Properties of Monoclonal Antibodies to Mouse Ig Allotypes, H-2, and Ia Antigens

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Advances in somatic cell hybridization techniques have made it possible to generate hybrid cell lines producing monospecific antibodies directed at desired antigenic determinants (1). In this paper a modification of the cell fusion procedure (2,3) was used to recover stable hybrid cell lines secreting IgG antibodies to: (a) mouse major histocompatibility complex (MHC) alloantigens (H-2K and I-A); and (b) mouse immunoglobulin (Ig) allotypes (Ig-1b, Ig-5a, and Ig-5b).

I. Production of NS-1 Derived Hybrid Cell Lines Secreting Monospecific Antibodies

The parental myeloma cell line used was the NS-1 variant of the P3 (MOPC 21) line (4). Cells of the NS-1 line do not synthesize the MOPC 21 γ_1 heavy chain; although NS-1 synthesizes the MOPC 21 κ chain, it is not secreted. NS-1 derived hybrid cell lines secreting the parental spleen cell Ig's will also secrete hybrid molecules containing the MOPC 21 κ chain.

Immune and hyperimmune mice were compared as sources of spleen cells for generating antibody-producing hybrid cell lines. This was examined with BALB/c mice immunized with C57BL/10 (Ig^b) anti-*B. pertussis*-pertussis complexes, an immunization that generates anti-Ig^b allotype antibodies (5). Spleen cells from hyperimmune animals three, six and eight days after their last antigen boost, and spleen cells from mice primed with a single antigen dose, also three, six, and eight days after a single boost were used for hybridization. Table 1 summarizes the recovery frequencies of antibody-producing hybrid cell lines from these experiments. The data suggest that primed and once-boosted spleen cells may be more effective in generating antibody-producing hybrids. The subsequent anti-spleen cell alloantigen immunizations consisted of an initial i.p. injection of $\sim 2 \times 10^7$ spleen cells per animal and a single boost also of $\sim 2 \times 10^7$ cells (Table 1).

Using polyethylene glycol (PEG) 1500 (BDH Chemicals Ltd., Poole, England) as the fusion agent, NS-1 cells from log-phase growth were fused with immune spleen cells at a 1:4 or 1:2 ratio. A total of 3×10^8 cells were treated with 1 ml of 50% PEG in serum-free RPMI-1640 medium. The cell mixture was plated in three 96-well microculture plates (Costar, Cambridge, MA) in 0.1 ml of 15% FCS-RPMI-1640 per well. Progressive HAT selection was carried out for two weeks. HAT medium, 0.1 ml, was added on day 1 and half the medium (0.1 ml) was replaced with fresh HAT medium on days 2, 3, 5, 8, 11 and 14.

During the third and fourth weeks, supernates from those microcultures containing growing hybrid cells were tested for antibody activity. Reactivity against Ig's was measured by a plate-binding assay (5). Ig adsorbed onto wells of flexible plastic microtiter plates (Cooke Lab. Prod., Alexandria, VA) was reacted with 5-20 µl of culture supernate for 1 hr at room temperature. ^{125}I-labeled anti-allotype antibodies (either a mixture of anti-Ig-1b and anti-Ig-4b or anti-Ig-1a

TABLE 1

PRODUCTION OF NS-1 (MYELOMA) HYBRIDS

	Priming (i.p.)	Days past boost (i.p.)	No. of Expts.	Wells $\bar{c}$ hybrids* / Total wells		Antibody: Initial pos. wells / Total hybrids		Antibody: Final pos. hybrids / Total hybrids	
				No.	(%)	No.	(%)	No.	(%)
αIg[b]	Hyper-immune	3	2	187/271	(69)	2/187	(1)	0	
"	"	6	2	218/346	(63)	4/218	(2)	0	
"	"	8	1	180/180	(100)	0		0	
"	1 wk	3	2	120/288	(42)	18/120	(15)	4/120	(3)
"	"	6	1	93/182	(51)	4/93	(4)	0	
"	"	8	2	75/288	(26)	3/75	(4)	0	
αspleen cell	3 wk	3	2	576/576†	(100)	42/576	(7)	12/576	(2)

*10^6 cells/well (2×10^5 NS-1, 8×10^5 spleen cells/well)

†10^6 cells/well (3.3×10^5 NS-1, 6.6×10^5 spleen cells/well)

and anti-Ig-4a) were used to detect bound antibody. Labeling with ^{125}I was done with antibodies adsorbed to immunoadsorbants to protect the combining sites, and then eluted with 0.2 M glycine-HCl (pH 2.3) containing carrier protein (5). Antibodies to spleen cell alloantigens were detected by reacting 4×10^5 spleen cells with culture supernates in microtiter wells for 1 hr. Bound antibodies were also detected with ^{125}I-anti-allotype antibodies or with ^{125}I-protein A. Screening for IgM antibodies was not done.

Microcultures producing desired antibodies were transferred into 1 ml cultures (Costar, Cambridge, MA) with 5×10^6 thymocytes as feeder cells. These cultures were expanded into flasks (6-14 days) and assessed for continued antibody production. Cells from 10 ml of high density cultures (containing $2\text{-}5 \times 10^6$ cells) were frozen in 0.5 ml of 90% FCS-10% DMSO. The anti-spleen cell hybrids were cloned by limiting dilution from the initial microculture wells. Cultures of 0.1 ml were plated with 10^6 thymocytes/well as feeder cells. These were fed with 0.1 ml of medium on days 7 and 12. At the beginning of the third week, microcultures with growing clones were tested for activity. The anti-Ig^b hybrid cell lines were cloned from cells grown in flasks either by limiting dilution or in soft agar. It was generally observed that those hybrid cell lines that maintained antibody production in 1 ml and flask cultures had high cloning efficiencies and good recoveries of antibody-producing clones.

At least four antibody-producing clones from each hybrid cell line were expanded and frozen (6 vials, each containing $2\text{-}5 \times 10^6$ cells). A total of 24 clones were injected subcutaneously into syngeneic mice (at least five mice/clone, each receiving $2\text{-}5 \times 10^6$ cells). Twenty-two (92%) of these clones produced tumors (hybridomas) within 10-30 days. All hybridoma-bearing mice produced myeloma-like proteins and 20 of the 22 hybridomas (91%) produced the desired antibody activity. These tumors were transplantable and continued to produce antibody.

The chain composition of the antibodies produced by these hybrid cell lines was assessed by two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE) (see below).

II. Antibodies to Alloantigens of the Major Histocompatibility Complex

Two hybridizations were done with spleen cells from mice immunized with allogeneic cells with the goal of obtaining monospecific antibodies reacting with antigens controlled by the H-2 complex or with cell surface Ig's. Antibodies from one hybridization (H10), in which the donor spleen cells were from CWB mice immunized with C3H spleen cells, should react only with $H\text{-}2^k$ or Ig^a determinants. Antibodies from the second hybridization (H11), in which the BALB/c spleen cell donor had been immunized with CKB cells, could potentially react with $H\text{-}2^k$ or Ig^b determinants or with the products of other genes.

Antibody production by hybrid cells was determined by testing the reactivities of supernates from the initial microcultures in the cell-binding assay, using as targets spleen cells from H-2 and Ig congenic strains of mice. As shown in Table 2, the reactivities of five of the H11 and two of the H10 supernatant antibodies are against antigens linked to the MHC, while one antibody each from H10 and H11 seems to react with cell surface Ig. The 10.1 hybrid supernate reacted with cells from all four congenic strains, including CWB, the spleen cell donor. This hybrid cell line apparently is producing an autoantibody against an undefined cell surface antigen.

TABLE 2

LINKAGE ANALYSIS OF HYBRID CELL ANTIBODY REACTIVITY

			BALB anti-CKB (H11) (anti-$H\text{-}2^k$, Ig^b, . . .)						CWB anti-C3H (H10) (anti-$H\text{-}2^k$, Ig^a)			
Strain	H-2	Ig	11-1*	11-2*	11-3*	11-4†	11-5†	11-6†	10-1*	10-2*	10-3Ψ	10-4Ψ
C3H	k	a	924§	1216	2672	1615	165	74	1761	1349	nd	nd
CWB	b	b	30	64	176	83	13	567	921	71	nd	nd
CKB	k	b	1278	1236	3072	nd	nd	nd	2213	1290	1896	66
CSW	b	a	136	142	244	nd	nd	nd	1790	157	139	1409
Linkage			MHC	MHC	MHC	MHC	MHC	Ig	Auto	MHC	MHC	Ig

nd: not determined

* ^{125}I-protein A

† ^{125}I-anti-Ig-1a + anti-Ig-4a

Ψ ^{125}I-anti-Ig-1b + anti-Ig-4b

§ Counts per minute bound to 4×10^5 spleen cells

The reactivity of the seven antibodies directed against products of the MHC were mapped to regions of the complex using a series of recombinant strains of mice (Table 3). The reactivity patterns of five of these antibodies are consistent with their detecting $I\text{-}A^k$ antigens. The remaining pair of antibodies apparently react with $H\text{-}2K^k$ antigens. None of these antibodies detect H-2D antigens or the products of other I subregions.

TABLE 3

MHC MAPPING OF HYBRID CELL ANTIBODY REACTIVITY

		I									H10		H11				
Strain	K	A	B	J	E	C	S	G	D	Medium	10-3.6	10-2.15	11-4.1	11-5.2	11-2.12	11-1	11-3
CKB	k	k	k	k	k	k	k	k	k	86*	1636	891	1358	736	1390	1634	2586
A.TL	s	k	k	k	k	k	k	k	d	70	656	338	58	196	493	112	1166
B10.A(4R)	k	k	b	b	b	b	b	b	b	64	1624	766	1081	606	1280	1436	2446
B10.A(3R)	b	b	b	k	k	d	d	d	d	41	100	54	84	69	76	69	77
C3H.OH	d	d	d	d	d	d	d	d	k	141	160	154	126	223	188	170	161
		Reactivity									I-A	I-A	H-2K	I-A	I-A	H-2K	I-A

*^{125}I-protein A counts per minute bound to 4 x 10^5 spleen cells

To confirm the specificities of these anti-MHC antibodies, the proteins they react with were analyzed biochemically. Antibodies produced by cloned cell lines were used for this analysis; evidence that the antibodies themselves are clonal products is presented below. Earlier studies have shown that 2-D PAGE of H-2 and Ia antigens produces patterns that are characteristic both of the region or subregion coding for the precipitated antigen and of the haplotype (6,7). As shown in Fig. 1, antibody from the cloned hybrid cell line 11-4.1 precipitates molecules from ^{35}S-methionine-labeled CKB extracts identical to those precipitated by an alloantiserum, (A.TL x C3H.OL)F_1 anti-C3H, which is directed against H-2K^k. Similarly, antibody from the cloned line 10-2.16 precipitates the same I-A^k molecules from C3H extracts as does A.TH anti-A.TL (anti-I^k) alloantiserum from B10.A(4R) (Fig. 2). It is interesting to note that all of the I-A^k molecules precipitated by the A.TH anti-A.TL serum, which probably represent three distinct gene products (P.P. Jones, D.B. Murphy and H.O. McDevitt, in preparation), are also precipitated by monospecific anti-I-A antibodies. Unless all three proteins have in common the antigenic determinant recognized by the 10-2.16 antibody and are independently precipitated, the three polypeptide chains probably are precipitated because they exist as a molecular complex on the cell surface. Anti-I-A antibodies produced by clones 10-3.6 and 11-5.2 precipitate the same molecules as 10-2.16; the other two, 11-2.12 and 11-3.25, are currently being examined.

The reactivities of the anti-H-2K and the anti-I-A antibodies were also analyzed by two-color immunofluorescence, using the fluorescence-activated cell sorter (FACS) (9). C3H spleen cells were stained with rhodamine-conjugated rabbit anti-μ antibodies, to label B lymphocytes, and with either anti-H-2K^k (11-4.1) or anti-I-A^k (10-2.16), followed by a fluorescein-conjugated rabbit anti-γ. As shown in Fig. 3-b, the 11-4.1 anti-H-2K stains most of the spleen cells, as would be expected for an anti-H-2K antibody. Fig. 3-c indicates that the μ^- population contains a small population of cells that have little or no H-2K, but the vast majority of μ^- (Fig. 3-c) and μ^+ (Fig. 3-d) cells have H-2K determinants recognized by this antibody. The other anti-H-2K antibody (11-1.23) shows a similar reactivity pattern.

The FACS analyses of C3H cells stained with rhodamine-conjugated anti-μ and 10-2.16 anti-I-A^k followed by fluorescein-conjugated anti-γ are presented in Fig. 4. The μ^+ peak in Fig. 4-a and the I-A^+ peak in

Figure 1

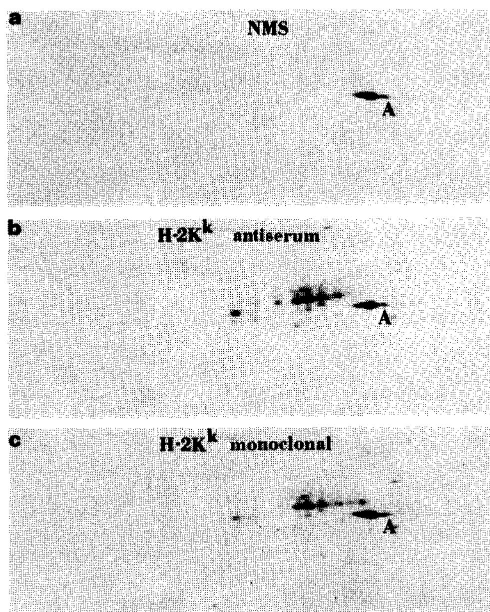

Figure 2

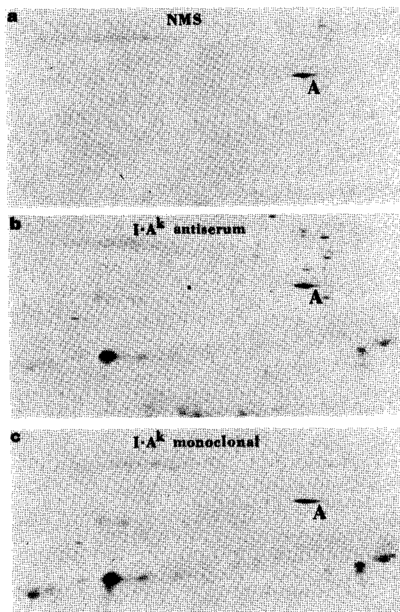

Fig. 1. Autoradiograms of 2-D gels of H-2K^k antigens. Proteins were precipitated from NP-40 extracts of ^{35}S-methionine labeled CKB spleen cells (6) by (a) normal mouse serum, (b) (A.TL x C3H.OL) anti-C3H, and (c) antibody from clone 11-4.1. The first dimension separation was by non-equilibrium pH-gradient electrophoresis (8) (acidic proteins are on the right and basic proteins on the left). The second dimension separation was by SDS PAGE (from top to bottom). Both separations were done under reducing conditions. Only the relevant portions of the autoradiograms are shown. The position of actin is indicated by the letter A.

Fig. 2. Autoradiograms of 2-D gels of I-A^k antigens. Proteins precipitated from (a) C3H extract by normal mouse serum, (b) B10.A(4R) extract by A.TH anti-A.TL, and (c) C3H extract by antibody from clone 10-2.16 were electrophoresed as described in the legend to Fig. 1.

* * *

Fig. 4-b each contain 60-65% of the cells. Analysis of the I-A profiles of μ^- cells (Fig. 4-c) and μ^+ cells (Fig. 4-d) indicates that nearly all the μ^+ cells also are I-A$^+$, while few of the μ^- cells have detectable I-A determinants. Two other anti-I-A^k antibodies reacted in a similar fashion; the final two anti-I-A antibodies are currently being examined.

Several unique antigenic determinants have been described for both H-2K^k and I-A^k based on the cross-reactivities of molecules of k haplotype with those of other haplotypes as detected by alloantisera. To test whether the determinants detected with monospecific antibodies correspond to those described previously, the five anti-I-A^k and the two anti-H-2K^k antibodies were tested for reactivity against cells of eight independent H-2 haplotypes. As seen in Table 4, three of the

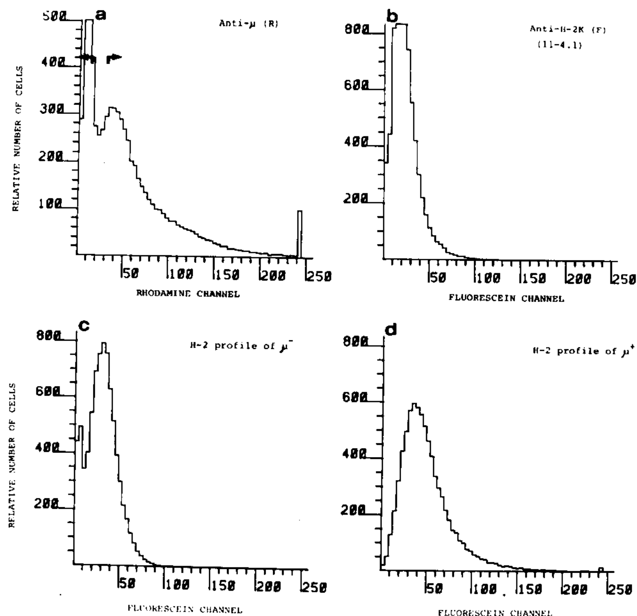

Fig. 3. Anti-H-2K and anti-μ chain immunofluorescence histograms. C3H spleen cell stained with rhodamine (R) conjugated rabbit anti-μ and anti-H-2K antibody from clone 11-4.1 followed by fluorescein (F) conjugated rabbit anti-γ were analyzed with the FACS; (a) R-anti-μ profile, (b) F-anti-H-2K profile, (c) F-anti-H-2K profile of μ^- cells (cells to the left of channel 15 in panel a, indicated by the left arrow), and (d) F-anti-H-2K profile of μ^+ cells (cells to the right of channel 30 in panel a, indicated by the right arrow).

* * *

anti-I-A^k antibodies react with cells of f, k, r, and s haplotypes, but not with b, d, p or q haplotypes. This pattern of reactivity corresponds to specificity Ia.17 previously described with alloantisera (10). The other two anti-I-A^k antibodies react only with cells of k haplotype, consistent with specificity Ia.2, which is unique to I-A^k (10). One of the anti-H-$2K^k$ antibodies binds to cells of k, p, q and r haplotype strains; the other reacts with k and perhaps f haplotype strains. Neither of these reactivity patterns corresponds to an H-2K antigenic specificity previously identified by alloantisera.

One additional point can be made from Table 4. These monospecific antibodies give different levels of binding with different haplotypes. In some cases, the antibodies seem to react more strongly with cells of haplotypes other than H-2^k. It is possible that the same determinant may be exposed to different degrees on cells of different haplotypes. Alternatively, the antigenic determinants might be similar but non-identical, allowing differential binding of the monospecific antibodies to molecules of different haplotypes.

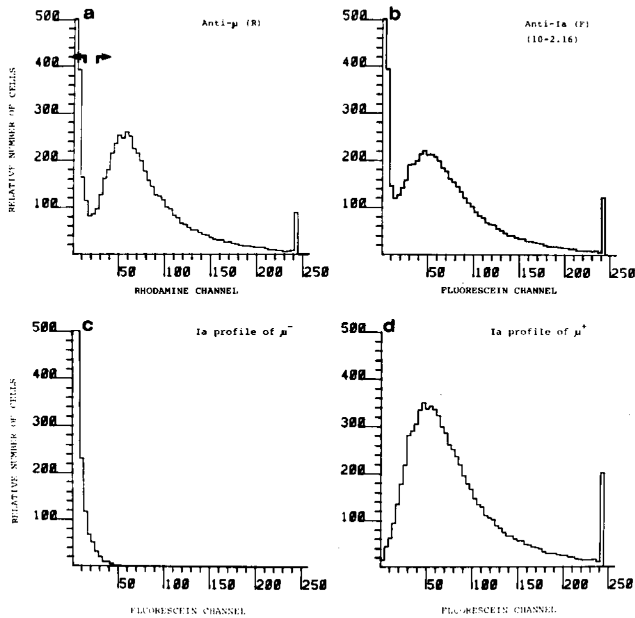

Fig. 4. Anti-I-A^k and anti-μ chain immunofluorescence histograms. C3H spleen cells stained with R-anti-μ and anti-I-A antibody from clone 10-2.16 followed by F-anti-μ were analyzed with the FACS; (a) R-anti-μ profile, (b) F-anti-I-A profile, (c) F-anti-I-A profile of μ^- cells (cells to the left of channel 15 in panel a, indicated by the left arrow), and (d) F-anti-I-A profile of μ^+ cells (cells to the right of channel 25 in panel a, indicated by the right arrow).

TABLE 4

HYBRID CELL ANTIBODY REACTIVITY* WITH DIFFERENT H-2 HAPLOTYPES

Strain	H-2K, I-A Haplotype	Anti-I-A 10-3.6	Anti-I-A 10-2.16	Anti-I-A 11-5.2	Anti-I-A 11-2.12	Anti-I-A 11-3.25	Anti-H-2K 11-4.1	Anti-H-2K 11-1.23
B10.D2	d	1.7	1.4	0.9	1.0	1.9	0.9	1.0
B10	b	1.4	1.3	0.8	1.0	2.1	1.0	1.1
B10.A	k	14.7	10.0	5.8	14.2	22.4	16.7	5.9
B10.M	f	22.2	13.6	1.0	1.4	36.5	1.3	3.7
B10.RIII	r	12.9	5.0	1.3	1.3	15.5	7.4	1.8
B10.S	s	13.1	5.4	1.0	1.4	18.8	1.1	1.4
B10.P	p	3.1	2.7	1.2	1.0	1.9	4.7	2.4
B10.T(6R)	q	1.6	1.6	1.1	1.5	2.4	4.4	1.5

*Ratio $\dfrac{\text{Specific } ^{125}\text{I-protein A cpm bound}}{\text{Control } ^{125}\text{I-protein A cpm bound}}$

Results are means for three experiments (d, p, q haplotypes) or four experiments (b, k, f, r, s haplotypes)

III. Isolation and Characterization of the Products of Hybrid Cell Lines

The majority of the products of these hybrid cell lines bound staphylococcal protein A in the cell binding assay; therefore affinity chromatography on protein A-Sepharose (11) provided a simple and rapid one-step procedure for their isolation from serum or culture supernates. Yields of IgG from culture supernates were typically 10-20 μg/ml, but were occasionally as high as 50 μg/ml.

Fig. 5 shows the analysis of Ig's by 2-D PAGE. Both heavy and light chains of IgG isolated from normal mouse serum (Fig. 5-a) are heterogenous. In contrast, the pattern obtained for MOPC 21 myeloma protein (Fig. 5-b) is simpler. The presence of 2-3 light and heavy chain spots probably is due to post-translational modifications such as deamidation. These modifications result in horizontal shifts toward the acidic end of the gel. A similar analysis of Ig purified from the culture supernate of clone 10-3.6 is shown in Fig. 5-c. As expected from the contribution of the NS-1 parent, one of the two pairs of light chain spots corresponds to the position of the MOPC 21 κ chain. On the other hand, the single pair of heavy chain spots is distinct from the expected position of the MOPC 21 heavy chain, which is clearly absent.

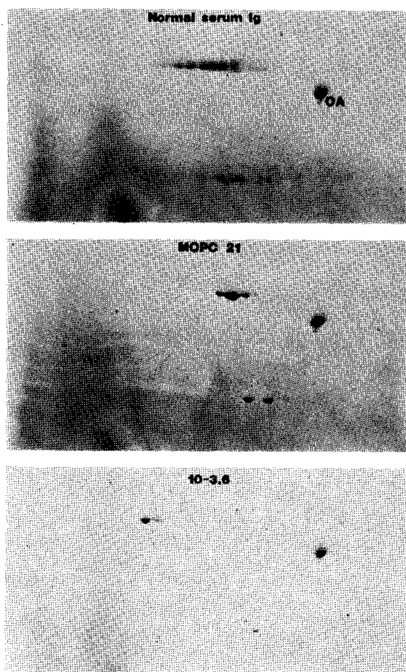

Fig. 5. 2-D PAGE analysis of light and heavy chains from (a) normal BALB/c serum Ig purified on protein A-Sepharose, (b) MOPC 21 myeloma protein, and (c) antibody from clone 10-3.6 (anti-I-A^k) purified from culture supernates on protein A-Sepharose. The gels were run as described in the legend to Fig. 1, and the proteins visualized by Coomassie Blue staining. Ovalbumin (OA), 45,000 daltons, was added as a molecular weight marker.

Schematic representations of the 2-D PAGE analyses of the anti-H-2K and I-A antibodies are shown in Fig. 6. All of these proteins appear to be clonal products. Also shown in Fig. 6 are the chain compositions of the isolated proteins as determined by Ouchterlony gel diffusion using class-specific antisera.

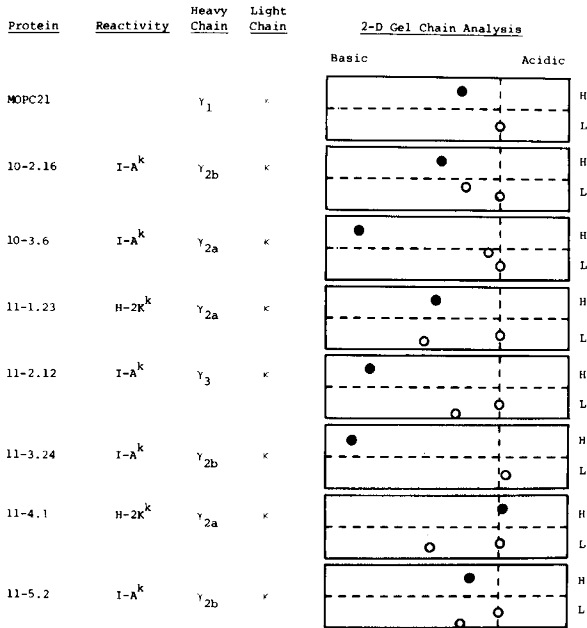

Fig. 6. Heavy and light chain composition of anti-H-$2K^k$ and anti-I-A^k antibodies from hybrid cell lines. The mobilities of heavy and light chains from MOPC 21 protein and hybrid cell antibodies in 2-D gels (see Fig. 5) are schematized on the right. The spots represent the most basic (unmodified) molecular species of each chain. The vertical dotted line marks the position of the MOPC 21 (NS-1) light chain spot. The heavy and light chain isotypes of each antibody, determined by Ouchterlony analysis, are also given.

IV. Antibodies to Ig Allotypes

A. Ig-5a and Ig-5b δ Heavy Chain Allotypes

In the analysis of hybrids derived from mice immunized with allogeneic spleen cells, two cell lines were found to produce antibody that reacts

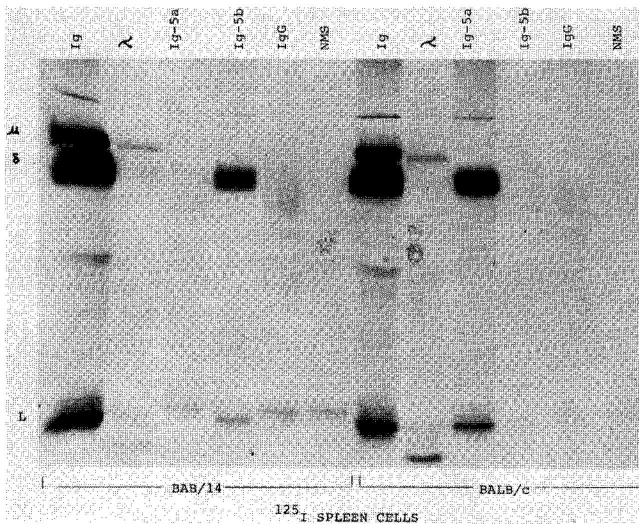

Fig. 7. Autoradiogram of SDS PAGE analysis of immunoprecipitates of NP-40 solubilized BALB/c (Ig^a) and BAB/14 (Ig^b) spleen cells, labeled with ^{125}I by the lactoperoxidase technique. Reactivities of antibodies used for immunoprecipitation are indicated at the top of the gel. Anti-Ig-5a is from clone 10-4.22 and anti-Ig-5b from clone 11-6.3. Fixed S. aureus bacteria were used to bring down the antigen-antibody complexes. Since the 11-6.3 antibody (IgG_1) does not bind to S. aureus protein A, a small amount of rabbit anti-mouse γ chain was added to facilitate the binding of the 11-6.3 complexes to the bacteria.

with cell surface antigens controlled by genes linked to the Ig heavy chain gene complex (Table 2). These antigens were likely to be δ or μ allotypic determinants. The proteins precipitated by these antibodies from NP-40 extracts of ^{125}I-labeled BALB/c (Ig^a) and BAB/14 (Ig^b) spleen cells were analyzed by SDS PAGE (see Fig. 7). Both antibodies precipitate molecules containing chains with mobilities of light chains and δ heavy chains. However, only molecules of the appropriate heavy chain allotype are precipitated.

The anti-δ antibodies also were analyzed by two-color fluorescence, as described above. Approximately 60% of C3H spleen cells are μ^+ (Fig. 8-a) and a similar number are stained by the 10-4.22 anti-δ (Fig. 8-b). The great majority of μ^+ cells are also stained by 10-4.22 antibody (Fig. 8-d), while virutally no μ^- cells are stained (Fig. 8-c). Analysis of clone 11-6.3 antibody showed identical results when tested on BAB/14 cells. These results are consistent with clones 10-4.22 and 11-6.3 recognizing allotypes of murine δ chain. Clone 10-4.22 recognizes the Ig-5a allele and 11-6.3, the Ig-5b allele.

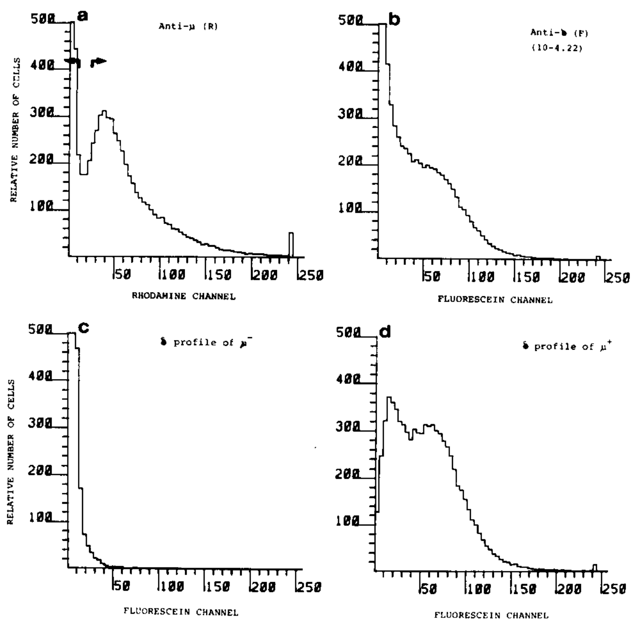

Fig. 8. Anti-δ chain and anti-μ chain immunofluorescence histograms. C3H spleen cells were stained with R-anti-μ and anti-Ig-5a antibody from clone 10-4.22 followed by F-anti-γ was analyzed with the FACS. (a) R-anti-μ profile, (b) F-anti-δ profile, (c) F-anti-δ profile of μ^- cells (cells to the left of channel 10 in panel a, indicated by the left arrow), and (d) F-anti-δ profile of μ^+ cells (cells to the right of channel 30 in panel a, indicated by the right arrow).

TABLE 5

GENETIC ANALYSIS OF ANTI-IgD ANTIBODIES

	Ig Haplotype					
	BALB/c	B10	DBA/2	AKR	A/J	CE
	a	b	c	d	e	f
NMS	62	42	50	39	66	53
11-6.3*	102	284	44	44	97	92
NMS	48	23	56	52	32	43
10-4.22†	272	57	349	186	27	327

Data presented as counts/min. Mean of 4 replicates. Standard error was typically 10% of mean.

*Second step was ^{125}I-anti-Ig-4a †Second step was ^{125}I-protein A

Table 5 shows the results of a more extensive genetic analysis of the reactivities of antibodies 10-4.22 and 11-6.3. On the basis of these results and previous findings using alloantisera, four IgD specificities are defined (Table 6). Specificities 1 and 2 are defined by alloantisera (12). Specificity 2 is also defined by the 11-6.3 antibody. Specificity 3 is defined by the H6/31 antibody of Pearson et al. (13, and T. Pearson and L. A. Herzenberg, unpublished). Specificity 4 is defined by antibody from clone 10-4.22.

TABLE 6

IgD SPECIFICITIES

	1	2	3	4
a	1	-	-	4
b	-	2	3	-
c	n.d.	-	-	4
d	n.d.	-	-	4
e	1	-	3	-
f	n.d.	-	n.d.	4

B. Ig-1b (γ_{2a}) Heavy Chain Allotype

Only one allotype specificity has been described with conventional alloantisera that distinguishes the γ_{2a} heavy chain of the b (Ig-1b) from the a (Ig-1a) allelic products. The five clones producing anti-Ig-1b antibodies (Ig(1b)1.2, 1.7, 2.4, 2.9, and 3.1) that were recovered from hybridizations with BALB/c spleen cells immunized with Ig^b immunoglobulins define three specificities on C.BPC101 (Ig-1b) myeloma protein. This was determined by testing whether different antibodies could block the subsequent binding of a second antibody to the Ig-1b molecule. Various concentrations of unlabeled antibodies were reacted with C.BPC101 myeloma protein that had been fixed with glutaraldehyde onto wells of flexible plastic microtiter plates. The reaction was allowed to proceed overnight at 4°C. The plates were then washed and a constant amount of ^{125}I-labeled antibody was added for 1 hr at room temperature to react with the remaining available (i.e., unblocked)

determinants on the myeloma protein. A typical blocking curve using a constant amount of ^{125}I-labeled Ig(1b)2.9 protein, known to be in excess of the amount of antigen available on the plate, is presented in Fig. 9.

Clearly, unlabeled Ig(1b)2.9 is able to block itself (i.e., the ^{125}I-Ig(1b)2.9), while the same amounts of unlabeled Ig(1b)2.4 and Ig(1b)3.1 proteins did not block the subsequent binding of ^{125}I-Ig(1b)2.9. The same amounts of unlabeled Ig(1b)2.4 and Ig(1b)3.1 antibodies were also capable of blocking themselves (curves not shown), but not each other. Another antibody, Ig(1b)1.2, duplicates the Ig(1b)2.9 specificity profile. The enhancing effect of unlabeled Ig(1b)2.4 on the subsequent binding of ^{125}I-Ig(1b)2.9 is a reproducible observation and also occurs with other antibody combinations. The nature of this interaction is unknown at this point. It may involve an induced conformational change of the C.BPC101 myeloma protein by one antibody, making other allotypic determinants available for other antibodies to bind. This problem is presently being investigated.

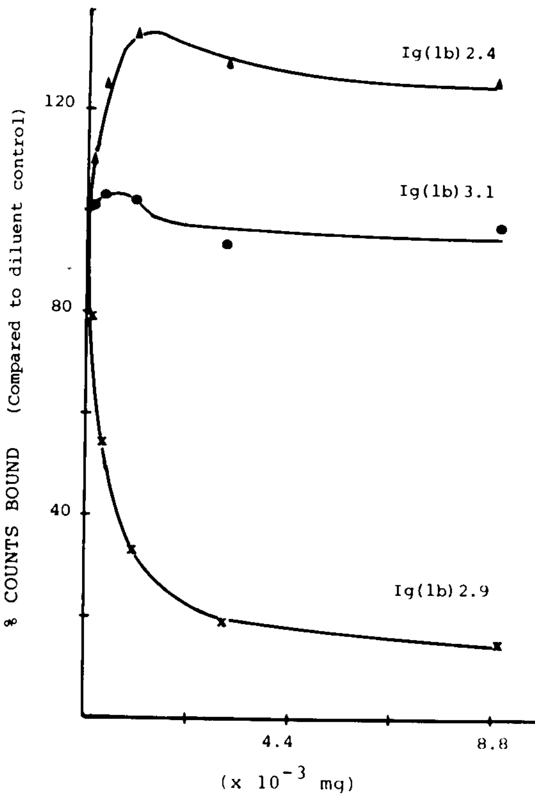

Fig. 9. Anti-Ig-1b blocking curve. Various concentrations of unlabeled Ig(1b)2.4, 3.1, and 2.9 were used (abscissa) to block the subsequent binding of ^{125}I-Ig(1b)2.9. One hundred percent of counts bound represents the number of ^{125}I-Ig(1b)2.9 bound when medium (1% BSA-PBS, pH 7.5) was used in the blocking step of the assay (as described in the text).

These new specificities, defined by the inability of the antibodies to block each other, represent distinct antigenic determinants. The members of pairs of determinants are far enough apart not to cause steric hindrance of antibodies binding to both determinants.

The chain compositions of the anti-Ig-1b and the anti-Ig-5a antibodies were examined by 2-D PAGE analysis (Fig. 10). The pattern obtained with (Ig(1b)1.7 protein shows that it actually contains two different heavy chains and three different light chains, despite the fact that it was "cloned" in soft agar. The other antibodies appear to be clonal products. The Ig(1b)2.9 protein and the Ig(1b)1.2 protein (not shown) do not have the MOPC 21 κ chain. Closer examination of the mobility patterns reveal that Ig(1b)2.9 and one of the proteins of Ig(1b)1.7 are identical. These products have similar specificity profiles as determined in the blocking assay. The other protein in Ig(1b)1.7 appears to be identical to Ig(1b)2.4. These could have been derived from independent fusions of cells derived in vivo from the same clone. The anti-Ig-5b antibody, 11-6.3, has not been purified but has been shown by Ouchterlony analysis to be an IgG_1 immunoglobulin.

V. Conclusion

This paper describes the methods that were used to generate stable, cloned hybrid cell lines secreting antibodies to $H\text{-}2K^k$, $I\text{-}A^k$, Ig-5a, Ig-5b, and Ig-1b antigens. The importance of the results presented here is that they illustrate the used of these monospecific reagents as new serological probes. The monoclonal anti-I-A antibodies provide a means for examining the polypeptide chain structure of Ia antigens.

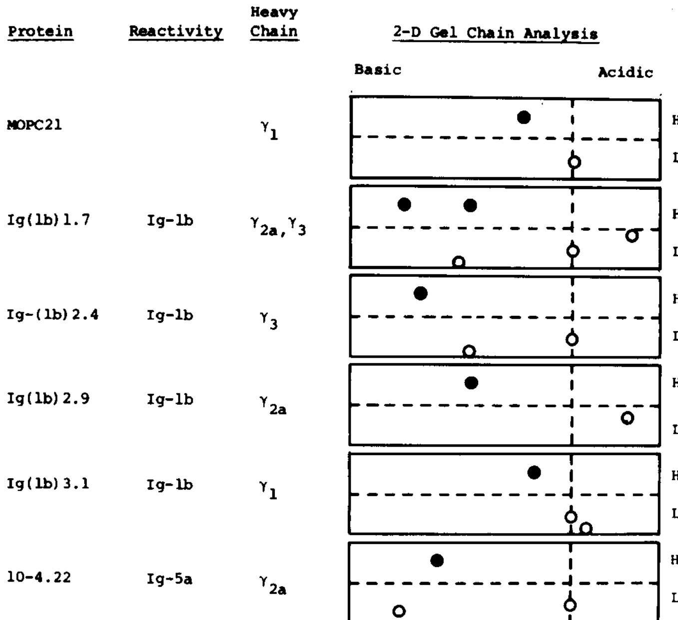

Fig. 10. Heavy and light chain compositions of anti-Ig-1b and Ig-5a antibodies from hybrid cell lines (see legend to Fig. 6).

With the monospecific anti-Ig-1b antibodies, the genetic polymorphisms and antigenic complexities of the γ_{2a} heavy chains can be dissected. Finally, the phenomenon of the enhanced binding of sets of anti-Ig-1b antibodies to Ig-1b protein may provide new information on antigen-antibody interactions. The availability of monospecific antibody reagents in general seems certain to introduce additional new uses of antibodies as molecular probes.

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