MONOCLONAL RAT ANTI-MAJOR HISTOCOMPATIBILITY COMPLEX ANTIBODIES DISPLAY SPECIFICITY FOR RAT, MOUSE, AND HUMAN TARGET CELLS*

By DAWN E. SMILEK, HEATHER C. BOYD, DARCY B. WILSON, CHESTER M. ZMIJEWSKI, FRANK W. FITCH, and THOMAS J. McKEARN

From the Department of Pathology, Divisions of Research Immunology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; and the Department of Pathology, University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637

Gene products of the major histocompatibility complex (MHC)[1] display a complex pattern of serologically defined antigenic determinants (1, 2). Attempts to resolve these antigenic complexities with conventional alloantisera have been complicated by the fact that these sera represent heterogeneous populations of antibody specificities. Often, such sera must be rendered functionally monospecific by selective absorption, and they frequently may display low cytotoxicity titers. In addition to these problems, for human studies, the preparation of anti-HL-A sera by conventional immunization procedures is not permitted for ethical reasons. Monoclonal antibodies derived from hybridoma cell lines offer an important alternative to conventional alloantisera because they represent homogeneous antibody populations that can be prepared with specificity for a wide variety of determinants (3, 4), which include antibodies reactive with MHC determinants.

For reasons that remain unclear, it is difficult at present to obtain large numbers of different hybridomas that secrete anti-H-2 antibodies by fusing lymphoid cells from alloimmunized mice to any of the mutant murine myelomas. A collection of anti-H-2 monoclonal antibodies was recently described, the production of which required several years' effort (5–7). This comparatively rare occurrence of anti-MHC hybridomas, however, does not appear to be a general case. In this communication, we describe our experience with the production of large numbers of anti-rat MHC (Rt-1 and Ag-B) hybridomas derived from interspecies fusion of alloimmune rat spleen cells with several mutant mouse myelomas. Antibodies from the supernates of 24 such cloned hybridoma cell lines were tested for their reactivity against rat MHC gene products with appropriate congenic rat strains. They were also tested for their cross-reactivity on target cells from various rats, mice, and humans. By analyzing the reactivity patterns of these monoclonal antibodies it is possible to derive some initial estimates of the degree of heterogeneity of anti-MHC antibodies.

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[1] *Abbreviations used in this paper:* BN, Brown Norway; Con A, concanavalin A; HAT, hypoxanthine, amniopterin, and thymidine; L, Lewis; MHC, major histocompatibility complex; V_H, variable region of the immunoglobulin heavy chain; V_L, variable region of immunoglobulin light chain.

Materials and Methods

Animals

RAT STRAINS. The rat strains used in this study are indicated in Table I along with their MHC haplotypes and sources.

MOUSE STRAINS. All mice were purchased from The Jackson Laboratory, Bar Harbor, Maine with the following exceptions: B10.S mice were obtained from Dr. D. Götze (Max Planck-Institut für Biologie, Tübingen, Federal Republic of Germany), D2.GD mice from Dr. F. Lilly (Albert Einstein Medical School, New York), and A.TL mice from Dr. P. Doherty (Wistar Institute, Philadelphia, Pa.).

Production and Maintenance of Hybridomas. Table II shows the derivation of the hybridomas used in these studies, some of which have been described previously (8, 9). This communication will deal with the first 24 cloned hybridomas, which were derived from 6 different fusions involving alloimmune spleen cells from 5 different Lewis (L) rat donors and 3 HAT-sensitive mutant myeloma cell lines. Many additional positive hybridomas were also derived in these fusions; these have been placed into frozen storage and will be examined later.

Techniques for the immunization of spleen cell donors, cell fusion, hybridoma selection, maintenance, and cloning have all been described in detail elsewhere (8, 9). In brief, spleen cells from adult L rats immunized one to three times with spleen and lymph node cells from (L/Brown Norway [BN])F_1 donors were fused to mutant myeloma cells with polyethylene glycol and seeded into microtiter dishes in selective hypoxanthine, amniopterin, and thymidine (HAT) medium (10). Supernates from wells that contained macroscopic colonies were assayed in the complement-mediated cytotoxicity assay, and cells from positive wells were expanded, cloned in semisoft agarose (11), and/or frozen to await further studies. Criteria for cloning have been published (9) and antibodies from cloned lines were used in all subsequent assays reported in this study.

To obtain fluids that contained high concentrations of the hybridoma antibody, D4.69A, hybridoma cells were grown in immunosuppressed animals as subcutaneous tumors. This required profound immunosuppression; for example, BALB/c mice were treated with 40 μl of anti-thymocyte serum (Microbiological Associates, Inc., Walkersville, Md.) intraperitoneally on 2 consecutive d. 3 d later, the animals were irradiated (600 rad), reconstituted with $10–20 \times 10^6$ anti-Thy 1.2-treated BALB/c bone marrow cells given intravenously, and $10–20 \times 10^6$ hybridoma cells were inoculated subcutaneously between the scapulae. Tumors were palpable after ~2 wk, at which time sera and/or thoracic duct lymph were obtained.

Serological Assays

COMPLEMENT-MEDIATED CYTOTOXICITY. This assay, used both for initial identification of positive hybridomas and for characterization of their specificities, has been described in detail elsewhere (9). The complement sources were selected lots of rabbit serum (diluted 1:8 to 1:12) that were chosen by screening samples from many individual young rabbits; they required absorption with Noble agar (Difco Laboratories, Detroit, Mich.) and rat spleen cells before use.

Supernates from the mutant myelomas were used as background controls. Maximum lysis was obtained with a hybridoma antibody known to lyse 100% of the relevant targets as verified with trypan blue. The positive control for L target cells was supernate from the hybridoma I1.69.1, which is specific for L and BUF target cells and was a gift from Dr. Donald Bellgrau (University of Pennsylvania School of Medicine, Philadelphia, Pa.). In those cases where ^{51}Cr-release was intermediate between the positive and negative control values, the results were verified with a trypan blue dye exclusion assay.

MICROLYMPHOCYTOTOXICITY ASSAY. The procedure of Zmijewski (12) was used to test the specificities of the hybridoma antibodies on human cells.

HEMAGGLUTINATION ASSAY. A modification of the method of Severson and Thompson (13) was used. An enhancing antibody (goat anti-rat Ig) was included in all assays; its presence was necessary to observe hemagglutination with IgG hybridoma antibodies.

Ammonium Sulfate Precipitation. Certain hybridoma culture supernates (Table V) were concentrated by precipitation with an equal volume of saturated ammonium sulfate. Precipitates were reconstituted to one-tenth the original volume and generally had antibody titers that were 10 times higher than the original supernates.

Isotype Characterization. The heavy-chain isotypes of the γ_{2b}- and γ_{2c}-hybridoma antibodies

TABLE I

Rat Strains Used in This Study

Strain	Designation	Rt-1	(Ag-B)	Source*
Lewis	L	l	(1)	M
Wistar Furth	WF	u	(2)	M
Brown Norway	BN	n	(3)	M + A
DA	DA	a	(4)	A
August 28807	AUG	c	(5)	A
Buffalo	BUF	b	(6)	M
	L.B3	n	(3)	A
	BN.B1	l	(1)	A

* (M) Microbiological Associates, Inc. (A) Animal Colonies, University of Pennsylvania and The Wistar Institute, Philadelphia, Pa.

TABLE II

Summary of Rat × Mouse Fusions

Fusion series	Fusion partners		Number of wells positive for growth (total)	Number of wells positive for anti-BN (total tested)	Number of wells positive for anti-BN MHC (total tested)	Number of clones reported in this study
	Spleen	Myeloma (source)				
D4	L1	P3/X63-Ag8*	189 (288)	15 (140)	3 (3)	3
K2	L2	SP2/0-Ag14‡	19 (288)	11 (19)	5 (5)	3
N2	L3	P3/X63-Ag8	205 (281)	42 (205)	19 (19)	8
R6	L4	P3/X63-Ag8.653§	93 (288)	10 (93)	8 (8)	1
R9	L5	SP2/0-Ag14	169 (288)	50 (169)	37 (38)	8
R10	L5	P3/X63-Ag8.653	112 (288)	27 (112)	21 (21)	1

* Provided by Dr. C. Milstein, Medical Research Council, Laboratory of Molecular Biology, Cambridge, England.

‡ Provided by Dr. G. Köhler, Basel Institute of Immunology, Basel, Switzerland.

§ Provided by Dr. J. Kearney, University of Alabama Medical School, Birmingham, Ala.

were determined in Ouchterlony analysis with antisera generously provided by Dr. H. Bazin (University of Louvain, Brussels, Belgium). Hybridoma D4.37.25.24 was typed as "μ" with this same method. All other hybridoma antibodies were subsequently typed for the μ-isotype by their ability to inhibit the binding of ^{125}I-labeled rabbit anti-mouse-μ to D4.37.25.24, with a modification of the method of Press and Klinman (14). ^{125}I-labeled anti-μ was kindly provided by Dr. M. Cancro (University of Pennsylvania School of Medicine).

Results

Frequency of Anti-MHC Hybridoma Antibodies. Table II shows that a large proportion of microtiter wells seeded after fusion displayed growth of proliferating hybrids. When supernates from these cultures were tested for cytotoxicity on concanavalin A (Con A) blast cells derived from BN lymph node cells, 11–58% of them were positive. Moreover, of the 94 hybridomas tested that were positive on BN target cells, 93 were positive on cells from the MHC congenic strain L.B3.

Table III describes the first 24 clones derived from this series of 6 fusions in terms of heavy-chain isotypes and antibody activities as culture supernates in cytotoxicity and hemagglutination assays. Several points emerge: (*a*) Most (21 of 24) of the monoclonal antibodies are IgM. (*b*) All of them are cytotoxic on Con A blasts from BN and L.B3 rats, but have no activity against cells of the reciprocal congenic rat

TABLE III

Heavy-Chain Isotypes, Cytotoxicity, and Hemagglutination Titers of Monoclonal Antibodies from Culture Supernates

Hybridoma	Heavy-chain isotype	Reciprocal cytotoxicity titers			Reciprocal HA titer on BN erythrocytes
		BN	L.B3	BN.B1	
D4.37.25.24	μ	6,200	4,700	0	4,094
D4.68.3.22	γ_{2b}	66	56	0	16
D4.69A*	γ_{2c}	530	240	0	125
K2.3.17	μ	870,000	240,000	0	8,192
K2.7.2	μ	220,000	660,000	0	4,096
K2.13.2	μ	8,200	1,400	0	1,024
N2.9.2	μ	510	220	0	0
N2.11.6	μ	1,350	890	0	32
N2.25.8	μ	590	290	0	16
N2.27.30	μ	625	860	0	16
N2.34.1	μ	590	290	0	0
N2.36.2	μ	390	220	0	0
N2.54.3	μ	110	74	0	16
N2.78.1	μ	590	190	0	64
R6.20.2	μ	29,000	5,400	0	256
R9.9.12	μ	5,060	1,640	0	128
R9.13.1	μ	1,400	2,660	0	1,024
R9.17.12	μ	48	48	0	64
R9.21.4	μ	1,350	1,000	0	32
R9.31.10	μ	340	130	0	8
R9.36.4	μ	590	510	0	256
R9.61.8	μ	1,400	2,260	0	512
R9.75.9	γ_{2c}	330	625	0	1,024
R10.14.2	μ	1,350	890	0	1,024

* Titers performed with lymph from tumor-bearing animal.

strain BN.B1, a finding that establishes the anti-MHC specificity of these monoclonal antibodies. In addition, all of the IgM hybridoma antibodies lyse >90% unstimulated BN lymph node cells (data not shown), thereby establishing that the determinants detected are expressed on both B and T cells. (*c*) Most (21 of 24) of these antibodies also agglutinated BN erythrocytes that display class I MHC determinants but lack class II determinants.

Cross-Reactivities of Hybridoma Antibodies on Target Cells from Allogeneic Rat Strains and from Different Mouse Strains. The cross-reactivity patterns of these monoclonal antibodies were tested on a panel of Con A blast cells from six different rat strains, each expressing a different MHC haplotype (Table IV), and from eight different mouse strains, expressing five different H-2 haplotypes (Table V). Hybridoma antibodies were used as undiluted culture supernates except where indicated. All of them caused complete lysis of BN target cells and showed no activity on L target cells. When tested on third-party targets of either rat or mouse origin, lysis was either complete or negligible, with few exceptions.

The extent of cross-reactivity of these monoclonal antibodies on different mouse strains was surprisingly high. 11 of the 24 antibodies showed no reactivity; 2, on the other hand, were lytic for every murine target tested. Further testing of these two

TABLE IV

*Cytotoxicity Patterns of Monoclonal Antibody Supernates on Target Cells from Six Rat Strains**

Hybridoma	BN Rt-1^n	L Rt-1^l	WF Rt-1^u	DA Rt-1^a	August Rt-1^c	Buffalo Rt-1^b
D4.37.25.24	100	0	77	100	0	100
D4.68.3.22	99	−1	3	−1	39	0
D4.69A‡	93	1	7	1	93	0
K2.3.17	97	−1	3	−3	1	−1
K2.7.2	91	−2	2	−2	0	−1
K2.13.2	88	−1	1	69	86	1
N2.9.2	105	0	4	−1	1	0
N2.11.6	100	0	8	−2	0	0
N2.25.8	92	0	−1	−1	1	−2
N2.27.30	91	−3	6	−3	1	−2
N2.34.1	101	−2	4	0	3	0
N2.36.2	101	−1	0	0	−1	−1
N2.54.3	100	−1	4	−2	1	−1
N2.78.1	95	−1	0	1	0	−1
R6.20.2	96	−1	1	44	100	−1
R9.9.12	100	0	1	0	80	−1
R9.13.1	102	0	3	2	6	−1
R9.17.12	113	0	2	1	1	0
R9.21.4	113	−1	3	2	82	−1
R9.31.10	112	−1	2	1	74	0
R9.36.4	116	−1	1	−1	93	0
R9.61.8	114	−1	3	−1	2	0
R9.75.9	104	−1	2	0	60	0
R10.14.2	115	0	3	−1	2	0
I1.69.1	−3	81	0	−4	7	90

* Data are presented as the percent cytotoxicity calculated in the ^{51}Cr-release assay.
‡ With thoracic duct lymph from tumor-bearing animal.

antibodies (D4.37.25.24 and N2.25.8) on a widely expanded panel of mouse target cells, including cells from wild mice, and several H-2 mutant mice, has without exception shown lysis of each mouse target (T. McKearn and D. Shreffler. Unpublished data.). The remaining 11 antibodies were cytotoxic for cells from certain mouse strains, and it is apparent that those antibodies that were cytotoxic for target cells of any mouse strain were always positive for BALB/c target cells.

Anti-Rat MHC Monoclonal Antibodies Also Detect Mouse H-2 Antigens. 11 of the 24 monoclonal anti-rat MHC antibodies tested on the panel of mouse targets detected polymorphic determinants in this species; i.e., they recognized determinants present in some murine strains that were absent in others. Target cells from H-2 congenic mouse strains were therefore used to determine if the polymorphic determinants might be linked to the H-2 locus (Table V).

All of the antibodies that were positive on BALB/c and DBA/2 ($H\text{-}2^d$) target cells and negative on C57BL/10 ($H\text{-}2^b$), were also positive on B10.D2 target cells, a congenic strain with the C57BL/10 background and the H-2^d haplotype of DBA/2. Similarly, other antibodies lysed C57BL/10 ($H\text{-}2^b$) but failed to lyse SJL ($H\text{-}2^s$) and the congenic strain B10.S ($H\text{-}2^s$).

Table VI shows the results from studies with cells from intra-H-2 recombinant mice

TABLE V

*Cytotoxicity Patterns of Monoclonal Antibody Supernates on Murine Target Cells**

Hybridoma	BALB/c H-2^d	DBA/2 H-2^d	B10.D2 H-2^d	CBA H-2^k	C57BL/10 H-2^b	B10.S H-2^s	SJL H-2^s	DBA/1 H-2^q
D4.37.25.24	100	100	100	100	100	100	100	100
D4.68.3.22	−10	−1	0	0	−6	0	−1	−2
D4.69A	−8	1	−1	8	−5	0	13	0
K2.3.17	98	104	106	100	−7	−2	−2	1
K2.7.2	91	87	70	100	−9	0	−3	−5
K2.13.2	−11	ND§	ND	−4	−10	ND	−3	−6
N2.9.2	0	4	0	10	−8	−2	−7	0
N2.11.6	92	107	99	5	−7	6	−1	0
N2.25.8	94	105	108	100	97	106	100	99
N2.27.30	86	101	100	1	−4	0	−4	−5
N2.34.1	−11	ND	ND	1	−9	ND	−2	0
N2.36.2	−13	ND	ND	2	−9	ND	−3	−7
N2.54.3	90	107	96	3	−9	−3	−2	−6
N2.78.1	90‡	105	96	103‡	−9‡	−2	−1‡	0‡
R6.20.2	97	105‡	105	101	91‡	6‡	−1	2
R9.9.12	−10	ND	ND	1	−10	ND	−3	−6
R9.13.1	91	102‡	107	88	95‡	2‡	11	14
R9.17.12	95	95‡	97	107	88‡	−1‡	−1	1
R9.21.4	−11	ND	ND	3	−8	ND	−1	2
R9.31.10	−6	ND	ND	0	−8	ND	−2	1
R9.36.4	−8	ND	ND	13	−4	ND	1	2
R9.61.8	96	95‡	88	94	98‡	4‡	0	6
R9.75.9	−4	ND	ND	8	−6	ND	−2	3
R10.14.2	103	107	109	103	90	1	0	2

* Data are presented as the percent cytotoxicity calculated in the ^{51}Cr-release assay, with hybridoma antibody D4.37.25.24 serving as the positive control.
‡ Assay done with ammonium sulfate precipitate.
§ Not done.

TABLE VI

*Cytotoxicity Patterns of Monoclonal Antibody Supernates on Target Cells from H-2 Recombinant Mice**

	K	I-A	I-B	I-J	I-E	I-C	S	G	D	D4.37.25.24	P3/X63-Ag8	K2.3.17	K2.7.2	N2.11.6	N2.27.30
B10.D2	d	d	d	d	d	d	d	d	d	+	−	+	+	+	+
D2.GD	d	d	d	b	b	b	b	b	b	+	−	+	+	+	+
C3H	k	k	k	k	k	k	k	k	k	+	−	+	+	−	−
A/J	k	k	k	k	k	d	d	d	d	+	−	+	+	−	−
B10.A(4R)	k	k	b	b	b	b	b	b	b	+	−	+	+	−	−
B10.A(5R)	b	b	b	k	k	d	d	d	d	+	−	−	−	−	−
A.TL	s	k	k	k	k	k	k	k	d	+	−	−	−	−	−
B10.S	s	s	s	s	s	s	s	s	s	+	−	−	−	−	−
C57BL/10	b	b	b	b	b	b	b	b	b	+	−	−	−	−	−

* Cytotoxicity titers on target cells bearing K^d alleles varied from 1:1,000 (N2.27.30) to 1:790,000 (K2.7.2). Titers on K^k target cells varied from 1:12,000 (K2.3.17) to 1:79,000 (K2.7.2).

TABLE VII

*Cytotoxicity Patterns of Monoclonal Antibodies on Human Peripheral T Lymphocyte Target Cells**

Hybrid-oma	Human lymphocyte donor number						
	47(5)‡	158(1)	239(25)	259(11)	282(3)	308(2)	319(1)
D4.37.25.24	++++	++++	++++	++++	++++	++++	++++
K2.3.17	++++	++++	−	++++	++++	++++	++++
K2.7.2	++++	++++	−	++++	++++	++++	++++
N2.11.6	−	+++	−	−	−	−	++
N2.25.8	++++	++++	++++	++++	++++	++++	++++
N2.27.30	−	++	−	−	−	−	−
R6.20.2	−	+++	−	++++	−	−	+++
R9.13.1	−	++++	−	++++	−	++	+++
R9.17.12	−	+++	−	++++	−	−	++
R9.61.8	−	++++	−	++++	−	++	+++
R10.14.2	−	++++	−	++++	++	+++	++++

* ++++, 80–100% cell lysis; +++, 60–79% cell lysis; ++, 40–59% cell lysis; +, 20–39% cell lysis; −, 0–19% cell lysis.

‡ The numbers in parentheses represent the number of individuals from the original panel of 48 donors who displayed this pattern of lysis.

used as targets in an attempt to localize more precisely the H-2-linked determinants detected by these monoclonal antibodies. Hybridoma antibodies K2.3.17 and K2.7.2, which are positive on both H-2^d and H-2^k target cells, detect determinants that are encoded to the left of I-A^k and to the left of I-J^d. Similarly, hybridoma antibodies N2.11.6 and N2.27.30, which lyse H-2^d target cells, also detect determinants that are encoded to the left of I-J^d. When positive, these 4 antibodies lyse >90% of unstimulated murine lymph node target cells.

Cross-Reactivities of Anti-Rat MHC Monoclonal Antibodies on Human Cells. Peripheral blood T lymphocytes from a panel of 48 healthy unrelated human individuals were tested in the microlymphocytotoxicity assay for their sensitivity to lysis by the L anti-BN hybridoma antibodies. Table VII shows that the lysis seen on this panel of 48 individuals could be reduced to 7 prototype patterns. As noted previously with mouse target cells, certain antibodies fail to lyse any human target cells and so, for simplicity, have been omitted from Table VII. The same two antibodies (D4.37.25.24 and N2.25.8) that lysed every mouse target cell also lysed every human target cell tested. A more extensive analysis of these 2 antibodies on >100 consecutive human donors has invariably shown lysis (data not shown). The remaining nine antibodies lysed only targets from certain individuals, which implies detection of polymorphic determinants in humans. Further investigations of the genetic linkage and biochemical nature of these polymorphic human determinants are in progress.

Discussion

This report describes the derivation and serologic characterization of 24 cloned rat-mouse hybridoma cell lines. These cell lines were all derived by fusion of spleen cells from alloimmunized L rats with mutant mouse myeloma cell lines and secrete cytotoxic antibodies specific for the products of the Rt-1^n (Ag-B3) MHC haplotype of the BN rat. Several comments and conclusions can be offered concerning these monoclonal antibodies.

First, the fusion of alloimmune rat spleen cells to mutant mouse myelomas resulted in the production of many (155 of 738) hybridomas that secreted antibody specific for cell-surface antigens of the BN strain (Table II). Of the hybridoma antibodies that were tested, most (93 of 94) were shown to be positive on target cells of the congenic L.B3 strain (Table II), a finding that establishes their specificity for MHC alloantigens of the Rt-1^n haplotype. The reasons for the apparent immunodominance of MHC alloantigens in this situation is not clear, but this finding may reflect the frequent predominance of anti-MHC antibodies in alloimmune sera. This approach to the preparation of anti-MHC hybridomas, therefore, stands in marked contrast to the experience of other groups with anti-H-2 hybridomas, where the number of anti-MHC hybridomas is quite low (5–7). The high yield of rat anti-MHC hybridomas may reflect the use of interspecies somatic cell hybrids or a heightened alloimmune response of rats compared with mice. The extensive cross-reactivities of the monoclonal anti-rat MHC antibodies on H-2 gene products (Table V) seemingly rules out the possibility that these mutant mouse myeloma cells are not permissive to the secretion of anti-H-2 antibodies.

Second, the patterns of reactivity on BN rat cells of the 24 cloned hybridoma antibodies suggest detection of class I gene products of the rat MHC locus (15); i.e., all of the IgG hybridomas and 18 of the 21 IgM antibodies displayed hemagglutinating activity for BN erythrocytes (Table III), and all of the IgM antibodies lysed >90% of unstimulated BN lymph node cells. Additional data derived from cross-reactivity studies on mouse and human cells suggest recognition of class I MHC determinants in those species: (*a*) those 11 antibodies that detect polymorphic determinants in the mouse were shown with congenic strains to detect H-2 gene products; (*b*) from mapping studies with H-2 recombinant mouse strains, two of these antibodies were shown to detect gene products of the H-2K region. Two additional antibodies detect gene products that map to the left of I-J^d and are expressed on >90% of H-2^d target cells (Table VI); and (*c*) several of the monoclonal antibodies lysed >90% of lymphoid cells from certain members of a panel of human donors (Table VII), but were negative on Daudi cells (data not shown), a human B lymphoblastoid cell line that does not express HLA-A, -B, or -C gene products and β_2-microglobulin (16). None of the 24 antibodies appears to detect class II rat MHC determinants, the homologues of the mouse I region. Because the expression of class II MHC antigens on rat Con A blasts may not parallel expression of I-region antigens on mouse Con A blast cells, failure to detect class II determinants could be the result of the screening tactics used, but could also reflect the relatively simple immunization protocols used for the alloimmunized spleen cell donors.

Third, all of the monoclonal antibodies detected polymorphisms of the rat MHC, but they could be further subdivided on the basis of their cross-reactivities on target cells from various mouse strains and human donors (Tables V and VII). It should be noted that the patterns of cross-reactivity were usually all or none. Three general patterns of cross-reactivity were noted: (*a*) 11 of the 24 monoclonal antibodies recognize determinants unique (thus far) to rat MHC haplotypes. The majority of these (8 of 11) detect determinants also present in the August 28807 rat, a crossreactivity that has been previously described with serum alloantibodies (17); (*b*) 2 of the 24 antibodies detect determinants on all normal mouse and human lymphoid cells tested to date. Although these antibodies detect class I MHC polymorphisms in rats, the nonpolymorphic expression of those determinants recognized in mouse and human

precludes their assignment by classical genetic analysis. These observations, however, raise the intriguing possibility that some MHC determinants may be polymorphic in one species and highly conserved or nonpolymorphic in another species; (*c*) 11 antibodies detect polymorphisms in the murine MHC, one unique to H-2^d, a second shared by H-2^k and H-2^d, and a third polymorphism shared by H-2^d, H-2^k, and H-2^b. Nine of these monoclonal antibodies also detect polymorphic determinants in human cells. These antibodies all recognize murine MHC gene products (Table V), and family studies in humans strongly suggest linkage to the HL-A gene complex (H. Boyd, D. Smilek, C. Zmijewski, R. Spielman, and T. J. McKearn. Monoclonal rat anti-MHC antibodies detect polymorphisims in humans which are linked to HL-A. Manuscript submitted for publication.).

It is of some interest that those monoclonal antibodies that react with human or mouse cells always react with H-2^d, the haplotype of the mutant mouse myeloma cell lines used for fusion. We do not currently know whether this is simply a result of the particular rat strain combinations used for alloimmunization or of some preferential selection of those cells that secrete antibodies cross-reactive with H-2^d gene products. Taken as a whole, these findings indicate an extensive sharing of class I MHC determinants in rats, mice, and humans, and therefore, certain of these antibodies may become useful reagents for both biochemical and genetic studies of MHC gene products in a variety of species.

Fourth, although the number of monoclonal antibodies described in this paper is somewhat limited, it is possible to derive some assessment of the heterogeneity of anti-MHC antibodies from the assembly of cross-reactivity patterns against rat, mouse, and human target cells observed with these hybridoma antibodies (Fig. 1) by using the strategy of reactivity pattern analysis. In such an analysis (18) one assumes that differences in the reactivity patterns between two monoclonal antibodies imply differences in fine specificities of those antibodies. Similarities in reactivity patterns, on the other hand, do not establish the two antibodies to be identical. Therefore, reactivity pattern analysis can provide a minimal estimate of the degree of antibody diversity in a collection of monoclonal antibodies. Two important points emerge from such a consideration: (*a*) a considerable number of different antibodies comprise the anti-MHC response (14 reactivity patterns among 24 hybridomas), and (*b*) when a given reactivity pattern recurs, it tends to do so within the individual (e.g., reactivity patterns 3, 5, and 12) rather than between individuals (e.g., reactivity pattern 2).

These data suggest that considerable heterogeneity exists within the B cell anti-MHC repertoire and imply that either the germ-line encodes for many anti-MHC specificities (19) or that some of this diversity is generated by somatic mechanisms. Reactivity pattern analysis measures the overall diversity that results from combinational assembly of the variable region of the immunoglobulin heavy chain (V_H) and the variable region of the immunoglobulin light chain (V_L) chains, and, therefore, the observed heterogeneity could reside in either V_H or V_L. Hence, it is possible that these antibodies represent a highly diverse set of V_L chains assembled with relatively few V_H chains, a situation that would reconcile these data to observations showing shared V_H idiotypes on T and B cells (20, 21). In addition, because the anti-MHC repertoire probably includes antibodies that recognize several distinct alloantigenic determinants, the heterogeneity seen in this collection of anti-MHC hybridomas is perhaps not entirely surprising.

The issue of recurring reactivity patterns within and between individuals deserves

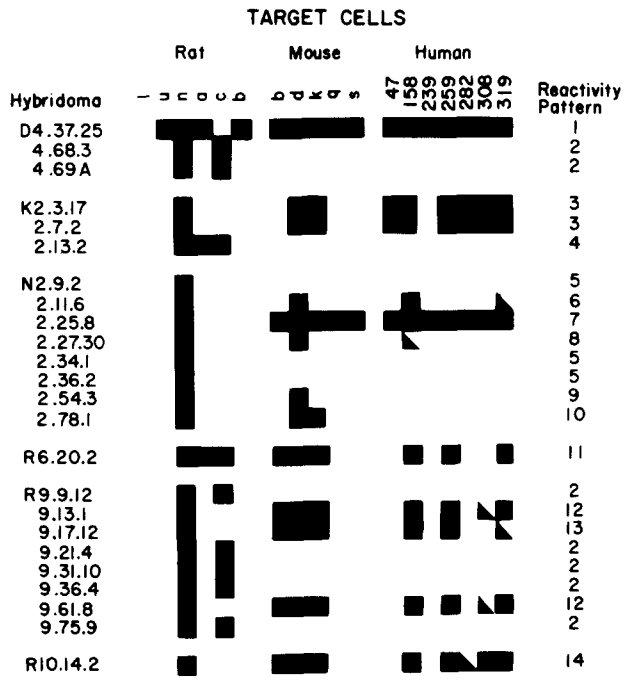

FIG. 1. Summary of the reactivity patterns of hybridoma antibodies, derived from 5 different L rats, on rat, mouse, and human target cells. Rat and mouse data are organized according to Rt-1 and H-2 haplotypes, respectively. Human data is organized as in Table VII. ■ implies positive reaction; □ implies negative reaction; and ◣ implies partial lysis on human target cells. 14 reactivity patterns are found with these monoclonal antibodies.

comment because it is a clear prediction that if true germ-line specificities exist in the anti-MHC antibody response, one should be able to demonstrate sharing of at least some reactivity patterns between individuals. The only pattern that is shared in this initial panel of 24 antibodies is reactivity pattern 2, and we already have preliminary evidence that not all members of that group share idiotypic determinants. In those situations where reactivity patterns are shared among hybridomas derived simultaneously from a single donor, we do not yet know whether such antibodies represent true sister clones and whether these represent (*a*) clonal dominance before antigen exposure, (*b*) selective expansion of certain clones by antigen challenge, (*a*) preferential fusion of certain clones to the mutant myeloma cells, or (*d*) preferential growth of certain clones after fusion. The use of anti-idiotypic antibodies prepared against these hybridomas should help resolve some of these basic questions.

Summary

24 monoclonal rat antibodies are described that are reactive with determinants encoded by the major histocompatibility complex (MHC) of the rat. These hybridoma antibodies were derived by fusing mutant mouse myeloma cells to spleen cells from Lewis rats immunized with allogeneic Brown Norway cells. All 24 antibodies are cytotoxic for both Brown Norway target cells and target cells from the appropriate MHC congenic rats. Patterns of cytotoxicity and hemagglutination strongly suggest

reactivity against class I (K or D equivalent) rat MHC determinants. Cytotoxic cross-reactivity patterns were generated for each monoclonal antibody on a panel of rat and mouse lymphoid cells and human peripheral T lymphocytes. A high degree of interspecies cross-reactivity was noted with approximately one-half of the antibodies positive on human and/or mouse target cells. 11 antibodies recognized polymorphic determinants in the mouse, and, by using target cells from MHC congenic mouse strains, it was shown that these determinants are encoded by genes within the H-2 complex. Finally, by considering the overall reactivity patterns of these monoclonal antibodies on all target cells, one can show that these 24 antibodies represent a minimum of 14 antibody specificities.

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