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Antigen presentation by Ia$^+$ B cell hybridomas to *H-2*-restricted T cell hybridomas

(T cell specificity/associative recognition/T cell clones/B cell clones)

JOHN KAPPLER*†, JANICE WHITE*†, DALE WEGMANN*†, ERIC MUSTAIN*†, AND PHILIPPA MARRACK*‡

*Department of Medicine, National Jewish Hospital and Research Center, Denver, Colorado 80206; and Departments of †Microbiology and Immunology and ‡Biochemistry, Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado 80262

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ABSTRACT **The Ia$^+$, *H-2*^{d} BALB/c lymphoma cell line L10.A 2J was fused to T cell-depleted spleen cells from mouse strains bearing other *H-2* haplotypes. A portion of the selected hybrids expressed Ia antigens of the normal spleen cell partner in the fusion as evidenced by their presentation of various antigens to a set of antigen-specific *I*-region-restricted T cell hybridomas. Three cloned hybrids were studied in detail. Antigen presentation was shown to be inhibited specifically by monoclonal anti-Ia antibodies. Both I-A and I-E molecules were expressed, including in the one case examined hybrid I-A and I-E molecules between the *H-2*^{d} and *H-2*^{b} haplotypes. These Ia$^+$ B cell hybridomas provide a useful set of tools for studying the role of *I*-region-encoded molecules in antigen presentation to T cells.**

Relatively little is known about how cells present antigens in association with products of the major histocompatibility complex (MHC) to antigen-specific MHC-restricted T cells. Recently we reported two tools that may be useful in the study of this problem. The first was a series of cloned, antigen-specific, *I*-region-restricted T cell hybridomas that grew constitutively but produced the lymphokine interleukin-2 (IL-2) only when presented with antigen by cells bearing the appropriate *I*-region gene products (1, 2). The second was a series of Ia$^+$ B cell lymphomas that were capable of presenting antigen to a number of T cell hybridomas (3). One of the shortcomings of this latter study was that all of the B cell lymphomas that successfully presented antigen were of *H-2*^{d} origin, thus limiting their use to antigen presentation involving either the I-A^d or the I-E^d molecule.

We report here three antigen-presenting cloned hybrids produced by the fusion of the *H-2*^{d}, Ia$^+$, BALB/c B cell lymphoma L10.A 2J (3–5) to normal T-cell-depleted spleen cells from *I*^{b}, *I*^{k}, and *I*^{s} strains. These hybrids were tested with an extended bank of *I*-region-restricted, antigen-specific T cell hybridomas and found to behave as F_1 antigen-presenting cells. This series of cloned antigen-presenting and T cell hybrids provides a useful set of tools to study the problem of *I*-region-restricted antigen presentation to T cells.

MATERIALS AND METHODS

Mice. C57BL/10 (B10) and B10.D2 mice were either purchased from The Jackson Laboratory or bred here at National Jewish Hospital and Research Center from stock obtained from Jackson. B10.BR mice were purchased from Jackson. B10.S (7R) mice were bred at National Jewish Hospital and Research Center from stock obtained from Donald Shreffler (Washington University, St. Louis, MO).

Culture Conditions. All cells were cultured in modified Mishell–Dutton medium as described (1–3, 6). Medium was supplemented with 10% fetal calf serum and 50 μM 2-mercaptoethanol.

Reagents and Antigens. Polyethylene glycol (M_r 6,000) was obtained from Baker. Hypoxanthine, aminopterin, thymidine, and hen ovalbumin (OVA) were obtained from Sigma. Keyhole limpet hemocyanin (KLH) was obtained from Calbiochem–Behring. Sendai virus (UV-inactivated) was purchased from Microbiological Associates (Walkersville, MD).

B Cell Lymphoma L10.A 2J. The BALB/c lymphoma cell line L10.A was originally produced by Kim, *et al.* (4). Michael Kuehl of the University of Virginia prepared an azaguanine-resistant subline of L10.A. This subline was recloned by David McKean (5), selecting for high Ia expression to yield the subclone L10.A 2J. We obtained this subclone from Noel Warner (University of New Mexico, Albuquerque, NM).

T Cell Hybridomas. The cloned T cell hybridomas used in these studies were produced and characterized as described (1–3). Briefly, normal T cell blasts enriched in antigen-specific, *I*-region-restricted cells were fused to either an azaguanine-resistant subline of the AKR thymoma, BW5147, or to azaguanine-resistant sublines of hybrids derived from BW5147: AO-40 or AO-40.10 (1). The resultant hybrids selected in hypoxanthine/aminopterin/thymidine (HAT) medium were tested for their ability to produce IL-2 when challenged with antigen presented by irradiated spleen cells syngeneic to the normal T cell blasts in the fusion. A high proportion of the hybrids (generally greater than 50%) tested positive in this assay. A few hybrids from each fusion were cloned at limiting dilution and the clones were tested similarly for IL-2 production. One clone of each hybrid was selected as standard, and its *I*-region restriction was determined with antigen and spleen cells from a bank of *H-2*-congeneic, recombinant, and F_1 mice. In most cases this specificity was confirmed by inhibition of antigen presentation with monoclonal anti-Ia antibodies. Table 1 lists the hybrids used in these studies with their origins and specificities. Except for 4AS-262, all were cloned.

B Lymphoma Fusion Protocol. Anti-T cell serum plus complement-treated spleen cells (7) were prepared from a number of *H-2* congeneic mouse strains. These were fused to L10.A 2J by standard methods (8, 9). Briefly, about 1–2 × 10^8 T cell-depleted spleen cells were fused to about 2–3 × 10^7 L10.A 2J cells for 2 min at 37°C in serum-free culture medium containing 40% (wt/wt) polyethylene glycol 6000. The mixture was slowly diluted with serum-free medium at 37°C to 50 ml, incubated for

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Abbreviations: Ia, surface structures associated with the products of the *I* region of the *H-2* complex; IL-2, interleukin-2; MAb, monoclonal antibody; Ag, antigen; OVA, hen ovalbumin; KLH, keyhole limpet hemocyanin; HAT, hypoxanthine/aminopterin/thymidine.

Table 1. T cell hybrids used in these studies

T cell hybrid	Fusion partners: Normal T cell blast Strain	I	Ag	Tumor T cell	Hybrid specificity: Ag + Self I	Allo I	Range of IL-2 production in response to Ag plus syngeneic irradiated spleen cells, units/ml
BDK-23.1	$B6D2F_1$	b/d	KLH	BW5147	KLH + $I\text{-}A^d$	—	160–640
AODK-1.16	B10.D2	d	KLH	AO-40	KLH + $I\text{-}E^d$	—	20–80
BDK-44.5*	$B6D2F_1$	b/d	KLH	BW5147	KLH + $I\text{-}A^{b/d}$	—	320–640
BDK-38.2†	$B6D2F_1$	b/d	KLH	BW5147	KLH + $I\text{-}E^{d/b}$	—	320–640
AODH-3.4‡	DBA/2	d	HGG	AO-40.10	OVA + $I\text{-}A^k$	$I\text{-}A^b$	160–640
4AS-262	B10.A	k/d	Sendai	BW5147	Sendai + $I\text{-}E^k$	—	160–320
SKK-17.1	$(C58 \times SJA)F_1$	k/s	KLH	BW5147	KLH + $I\text{-}A^s$	—	320–640
SKK-37.3	$(C58 \times SJA)F_1$	k/s	KLH	BW5147	KLH + $I\text{-}A^s$	—	160–640

Ag, antigen; HGG, human gamma globulin.
* Restricted by hybrid I-A molecule: $A^b_\alpha A^d_\beta$.
† Restricted by hybrid I-E molecule: $E^d_\alpha E^b_\beta$.
‡ Parental AO-40.10 tumor specific for $I\text{-}A^k$ + OVA and crossreactive on $I\text{-}A^b$ without OVA. AODH-3.4 retained this specificity but not that of the normal T cell parent. This hybrid was used in these studies because its responses to both $I\text{-}A^k$ + OVA and to $I\text{-}A^b$ were greater than those of AO-40.10. See ref. 1.

10 min at 37°C, pelleted by centrifugation, resuspended in 50 ml of balanced salt solution, and centrifuged again. The pellet was resuspended in complete culture medium and distributed in six to eight 96-well microculture plates at 100 μl/well. After 24 hr of culture HAT medium was added. The medium was changed every 5 days to fresh medium containing HAT and 30% medium conditioned by L10.A 2J. Hybrid growth was evident by 10 days. Cells from a portion of the wells showing confluent growth were transferred to larger vessels in order to obtain enough cells for testing antigen-presenting function.

Cloning of B Cell Hybrids. B cell hybrids selected for cloning were cultured at limiting dilution in 96-well microculture plates containing 100 μl of culture medium, 2×10^4 L10.A 2J cells as feeder cells, and HAT. Medium was changed and the clones were picked and tested for antigen presentation as with the primary fusion cultures.

Testing of Antigen Presentation. The ability of L10.A 2J and its hybrids to present antigen to T cell hybridomas was tested as described (1–3). Briefly, 200-μl microcultures were prepared containing 10^5 hybridoma T cells, $1–2 \times 10^5$ presenting cells and, if required, antigen. OVA and KLH were added to cultures as 200 μg of soluble protein. Sendai virus was added to cultures in the form of virus preadsorbed to antigen-presenting cells. This was accomplished by preincubating L10.A 2J or its hybrids at 10^6 per ml with UV-inactivated Sendai virus at 40 hemagglutinating units/ml in serum-free balanced salt solution for 10 min at 0°C, then 30 min at 37°C. The mixed T cell hybridoma cells, antigen, and antigen-presenting cells were cultured for 24 hr, at which time the culture supernate was harvested and assayed in 1:1 dilutions for the level of IL-2, using the IL-2-dependent T cell line HT-2 (10) as described. Results are presented as units of IL-2 per ml of undiluted supernate.

Monoclonal Anti-Ia Antibodies. Four monoclonal antibodies (MAbs) specific for Ia antigens were used in these studies. These are described below with their relevant properties. The ability of these antibodies to inhibit antigen presentation by irradiated spleen cells to the T cell hybrids listed in Table 1 was determined in preliminary experiments.

MK-D6. The MAb was produced in our laboratory (1). It is a γ2a antibody that reacts with $I\text{-}A^{d,p,q}$ but not $I\text{-}A^{s,f,b,k}$. Recently several lines of evidence, including immunoprecipitation data (P. Jones, Stanford University, personal communication) and T cell hybrid inhibition studies in our laboratory, indicated that this MAb detects as well hybrid molecules containing A^d_β chain in association with either A^s_α or A^b_α chain. This MAb hybridoma cell line can be obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Its antibody can be obtained from Becton Dickinson (Sunnyvale, CA).

MK-S4. This MAb was also produced in our laboratory (1). It is a γ2b antibody that detects $I\text{-}A^{s,f}$ but not $I\text{-}A^{b,d,k}$. The hybridoma cell line is also available from the ATCC.

11-5.2. This MAb was originally produced by Oi *et al.* (9), who kindly made it available to the scientific community via the Salk Cell Distribution Center. It is a γ2b antibody that detects $I\text{-}A^k$ but not $I\text{-}A^{b,d,s,f,q}$.

Y-17. This MAb was produced by Lerner *et al.* (11), who kindly made the cell line available to us. It is a γ2b antibody that detects a variety of I-E molecules, including $I\text{-}E^k$, $I\text{-}E^{d/b}$, and $I\text{-}E^{d/s}$ but not $I\text{-}E^d$.

RESULTS

Three fusions were performed between T cell-depleted spleen cells of three different *H-2* haplotypes and L10.A 2J. Some of the details of the fusions are listed in Table 2. In each case some of the initial hybrids were tested for antigen presentation with a few T cell hybrids restricted by *I*-region products of the normal spleen cell partner in the fusion. A substantial portion of the hybrids presented antigen in this initial screen. A few of the best presenters were cloned at limiting dilution and the clones were retested for antigen presentation. A clone of one

Table 2. Results of L10.A 2J fusion of T cell-depleted spleen cells

Fusion	T cell-depleted spleen cells: Source	*I*-region type	No. of wells positive for growth	No. of hybrids tested for Ag presentation	No. presenting Ag with Ia of spleen cell cell fusion partner	Clone
LB	C57BL/10	b	75	34	7	LB-15.13
LK	B10.BR	k	≈300	44	20	LK-35.2
LS	B10.S (7R)	s	>300	29	19	LS-102.9

Table 3. Antigen presentation by L10.A 2J and its hybrids

T cell hybrid	Ag specificity		*I* restriction	IL2, units/ml*							
				L10.A 2J		LB-15.13		LK-35.2		LS-102.9	
				No Ag	With Ag	No Ag	With Ag	No Ag	With Ag	No Ag	With Ag
BDK-23.1	KLH		I-A^d	—	640 ± 140	—	510 ± 90	—	530 ± 70	—	430 ± 70
AODK-1.16	KLH		I-E^d	—	200 ± 40	—	200 ± 40	—	170 ± 50	—	170 ± 50
BDK-44.5	KLH		I-$A^{b/d}$	—	—	—	530 ± 70	—	—	—	—
BDK-38.2	KLH		I-$E^{d/b}$	—	—	—	750 ± 110				
AODH-3.4	OVA	or	I-A^k	—	—	800 ± 220	1,070 ± 140	—	1,070 ± 140	110 ± 20	80 ± 0
	None		I-A^b								
4AS-262	Sendai		I-E^k	—	—	—	—	—	170 ± 30	—	—
SKK-17.1	KLH		I-A^s	—	—	—	—	—	—	—	530 ± 70
SKK-37.3	KLH		I-A^s	—	—	—	—	—	—	—	400 ± 80

* Mean ± SEM of six determinations in three experiments. A — indicates no detectable IL-2 (<10 units/ml).

hybrid from each fusion was selected for extensive characterization.

The three cloned B cell hybrids and L10.A 2J were tested for antigen presentation with the bank of T cell hybrids of various *I*-region and antigen specificities shown in Table 1. The results of these assays are shown in Table 3. All of the hybrids retained the ability received from L10.A 2J to present antigens in association with either the I-A^d or I-E^d molecule. In all but one case the ability of the B cell hybrids to present antigen was predicted from the known specificities of the T cell hybrids. Thus L10.A 2J failed to present antigen to any hybrids but BDK-23.1 and AODK-1.16. LB-15.13 stimulated I-A^b-reactive AODH-3.4 without antigen, and presented KLH to *I*-$A^{b/d}$-restricted BDK-44.5 and *I*-$E^{d/b}$-restricted BDK-38.2. LK-35.2 presented OVA to *I*-A^k-restricted AODH-3.4 and Sendai virus to *I*-E^k-restricted 4AS-262. LS-102.9 presented KLH to the *I*-A^s-restricted hybrids SKK-17.1 and SKK-37.3. The only surprise among the combinations tested was the stimulation of a modest but significant response by LS-102.9 from AODH-3.4 in the absence of antigen. AODH-3.4 was characterized with spleen cell presenting cells to respond to I-A^k plus OVA and to I-A^b without antigen. No response has ever been seen with H-2^d or H-2^s spleen cells. It is possible that the response to LS-102.9 represents an "allo" crossreaction with a hybrid I-A molecule between I-A^d and I-A^s.

The presentation of antigen to various T cell hybrids shown in Table 3 indicated the expression in the B cell hybrids of functional *I*-region molecules derived from the normal partner in the fusion. This expression was confirmed in an additional experiment in which monoclonal anti-Ia antibodies were used to inhibit specifically the responses of various T cell hybrids to antigen presented by the B cell hybrids. The results are shown in Table 4. MK-D6 specifically inhibited KLH presentation to BDK-23.1 by any of the hybrids and KLH presentation to BDK-44.5 by LB-15.13. MK-S4 specifically inhibited KLH presentation to SKK-37.3. 11-5.2 specifically inhibited OVA presentation to AODH-3.4. Y17 specifically inhibited antigen presentation to 4AS-262 and BDK-38.2 but not as completely as the specific inhibition seen with the I-A-specific MAbs. Relatively little nonspecific inhibition was seen with the MAb, even including the fact that MAb reaction with one *I*-region restricting element on a B cell hybrid did not affect presentation via a different restricting element. These results taken together confirmed the expression in the B cell hybrids of *I*-region-encoded antigens and restricting elements derived from the normal partner in the fusion.

DISCUSSION

The results presented here support the conclusion that hybrids produced between the *H-2*^{d} B cell lymphoma and normal T-cell-depleted spleen cells taken from mice of various other *H-2* types express the functional *I*-region gene products of both fusion partners. This was shown for both the I-A and the I-E molecule, and in one case shown to include hybrid I-A and I-E molecules composed of an α chain from one partner and a β chain from the other. Although the demonstration of the expression of *I*-

Table 4. Inhibition of T cell hybrid response with monoclonal anti-Ia antibodies

T cell hybrid	Ag	*I*-region restricting element	Ag-presenting B cell hybrid	% IL-2 production remaining in presence of monoclonal anti-Ia inhibitor*			
				MK-D6 (I-A^d, I-$A^{b/d}$)	MK-S4 (I-A^s)	11-5.2 (I-A^k)	Y17 (I-$E^{d/b}$, I-E^k)
BDK-23.1	KLH	I-A^d	LB-15.13	<3	127 ± 14	104 ± 20	100 ± 0
BDK-44.5	KLH	I-$A^{b/d}$	LB-15.13	<2	100 ± 0	114 ± 14	81 ± 10
BDK-38.2	KLH	I-$E^{d/b}$	LB-15.13	127 ± 14	114 ± 14	114 ± 14	25 ± 0
AODH-3.4	None	I-A^b	LB-15.13	114 ± 14	100 ± 0	100 ± 0	100 ± 0
BDK-23.1	KLH	I-A^d	LK-35.2	<2	87 ± 28	104 ± 20	94 ± 23
AODH-3.4	OVA	I-A^k	LK-35.2	104 ± 21	81 ± 10	<1	70 ± 0
4AS-262	Sendai	I-E^k	LK-35.2	90 ± 10	90 ± 10	90 ± 10	34 ± 9
BDK-23.1	KLH	I-A^d	LS-102.9	<3	104 ± 20	104 ± 20	104 ± 20
SKK-37.3	KLH	I-A^s	LS-102.9	100 ± 0	<3	100 ± 0	100 ± 0

* Percent of IL-2 production remaining compared to control cultures containing no MAb inhibitor; mean ± SEM of three determinations. Control responses are shown in Table 3. Inhibitor concentration was 1% ascitic fluid in the case of MK-D6, MK-S4, and 11-5.2 and 1% of 100-fold-concentrated culture supernate in the case of Y-17. Relevant specificities are shown in parentheses. See *Materials and Methods* for more complete description of the specificities of these MAbs.

region products from both partners is *a priori* evidence that these cells are hybrids, we have not formally shown that these are B cell–B cell hybrids. It seems likely that the normal cell in the fusion was a B cell; however, other Ia^+ cell types are present in spleen [e.g., macrophages (12) or dendritic cells (13)]. Although these cells are present in considerably lower concentrations than B cells, it is possible that one of these other cell types fused preferentially to the B cell lymphoma, L10.A 2J. Further characterization of these hybrids with, for example, antibodies to immunoglobulin allotypes or to allelic forms of B cell differentiation antigens should allow us to decide between these possibilities.

The study of the phenomenon of *I*-restricted antigen recognition by T cells has been hampered by the lack of suitable cloned responding and antigen-presenting cell lines. Recently the development of techniques for the production of cloned normal (10, 14–16) and hybridoma (1–3) T cell lines bearing *I*-restricted antigen-specific receptors has made available uniform populations of responding T cells to study this phenomenon. Although we (3, 17) and others (5, 18, 19) have reported B cell and macrophage tumor cell lines capable of antigen presentation to cloned T cell lines, these have all been of the $H\text{-}2^d$ haplotype. The production of a set of cloned hybridomas bearing Ia antigens of various haplotypes represents an important addition to this set of cellular tools, useful in the study not only of antigen presentation but also perhaps of other aspects of Ia antigen biosynthesis and expression and in T cell–B cell interactions. To this end we have made this group of Ia^+ hybrids available to the scientific community via the American Type Culture Collection in Rockville, MD.

Note Added in Proof. The L10.A origin of the L10.A 2J B cell lymphoma line has recently been called into doubt. A characteristically abnormal heavy chain and Southern blot analysis of DNA restriction enzyme fragments with a C_κ probe demonstrate that L10.A 2J is derived from the BALB/c B lymphoma A20, not from L10.A. Consequently, this line has been renamed A20-2J (Michael Kuehl, University of Virginia, personal communication).

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