Immunogenetics 23: 137–139, 1986

Immuno-genetics

A Monoclonal Antibody Against Igh6-4 Determinant

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In order to investigate B-cell differentiation in a multicellular experimental system such as a stromal cell-dependent culture (Whitlock et al. 1984) or a radiation chimera, markers that are able to discriminate the B cells being pursued from other B cells are required. Among such markers, allotypic variations of immunoglobulin M (IgM) molecules are of particular importance, because IgM is the functional molecule that is expressed in all B cells from an early stage of differentiation. Anti-allotype sera are difficult to prepare, however, and used to be contaminated with antibodies of other specificities. Several monoclonal antibodies specific for allotypic determinants of mouse IgM have recently been produced as a solution to this problem (Kincade et al. 1981, Kung et al. 1981, Leptin et al. 1984, Stall and Loken 1984). Here, we report another monoclonal antibody, MB86, the allotypic specificity of which is different from that of other antibodies.

MB86 was produced by fusing X63-Ag8/653 myeloma cells with the spleen cells from a BALB/c mouse which had been immunized and boosted with C57BL/6 spleen cells. Because MB86 bound to B1–8 ($\mu\lambda_1$, IghC^b) protein but not to P8-86.9 ($\gamma_1\lambda_1$, IghC^b) protein which has the same V_H and V_L as B1-8 (Reth et al. 1978, Takemori et al. 1982) or to 20.2.267 ($\mu\lambda_1$, IghC^a) protein (White-Scharf and Imanishi-kari 1981), we concluded that MB86 recognizes an allotypic determinant of mouse IgM. We then tested the allotypic specificity of MB86 on eight mouse strains. Instead of using purified IgM, we used mouse spleen cells as antigens. One million spleen cells were incubated with either ^{125}I-labeled MB86 or ^{125}I-labeled rabbit mouse μ-specific antibody for 1 h. They were then washed, and the cell-bound radioactivity was determined (Table 1). The μ-specific antibody bound to the spleen cells of all strains to the same extent, whereas MB86 bound to B6, AKR, NZB, A/J, and CAL/20 cells, but not to BALB/c, DBA/2, or CBA cells. This pattern of reactivity of MB86 suggests that MB86 is specific for an Igh6-4 determinant which was defined by an alloantiserum by Black and co-workers (1978). Interestingly, MB86 further categorizes the Igh6-4-positive IgM into two groups. The first comprises *IghC* haplotypes *b*, *d*, and *n* which are strongly positive in MB86 binding, and the other includes haplotypes *e* and *o* which are weakly reactive. We therefore concluded that at least three allelic forms of the *Igh6* (μ) gene exist for the Igh6-4 determinant. It is of interest to note that these three allelic forms also determine an Igh6-5 determinant which was defined by monoclonal antibody Bet 1 (Kung et al. 1981). This could simply be a linkage of two determinants at different sites on the μ chain. A second possibility is that these two specificities are the different manifestations of a polymorphism of the same region of the μ heavy chain. In order to determine the localization of these determinants, we carried out the following two experiments. First, because it was reported that all the currently available monoclonal IgM allotype-specific antibodies recognized the determinants which appear

Table 1. Allotypic specificity of MB86

Spleen cells		Bound cpm*		cpm MB86	
Strain	IghC	MB86	RaMμ†	cpm RaMμ	
C57BL/6	*b*	14720‡	5778	2.54	++
BALB/c	*a*	150	6297	0.02	−
CBA/J	*j*	108	6799	0.02	−
DBA/2	*c*	106	8106	0.01	−
A/J	*e*	6184	6786	0.91	+
AKR	*d*	15980	7053	2.27	++
CAL20	*o*	6157	5479	1.12	+
NZB	*n*	8260	4499	1.84	++

* 10^6 spleen cells were incubated with 50 μl of 0.5 μg/ml iodinated antibodies for 1 h at 4 °C. After incubation, the cells were washed four times

† Rabbit mouse μ-specific antibody

‡ Arithmetic mean of duplicate assay

only when the heavy chain is associated with the light chain (Leptin et al. 1984, Veraldi et al. 1984), we tested the binding of MB86 to IgM proteins fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions. As shown in Figure 1, MB86 bound only to complete IgM molecules (lanes 1 and 2, Figure 1), while mouse μ chain-specific antibody bound to the reduced μ heavy chain, as well as to complete IgM (lanes 5 and 6, Fig. 1). As has been demonstrated for other monoclonal antibodies, this result shows that MB86 is also specific for the determinant which is formed by the μ heavy chain and light chain association, and that the Igh6-4 determinant is located on the CH1 domain of the μ heavy chain.

In order to further investigate the spatial relation of the Igh6 determinants defined by monoclonal antibodies, we then chose three monoclonal antibodies reactive with the IgM of *IghC*^{b} haplotypes, MB86, Bet 1, and AF6-78, which bind to the Igh6-4, -5, and -6 determinants, respectively. We then cross-blocked the binding of an antibody to B1-8. In Table 2, the data are expressed as the amount of cold antibodies required for 50% inhibition of binding of 50 ng of the ^{125}I-labeled antibodies to B1-8. All of these three antibodies were mutually inhibitory. Although variations did exist among antibodies as regards the blocking efficiency, inhibition of the B1-8 binding of one antibody by another was always complete when the amount of inhibitors was increased (data not shown). Furthermore, MB86 was always the best inhibitor for the binding of all three antibodies to B1-8. Therefore, unlike the steric inhibition observed between the binding of two monoclonal antibodies to two determinants at different sites on the IgD heavy chain (Stall and Loken 1984), the inhibition observed here is not due to the steric hindrance of a determinant by an antibody binding to a different site on the same molecule. Thus, we conclude that the determinants recognized by these antibodies, Igh6-4, -5, and -6, are positioned close together in a small region of the IgM CH1 domain. As summarized in Table 3, we propose that three alleles defined by Kung and co-workers as *Igh6* (μ) *a*, *b*, and *e* (Kung et al. 1981) are different at least in this region, and

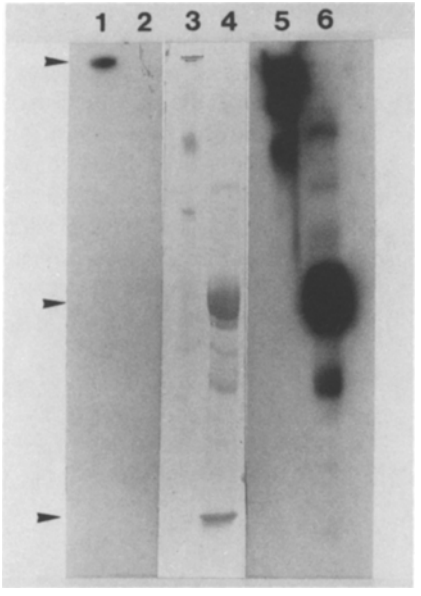

Fig. 1. Reactivity of MB86 to IgM subunits fractionated by SDS-PAGE. Ten micrograms of B1-8 was fractionated by 5–15% SDS-polyacrylamide gel without using stacking gel either in a nonreducing condition (lanes 1, 3, and 5) or a reducing condition (lanes 2, 4, and 6). Fractionated proteins in the gel were transferred to nitrocellulose membrane. Lanes 1 and 2 were incubated with ^{125}I-MB86, lanes 3 and 4 were stained with amido-black, and lanes 5 and 6 were incubated with ^{125}I-rabbit mouse μ-specific antibody. The *arrows* indicate the positions of intact IgM, the μ heavy chain, and the λ_1 light chain from the top, respectively

Table 2. Cross-blocking of B1-8 bindings by the antibodies specific for Igh6-4, -5, and -6 determinants*

Reaction	Inhibitors			
	MB86	Bet 1	AF6-78	Ls136†
MB86⟷B1-8	70	320	170	> 5000 ng
Bet 1⟷B1-8	40	110	110	> 5000 ng
AF6-78⟷B1-8	40	130	40	> 5000 ng

* Plates were coated with 50 μl of 10 μg/ml B1-8 solution and blocked with 1% bovine serum albumin-phosphate-buffered saline (PBS). 50 μl of 1 μg/ml labeled antibodies mixed with varying amounts of cold antibodies were added to the wells and incubated for 1 h at room temperature. After washing four times with PBS, the wells were cut for counting with a gamma counter. The absolute amount of cold antibody required for 50% inhibition of B1-8 binding of labeled antibody is calculated from the inhibition curve

† Ls136 is a monoclonal antibody specific for the λ_1 light chain (Reth et al. 1978)

Table 3. Strain distribution of Igh-4, -6, and -5 specificities defined by three monoclonal antibodies, MB86, Bet 1, and AF6-78

IghC haplotype	*Igh6* (μ) allele*	Strains	Specificities		
			6-4	6-5	6-6
a	*a*	BALB/c	–	+	–
j	*a*	CBA/J	–	+	–
c	*a*	DBA/2	–	+	–
b	*b*	C57BL/6	+	w†	+
d	*b*	AKR	+	w	+
n	*n*	NZB	+	w	+
e	*e*	A/J	w	–	–
o	*e*	CAL20	w	–	–

* Kung and co-workers (1981)

† Weakly positive

that three serotypes exist in this region. Igh6-$4^-5^+6^-$, Igh6-$4^+5^w6^+$, and Igh6-$4^w5^-6^-$, respectively. It still remains to be determined whether a fourth allele, *n* (Black et al. 1978, Kung et al. 1981) is different from others in this region. At present, the serotype of this allele is indistinguishable from that of allele *b* with these three monoclonal antibodies. Since the difference between alleles *b* and *n* was defined by Igh6-2 and Igh6-3 specificities (Black et al. 1978), further cross-blocking analysis using conventional antisera and monoclonal antibodies reactive to the IgM of haplotype $IghC^n$ will be needed to determine the location of these specificities.

Acknowledgments. We wish to thank Drs. K. Rajewsky, A. Radbruch, T. Saito, T. Tokuhisa, T. Takemori, A. M. Stall, M. Loken, M. White-Scharf, and W. E. Paul for providing antibodies and for helpful discussions. The excellent technical and secretarial help of Ms. Y. Takaoki is greatly appreciated. We are willing to distribute MB86 hybridoma cells to those investigators who need them. This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan, and a Shimizu Foundation Research Grant.

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Received October 10, 1985; revised version received November 4, 1985