T-CELL-SPECIFIC MURINE Ia ANTIGENS: SEROLOGY OF I-J AND I-E SUBREGION SPECIFICITIES*

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The I region of the murine major histocompatibility complex (H-2)[1] was first defined by immune response (Ir) genes governing antibody synthesis to chemically defined antigens in inbred mice (1, 2). Recent studies suggest that other immunoregulatory genes are localized to this region as well. Specifically, T-B cell collaboration in antibody production (3), macrophage–T-cell interaction (4), proliferation in mixed lymphocyte culture (5), graft-versus-host reactivity (6, 7), virus susceptibility (8), and antigenic determinants on suppressor T cells (9), as well as suppressive (10) and enhancing factors (11) are governed by I-region genes. Because of the importance of immunoregulatory genes, great interest currently centers on I-region gene product identification.

Reciprocal immunization between I-region congenic mice (12–15) has revealed a complex system of I-region-associated (Ia) antigens (16). Their expression is confined to lymphocytes, macrophages, and epidermal and sperm cells (17). If Ia antigens or the cells which express them are involved in immunoregulation, it appears paradoxical that these antigens have been found predominantly on B cells (12–18), since immunoregulatory functions (Ir genes) are expressed in T cells (2). Initially, a controversy existed regarding T-cell Ia antigen expression; some investigators were able to demonstrate Ia-positive T cells (12, 13, 15), whereas others could not (14, 18–20). Subsequent results suggest that a T-cell subpopulation bears Ia determinants in diminished density compared to B cells (21–25).

Limited evidence suggests restricted expression of certain Ia specificities to cells of either B or T lineage, whereas others are common to both. Specificities Ia.1–Ia.10 appear indistinguishable on T and B blasts (23, 26). On the other hand, I-J region specificities have been detected exclusively on T cells (9). Thus, while several Ia antigens are common to B and T lymphocytes, others are unique to one or the other cell type.

The findings that there are Ia antigens restricted in thymus-derived cells (9, 25), and that a minor fraction of peripheral T Cells express Ia antigens in lower density than peripheral B cells (21–24), may explain the observed discrepancies regarding T-cell Ia antigen expression (12–20). If the antisera employed varied either in titer, antibody-antigen affinity, or in content of relevant T-cell Ia antibody by virtue of differing immunization protocols or I subregions studied, conflicting results might be expected.

We have employed an alternative approach to the study of T-cell Ia antigens. I-region disparate mice were immunized with an Ia-positive T-cell population, concan-

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[1] *Abbreviations used in this paper:* C′, rabbit serum complement; Con A, concanavalin A; H-2, murine major histocompatibility complex; Ia, I-region-associated; Ir, immune response; PBS, phosphate-buffered saline.

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TABLE I

H-2 Haplotype of Origin of Strains Used in This Study (29)

Haplotype	Strain	K	H-2 Region I					S	G	D
			A	B	J	E	C			
H-2^{bi3}	B10.A(3R)	b	b	b	b	k	d	d	d	d
H-2^{bi5}	B10.A(5R)	b	b	b	k	k	d	d	d	d
H-2^{tu}	B10.HTT	s	s	s	s	k	k	k	k	d
H-2^{b}	B10	b	b	b	b	b	b	b	b	b
H-2^{d}	B10.D2	d	d	d	d	d	d	d	d	d
H-2^{k}	B10.BR	k	k	k	k	k	k	k	k	k
H-2^{s}	B10.S	s	s	s	s	s	s	s	s	s
H-2^{q}	B10.G	q	q	q	q	q	q	q	q	q

avalin A (Con A)-stimulated thymocyte blasts (26–28). The genetic disparity between donor, B10.A(5R), and recipient mice, (B10 × B10.D2)F_1, was restricted to the I-J and I-E subregions of the H-2 complex (9, 29). The H-2 haplotypes of origin of strains used in this study are given in Table I. This immunization protocol produced a strikingly T-cell-specific anti-I-J^kE^k serum of high titer and limited haplotype cross-reactivity. These results and implications for Ia antigen serology are discussed.

Materials and Methods

Mice. Mice were obtained from our colony at the University of Wisconsin. Both male and female animals were used.

Media. All media and supplements were obtained from Grand Island Biological Co., Grand Island, N. Y., except as otherwise noted.

Con A Stimulation of Thymocytes. Thymuses were removed from asphyxiated 4–6-wk-old mice into cold Hanks' balanced salt solution. Capsular tissue was carefully removed, the thymus was teased gently apart using a needle and forceps, and a single cell suspension was prepared. Thymocyte blasts were prepared according to David et al. (26). Briefly, cells were suspended (10–15 × 10^6/ml) in 25 mM Hepes-buffered RPMI-1640 containing fetal calf serum (2.5%), 2-mercaptoethanol (25 μM; Sigma Chemical Co., St. Louis, Mo.), L-glutamine (20 mM), and antibiotics (50 U/ml penicillin, 50 μg/ml streptomycin). Con A (5 μg/ml; Pharmacia Fine Chemicals, Uppsala, Sweden) was added, and cultures were incubated at 37°C for 48 h in a humidified atmosphere of 5% CO_2 in air. Stimulation was monitored by pulsing sample cultures (0.5–1.0 × 10^6 cells in 0.2 ml medium) with [^{3}H]thymidine (2 μCi; New England Nuclear, Boston, Mass.) for 6 h, and measuring isotope uptake. Cultures were harvested, washed three times in phosphate-buffered saline (PBS) containing methyl α-D-mannopyranoside (0.1 M; Sigma Chemical Co.), and resuspended in PBS.

Immunization. Each recipient mouse was injected intraperitoneally with 20 × 10^6 viable cells in 0.5 ml of an emulsion made from equal volumes of PBS-cell suspension and complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). Subsequent injections of 15–30 × 10^6 cells in 1 ml PBS were given intraperitoneally at weekly intervals. Mice were bled from the retro-orbital sinus 1 wk after the sixth and seventh injections. These two bleeds were employed throughout this investigation. Serum was stored in aliquots at −80°C.

Serum Absorption. Autoantibody in (B10 × B10.D2)F_1 anti-B10.A(5R) serum was removed as follows. Antiserum was diluted 1/5 with PBS and 0.5 ml absorbed three times (1 × 10^8 pelleted spleen plus lymph node cells per absorption, 15 min on ice) with B10 and three times with B10.D2. For absorption experiments, antiserum depleted of autoantibody was diluted 1/25 in Hanks' balanced salt solution, and incubated with increasing numbers of lymph node cells for 30 min on ice. The cells were then pelleted, and residual cytotoxicity was assayed as described.

Microcytotoxicity Test. Lymph node cell suspensions were prepared from the inguinal, axillary,

brachial, cervical, and mesenteric nodes of 8–12-wk-old mice. Lymph node, spleen, or thymus cell suspensions were sedimented on Ficoll-Hypaque (Sigma Chemical Co. (30) to remove erythrocytes and dead cells. The dye-exclusion microcytotoxicity test in Terisaki plates (Cooke Laboratory Products, Div. Dynatech Laboratories Inc., Alexandria, Va.) was performed as described by Frelinger et al. (21), with modifications suggested by D. B. Murphy (personal communication). Briefly, 2 μl of cell suspension (1×10^6/ml in medium with 5% fetal calf serum) was added to 2 μl of appropriately diluted antiserum, and plates were incubated for 15 min at 37°C. One drop of medium 199 was then added to each well and the plates were flicked after 10 min. After the addition of 2 μl of complement, lysis proceeded for 30 min at 37°C, whereupon a drop of nigrosine dye (0.1% in fetal calf serum-containing medium) was added to each well. Plates were again flicked after 10 min, wells were filled with medium (5% fetal calf serum), and the percentage of dead cells was determined. Rabbit serum, absorbed twice with mouse splenocytes (2×10^8 cells/ml serum) and diluted 1/20 with medium 199 served as the source of complement. Complement controls, omitting antiserum, were carried out in quadruplicate for each target cell. Lysis in complement (C′) controls was ≅10%. Percent of cells lysed was calculated according to the formula:

$$\% \text{ cells lysed} = 100 + \left[\frac{\% \text{ dead experimental} - \% \text{ dead C}' \text{ control}}{100\% - \% \text{ dead C}' \text{ control}}\right].$$

Cell Separation. Cell suspensions were enriched for T lymphocytes by passage through nylon wool columns (Fenwall Laboratories, Inc., Morton Grove, Ill.) (31). Nylon wool-passed cells were assayed immediately for Ia antigen expression, or alternatively, cultured in RPMI-1640 supplemented with 2.5% fetal calf serum and 25 μM 2-mercaptoethanol for 12 h at 37°C in a humidified atmosphere of 5% CO_2 in air. To obtain a cell population enriched for B lymphocytes, spleen cells (30×10^6/ml in RPMI-1640 with 50% fetal calf serum) were first incubated in plastic dishes for 1 h at 37°C, transferred to a second set of dishes for 1 h, and the nonadherent cells were collected. T cells were lysed using anti-Thy 1.2 serum (40×10^6 cells, 1 ml serum diluted 1/30, generously given by Doctors F-W. Shen and E. A. Boyse, Sloan-Kettering Institute for Cancer Research, New York) and C′ (absorbed and diluted as detailed above) in a two-step procedure.

Alloimmunization. Recipient mice received 2×10^7 x-irradiated (2,500 R) donor spleen cells in each hind footpad. 3 days later, spleen and lymph nodes (excepting the draining popliteal nodes) were excised and assayed for reactivity with antiserum.

Results

Con A stimulation of B10.A(5R) thymocytes resulted in a 10–20% recovery of the initial cell number; 20–40% of viable cells were blasts morphologically. The ratio of [^{3}H]thymidine uptake by stimulated cultures to that of controls was usually ≅50:1. Recipient (B10 × B10.D2)F_1 mice developed reasonable antibody titers (2,560–5,120) against B10.A(5R) or B10.BR lymph node cells after the sixth injection with B10.A(5R) thymocyte blasts. There was considerable cytotoxicity against B10 and B10.D2 lymph node cells. This was removed by absorption with B10 and B10.D2 splenocytes and lymph node cells before all further analysis.

The antiserum, hereafter designated anti-I-J^kE^k serum, was titrated in the standard dye exclusion microcytotoxicity assay on lymph node, spleen, and thymus cells from a representative haplotype panel. These results are presented in Table II. Only H-2^k, H-2^q, and H-2^s lymph node cells were lysed to a significant and approximately equal (27–30%) extent. Titration on H-2^k splenocytes consistently (five experiments) gave a low degree of lysis (5.3 ± 2% at dilutions <1/20). All other cell populations tested gave 0–5% lysis. Lysis of these cells was variable: it was sometimes negative and usually <3%. They are represented as <3% in Table II, and are considered negative. Thus, the Ia antigen(s) detected by this serum are expressed on lymph node cells and a very minor fraction of spleen cells.

TABLE II

Cytotoxicity of (B10 × B10.D2)F_1 Anti-B10.A(5R) Serum on a Haplotype Panel

Haplotype	Strain	Percentage of cells lysed		
		Lymph node	Spleen	Thymus
H-2^b	B10	<3	ND*	<3
H-2^d	B10.D2	<3	ND	<3
H-2^f	B10.M	<3	<3	<3
H-2^k	B10.BR	27.2 ± 3‡	5.3 ± 2	<3
H-2^q	B10.Q	30 ± 2	<3	<3
H-2^r	B10.RIII	<3	ND	<3
H-2^s	B10.S	28 ± 2	<3	<3

* ND, not determined.

‡ Mean and standard deviation from five experiments.

Titration curves for B10.BR (H-2^k) lymph node and spleen cells are shown in Figs. 1 A and D. When these cell populations were specifically enriched for T cells by nylon wool passage, and nonadherent cells were assayed immediately, they showed no reactivity with anti-I-J^kE^k serum (data not shown). If, however, a 12-h culture period preceded testing, the results shown in Figs. 1 B and E were obtained; there was no significant difference in either percentage of cells lysed (≅35–40%), nor antiserum titer between nylon wool nonadherent lymph node and spleen cells. Splenic B lymphocytes, obtained after removing macrophages by adherence to plastic, and T lymphocytes by anti-Thy1.2 plus C′ treatment, were not reactive with this serum (0–2% lysis), nor did they become reactive after a 12-h culture period. Therefore, the antigen(s) detected appear to be expressed only on T lymphocytes.

Lymphocytes from B10.BR mice immunized with irradiated, allogeneic B10.M spleen cells, showed greater reactivity with anti-I-J^kE^k serum than did lymphocytes from normal mice (Figs. 1 C and F). Specifically, antiserum cytotoxicity against lymph node cells increased from 27 ± 3% to 40%, against splenocytes from 5.3 ± 2% to 20 ± 2%. Lymphocytes, activated in a 5-day in vitro allogeneic mixed lymphocyte culture, likewise showed increased cytotoxicity with this antiserum (data not shown). Furthermore, Con A stimulation of spleen cells induced an increase in percentage of spleen cells lysed by antiserum from 5.3 ± 2% to 25 ± 2%. These results suggest that Ia antigen expression was either altered as a result of alloantigen or mitogen activation, or that such activation led to the selective expansion of one T-cell subpopulation.

Anti-I-J^kE^k serum contains antibody to the I-E^k subregion. B10.S-absorbed serum lysed ≅20% of recombinant B10.HTT lymph node cells (Fig. 2). Reaction of recombinant B10.HTT (I-$A^sB^sJ^sE^kC^k$) (9, 29) with B10.S-absorbed serum can be attributed to an I-E region-encoded antigen of the H-2^k haplotype, which is not shared by H-2^s strains (29). This conclusion was supported by antiserum reactivity with 20% of B10.A(3R) (I-$A^bB^bJ^bE^kC^d$) (9, 29) lymph node cells (Fig. 3 A). Because the antiserum was produced in (B10 × B10.D2)F_1 mice and absorbed with B10 and B10.D2 lymphocytes, the antigen detected seems to map within I-E^k.

(B10 × B10.D2)F_1 anti-B10.A(5R) serum also contains cytotoxic antibody for I-J^k subregion-encoded determinants. When the antiserum was absorbed with B10A.(3R) (I-$A^bB^bJ^bE^kC^d$) lymph node cells to remove I-E^k reactivity, 12 ± 3% cytotoxicity on

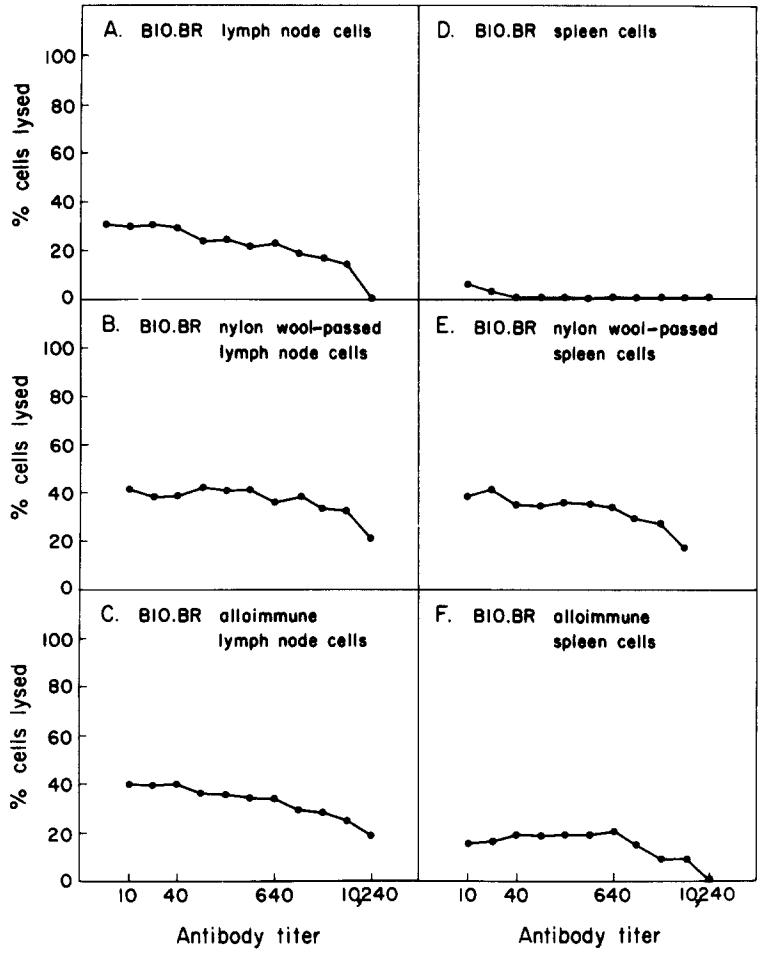

FIG. 1. Cytotoxicity of (B10 × B10.D2)F_1 anti-B10.A(5R) (anti-I-J^kE^k) serum on lymph node and spleen cells. Nylon wool-passed cells (B and E) were cultured 12 h before cytotoxicity assay. Alloimmune cells (C and F) were taken 3 days post-immunization with irradiated B10.M splenocytes (see Materials and Methods). Standard deviation was ± 10% of the mean.

B10.BR lymph node cells remained (four experiments; Fig. 3 B and Fig. 4 B). Additional absorption did not remove residual antibody. This determinant, therefore, appears to map within the I-J^k subregion (9).

Absorption studies were conducted to determine if haplotypes H-2^q and H-2^s cross-react with H-2^k by virtue of I-J^k antibody, I-E^k antibody, or both (Fig. 4). Absorption with B10.BR (H-2^k) lymph node cells removed cytotoxic antibody for B10.G (H-2^q), B10.S (H-2^s), and B10.A(3R) lymph node cells (Fig. 4 A). However, B10.A(3R) absorption did not remove cytotoxic antibody for B10.S or B10.G lymphocytes (Fig. 4 B), nor did B10.G (Fig. 4 C) or B10.S (Fig. 4 D) absorption remove cytotoxic antibody for B10.A(3R) cells. These results suggest that the I-E^k antigen described is not shared with haplotypes H-2^q and H-2^s. Strains B10.G and B10.S absorb some reactivity against B10.BR lymph node cells indicating cross-reactivity with an I-J^k

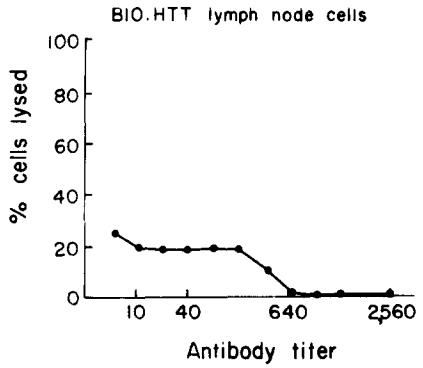

FIG. 2. Cytotoxicity of B10.S-absorbed anti-I-J^kE^k on lymph node cells of recombinant B10.HTT. Antiserum (100 μl of a 1/5 dilution) was absorbed with B10.S lymph node cells until no further activity remained (20 × 10^6 cells/absorption, three times).

subregion specificity; residual lysis of B10.BR cells may be attributed to an I-E^k subregion antigen(s), or possibly a second I-J^k antigen.

Discussion

These experiments illustrate the production of a T-cell specific, cytotoxic anti-I-J^kE^k serum using Con A-stimulated thymocyte blasts as immunogen. Con A-stimulated lymphoblasts have been used previously for Ia antiserum production (28). In contrast to the present report, the Ia antiserum described (28) lysed 95% of spleen or lymph node cells, exhibited extensive cross-reactivity with other haplotypes, and reacted with B lymphocytes as well as T cells. Götze (28) employed lymphoblasts from spleen and lymph node cells and studied I subregions A, B, J, and E—either or both of which may account for the difference in results obtained.

The induction of anti-I-J^k antibody is not surprising in view of the finding that I-J subregion Ia antigens are expressed on Con A-reactive T cells (29, 32). Moreover, I-E specificities have been reported on Con A-stimulated thymocytes (29). That high titered, T-cell specific antiserum can be raised against mitogen-stimulated T-cell blasts may be due to increased T-cell Ia antigen density on these cells, or alternatively, to selective expansion of that T-cell population which is Ia positive. Thus it has been proposed that activation, either by mitogen or alloantigen, may be required for full expression of T-cell Ia determinants (24, 26, 33). The results reported herein support this hypothesis.

The finding that nylon wool-nonadherent cells were not reactive with anti-I-J^kE^k serum immediately after column passage, but became reactive after 12 h in culture deserves comment. Two results argue that the antigens detected are probably not artifactually introduced as a consequence of culturing. First, lymph node cells taken directly from a donor mouse do not require culturing in order to express antigenic determinants. Second, B cells did not acquire antigenic determinants after 12 h in culture. If T-cell Ia antigens are adherent to nylon wool and are readily shed from the cellular membrane, it is possible that nylon wool columns may strip Ia antigens from the T-cell surface. This would convert Ia-positive cells into Ia-negative cells; a period of time in culture would then permit resynthesis and/or reexpression of these antigens.

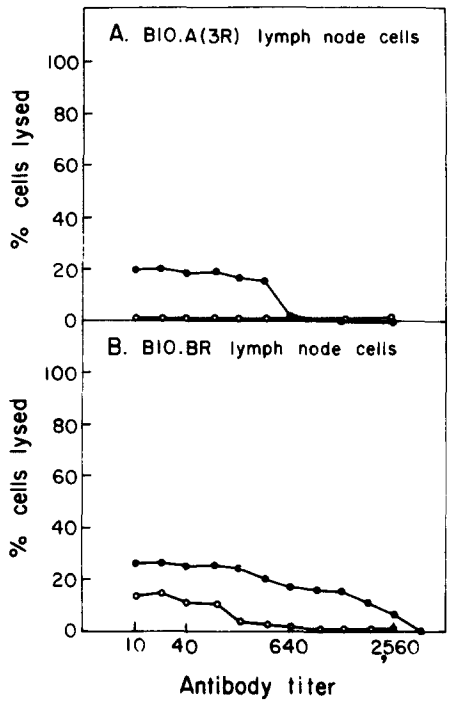

FIG. 3. Cytotoxicity of anti-I-J^kE^k serum on B10.A(3R) and B10.BR lymph node cells. Serum reactivity before absorption, (●); after twice absorbing 50 μl of 1/5 diluted serum with 20×10^6 B10.A(3R) lymph node cells, (○).

The conversion of Ia positive to negative cells by nylon wool-column passage has been suggested previously (21, 23).

Since (B10 × B10.D2)F_1 recipient mice (TL.2) differed from B10.A(5R) donor mice (TL.1, 2, 3, 5) at the T1a locus, and TL antigens are expressed on thymocytes, the possibility of anti-TL antibody must be considered (34–36). TL antigens are not expressed on lymph node cells (34), with which this antiserum reacts most strongly. Moreover, B10.HTT carries the same T1a allele as B10.D2. Therefore, the anti-I-E activity measured cannot be due to TL activity. Likewise, anti-I-J activity cannot be attributed to TL; B10.A(3R)-absorbed serum was tested on TL^- B10.BR cells.

A second T-cell antigen, Qa-1, might also have elicited antibody (37). B10.A(5R), derived from B10.A ($Qa\text{-}1^+$), differs at this locus from B10 and B10.D2 (both $Qa\text{-}1^-$) (37). However, test strains B10.HTT and B10.BR ($Qa\text{-}1^-$) cannot react by virtue of the Qa-1 antigen, indicating that the specificities detected are not products of this locus. B10.D2, B10, and B10.A recombinant B10.A(5R) share the Qa-2 allele, ruling out products of this locus having elicited antibody (38). To determine whether the antiserum contains antibody or other H-2-linked antigens will require further experimentation.

The I-J subregion of H-2, marked by locus Ia-4, controls antigenic determinants expressed on a subclass of T lymphocytes, suppressor T cells (9, 39). These determinants are not present on B lymphocytes (9, 39). Although suppressive function may be abolished by anti-I-J serum plus C′, conventional cytotoxicity assays have failed to demonstrate I-J antigens on either spleen or lymph node cells (9, 35). Thus, Murphy

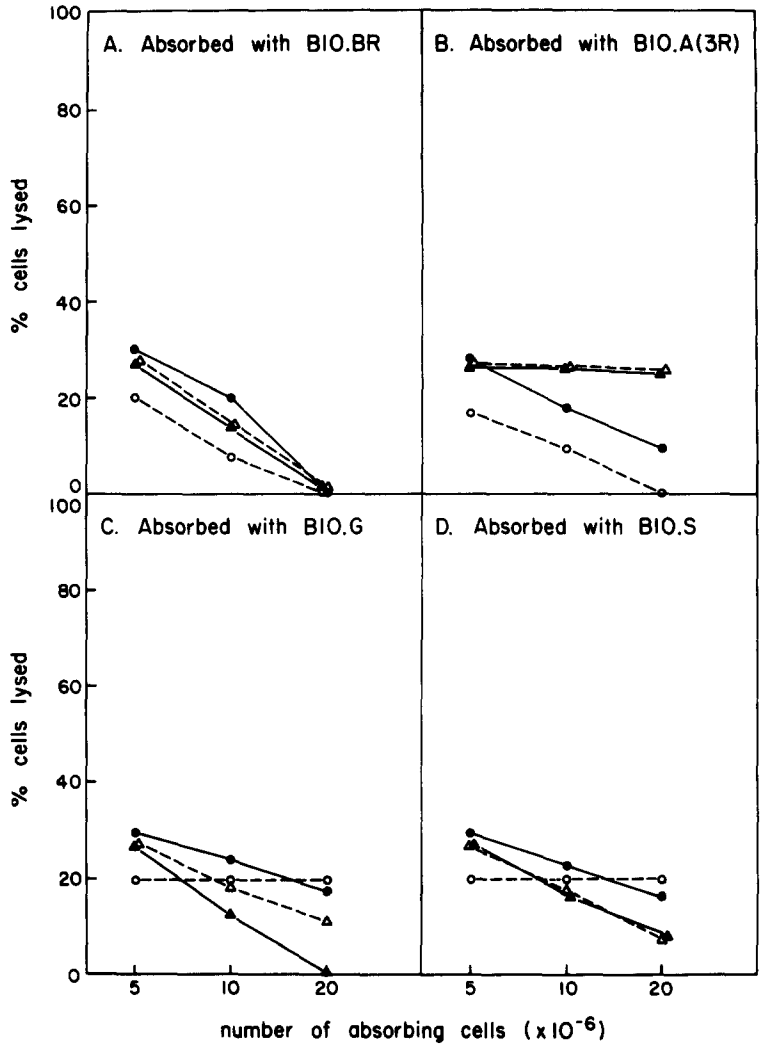

FIG. 4. Absorption of anti-I-J^kE^k serum with B10.BR, B10.A(3R), B10.G, and B10.S lymph node cells. Experimental details are described in Materials and Methods. B10.BR, (●); B10.A(3R), (○); B10.G, (▲); B10.S, (△).

et al. (9) estimated that I-J-bearing cells comprise <10% of spleen or lymph node lymphocytes. Parish and McKenzie (40), employing a rosetting assay, suggested that 8% of Ig^- splenocytes displayed I-J specificities. Finally, Okuda et al. (41) estimated that 10% of nylon wool-nonadherent lymph node cells express the I-J determinant. Although no estimate of the percentage of I-E positive T cells was given, these determinants were weakly expressed on some T cells (29). Unlike these previously described antisera, the antiserum described herein is very clearly cytotoxic for T lymphocytes from lymph node and spleen; 35–40% of nylon wool-nonadherent lymphocytes were lysed by anti-I-J^kE^k serum plus C′. This is a far higher proportion than that found in prior studies (9, 29, 39–41). Considering the results obtained with lymph node cells (27% lysed by anti-I-J^kE^k; 10% lysed by anti-I-J^k; 20% lysed by anti-

$I\text{-}E^k$), it appears possible that those cells expressing $I\text{-}J^k$ specificities and those expressing $I\text{-}E^k$ determinants may be nonoverlapping T-cell subsets. Okuda et al. (41) suggested that I-J-positive and I-E-positive cells were included in the same T-cell subset.

In the present report, the assignment of an Ia specificity to the I-E subregion of H-2 is based on the assumption that strain B10.A (from which B10.A(3R) and B10.A(5R) were derived) carries the $Ia\text{-}3^d$ rather than the $Ia\text{-}3^k$ allele (29). If this is not the case (B10.A carries the I-C subregion of the k parent), the specificity described herein might well be an I-C rather than I-E subregion-encoded antigen (42). Assuming that B10.A carries $I\text{-}C^d$, the specificity described in this report may be Ia.22 (29). This antigen was reportedly expressed on thymocyte blasts; however, Ia.22 was also detected on lipopolysaccharide blasts (29). The authors concluded that Ia.22 was predominantly expressed on B cells (29). In the present report, unstimulated B cells did not react with anti-$I\text{-}J^kE^k$ serum. Thus, it is not clear on the basis of cellular expression whether the antigen described is Ia.22. It is noteworthy that an anti-$I\text{-}A^kB^kJ^kE^k$ serum lysed T but not B lymphocytes of strain B10.HTT ($I\text{-}A^sB^sJ^sE^k$) (25). There are two possible interpretations of this result. Either anti-$I\text{-}A^kB^kJ^kE^k$ serum reacted with B10.HTT T cells by virtue of an $I\text{-}E^k$-encoded antigen expressed only on T cells, in which case the result supports our finding, or T-cell lysis was due to cross-reactivity between $I\text{-}A^sB^sJ^s$ of B10.HTT and $I\text{-}A^kB^kJ^kE^k$ of AQR (against which the serum was produced). The I-E-region specificity described in the present report is unique to the $H\text{-}2^k$ haplotype, as is Ia.22 (29).

The finding that an $I\text{-}J^kE^k$ serum, raised against Con A-stimulated thymocyte blasts, does not react with B lymphocytes has important implications for Ia antigen serology, as well as studies of Ia antigen expression on functionally distinct T-cell subpopulations. This result suggests that a group of I region-encoded antigens, unique to T lymphocytes, may not have been detected because they would have comprised only a minor antigenic component in conventional immunization protocols (skin grafting and/or spleen and lymph node cell immunization), in contrast to the B-cell Ia antigens. If this is the case, T cells that have been described as Ia negative (cytotoxic T lymphocytes (43), T lymphocytes mediating delayed hypersensitivity (44), and others) may express Ia antigens not yet described. Experiments examining this question are in progress. Conceptually, the demonstration of Ia determinants associated with different subregions of H-2 I, and present on substantial percentages of the T lymphocytes in lymph nodes and spleens (with different determinants probably present on different cell populations), establishes another potential link between Ia antigens and T-lymphocyte immune response function. Although these data are consistent with the hypothesis that molecules (or lymphocytes) carrying Ia determinants play a role in immunoregulatory communication among lymphocytes, a direct confirmation of this hypothesis awaits further exploration.

Summary

(B10 × B10.D2)F_1 mice were immunized with B10.A(5R) concanavalin A-stimulated thymocyte blasts. The genetic disparity between donor and recipient was restricted to the I-J and I-E subregions of the murine major histocompatibility (H-2) complex. A high-titered, T-cell-specific anti-$I\text{-}J^kE^k$ serum was obtained. The antiserum lysed 27–30% of haplotype k, q, or s lymph node cells, 5.3 ± 2% of haplotype k spleen

cells, and did not lyse thymocytes. Nylon wool-passed lymph node or spleen cells (H-2^k) showed considerable reactivity with anti-I-J^kE^k serum (35–40% lysis); anti-Thy1.2 plus complement-treated spleen cells did not react (<5% lysis). I-E^k antibody was detected by B10.A(3R) lymph node cell reactivity (20% lysis), whereas reaction with H-2^k lymph node cells after B10.A(3R) absorption demonstrated I-J^k antibody (12% lysis). Lymphocyte activation with alloantigen or mitogen led to increased anti-I-J^kE^k serum reactivity. These results, showing antibody production to at least two T-cell Ia antigenic determinants by concanavalin A thymocyte blast immunization, suggest that a group of I-region-encoded T-cell specificities may not have been detected using conventional immunization protocols because they would not have comprised a major antigenic component of the immunizing cell population. The existence of multiple Ia antigenic determinants unique to T lymphocytes would have important implications for serological and functional studies of T-cell subpopulations.

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