtually completely removed the cytotoxic activity detectable with unabsorbed serum in the dye-exclusion microcytotoxicity test (Fig. 1). The residual antibody activity at low dilutions in the A.TH anti-A. TL serum was removed by a second absorption with B cells. It should be noted that with the A.TL anti-A.TH serum (due to fortuitously low C controls) there were indications that, after B-cell absorption, cytotoxic antibodies remained against a small subpopulation of splenocytes (approximately 3%). This antibody could not be removed by further absorption with Ig$^+$ spleen cells.

The absorbed and unabsorbed anti-Ia sera were then tested, by the rosetting system, for their ability to react with Ig$^-$ spleen cells (Fig. 2). The unabsorbed sera reacted with approximately 8–12% of the Ig$^-$ splenocytes, whereas the B-cell-absorbed sera bound to from 6 to 8% of the Ig$^-$ cells. The rosette background in this experiment was approximately 1%. Thus it appears that both antisera (i.e., A.TH anti-A.TL and A.TL anti-A.TH sera) recognize a subpopulation of Ig$^-$ cells which carry antigenic specificities shared by B cells. The nature of this

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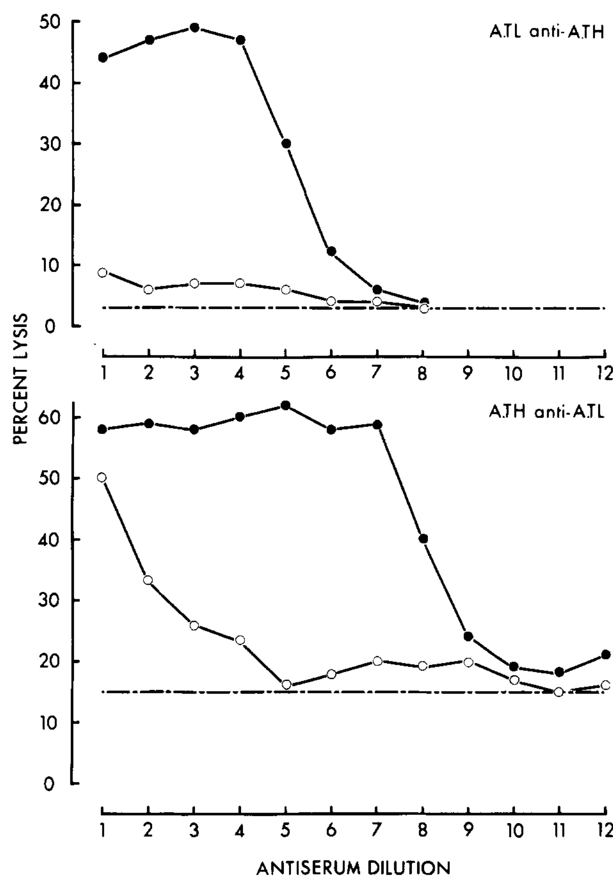

FIG. 1. Ability of serial dilutions of two anti-Ia sera to lyse spleen cells before (●) and after (○) absorption with B (Ig^+) spleen cells. The A.TL anti-A.TH serum was tested on A.TH spleen cell targets and was absorbed with A.TH Ig^+ cells, whereas the A.TH anti-A.TL serum was tested on CBA/H spleen cell targets and was absorbed with CBA/H Ig^+ cells. The discontinuous line represents the percent lysis by rabbit C alone. Tube 1, 1/20 dilution of antiserum.

subpopulation and the genetic mapping of the antigens it carries remains to be determined.

The genetic origin of the antigenic specificities recognized by the B-cell-absorbed antisera was then determined. It was found that both antisera showed haplotype specificity in their reactivity (Fig. 3). Thus, the B-cell-absorbed A.TL anti-A.TH serum only reacted with A.TH Ig^- spleen cells (I^s, S^s, G^s), whereas the B-cell-absorbed A.TH anti-A.TL serum only bound to CBA/H Ig^- cells (I^k, S^k, G^k). These results eliminated the possibility of Qa and Tl antigens being the relevant specificities and suggested intra-*H-2*-controlled antigenic specificities were being recognized by the antisera (see Table I).

Subsequent genetic mapping studies revealed that both antisera were recognizing determinants controlled by the *I-J* subregion (Fig. 4). In the case of the A.TL anti-A.TH serum (anti-I^s, S^s, G^s), the recombinant strain B10.HTT mapped the relevant gene(s) to the left of the *I-E* subregion, whereas B10.S(9R)

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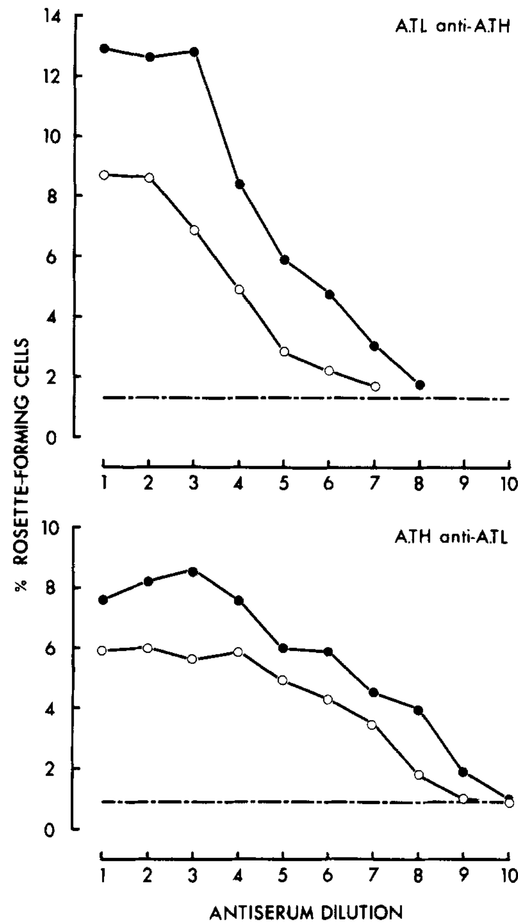

FIG. 2. Ability of two anti-Ia sera to bind to Ig^- spleen cells before (●) and after (○) absorption with B (Ig^+) spleen cells as detected by the rosetting assay. Target cells and antisera absorptions as in Fig. 1. The discontinuous line represents the percent rosetting in the absence of antiserum. Tube 1, 1/40 dilution of antiserum.

mapped the gene(s) to the right of the *I-A* subregion. Since there is no evidence for *I-B*-subregion-controlled Ia antigens it seems reasonable to conclude that the *I-J* subregion controls the determinants recognized by this antiserum. Furthermore, the specificity or specificities detected by the antiserum are unique to the $I\text{-}J^s$ haplotype, i.e., $Ia\text{-}4^s$ locus.

On the other hand, with the A.TH anti-A.TL serum (anti-I^k, S^k, G^k), the recombinant strains B10.HTT, A, and AQR mapped reactivity to the left of the *I-E/I-C* subregion, B10.A(4R) mapped reactivity to the right of the *I-A* subregion, and B10.A(5R) and B10.S(9R) directly mapped the relevant gene(s) to the *I-J* subregion.

Based on our rosetting studies the haplotype origin of the *I-J* subregion in the various mouse strains examined could be deduced (Table II). In all cases our haplotype designations agree with those reported by Murphy et al. (6) using a different assay system, namely elimination of suppressor T cells by antiserum

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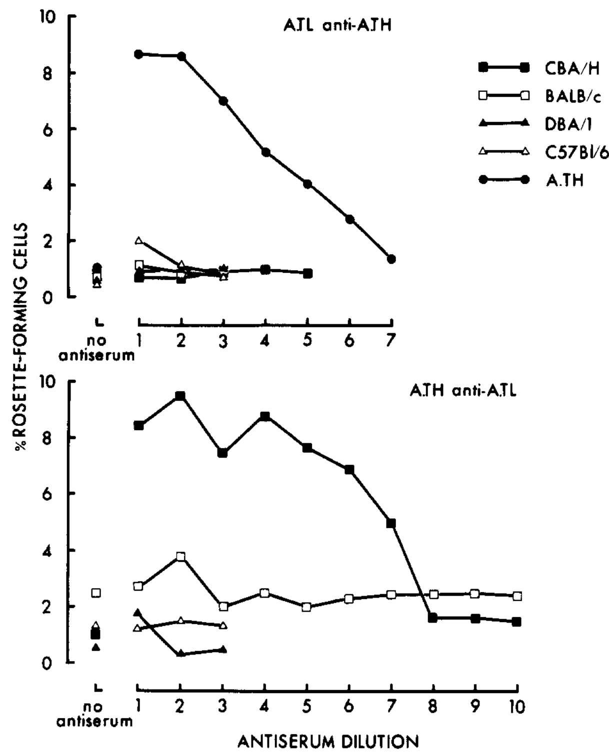

FIG. 3. Reactivity of two B-cell-absorbed anti-Ia sera with mouse strains of different *H-2* haplotypes as measured by the rosetting assay. Tube 1, 1/40 dilution of antiserum.

and C. For clarification, our method for detecting lymphocytes bearing Ia antigens controlled by the *I-J* subregion is briefly summarized in Table III.

Nature of Cells Bearing I-J-Subregion-Controlled Antigens. In the previous section it was found that anti-I-J sera only react with a subpopulation of Ig^- spleen cells. In the next series of experiments we attempted to characterize this subpopulation of Ig^- cells. It was found that when T cells were removed from the Ig^- population by treatment with anti-Thy-1.2 serum and C, virtually all (97%) of the cells which bound anti-I-J antibodies were eliminated (Table IV). The cells bearing *I-J*-subregion-defined antigens were also almost completely removed by anti-Ly-2.1 treatment (approximately 90%) but they resisted anti-Ly-1.1 treatment (Table IV). Thus, the *I-J*-subregion-controlled antigenic specificities of CBA/H mice are predominantly expressed on a subpopulation of T lymphocytes which are $Ly\text{-}1.1^-$ and $Ly\text{-}2.1^+$.

It was also found that at least a proportion of suppressor T lymphocytes react with anti-I-J serum (Table V). The experimental approach was to rosette for cells bearing *I-J*-subregion-controlled determinants and then separate the rosetting and nonrosetting cells on Isopaque/Ficoll (17). The suppressive activity of the rosetting and nonrosetting cells was then assayed. The suppressor T cells were HRBC specific and were derived from HRBC-tolerant mice. The general properties of these cells are described elsewhere (21). In the experiment presented in Table V either unfractionated or fractionated suppressor T-cell popula-

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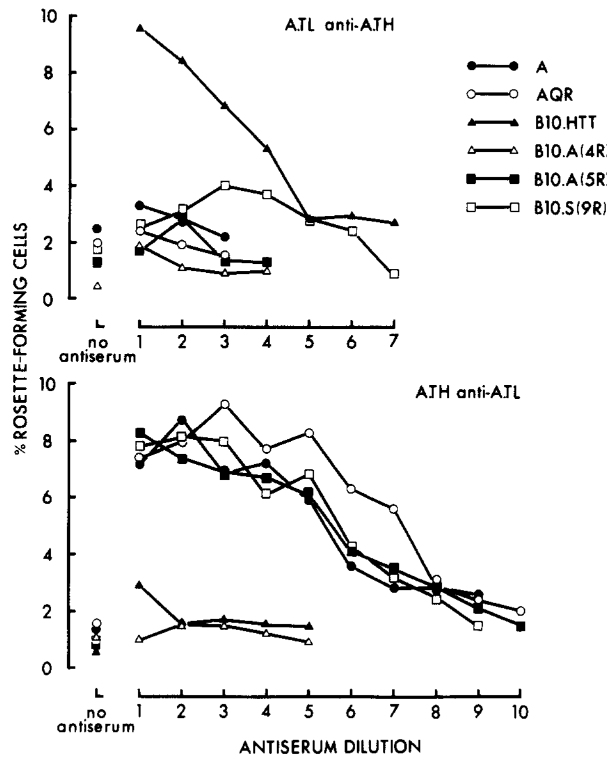

FIG. 4. Reactivity of two B-cell-absorbed anti-Ia sera with a range of *H-2*-recombinant mouse strains as measured by the rosetting assay. Tube 1, 1/40 dilution of antiserum.

TABLE II
Haplotype Origin of I–J Subregion in Various Mouse Strains as Judged by Rosetting Assays

Strain	I–J^k	I–J^s
C57BL/6	– (–)*	– (–)
BALB/c	– (–)	– (–)
CBA/H	+	–
DBA/1	–	–
A/Tb	+ (+)	– (–)
B10.A(5R)	+ (+)	– (–)
A.TL	+ (+)	– (–)
B10.S(9R)	+ (+)	– (–)
AQR	+ (+)	– (–)
B10.A(4R)	– (–)	– (–)
A.TH	– (–)	+ (+)
B10.HTT	– (–)	+ (+)

* Haplotype origin of *I–J* subregion according to Murphy et al. (reference 6, Table I) based on suppressor T-cell function.

tions were mixed with normal spleen cells and transferred into irradiated mice. The recipient mice were then challenged, i.p., 1 day and 4 days later with 5×10^8 HRBC. Anti-HRBC PFC were assayed 7 days after the first challenge.

Ig⁻ spleen cells from tolerant mice very effectively suppressed the anti-HRBC

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TABLE III

Summary of Method for Detecting Lymphocytes Bearing Ia Antigens Controlled by the I-J Subregion

1. Production of anti-Ia sera by immunizing recipients with pooled spleen and lymph node cells
2. Absorption of antisera with donor B (Ig^+) spleen cells
3. Reaction of serial dilutions of absorbed antisera with Ig^- donor spleen cells
4. Formation of rosettes by the addition of SRBC coated with sheep anti-mouse Ig

TABLE IV

Characterization of the Ig^- Lymphocytes Bearing I-J-Controlled Antigenic Determinants

Treatment	Lysis	I-J$^+$ cells after treatment*	Recovery I-J$^+$ cells
	%	%	%
GPC‡ control	<0.5	6.6	100
Anti-Thy-1.2 + GPC	63	0.6	3
RC‡ control	1	7.6	100
Anti-Ly-1.1 + RC	52	22.6	143
Anti-Ly-2.1 + RC	6	1.0	12

* % of Ig^- cells from CBA/H spleen binding anti-I-J^k antibodies as detected by rosetting. Before rosetting, lysed cells were removed by spinning the cell suspensions on Isopaque/Ficoll (19).

‡ GPC, guinea pig complement, RC, rabbit complement.

PFC response of normal spleen cells (Table V). When the population of suppressor T cells was rosetted with an antiserum against the complete *I* region, suppressive activity, particularly of the indirect PFC response, was recovered in both the rosette-enriched and rosette-depleted fractions (Table V). Similarly, when rosetting was performed with an anti-I-J serum, suppressive activity was obtained in both fractions of cells. Thus, the small subpopulation of T cells which react with anti-I-J serum have suppressor T-cell activity. The incomplete depletion of suppressor T cells probably has a technical explanation as only complete rosettes were recovered in the rosette fraction. Incomplete rosettes, which constituted approximately 20% of all rosettes, were recovered in the "rosette-depleted" fraction. Consistent with this conclusion was our observation that the unabsorbed A.TH anti-A.TL serum was, in the presence of C, cytotoxic for all of the suppressor T cells detected in this system.[2] Furthermore, the HRBC-specific suppressor T cells have been found to be Ly-1.1^- and Ly-2.1^+.[2]

Organ Distribution of Cells Bearing I-J Subregion-Controlled Antigens. A range of lymphoid tissues from CBA/H mice were assayed for their content of cells reactive with an anti-I-J^k serum (Table VI). As described earlier, each suspension of lymphoid cells was depleted of Ig^+ cells before anti-I-J rosetting. The values presented in Table VI were, in each case, derived from a complete anti-I-J titration.

[2] Ramshaw, I. A., I. F. C. McKenzie, P. A. Bretscher, and C. R. Parish. Discrimination of suppressor T cells of humoral and cell-mediated immunity by anti-Ly and anti-Ia sera. *Cell. Immunol.* In press.

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TABLE V

Suppressor T Lymphocytes Carry I–J Subregion Controlled Determinants Detected by Rosetting

CBA/H spleen cells transferred*	Cell no.	HRBC PFC/spleen	
		Direct	Indirect
Normal	1×10^7	7,380 ± 1,890	7,163 ± 2,454
Normal + Ig^- tolerant	1×10^7 2×10^7	312 ± 111	162 ± 60
Normal + Ia^-, Ig^- tolerant	1×10^7 1.8×10^7‡	2,736 ± 939	438 ± 231
Normal + Ia^+, Ig^- tolerant	1×10^7 2×10^6‡	2,124 ± 594	1,170 ± 384
Normal + $I\text{–}J^-$, Ig^- tolerant	1×10^7 1.8×10^7‡	1,245 ± 207	233 ± 117
Normal + $I\text{–}J^+$, Ig^- tolerant	1×10^7 2×10^6‡	3,450 ± 672	390 ± 378

* Ig^- spleen cells from mice made tolerant to HRBC by treatment with HRBC and cyclophosphamide (21). Rosetted Ig^- cells with either unabsorbed A.TH and A.TL serum (anti-I^k) or B-cell-absorbed A.TH and A.TL serum (anti-$I\text{–}J^k$). The rosetting and nonrosetting cells were separated on Isopaque/Ficoll (17) and the rosette-depleted (Ia^- and $I\text{–}J^-$ cells) and rosette-enriched populations (Ia^+ and $I\text{–}J^+$ cells) recovered.

‡ Recovery of cells when 2×10^7 cells were separated on Isopaque/Ficoll.

TABLE VI

Organ Distribution of Cells which Bear I–J-Subregion-Controlled Antigenic Determinants

Lymphoid organ*	$Thy\text{-}1^+$ cells	Cells bearing I–J-controlled determinants	
		Total	$Thy\text{-}1^+$
	%	%	%
Thymus	95	1.4	1.5
Lymph node	65	2.7	4.2
Spleen	24	3.2	13.3
Bone marrow	2.3	0.8	34.8

* Organs from CBA/H mice.

‡ Detected by rosetting Ig^- cells from each lymphoid organ with an anti-$I\text{–}J^k$ serum (A.TH anti-A.TL absorbed with B cells).

The content of anti-I-J-reactive cells was spleen, lymph node > thymus, bone marrow. It was subsequently found that all the anti-I-J-reactive cells in the different lymphoid organs were T cells as they were destroyed by anti-Thy-1.2 and C treatment. Thus, it was possible to compute the proportion of T cells in each organ which expressed *I-J*-controlled determinants (Table VI). It was found that a high proportion (35%) of the T cells in bone marrow reacted with

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anti-I-J antibodies, a substantial proportion (13%) of T cells from spleen were reactive, whereas the lymph node and thymus T-cell populations contained only a small proportion of reactive cells.

Discussion

The *Ia-4* locus within the *I-J* subregion of the *H-2* complex appears to code for surface antigens which are selectively expressed on T lymphocytes which suppress antibody responses (6). Thus, the proportion of cells in most lymphoid populations which react with anti-I-J sera is small and difficult to detect by the conventional dye-exclusion microcytotoxicity assay (6). To overcome this problem we developed a sensitive rosetting assay for detecting the binding of anti-Ia antibodies to T (Ig^-) lymphocytes. This assay system, unlike the C lysis technique, has a low background and since it represents a direct binding assay has the potential of detecting subpopulations of lymphocytes which react with the anti-I-J sera but which are not lysed by C.

Two anti-Ia sera were used in these studies, A.TH anti-A.TL and A.TL anti-A.TH both of which were absorbed with B (Ig^+) lymphocytes to eliminate antibodies directed against Ia antigens carried by B lymphocytes. The antibodies remaining after these B-cell absorptions were found to be directed against *I-J*-subregion-controlled antigens. This conclusion was based on genetic mapping studies using a range of recombinant mouse strains which had been previously mapped for *I-J* subregion haplotype by Murphy et al. (6).

It should be noted, however, that Murphy et al. (6) defined two antigenic specificities controlled by the *Ia-4* locus. One specificity was unique to the $Ia\text{-}4^s$ haplotype, whereas the other determinant was shared by the $Ia\text{-}4^s$ and $Ia\text{-}4^k$ haplotypes. The anti-I-J sera we used would be unable to detect this cross-reacting determinant. The antigenic specificities which were detected by our two anti-I-J sera appeared to be highly haplotype specific for $H\text{-}2^k$ and $H\text{-}2^s$.

Virtually all of the spleen cells which bound anti-I-J antibodies were found to be T lymphocytes which were $Ly\text{-}1.1^-$ and $Ly\text{-}2.1^+$. This is generally recognized as the surface phenotype of T lymphocytes which suppress antibody responses (16, 23). Consistent with this observation was our finding that anti-I-J-rosetting cells can possess suppressor T-cell activity (Table V). It is noteworthy that the bulk (>80%) of the $Ly\text{-}2.1^+$ T cells in normal CBA/H spleen appeared to carry *I-J*-subregion-controlled determinants (Table IV). Thus, killer T cells ($Ly\text{-}1^+$, 2^+, 3^+, Ia^-) and their precursors must represent a very minor T-cell subpopulation in normal CBA/H spleen (24).

Lymphoid organs differed in their content of cells bearing *I-J*-subregion-controlled antigens (Table VI). Spleen and lymph node contained the highest proportion of anti-I-J-reactive cells, whereas bone marrow and thymus had fewer reactive cells. In contrast, the proportion of T cells in each lymphoid organ reacting with anti-I-J antibodies was bone marrow > spleen > lymph node > thymus. Suppressor T cells of antibody responses have a similar anatomical distribution (25, 26). In fact, T cells from bone marrow appear to be highly suppressive.

Summary

Previous studies have demonstrated that *I-J*-subregion-controlled Ia antigens are only expressed on a small subpopulation of peripheral T lymphocytes which

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includes the suppressor T cells of antibody responses (6). This subpopulation of T cells cannot be detected by conventional dye-exclusion cytotoxicity tests. A sensitive rosetting procedure therefore was developed for detecting the binding of anti-Ia antibodies to T lymphocytes. This assay system, unlike the complement lysis technique, has a low background and since it represents a direct binding assay could detect noncomplement-fixing antibodies in the antisera.

Anti-Ia sera were absorbed with B cells and using the rosetting procedure in genetic mapping studies the remaining antibodies were found to be directed against *I-J*-subregion-controlled determinants. These determinants were shown to be highly haplotype specific for $H\text{-}2^k$ and $H\text{-}2^s$ and appeared to be exclusively expressed on $Ly\text{-}1.1^-$, $Ly2.1^+$, T lymphocytes, at least some of which were suppressor T cells. Lymphoid organs differed in their content of anti-I-J-reactive cells, the hierarchy being spleen, lymph node > thymus, bone marrow. In contrast, on a T-cell basis, a high proportion (35%) of the T cells in bone marrow reacted with anti-I-J antibodies, a substantial proportion (13%) of T cells from spleen were reactive, whereas the lymph node and thymus T-cell populations contained only a small proportion of positive cells (1–4%).

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