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Presence of natural autoantibodies in hyperimmunized mice

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Summary. Mice were immunized with various antigens in complete Freund's adjuvant following various injection schedules. Hybridomas were produced from the spleens of these immunized mice and examined for production of antibodies directed against the antigen injected and against a panel of self (tubulin, actin, myosin, DNA) and non-self antigens (myoglobin, spectrin, peroxidase, trinitrobenzene). Two to five percent of the hybrids were found to secrete polyspecific antibodies able to react with two or more antigens of the panel. Several of these hybrids were subcloned and expanded into ascites. The monoclonal immunoglobulins they secreted were isolated and shown to be IgM (κ) and to possess the polyspecific antibody function. Several hybrids were also found to secrete antibodies reacting with the immunizing antigen as well as one or more antigens of the panel. The antibody secreted by one subclone which reacts with both the immunizing antigen, prolactin and one of the panel antigens, TNP, has been isolated using a DNP-immunoadsorbent. The isolated antibody was found to be a monoclonal IgM (κ) immunoglobulin and to react both with prolactin and TNP.

The hypothesis is advanced that cells carrying polyspecific natural antibodies as receptors after a given antigenic stimulation proliferate into cells producing highly specific antibodies for epitopes of that given antigen; the cells with polyspecific receptors will be continuously replaced by new cells probably on bone-marrow origin.

Abbreviations: OV, egg ovalbumin; HSA, human serum albumin; BSA, bovin serum albumin; TNP, trinitrobenzenesulphonic acid; HIST, histamine; BU, 5-bromouridine; IAg, antigen used for immunization; PAg, antigen of the panel.

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INTRODUCTION

The presence of 'natural' antibodies, in the sera of physiologically healthy animals, directed against a variety of antigens has been well established and documented (Boyden, 1964; Cunningham, 1974; Shinomiye & Koyame, 1974; Martin & Martin, 1975; Mead, Gowin & Whitehouse, 1977; Steele & Cunningham, 1978; Longenecker & Mosmann, 1980; Ivanyi *et al.*, 1982; Lutz & Wipf, 1982; Avrameas *et al.*, 1983; Grabar, 1983; MacKay, 1983; Cairns, Block & Bell, 1984; Galili *et al.*, 1984; Lutz, Flepp & Stringaro-Wipf, 1984; Prabhakar *et al.*, 1984).

In the laboratory, while studying the preparation of specific anti-tubulin antibodies, it was realized that, in fact, anti-tubulin antibodies were present in the sera of various animal species before any experimental immunization (Karsenti *et al.*, 1977). This initial observation was followed by a series of experiments performed in man and mouse, intending to establish the existence, specificity and significance of these natural antibodies. Thus, we found and isolated from normal sera, natural antibodies to tubulin, actin, thyroglobulin, albumin, transferrin, cytochrome *c*, fetuin and collagen (Avrameas, Guilbert & Dighiero, 1981). The main charac-

teristic of these natural antibodies was that, in contrast to what it is known about experimentally induced antibodies, the great majority of natural antibodies were able to react with more than two antigens, sometimes corresponding to self antigens (Guilbert, Dighiero & Avrameas, 1982). Additional experiments performed with 600 human monoclonal immunoglobulins derived from patients suffering from Köhler's disease or Waldrenström's macroglobulinaemia showed that some of them possessed antibody specificities similar to those of the natural antibodies (Dighiero, Guilbert & Avrameas, 1982; Dighiero *et al.*, 1983a).

Using the spleen cells from normal adult unimmunized BALB/c mice, we prepared hybridomas and demonstrated that several clones secreted monoclonal immunoglobulins with specificites similar to those of humans, able to recognize various self and non-self antigens (Dighiero *et al.*, 1983b). Finally, we studied hybridoma clones derived from the spleen cells of 6-day-old unimmunized BALB/c mice and found a high incidence of clones producing natural antibodies and possessing broad specificities and autoreactivities similar to those of the natural antibodies found in adult mice (Dighiero *et al.*, 1985).

This investigation was undertaken in order to determine if cells producing natural antibodies were present in hyperimmunized mice. The results obtained demonstrate that these cells, in amounts of equal to two times greater than those in normal unimmunized animals, are found in the spleens of hyperimmunized mice. Their presence suggest that an extensive antigen stimulation is not sufficient to cause their negative selection.

MATERIALS AND METHODS

Antigens

Egg albumin (OV), human serum albumin (HSA), bovine serum albumin (BSA), double stranded DNA, human prolactin, whale skeletal muscle myoglobin, trinitrobenzenesulphonic acid (TNP), histamine (HIST) and 5-bromouridine (BU) were purchased from Sigma Chemical Co (St Louis, MO). TNP-BSA conjugates were prepared according to Little & Eisen (1966), HIST-OV using the *p*-benzoquinone method of Ternynck & Avrameas (1977) and BU-BSA using *m*-periodate following the protocol of Erlanger & Beiser (1964).

Horseradish peroxidase Grade 1 was purchased from Böehringer (Mannheim, FRG) and human spectrin was a gift from Dr E. Lazarides (Caltech, Pasadena, CA). BALB/c brain tubulin, muscle actin and myosin were prepared as previously described (Dighiero *et al.*, 1983b). All antigens were examined by SDS-polyacrylamide gel electrophoresis and each of them was found to be free of contamination by the others.

Antibodies

Sheep antibodies to mouse or rabbit Ig and rabbit antibodies to goat Ig were isolated on Ultrogel 34 Ig-immunoadsorbents (Ternynck & Avrameas, 1976). The purified antibodies were coupled to peroxidase and to *E. coli b*-galactosidase (kindly provided by Dr Ullmann, Institut Pasteur, Paris, France) according to procedures described previously (Avrameas, Ternynck & Guesdon, 1978). Rabbit or goat antisera specifically directed against mouse IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, κ and λ were purchased from Nordic Immunology Laboratory (Tilburg, The Netherlands).

Cell fusions

Somatic cell hybridization was carried out 3 days after the last antigen administration, according to the protocol of Köhler & Milstein (1976) as previously described in detail, using the non-secreting hybridoma line Sp2 (Dighiero *et al.*, 1983b). The fusions were distributed into microwells at concentrations resulting in 70–80% growing cultures.

Lines secreting immunoglobulins with 'natural' antibody-like specificity were subcloned, at least twice, by limiting dilution as previously described (Dighiero *et al.*, 1983b). All hybrids were expanded in culture and some of them grown as ascitic fluids in BALB/c or (BALB/c × C57BL/6) F_1 hybrids.

Animals and immunization

BALB/c mice were immunized with either HSA, rat C57BL/6 myosin or HIST-OV (13:1 molar ratio), while (BALB/c × C57/BL6) F_1 mice were immunized with BU-BSA (28:1 molar ratio). High responder mice from the Biozzi line (Biozzi *et al.*, 1982) were immunized with human prolactin.

For immunization with HSA, each mouse was given 40 μg of the antigen by subcutaneous injection and 20 μg of HSA in complete Freund's adjuvant in each hind footpad on Days 0 and 15. On Day 23, 3 days before the fusion was performed, the mouse was injected intravenously with 30 μg of HSA.

For immunization with rat myosin, each mouse was injected subcutaneously with 20 μg of the antigen in complete Freund's adjuvant on Days 0, 6, 13, 20 and 27. On Day 34 the mouse received intraperitonal 20 μg of the antigen and the fusion was carried out on Day 37.

For immunization with HIST-OV, each mouse was injected subcutaneously with 100 μg of the antigen in complete Freund's adjuvant on Day 0. On Day 14, the mouse was injected subcutaneously with 100 μg of HIST-OV in incomplete Freund's adjuvant and on Day 21, it was inoculated intravenously with 50 μg of HIST-OV. Its spleen cells were fused on Day 25.

For immunization with BU-BSA each mouse was given subcutaneously 100 μg of the antigen in complete Freund's adjuvant on Days 0 and 35 and the fusion was carried out on Day 41.

For immunization with prolactin, each mouse was given subcutaneously 4 μg of the antigen in complete Freund's adjuvant on Days 0, 10, 20, 30, 40, 50, 60, 70. On Day 80, 3 days before the fusion was performed, the mouse was injected intravenously with 4 μg of prolactin.

Screening of the culture supernatants for natural antibody activity by enzyme-immunoassay

Polystyrene flat-bottomed Nunc plates (Roskilde, Denmark) coated with the antigen used for immunization (IAg) and with each of the antigens of the panel (PAg): actin, tubulin, myosin, TNP-BSA, spectrin, DNA, myoglobin and peroxidase, were incubated with each supernatant. After washing, the plates were incubated with the *b*-galactosidase-labelled sheep anti-mouse Ig antibody, further washed and the enzyme substrate added. The colour which developed was determined with a Titertek multiskan. The values obtained with the culture medium were considered as background. As negative controls three mouse monoclonal IgM without known antibody activity were used; none of them exhibited values higher than those of background. A murine monoclonal IgM, found to possess natural antibody specificity, was used as the positive control. The detailed procedures followed have previously been described (Guilbert, Dighiero & Avrameas, 1982; Dighiero *et al.*, 1983b; Dighiero *et al.*, 1985).

Isotypes of the monoclonal antibodies secreted by the hybridomas

Antibody isotypes were determined by incubating the antigen coated plates with culture supernatants (1:5 dilution), ascitic fluids (1:10000) or the isolated monoclonal antibody (1–2 μg/ml). Goat or rabbit antiserum against mouse μ, γ1, γ2a, γ2b, γ3, α, κ and λ immunoglobulins were added and followed by peroxidase coupled anti-goat or anti-rabbit Ig antibody (1 μg/ml). The isotypes of the monoclonal antibodies in ascitic fluid were also assessed by immunoelectrophoresis using a rabbit anti-mouse Ig antiserum.

Isolation and characterization of mouse monoclonal IgM from ascitic fluids

All the monoclonal antibodies in the ascitic fluids were found to belong to the IgM (κ) isotype. They were separated, following a procedure described for the isolation of human monoclonal IgMs, by passing 3 ml of ascites through a Sephacryl S-200 column (2 × 25) equilibrated with 5 mM phosphate buffer pH 8 (Bouvet, Pires & Pillot, 1984). The isolated IgMs, following procedures previously described, were reduced and analysed either by SDS-polyacrylamide gel (10%) or by isoelectrofocusing (pH 3–10 gradient) in polyacrylamide gel (4%) containing urea (9·5 M) (Weber & Osborn, 1969); O'Farrell, 1975).

In order to examine their specificities, nitrocellulose immunoprints were made with some of the isolated IgMs. Actin, tubulin, myosin and TNP-BSA were separated by electrophoresis of SDS-polyacrylamide gels, transferred onto nitrocellulose, and the non-specific nitrocellulose sites were blocked by incubation with BSA. The nitrocellulose filters were then incubated with the monoclonal IgM (5–100 μg/ml), washed and exposed to peroxidase-labelled rabbit anti-mouse Ig antibody. The enzyme activity was revealed with diaminobenzidine. The detailed procedures have previously been published (Dighiero *et al.*, 1985).

The specificities of the isolated monoclonal IgM were further examined following a previously described competitive enzyme-immunoassay procedure. Briefly, the 50% fixation point of each monoclonal IgM bound to the antigens immobilized on the polystyrene plates was determined by the enzyme-immunoassay described above. The IgM was then incubated at this concentration with increasing concentrations of soluble competing antigen and the inhibition of IgM fixation to the plates was determined. The procedures followed have been described in detail elsewhere (Guilbert *et al.*, 1982; Dighiero *et al.*, 1985).

RESULTS

The hybrids, obtained with the spleens from mice immunized with the various antigens, were tested by enzyme-immunoassay for Ig secretion, for antibody activity against the IAg and for all the PAg: actin, tubulin, myosin, spectrin, myoglobin, peroxidase, DNA and TNP-BSA. The results obtained are summarized on Table 1, where only the hybrids which secrete antibodies with an evident binding for one or more of the PAg are given. Thus, only hybrid supernatants giving an optical density (OD) exceeding the background (OD < 0·020) by at least five times are included. Two to five percent of the Ig secreting hybrids were found to possess natural antibody activity and to be able to react with two or more PAg.

Furthermore, a large number of cells secreting antibodies with a single specificity for one of the PAg is observed. The greater majority of these hybrids was found to recognize TNP: three out of three clones derived from mice immunized with HSA; two out of four from mice immunized with HIST-OV; one out of two immunized with BU-BSA; and four out of six immunized with prolactin. The remaining antibodies with single specificities recognized either tubulin, DNA, myosin, actin or peroxidase.

From Table 1, it appears that hybrids which recognize the IAg and one or more of the PAg are also present. Among the nine such hybrids found: five recognized the IAg and one of the PAg (four reacted with TNP and one with DNA), one recognized the IAg and two PAg (TNP and tubulin) and the remaining two reacted with more than three PAg.

Cells were subcloned at least twice and their specificities were re-examined. In general, it was found that the subcloned cells possessed the initial specificities of the hybrids, although it was also noted, that some of the subclones derived from hybrids recognizing more than 3 PAg were able to bind to one or two additional PAg.

During culture and subcloning, several clones lost their capacities to synthesize antibodies. This was the case for all the clones recognizing only one PAg and for those recognizing the IAg and one or more PAg, with the exception of the clone reacting with prolactin

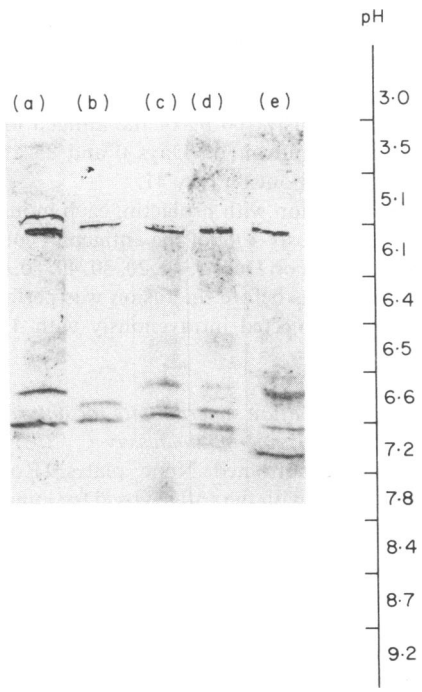

Figure 1. Isoelectrofocusing of five IgMs isolated from ascites: (a) IgM1; (b) IgM2; (c) IgM6; (d) IgM7; (e) IgM8. The specificities of these IgM are found in Table 2.

Table 1. Antibody activity of hybrids derived from the spleens of mice immunized with various antigens

Immunizing antigen (IAg)	Clones obtained	Clones secreting Ig	Clones reacting only with IAg	Clones reacting with IAg and with at least one test panel antigen (PAg)	Clones reacting with only one PAg	Clones reacting with two PAg	Clones reacting with three or more PAg
HSA	240	43	14	3	3	1	0
Rat myosin	187	138	15	3	0	0	5
HIST-OV	153	121	30	1	4	1	5
BU-BSA	74	64	5	1	2	0	2
Prolactin	355	355	28	1	6	0	13

and TNP which continues to produce antibody even after several subclonings. Supernatants from this latter clone were concentrated ten times by ultrafiltration, passed through a DNP-Lysine-Sepharose immunoadsorbent equilibrated with 0·2 M H_3BO_3-NaCl buffer, pH 8·0. The column was washed with the buffer, and the adsorbed proteins were eluted with 0·1 M DNP-glycine, pH 8, (Jaffe *et al.*, 1969). The eluate was concentrated and dialysed extensively until it became colourless. The monoclonal Ig isolated in this manner was found to be an IgM (κ) and to react, by enzyme-immunoassay, with both prolactin (sensitivity limit: 20 μg/ml of antibody) and TNP-BSA (sensitivity limit: 1·25 μg/ml of antibody).

Ascites were prepared in mice with two subclones derived from animals immunized against HSA, six subclones from myosin immunizations, eleven from HIST-OV injected mice and three from BU-BSA inoculated animals. Antibody activity was not found in ten out of the twenty-two ascites produced. The activities of the remaining positive ascites were found to be associated with an IgM (κ) isotype. These monoclonal IgMs were purified as described in 'Materials and Methods', using Sephacryl chromatography. The material isolated was tested by immunoelectrophoresis using a rabbit anti-mouse Ig polyspecific antiserum and was found to contain only IgM with restricted electrophoretic mobility, characteristic of monoclonal type IgM. The isolated IgMs analysed under reducing conditions by SDS-polyacrylamide gel electrophoresis were found to contain bands corresponding to μ and κ chains. When the IgMs were examined by isoelectrofocusing, they were found to contain a restricted number of bands (three to five), which is characteristic of monoclonal immunoglobulins (Fig. 1).

The antibody specificities of the isolated IgMs were compared to those of the subclones (Table 2). In general, it was found that the specificity of the isolated monoclonal IgM corresponded well with that of the sub-clones, from which it originated, although occasionally several differences were noted: some isolated monoclonal IgM were found to react with one or two additional antigens even though the supernatants from the corresponding subclones were either negative or only weakly positive with these additional antigens.

Table 2. Specificities of subclones and isolated monoclonal IgM derived from mice immunized with various antigens

IAg	Test preparation	Actin	DNA	Myosin	Peroxidase	TNP-BSA	Tubulin	Spectrin	Myoglobin
HSA	Subclone 1	+†	−	−	−	++	++	−	−
	IgM1*	++	−	−	−	++	++	+	−
	Subclone 2	+	−	++	−	++	++	−	−
	IgM2	++	+	++	−	++	++	+	−
Rat Myosin	Subclone 3	+	−	+	−	−	−	++	++
	IgM3	++	−	+	−	++	−	++	++
	Subclone 4	−	++	−	−	++	−	−	−
	IgM4	−	++	−	−	++	−	−	−
	Subclone 5	+	++	−	−	++	−	+	+
	IgM5	++	++	+	−	++	−	++	++
	Subclone 6	−	++	−	−	++	++	−	++
	IgM6	++	++	−	−	++	++	−	++
	Subclone 7	++	++	−	−	++	++	++	++
	IgM7	++	++	+	−	++	+	++	++
HIST-OVA	Subclone 8	++	++	−	−	++	−	++	++
	IgM8	++	++	++	−	++	−	++	++
	Subclone 9	−	++	−	−	++	−	−	−
	IgM9	−	++	−	−	++	−	−	−
	Subclone 10	++	++	++	++	++	++	++	++
	IgM10	++	++	++	++	++	++	++	++

* The number denotes the origin of the IgM.
† ++ Supernatants from subclones (1:5 dilution) or isolated IgM (5 μg/ml) giving an OD > 0·500.
\+ 0·500 > OD > 0·100 for a background < 0·020.

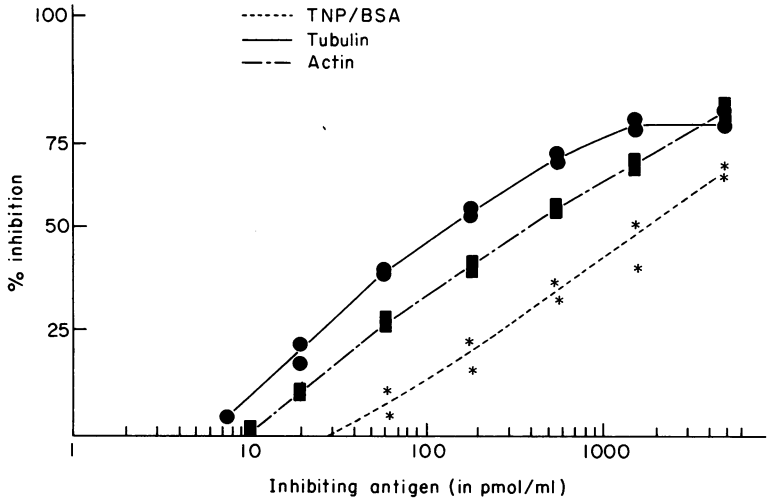

Figure 2. Inhibition of binding of a monoclonal IgM antibody to tubulin-coated plates. The antibody (IgM1 of Table 2) which was found to recognize mainly immobilized actin, TNP-BSA and tubulin is inhibited by the same soluble antigens. Soluble myosin, DNA, peroxidase, spectrin and myoglobin do not inhibit this binding.

In order to examine further their specificities, inhibition and immunoblotting experiments were carried out with five isolated monoclonal-IgMs. The enzyme-immunoassay inhibition experiments performed showed that an IgM antibody which bound primarily to a given number of antigens was also inhibited, for the most part, by these same antigens, although sometimes an additional antigen was also found to block the reaction. Representative results of these inhibition experiments, obtained with one of the monoclonal IgM antibodies are given on Fig. 2. The immunoblotting experiments, carried out with the isolated IgM, confirmed the results obtained with the enzyme-immunoassay. Thus, a given monoclonal IgM, exhibiting preferential binding with a certain number of antigens by enzyme-immunoassay also showed a similar order of reactive preference by immunoblotting.

DISCUSSION

The results obtained in this study show that in the spleens of mice extensively immunized with various antigens and injection schedules, immunocytes are present and they secrete antibodies which are able to recognize more than two PAg. The experiments performed demonstrate that, as in normal adult non-immunized mice (Dighiero *et al.*, 1983b), all these antibodies belong to the IgM (K) family. Furthermore, they indicate that these antibodies possess specificities similar to those secreted by the immunocytes found in non-immunized mice. It can therefore be concluded, that cells which synthesize immunoglobulins with polyspecific natural antibody-like function i.e., capable of reacting with more than two apparently immunologically unrelated antigens, are present in both immunized and non-immunized mice. In previous studies, we advanced the hypothesis that similar, but not necessarily identical, three-dimensional structures present on these antigens are responsible for these reactions (Avrameas *et al.*, 1983; Dighiero *et al.*, 1983b; 1985). In addition, cells synthesizing antibodies with specificities for only one PAg were noted in spleens from immunized mice, and this is in contrast to the non-immunized animals, where such cells were absent. The clones synthesizing these mono-specific antibodies were lost during culture, and therefore their further characterization was not possible.

Compared to the results previously obtained with adult non-immunized BALB/C mice (Dighiero *et al.*, 1983b), where approximately 2% of the hybrids were found to secrete immunoglobulins with natural antibody function, 2–5% of the hybrids derived from the immunized mice were found to synthesize natural antibodies. This suggests that the splenocytes producing these antibodies are only slightly, if at all, stimulated by the various factors released during the *in vivo*

immunization process with antigens incorporated into Freund's adjuvant. Furthermore, these results indicate that an extensive antigen stimulation is not sufficient to select negatively the splenocytes synthesizing the natural antibodies. This implies either that the pool of cells synthesizing natural antibodies is insensitive to the events occurring during active immunization of the mice, or to the contrary, that this pool of cells responds to the antigen. In this latter case it can be hypothesized that cells carrying the polyspecific natural antibodies as receptors, during an active immunization with a given antigen and under the selective pressure of this antigen undergo a series of divisions and mutations which result in cells producing a highly specific antibody for a given epitope of that antigen. Because the number of cells synthesizing natural antibodies remains constant or is only slightly increased during immunization it can be inferred that these cells are continuously replaced by new ones probably of bone-marrow origin.

The hypothesis that cells synthesizing polyspecific natural antibodies are implicated in the subsequent production of highly specific antibodies is supported by the results of the present study which indicates that some hybrids secrete immunoglobulins which react with the immunizing antigen as well as with one or more PAg. Since, almost all of the hybrids producing these antibodies lost their capacities to synthesize immunoglobulins during culture and subcloning, they cannot be further characterized. However, this analysis has been achieved with one immunoglobulin, synthesized by a subcloned hybrid, which reacts with both prolactin, the IAg and TNP-BSA. The antibody secreted by this subclone was isolated using a DNP-immunoadsorbent, was found to be a monoclonal IgM (κ) and was shown to react with both prolactin and TNP, the latter antigen being recognized by almost all the natural monoclonal antibodies we have examined so far. It is evident, however, that extensive molecular biology studies (now in progress in our laboratory) are needed in order to confirm or disprove the above hypotheses.

REFERENCES

AVRAMEAS S., DIGHIERO G., LYMBERI P. & GUILBERT B. (1983) Studies on natural antibodies and autoantibodies. *Ann. Immunol.* **134D,** 103.

AVRAMEAS S., GUILBERT B. & DIGHIERO G. (1981) Natural antibodies against tubulin, actin, myoglobin, thyroglobulin, fetuin, albumin and transferrin are present in normal human sera and monoclonal immunoglobulins from multiple myeloma and Waldrenström's macroglobulinemia may express similar antibody specificities. *Ann. Immunol.* **132C,** 231.

AVRAMEAS S., TERNYNCK T. & GUESDON J.L. (1978) Coupling of enzymes to antibodies and antigens. *Scand. J. Immunol.* **8,** 7.

BIOZZI G., CABRERA W.H., MOUTON D. & IBANEZ O.M. (1982) Restricted and general polygenic regulation of antibody responsiveness. In: *Immunogenetics and Immune Regulation* (ed. B. Benacerraf), p. 31. Masson, Milan.

BOUVET J.P., PIRES R. & PILLOT J. (1984) A modified gel filtration technique producing an unusual exclusion volume of IgM: a simple way for preparing monoclonal IgM. *J. immunol. Meth.* **66,** 299.

BOYDEN S.V. (1964) Autoimmunity and inflammation. *Nature* (Lond.), **201,** 200.

CAIRNS G., BLOCK J. & BELL D.A. (1984) Anti-DNA autoantibody-producing hybridomas of normal human lymphoid cell origin. *J. clin. Invest.* **74,** 880.

CUNNINGHAM A.J. (1974) Large numbers of cells in normal mice produce antibody components of isologous erythrocytes. *Nature* (Lond.), **252,** 749.

DIGHIERO G., GUILBERT B. & AVRAMEAS S. (1982) Naturally occurring antibodies against nine common antigens in human sera, II. High incidence of monoclonal Ig exhibiting antibody activity against actin and tubulin and sharing antibody specificities with natural antibodies. *J. Immunol.* **128,** 2788.

DIGHIERO G., GUILBERT B., FERMANT J.P., LYMBERI P., DANON F. & AVRAMEAS S. (1983a) Thirty-six human monoclonal immunoglobulins (MIg) with antibody activity against cytoskeleton proteins, thyroglobulin, and native DNA. Immunological studies and clinical correlations. *Blood,* **62,** 264.

DIGHIERO G., LYMBERI P., MAZIÉ J.C., ROUYRE S., BUTTLER-BROWN G.S., WHALEN R.G. & AVRAMEAS S. (1983b) Murine hybridomas secreting natural monoclonal antibodies reacting with self antigens. *J. Immunol.* **131,** 2267.

DIGHIERO G., LYMBERI P., HOLMBERG D., LYNDQUIST I., COUTINHO A. & AVRAMEAS S. (1985) High frequency of natural autoantibodies in normal newborn mice. *J. Immunol.* **134,** 765.

ERLANGER B.F. & BEISER S.M. (1964) Antibodies specific for ribonucleosides and ribonucleotides and their reaction with DNA. *Proc. Natl. Acad. Sci. USA.* **52,** 68.

GALILI U., RACHMILEWITZ E.A., PELEG A. & FLECHNER I. (1984) Unique natural human IgG antibody with anti-α-galactosyl specificity. *J. exp. Med.* **160,** 1519.

GRABAR P. (1983) Autoantibodies and the physiological role of immunoglobulins. *Immunol. Today,* **4,** 337.

GUILBERT B., DIGHIERO G. & AVRAMEAS S. (1982) Naturally occurring antibodies against nine common antigens in humans sera. I. Detection, isolation and characterization. *J. Immunol.* **128,** 2779.

IVANYI, P., VAN MOURIK P., BREUNING M., KRUISBEEK A.M. & KRÖSE C.J.M. (1982) Natural H-2-specific antibodies in sera of aged mice. *Immunogenetics,* **15,** 95.

JAFFE B.M., EISEN H.M., SIMMS E.S. & POTTER M. (1969) Myeloma proteins with anti-hapten antibody activity ε-2-4 dinitrophenyl binding by the protein produced by mouse plasmocytoma MOPC 460. *J. Immunol.* **103,** 872.

KARSENTI E., GUILBERT B., BORNENS M. & AVRAMEAS S. (1977) Antibodies to tubulin in normal nonimmunized animals. *Proc. Natl. Acad. Sci. USA.* **74,** 3997.

KÖHLER G. & MILSTEIN C. (1976) Derivation of specific antibody tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* **6,** 511.

LITTLE J.R. & EISEN H.N. (1966) Preparation and characterization of antibodies specific for the 2-4-6 Trinitrophenyl group. *Biochemistry*, **5,** 3385.

LONGENECKER B.M. & MOSMANN T.R. (1980) Natural antibodies to chicken MHC antigens are present in mice, rats, humans, alligators and allogeneic chickens. *Immunogenetics*, **11,** 293.

LUTZ H.U. & WIPF G. (1982) Naturally occurring auto-antibodies to skeletal proteins from human red blood cells. *J. Immunol.* **128,** 1695.

LUTZ H.U., FLEPP R. & STRINGARO-WIPF G. (1984) Naturally occurring autoantibodies to exoplasmic and cryptic regions of band 3 protein, the major integral membrane protein of human red blood cells. *J. Immunol.* **133,** 2610.

MACKAY I.R. (1983) Natural autoantibodies to the fore-forbidden clones to the rear. *Immunol. Today*, **4,** 340.

MARTIN W.J. & MARTIN S.E. (1975) Thymus reactive IgM auto-antibodies in normal mouse sera. *Nature (Lond.)*, **254,** 716.

MEAD G.M., COWIN P. & WHITEHOUSE, J.M.A. (1977) Antitubulin antibody in healthy adults and patients with infectious mononucleosis and its relationship to smooth muscle antibody (SMA). *Clin. exp. Immunol.* **39,** 328.

O'FARRELL P.H. (1975) High resolution two dimensional electrophoresis. *J. Biol. Chem.* **250,** 4007.

PRABHAKAR S.B., SAEGUSA J., ONODERA T. & NOTKINS A.L. (1984) Lymphocytes capable of making monoclonal autoantibodies that react with multiple organs are a common feature of the normal B-cell repertoire. *J. Immunol.* **133,** 2815.

SHINOMIYE T. & KOYAME J. (1974) Naturally occurring antibodies reacting with some buried determinants of homologous IgG exposed by spleen protease digestion. *Immunochemistry*, **11,** 467.

STEELE E.J. & CUNNINGHAM A.J. (1978) High proportion of Ig-producing cells making autoantibody in normal mice. *Nature (Lond.)*, **274,** 483.

TERNYNCK T. & AVRAMEAS S. (1977) Conjugation of p-benzoquinone treated enzymes with antibodies and Fab fragments. *Immunochemistry*, **14,** 767.

TERNYNCK T. & AVRAMEAS S. (1976) Polymerization and immobilization of proteins using ethylchloro-formate and glutaraldehyde. *Scand. J. Immunol.* **3,** 29.

WEBER K. & OSBORN M. (1969) The reliability of molecular weight determination by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **144,** 4406.