(Reprinted from Nature, Vol. 265, No. 5593, pp. 447–449, February 3, 1977)

Allotypes of mouse IgM immunoglobulin

GENETIC polymorphism of the structural genes encoding the class-specific (heavy) polypeptide chains of the immunoglobulin (Ig) molecules provides a useful set of markers for elucidating the arrangement and expression of these genes. On the basis of various antigenic, physiochemical and biological properties, the immunoglobulins of the mouse have been divided into eight distinct classes, IgM, IgD, IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgE, with a specific structural gene determining the heavy chain (H) for each class. Previous studies have documented the existence of a genetic polymorphism for five of these H-chain genes[1–3], with all the genes being closely linked constituting a heavy chain chromosome region. The most recent of these loci to be demonstrated (Ig-5, determining the δ chain)[3] was detected by the reaction of alloantisera prepared against spleen cells from mice differing in both *H–2* and allotype. These sera reacted with B lymphocytes of the appropriate allotype congenic strains, as assessed by either immunofluorescence, or cell surface iodination and polyacrylamide gel electrophoresis (PAGE). As most B lymphocytes express cell surface IgM as well as IgD (refs 4–6), we have further pursued these various approaches to identify Ig allotypes and have found a polymorphism of the heavy chain (μ) of murine IgM, a molecule found in pentameric form (19S) in serum, and in monomeric (8S) form on the surface of lymphocytes. This defines a locus, Ig-6, which encodes the μ chain of most mouse IgM immunoglobulin.

Alloantisera containing anti-IgM antibodies were raised in various strain combinations by two different methods. A small proportion of anti-allotype sera raised by repeated immunisation with pertussis anti-pertussis complexes[1], were found to contain antibodies to allotypic determinants on IgM molecules. However, the most effective antisera against IgM allotype have been raised by spleen cell immunisation (4–6 weekly injections of 10^7 spleen cells, with or without adjuvants) between strain combinations which differ in *H–2* type as well as allotype.

The binding capacities of these antisera against purified mouse immunoglobulins were assessed by radioimmunoassay[1] using purified ^{125}I-labelled mouse myeloma proteins[2]. Each labelled myeloma protein was more than 80% precipitable by the appropriate heterologous anti-mouse heavy-chain serum, while less than 10% was precipitated by all other anti-H-chain sera. The IgM proteins used were HPC-76 and MOPC104 (IG-6a) of BALB/c origin, and C.BPC112 (IG-6b) of C.B/20 (BALB/c Ig^b) origin (kindly provided by Dr M. Potter). The allelic designations for Ig-6 are assigned as for other allotype loci according to the Ig-1 allele of the type strains in which they are found[2]. Thus, Ig-6a is from BALB/c and Ig-6b is from C57BL/6. The precipitating activity of such antisera against ^{125}I-labelled HPC-76 is shown in Fig. 1. Antisera GA20 and GA26 were both made in C57BL/6 (C57) against pertussis anti-pertussis (of CBA origin) complexes. Each contained anti-Ig-1a antibodies (Fig. 1*b*), but serum GA20 also contained anti-Ig-6a antibody (Fig. 1*a*). In contrast, the serum MA3 prepared in C57 mice against CBA spleen cells, contained no anti-Ig-1a antibody, but did contain anti-Ig-6a antibody (Fig. 1*a* and *b*).

The anti Ig-6a sera were specific since they did not precipitate an IgM myeloma of C.B/20 (Ig^b) origin, C.BPC112 (see Table 1). Their specificity was confirmed by inhibition studies in which no myeloma protein of other classes (IgA, IgG_1, IgG_2) inhibited precipitation of ^{125}I-labelled HPC-76. Furthermore, unlabelled HPC-76, MOPC104 and normal serum from BALB/c did inhibit this precipitation, although MOPC104 was considerably less effective in inhibiting than the other inhibitors.

Antisera to another IgM allotype, Ig-6a, were prepared in SJA (Ig^a) against spleen cells of Ig-1b positive strains BALB/c.Ig^b (BAB/14) or CWB (C3H.SW.Ig^b). These sera precipitated C.BPC112 but precipitated neither HPC-76 nor MOPC104 (Table 1). Inhibitions of precipitation were found with Ig-6b containing sera and not with Ig-6a containing sera or myelomas. Similarly, no non-IgM myeloma protein inhibited ^{125}I-C.BPC112 precipitation with these alloantisera (data not presented).

Close linkage of Ig-6 to the Ig heavy chain region is implied in the use of allotype congenic strains for antiserum testing. Anti-Ig-6a (for example, antiserum MA-3 in Fig. 1*c*) is inhibited in its precipitation of HPC-76 by serum from BALB/c (Ig^a) and not by serum from the congenic

Table 1 Anti-Ig-6 antibody detected by radioimmunoassay, immunofluorescence and cell surface radioiodination/polyacrylamide gel electrophoresis (PAGE)

Alloantiserum	Precipitation of ^{125}I IgM†		Positive immunofluorescence‡		PAGE analysis‖	
	HPC-76 (Ig-6a)	C.BPC112 (Ig-6b)	Strain	Splenic B cells	Strain	Heavy chain detected
GA20: C57BL anti-CBA* serum	Yes	No	BALB/c	Yes§	BALB/c	μ
			BALB/c.Ig^b	No§		
GA26: C57 anti-CBA serum	No	No	BALB/c	No	BALB/c	—
MA3: C57BL anti-CBA spleen	Yes	No	C57.Ig^a	Yes	CBA	μ,δ
			C57BL	No		
MB1: SJA anti-BALB/c.Ig^b spleen	No	Yes	SJL	Yes	C57BL	μ,δ
			SJA	No		
MB2: SJA anti-BALB/c.Ig^b spleen	No	No	SJL	Yes	C57BL	δ
			SJA	No		
MB3: SJA anti-CWB spleen	No	Yes	SJL	Yes	C57BL	μ
			SJA	No		

*CBA anti–pertussis serum/pertussis complex.
†Determined by radioimmunoassay as previously described[1].
‡Revealed by indirect immunofluorescence using a fluoresceinated goat anti-mouse IgG (refs 3 and 9).
§Analysis was by visual fluorescence microscopy and/or with a fluorescence-activated cell sorter (FACS).
Yes: almost all B cells were stained. No: few, if any, B cells were stained.
‖PAGE analysis of ^{125}I-labelled membrane protein precipitated by antisera[3].
Mouse strains used in these experiments are of the following allotypes: Ig^a-CBA, BALB/c, SJA, C57.Ig^a; Ig^b-C57BL/6, BAB/c.Ig^b (BAB/14 or C.B/20), SJL, CWB (C3H.SW.Ig^b).

MOPC104, an IgM myeloma from the Ig^a strain BALB/c (data not shown). Neither conjugate removed any staining activity from the opposite antisera. Heterologous anti-mouse μ chain blocked staining by both these antisera as well as by serum GA20. These same allotype antisera also precipitate cell surface IgM shown by PAGE of radioiodinated membrane proteins[3,7]. Thus, these alloantisera detect the allotypes Ig-6a and Ig-6b on cell-surface IgM.

Some antisera prepared by allogeneic spleen cell injections also have antibodies against IgD heavy-chain allotypes (Ig-5)[3,9]. Serum MB2 stained SJL spleen cells, although it contained no anti-Ig-6b reactivity as shown by radioimmune precipitation. This staining activity of SJL cells by serum MB2 was not removed by adsorption with insolubilised C.BPC112 nor was it blocked by pretreatment with heterologous anti-mouse μ. MB2 (Table 1) also precipitates radioiodinated cell surface IgD demonstrated by PAGE (ref. 3). Anti-Ig-5b activity also accounts for the small residual staining of IgM-adsorbed serum MB1 (Fig. 2*c*). The μ and δ precipitation activity of each of the sera detected by PAGE is given in the last column of Table 1. These data show that sera made against allogeneic spleen cells may have anti-allotype antibodies to δ chains (Ig-5), μ chains (Ig-6) or both in varying amounts. These new allotypes will serve as useful markers in following B-cell differentiation and gene expression. They may also aid in fine structure mapping of the Ig heavy-chain region which contains genes for both the constant and variable parts of the polypeptides. Some of these data has been presented[9]. A more detailed immunogenetic analysis of these loci will be published elsewhere.

This work was supported by research grants from the US National Institutes of Health; the Wellcome Foundation; and the National Health and Medical Research Council, Canberra, Australia. G.A.G. is an Arthritis Foundation Fellow, G.W.W. is in receipt of a postdoctoral fellowship from the Science Research Council, S.J.B. is a fellow of the American Cancer Society, California Division, and W. vdL. is supported by a NATO research fellowship. We thank Mr D. Hewgill and Mr F. T. Gadus for technical assistance.

NOEL L. WARNER
JAMES W. GODING
GEORGE A. GUTMAN
GREGORY W. WARR

The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital Post Office, Victoria 3050, Australia

LEONARD A. HERZENBERG
BARBARA A. OSBORNE
WESSEL VAN DER LOO
SAMUEL J. BLACK
MICHAEL R. LOKEN

Department of Genetics, Stanford University School of Medicine, Stanford, California, 94305

Received November 11; accepted December 6, 1976.

[1] Herzenberg, L.A., & Warner, N. L., in *Regulation of the Antibody Response* (ed. Cinader, B.), 322–348 (C. C. Thomas, Springfield, Illinois, 1968).
[2] Herzenberg, L. A., McDevitt, H. O. & Herzenberg, L. A. *A. Rev. Genet.* **2**, 209–244 (1968).
[3] Goding, J. W., Warr, G. W. & Warner, N. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1305–1309 (1976).
[4] Pernis, B., Brouet, J. C. & Seligmann, M., *Eur. J. Immun.* **4**, 776–778 (1974).
[5] Goding, J. W. & Layton, J. *J. exp. Med.* (in the press).
[6] Abney, E. R., Hunter, I. R. & Parkhouse, R. M. E. *Nature* **259**, 404–406 (1976).
[7] Kessler, S. W. *J. Immun.* **115**, 1617–1624 (1976).
[8] Weigert, M., Potter, M. & Sachs, D. *Immunogenetics* **1**, 511–523 (1975).
[9] Herzenberg, L. A. *et al. Cold Spring Harb. Symp. quant. Biol.* **41** (in the press).

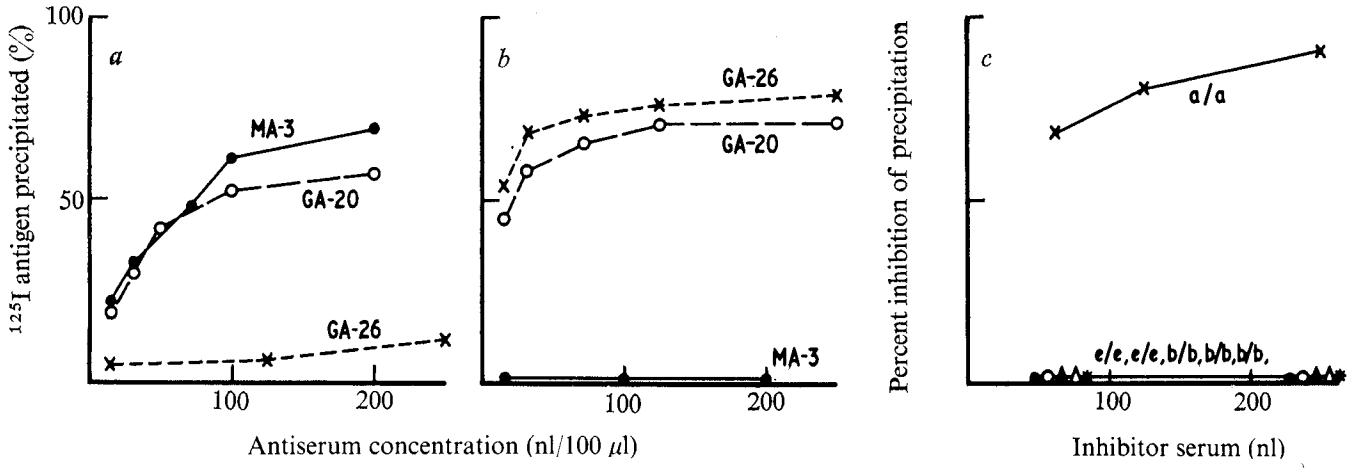

Fig. 1 Radioimmunoassay analysis of anti-Ig-6a antisera. The ability of alloantisera GA20, GA26 and MA3 to precipitate ^{125}I-labelled IgM (HPC-76 macroglobulin) or IgG_{2a} (HPC-149) of BALB/c origin is shown in *a* and *b*, respectively, as the per cent precipitation of the labelled antigen against the amount of antiserum used. For panel *c* cell assays were performed in a volume of 100 μl with 5 ng of ^{125}I-labelled HPC-76 (Ig-6a). All inhibitor sera derived from 8-week-old mice of the following allotype congenic strains: BALB/c (Ig^a), BALB/c.Ig^b; C57BL(Ig^b), C57BL.Ig^e NZB-(Ig^e), NZB.Ig^b. Note that the lack of inhibition by Ig^e sera indicates that Ig-6^a and Ig-6^e do not cross react with this antiserum. Further details of antigenic specificity will be published.

mice BALB/c.Ig^b (BAB/14) (Fig. 1*c*) nor from C.B/20 (data not shown) which have all the alleles of BALB/c except these closely linked to Ig-1. Since something more than 21 backcross generations are involved in the derivation of these strains (they are not completely independently derived), very close linkage is indicated for Ig-6 to Ig-1 and the other heavy-chain loci[2].

These results with radioimmunoassay analysis demonstrate that antisera prepared against either cell-surface 8S IgM or serum 19S IgM, are capable of binding to 19S IgM molecules. We have also shown by immunofluorescence, that these antisera can react with cell-surface 8S IgM. Spleen cells from 8-week-old BALB/c (Ig^a), BALB/c.Ig^b, SJL (Ig^b), or SJL.Ig^a (SJA) allotype congenic strains were treated with a 5–10% (v/v) concentration of the alloantisera, washed and then reacted with a fluorescein-conjugated goat anti-mouse γ_1 and γ_2 antiserum. The latter serum was completely non-reactive to IgM, as shown by immunofluorescence and radioimmunoassay. Fluorescence was detected by fluorescence microscopy as previously described[3], or by flow fluorometry with a fluorescence-activated cell sorter (FACS)[9]. These data are summarised in Table 1.

Table 1 shows that the same sera which precipitate secreted IgM also bind to cell surface IgM. Antisera made against pertussis–anti-pertussis complexes (that is, against secreted IgM) could be tested on spleen cells of any strain whereas antisera made against spleen cells could not because of the possible presence of antibodies to other cell surface alloantigens. With these latter antisera, spleen cells from mice congenic but for allotype with the antibody produced were used.

The antisera made in C57(Ig^b) against CBA(Ig^a) stained C57.Ig^a spleen cells and, of course, did not stain C57 cells (Table 1). Similarly, antisera made in SJA(Ig^a) against Ig^b cells stained SJL(Ig^b) but not SJA cells (Table 1 and Fig 2, *a* and *b*). Thus the surface molecules detected by these antisera are coded by genes in the Ig heavy-chain chromosome region.

The immunoglobulin class of the cell-surface molecules detected in these immunofluorescent reactions was determined by adsorption and blocking studies as well as by gel electrophoresis (PAGE). Serum MB1, SJA anti-BALB/c.Ig^b spleen, passed through a column of Sepharose-conjugated C.BPC112, an IgM of *b* allotype (Ig-6b), lost most of its staining activity for SJL (Fig. 2*c*). Therefore most of the reactivity is directed towards IgM. The antithetical serum against Ig-6a was similarly adsorbed by Sepharose

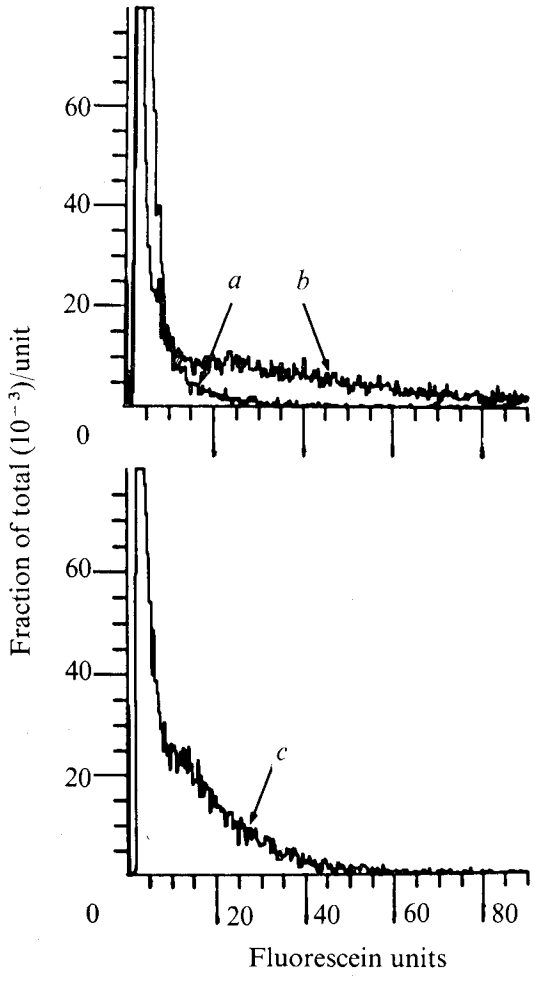

Fig. 2 FACS fluorescence analysis of anti-Ig.6^b serum. Fluorescence profile of SJA(Ig^a) anti-BALB/c.Ig^b serum on (*a*) SJA spleen cells (Ig^a), (*b*) SJL spleen cells (Ig^b), (*c*) SJL spleen cells after adsorption of serum with Sepharose-bound IgM myeloma C.BPC112 (Ig-6b). Cells (2×10^6) were reacted with 2 μl (diluted to 40 μl with buffered saline containing 0.2% sodium azide) SJA anti-BALB/c.Ig^b serum for 20 min at room temperature. These cells were washed and stained with fluoresceinated goat anti-mouse IgG for a further 20 min at room temperature and analysed on the FACS. Adsorption of this anti-Ig-6b serum by Sepharose-bound Ig-6a did not reduce its staining activity for SJL spleen cells.