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Immunoglobulin Classes in Antibody Responses in Mice

I. ANALYSIS BY BIOLOGICAL PROPERTIES

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Summary. The formation of different immunoglobulin classes of mouse antibodies to dinitrophenol (DNP) has been qualitatively determined by biological assays which discriminate between some immunoglobulin classes. Antibody responses to two other antigens were compared in five mouse strains. Mice of five different strains immunized with DNP–haemocyanin (DNP–Hcy) in complete Freund's adjuvant made both γG_1 and γG_{2a} antibodies. The amounts of antibody are less in DBA and C57 mice and at 80 days after primary immunization a 2-mercaptoethanol sensitive (2MES) antibody is still present in these strains (but not in A, AKR and C3H). When DNP–Hcy is given in $Al(OH)_3$, very little γG_{2a} is produced in any strain, although γG_1 antibodies are produced as in mice given DNP–Hcy in complete Freund's adjuvant. Possible interpretations of these observations are discussed.

INTRODUCTION

In all mammalian species extensively studied, the immunoglobulins have been found to be heterogeneous in physico-chemical properties, biological activities and antigenic determinants (Cohen and Porter, 1964; Fudenberg, 1965; Ovary, 1966). Electrophoresis of guinea-pig antisera demonstrated that homologous anaphylactic sensitization properties were present only in the γ_1 (fast migrating fraction), whereas complement fixation is a property of the slower moving γ_2 fraction (Benacerraf, Ovary, Bloch and Franklin, 1963; Ovary, Benacerraf and Bloch, 1963). Using similar electrophoretic analysis of mouse antisera, it was demonstrated that mouse antibodies are also separable into γ_1 and γ_2 components having different biological activities (Nussenzweig, Merryman and Benacerraf, 1964). Antigenic studies of various mouse myeloma proteins and sera have shown that there are five distinct mouse immunoglobulin classes (Fahey, Wunderlich and Mishell, 1964a, b) which will be termed in this paper γM, γA, γG_1, γG_{2a} and γG_{2b} (Warner, Herzenberg and Goldstein, 1966).

Antibody responses can be measured by various techniques, many of which make use of specific biological properties, often characteristic of only one or a limited number of immunoglobulin classes. In mice, only γG_1 immunoglobulins will sensitize mouse tissues either *in vivo* (Nussenzweig *et al.*, 1964; Fahey and Barth, 1965; Vaz, Warner and Ovary, 1968) or *in vitro* (Vaz *et al.*, 1968). Passive cutaneous anaphylaxis (PCA) in mice is accordingly used as a specific measurement of γG_1 antibodies. Sensitization of heterologous skin of the guinea-pig is a property of, and therefore measurement for, γG_{2a} mouse antibodies (Ovary, Fahey and Barth, 1965). Complement fixation (passive lysis) is given by γM and

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by both γG_{2b} and γG_{2a} (Müller-Eberhard and Grey, 1967 personal communication) mouse antibodies, although generally far more efficiently by γM antibody.

The mouse immunoglobulin classes are products of different immunoglobulin heavy chain genes (Herzenberg, Warner and Herzenberg, 1965; Herzenberg and Warner, 1968) and it is of potential significance for understanding their genetic control and regulation to determine the effect of variations in immunization procedure (e.g. adjuvant, antigen, mouse strain, etc.), on the expression of the different immunoglobulin H chains in a specific immune response. This paper reports on the immune response of five different mouse strains to a simple hapten dinitrophenol (DNP), coupled to haemocyanin, as measured by different antibody assays. Antibody responses to *Brucella* and sheep red blood cells in the five strains were also compared. Previous studies have indicated that mineral gel adjuvants may favour production of γG_1 antibodies (Vaz and Peixoto, 1963), and this adjuvant has been compared with complete Freund's adjuvant.

MATERIALS AND METHODS

Mice

Female adult mice were obtained from the Jackson Laboratories (Bar Harbor, Maine) The following strains were used: DBA/1J, C3H/HeJ, C57BL/6J, AKR/J and A/HeJ hereon referred to as DBA, C3H, C57, AKR and A. Random bred Swiss Webster mice were obtained from a local dealer and used as recipients for PCA tests in mice.

Guinea-pigs

Random bred Hartley strain guinea-pigs weighing 250–350 g were used as recipients for PCA tests in guinea-pigs.

Antigens

Crystalline bovine serum albumin (BSA, Armour lot 2266), five times crystallized hen's egg albumin (Ov, Pentex, Inc., Kankakee, Illinois, lot F61) and haemocyanin from the horseshoe crab (*Lymulus polyphemus*) obtained in the laboratory as described by Campbell, Garvey, Cremer and Sussdorf (1963) were dinitrophenylated as previously described (Ovary and Benacerraf, 1963). The resulting dinitrophenylated proteins ($DNP_{13}Ov$, $DNP_{37}BSA$, $DNP_{240}Hcy$) contained an average of 13, 37 and 240 DNP groups per molecule of protein. (The molecular weight of haemocyanin was taken to be 1,000,000 for the calculation of groups per molecule.) Freshly washed sheep red blood cells (SRBC) (Certified Blood Donor Service, Woodbury, New York) and a suspension of *Brucella abortus* strain 1119–3, obtained from the U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C., were also used as antigens.

Adjuvants

Complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) and $Al(OH)_3$ gel were used as adjuvants for immunization. The latter was prepared by adding dropwise a 2 N NaOH solution to a 2 N $Al_2(SO_4)_3$ solution until gelification; the resulting gel was washed five times in 0·15 M NaCl. It contained 60 mg $Al(OH)_3$/ml.

Immunization and bleedings

Three groups of ten mice of each strain were immunized with a single injection of 50 μg DNP–Hcy in 0·2 ml by the intraperitoneal route. The first group received the antigen in

complete Freund's adjuvant emulsion (1 part antigen to 1 part adjuvant). The second group received the antigen mixed in mineral gel containing 9 mg $Al(OH)_3$. The third group was immunized with 50 μg DNP–Hcy in complete Freund's adjuvant, the emulsion also containing 0·02 ml of *Brucella* suspension and 0·1 ml of packed SRBC per 0·2 ml per mouse.

Blood samples of approximately 0·2 ml were collected by retro-orbital puncture from each individual mouse at 5, 8, 14, 21, 25, 39, 50 and 79 days after immunization. Within each strain and group receiving a single treatment an equal amount of serum from individual mice was pooled, and this was used in subsequent antibody assays.

Antibody assays

PCA in mice. PCA tests in Swiss Webster mice were done as previously described (Vaz and Ovary, 1968). Recipient mice were lightly anaesthetized with ether, shaved with an electric clipper on the back, and injected at two sites on either side of the mid line with 0·03 ml of the antiserum dilution to be tested. Two hours later the mice were challenged intravenously with 0·1 mg protein of $DNP_{13}Ov$ in 0·2 ml of 0·5 per cent Evans blue dye (Eastman Kodak). Fifteen to 30 minutes later the animals were killed, the skin reversed, and the magnitude of the PCA reaction scored using an arbitrary scale of intensities and diameters ranging from 0 to 4 (Vaz and Ovary, 1968). Each dilution of each antiserum was tested in at least five recipient mice. The PCA sensitizing activity of each particular antiserum was represented as the dilution which produced a PCA reaction of mean score 2, although threshold reactions were obtained with two- to four-fold higher dilutions.

PCA in guinea-pigs. PCA tests in guinea-pigs (groups of 4–5) were made as previously described (Ovary, 1964) using 0·1-ml dilutions of mouse antisera in saline for intradermal injections, followed after a sensitization period of 4 hours by intravenous challenge with 400 μg of DNP_{37} BSA in 0·5 per cent Evans blue in saline. The magnitude of the PCA reaction was scored and is shown as the endpoint dilution giving a significant reaction (>6 mm diameter) in at least two out of four recipients tested.

Passive lysis

$DNP_{37}BSA$ was coupled to three times washed sheep red blood cells by the method of Boyden (1951) as previously described (Bloch, Kourilsky, Ovary and Benacerraf, 1963). Passive lysis tests were performed as previously described using guinea-pig serum as the complement source.

Haemagglutination assays

DNP determinants were coupled to thrice washed sheep red blood cells as described by Bullock and Kantor (1965) using 1,3-difluoro-4,6-dinitrobenzene. Haemagglutination assays were performed in microtitre (Cooke Engineering) trays with EDTA–glucose buffer (Bullock and Kantor, 1965) as diluent. For 2-mercaptoethanol (2ME) treatment, samples of the sera were incubated for 1 hour at 37° with a final concentration of 0·1 M 2ME. Antibodies sensitive to the 2ME treatment are referred to as 2MES, and those resistant, as 2MER antibodies. Antisera to sheep red blood cells were directly titrated against thrice washed sheep red blood cells in saline.

Brucella *agglutination*

Antisera to *Brucella* were titrated in microtitre trays against a 1:250 dilution of the stock *Brucella abortus* antigen used for immunization (method as described by Claflin, Smithies and Meyers, 1966).

RESULTS

IMMUNE RESPONSE TO DNP–HAEMOCYANIN

The basic immune response of three strains of mice to DNP–haemocyanin is shown in Fig. 1. In all cases, only specific antibodies to the haptenic determinant are measured, as an unrelated carrier protein ($DNP_{37}BSA$ or $DNP_{13}Ov$) has been used as test antigen. All mice in this experiment were given a primary immunization in complete Freund's adjuvant, and were then bled on many occasions until 79 days after immunization. The immune responses were determined by PCA in mice, PCA in guinea-pigs and passive lysis (respectively measuring γG_1, γG_{2a}, and mixed γM, $\gamma G2_a$ and γG_{2b} antibodies). The maximum titre is reached in 3–5 weeks for all parameters, and only a mild decline in titre is evident between 6 and 12 weeks, except for DBA/1, in which a more rapid decline in

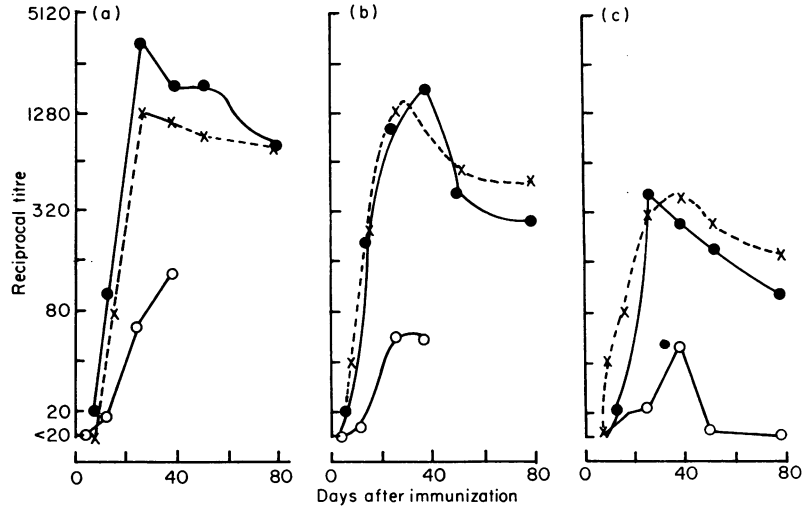

FIG. 1. Anti-DNP antibody responses in (a) C3H, (b) AKR, and (c) DBA mice after immunization with DNP–Hcy in Freund's complete adjuvant, as measured by PCA in guinea-pigs (●), PCA in mice (○) and passive haemolysis of DNP-coated cells (×).

γG_1 antibody was observed. The immune response of A mice was quite similar to that of C3H and is plotted in Fig. 3. The response of C57 mice was quite identical to that of the DBA mice. A basically similar pattern in titre for all types of response was therefore observed in the five strains, although quantitatively a lower response was given by the DBA and C57 mice.

STRAIN VARIATION IN RESPONSE TO DNP, *Brucella* AND SRBC

The immune response of mice simultaneously immunized with three different antigens showed only slight strain differences in the response to SRBC. A maximum of two to three two-fold dilution endpoints was observed at any given bleeding time, DBA responding maximally to this antigen. Responses to *Brucella* were also similar in the five strains, with DBA being, however, the least responsive again with only a two to three two-fold dilution endpoint difference. The immune response to DNP was, however, quite significantly influenced by the strain of mouse immunized. The results in Fig. 2 show that, whereas

A and C57 mice made identical agglutinin responses to *Brucella*, marked differences in response to DNP were observed in these strains. The antibody to DNP was determined by PCA in guinea-pigs and similar differences were observed between A and C57 in passive lysis and in assays by PCA in mice. Fig. 2(b) shows that the lower response of C57 mice (relative to A mice) to DNP was shown by both groups, whether the DNP–haemocyanin in Freund's adjuvant was injected with or without *Brucella* and SRBC antigens. The lowered response of both strains to DNP injected with other antigens will be discussed below. The responses of C3H and AKR mice to DNP were respectively similar and slightly lower than A, whereas the response of DBA mice was wholly identical to that of the C57 mice.

The response to DNP is, therefore, influenced by the strain of mouse, DBA and C57 being poor responders to this antigen, although very good responders (relative to A) to sheep red cells and *Brucella* respectively. A qualitative difference in the immune response of these two strains has also been observed and is described below.

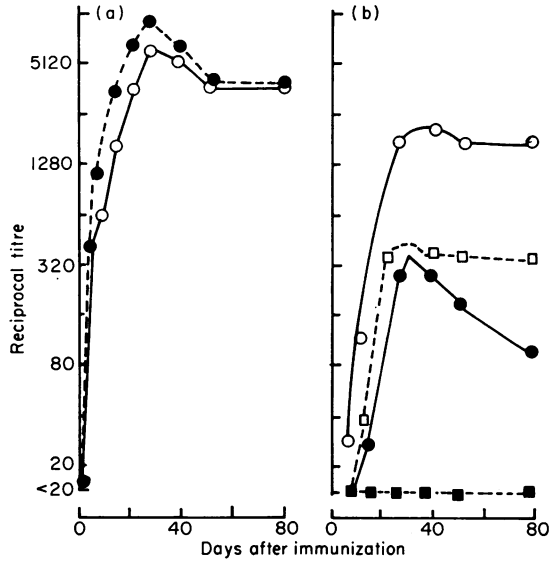

FIG. 2. Antibodies to *Brucella* and to DNP in C57 and A mice. (a) Agglutinins to *Brucella* after immunization with a mixture of DNP–Hcy+SRBC+*Brucella* in complete Freund's adjuvant. ●. C57Bl mice; ○, A mice. (b) Anti-DNP antibodies, as measured by PCA tests in guinea-pigs, in mice injected with DNP–Hcy in Freund's adjuvant (——) or with DNP–Hcy+SRBC+*Brucella* in Freund's adjuvant (- - - -). ○, □, A mice; ●, ■, C57Bl mice.

EFFECT OF ADJUVANTS ON THE IMMUNE RESPONSE OF MICE TO DNP

The distribution of antibody in immunoglobulin classes is influenced by the type of adjuvant used. The results in Fig. 3 show the immune response of A and DBA mice immunized with DNP–Hcy in complete Freund's adjuvant, or in $Al(OH)_3$ gel. The quantitative response of DBA mice is less than that of A mice by all three parameters and with both adjuvants. The qualitative response, as judged by comparisons of the three different parameters of antibody production, is influenced by the type of adjuvant in both DBA and A. The passive lysis titres of the A and DBA mice given DNP–Hcy is approximately the same for the two adjuvant groups (comparison between solid and open symbols, Fig. 3a).

As measured by PCA in guinea-pigs, however, the antibody response is very markedly reduced in the $Al(OH)_3$ group, a brief response appearing in A mice between about 3 weeks and 50 days, whereas the Freund's adjuvant group had titres of 1280 at 80 days. In the DBA mice, no antibody detectable by PCA in guinea-pigs appeared at all in the

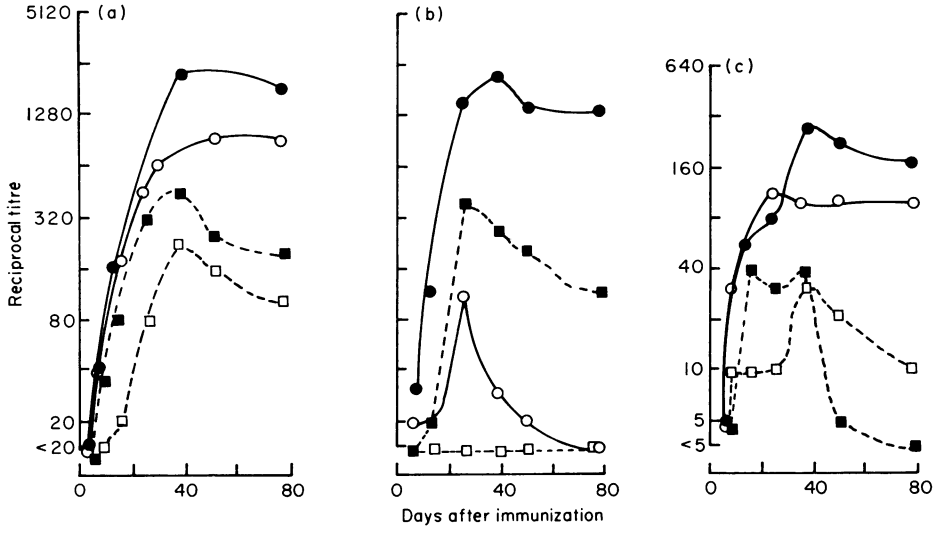

FIG. 3. Anti-DNP antibodies in A (——) and DBA (- - - -) mice after immunization with DNP–Hcy in Freund's complete adjuvant (●, ■) or with DNP–Hcy with $Al(OH)_3$ gel (○, □). Antibody titres measured by passive haemolysis of DNP-coated SRBC (a), PCA in guinea-pigs (b) and PCA in mice (c).

TABLE 1

2ME TREATMENT OF MOUSE ANTI-DNP ANTIBODY

Variables		Haemagglutinin titre* (log_2)					
		25 days†		50 days†		79 days†	
Strain	Adjuvant	MES‡	MER§	MES	MER	MES	MER
A	Complete Freund's	1·0	9·2	0·5	9·2	0·1	9·2
A	$Al(OH)_3$	NT	NT	2·2	6·0	4·0	4·0
C3H	Complete Freund's	NT	NT	0·2	8·0	0·0	8·2
DBA	Complete Freund's	NT	NT	2·0	4·2	3·0	3·3

* log_2-titre of agglutination of DNP-coated SRBC.
† Days after immunization.
‡ Titre of 2-mercaptoethanol sensitive (MES) antibody (log_2 titre; control minus 2MER titre)
§ Titre of 2-mercaptoethanol resistant (MER) antibody (log_2 titre). NT = Not tested.

$Al(OH)_3$ group (Fig. 3b). For each strain, antibody responses measured by PCA in mice were approximately identical with both adjuvants. Results essentially identical to A mice were obtained for C3H and AKR strains, while C57 mice gave results similar to DBA mice. The γG_1 response is, therefore, similar in both types of immunization, whereas the γG_{2a} response is very much influenced by the adjuvant, high titres occurring only with complete Freund's adjuvant.

Sera from the last two to three bleedings of the A mice were tested for haemagglutinating activity of DNP-coupled cells (Table 1). Agglutinin titres of the group immunized with Freund's adjuvant were unaffected by prior 2ME treatment, whereas the $Al(OH)_3$ treated mice showed both 2MES and 2MER antibody. Two sera from the preceding experiment were also tested in this system (Table 1). Antisera from the C3H mice given DNP–Hcy in Freund's adjuvant behaved exactly like the A mice, whereas antisera from the DBA mice given DNP–Hcy, in Freund, showed both 2MES and 2MER antibody.

EFFECT OF SIMULTANEOUS IMMUNIZATION WITH THREE ANTIGENS

The immune response to DNP–Hcy was lower in mice simultaneously immunized with two other antigens in Freund's adjuvant (Fig. 2b); a similar result was seen with C3H and C57 mice as determined by three antibody assays (Fig. 4).

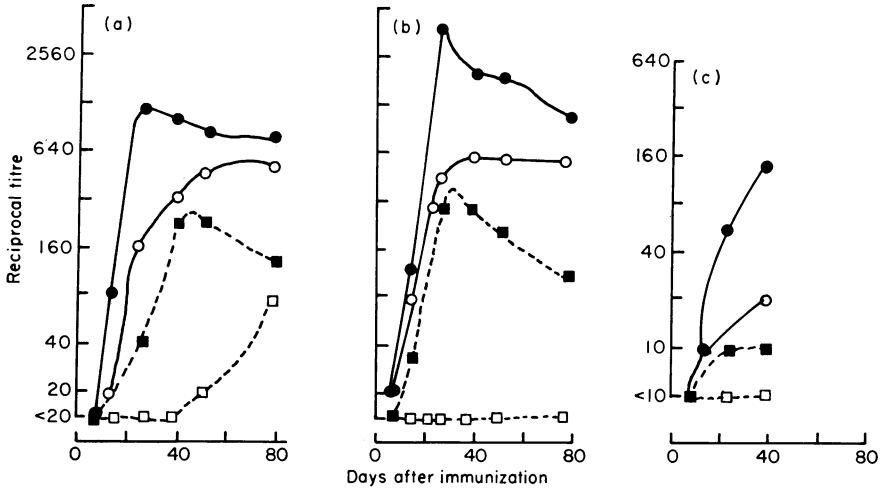

FIG. 4. Anti-DNP antibodies in C3H (——) and C57 mice (- - -) after injection of DNP–Hcy in Freund's complete adjuvant (●, ■) or with a mixture of DNP–Hcy+SRBC+*Brucella* in Freund's complete adjuvant (○, □). Antibodies measured by passive haemolysis of DNP-coated SRBC (a), PCA in guinea-pigs (b) and PCA in mice (c).

DISCUSSION

The results presented here reinforce the concept that variations in immunizing conditions markedly affect the quantity and class of antibody formed. The dose of antigen is known to be important (Jerne, 1965; Barth, McLaughlin and Fahey, 1965), and we have not re-investigated this point.

Several mouse strains immunized with DNP–Hcy in complete Freund's adjuvant, show a simultaneous increase in titre of all antibody classes. Coe (1966) found a γG_1 to γG_2 shift but no indication of this was found in any strain in the present studies with anti-DNP antibodies and this agrees with findings in guinea-pigs (Benacerraf *et al.*, 1963; Ovary *et al.*, 1963, Nussenzweig and Benacerraf, 1967). In fact, in guinea-pigs, a later increase of γ_1 often occurs (Nussenzweig and Benacerraf, 1967).

The immune response to DNP–Hcy in the five strains was reduced in the presence of two other antigens and this is presumably a manifestation of antigenic competition (Adler, 1964).

Several studies have demonstrated that mouse strains respond differently to identical immunization (Fink and Quinn, 1953; Rothberg and Talmage, 1961; Farr, Grey, Dickinson and Rosenstein, 1963; Coe, 1966). Our results show that such differences do not indicate a general hyporeactivity of a particular strain. DBA mice responded better than other strains to sheep red cells, but gave a lower response to DNP. Similarly, C57 mice responded as well as A to *Brucella*, but far less to DNP–Hcy. The response to DNP–Hcy was in fact the one most clearly influenced by strain. Furthermore, in DBA and C57 mice given DNP–Hcy in complete Freund's adjuvant, a 2MES antibody was produced over a period not observed in the other three strains. Late bleedings from these mice (DBA and C57—DNP–Hcy in complete Freund's adjuvant), showed only trace amounts of γG_1 antibody, and low titres of γG_{2a} and haemolytic antibody. Although the titres in the passive lysis test and the PCA tests in guinea-pigs were similar, a γM component may be involved in the lysis test, since γM is far more efficient for C′ fixation than γG (Borsos and Rapp, 1965). A γM component would, therefore, explain the presence of 2MES antibody (Table 1). Other alternatives cannot be excluded: the 2MES antibody could be a γG (Szenberg, Lind and Clarke, 1965) or even a non-haemolytic γM (Hyslop and Matheson, 1967).

Several interpretations could explain the different titres of antibody in different mouse strains. Even though the antigen is probably complex, a genetic unresponsiveness, as described in guinea-pigs (Levine, Ojeda and Benacerraf, 1963) and mice (Maurer, 1964; McDevitt and Sela, 1965; Lennox ,1966), possibly to one of many components might be involved. Alternatively, some antigenic components might cross react with a strain specific tissue antigen in poorly responsive strains (Rowley and Jenkins, 1962; Rapaport and Chase, 1965).

The importance of complete Freund's adjuvant is eliciting the γ_2 class of antibodies has been previously documented (Benacerraf *et al.*, 1963; White, Jenkins and Wilkinson, 1963). Our results demonstrate that in A mice immunized with DNP–Hcy in $Al(OH)_3$, γG_{2a} antibodies appear only transiently, and in lower responding strains, e.g. DBA, no detectable γG_{2a} is formed, although γG_1 antibodies are still produced. Antibodies capable of passive lysis were also formed (Fig. 3a). Since late bleedings of all strains immunized with antigen in $Al(OH)_3$ contained no $\gamma G2_a$ antibody, the lysis could only be given by γM or γG_{2b}. Furthermore, since γG_1 was present and might account for the 2MER antibody detected (Table 1), all the lytic antibody may have been γM. Alternatively, dissociation between γG_{2a} and γG_{2b} would have to occur. In either event, a marked alteration in the expression of H chain genes has been induced by the type of adjuvant used.

It is not definitely known whether specific cells are restricted to the synthesis of a single class of H chain, or whether a sequential shift in H chain expression occurs within an antibody forming clone (Nossal, Szenberg, Ada and Austin, 1964). If the first alternative is correct, it would seem that the variables which can affect the production of different antibody classes may do so by initial selection of cells. Alternatively the variables must affect the sequential shift of H chain production within cells from a single clone.

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REFERENCES

ADLER, F. L. (1964). 'Competition of antigens.' *Progr. Allergy*, **8**, 41.

BARTH, W. F., MCLAUGHLIN, C. L. and FAHEY, J. L. (1965). 'The immunoglobulins of mice. VI. Response to immunization.' *J. Immunol.*, **95**, 781.

BENACERRAF, B., OVARY, Z., BLOCH, K. J. and FRANKLIN, E. C. (1963). 'Properties of guinea pig 7S antibodies. I. Electrophoretic separation of two types of guinea pig 7S antibodies.' *J. exp. Med.*, **117**, 937.

BLOCH, K. J., KOURILSKY, F. M., OVARY, Z. and BENACERRAF, B. (1963). 'Properties of guinea pig 7S antibodies. III. Identification of antibodies involved in complement fixation and hemolysis.' *J. exp. Med.*, **117**, 965.

BORSOS, T. and RAPP, H. J. (1965). 'Complement fixation on cell surfaces by 19S and 7S antibodies.' *Science*, **150**, 505.

BOYDEN, S. V. (1951). 'The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera.' *J. exp. Med.*, **93**, 107.

BULLOCK, W. E. and KANTOR, F. S. (1965). 'Hemagglutination reactions of human erythrocytes conjugated covalently with dinitrophenyl groups.' *J. Immunol.*, **94**, 317.

CAMPBELL, D. H., GARVEY, J. S., CREMER, N. E. and SUSSDORF, D. H. (1963). *Methods in Immunology*, p. 69. Benjamin, New York.

CLAFLIN, A. J., SMITHIES, O. and MEYERS, R. K. (1966). 'Antibody responses in bursa-deficient chickens.' *J. Immunol.*, **97**, 693.

COE, J. E. (1966). '$7S_{\gamma 1}$ and $7S_{\gamma 2}$ antibody response in the mouse. I. Influence of strain, antigen and adjuvant.' *J. Immunol.*, **96**, 744.

COHEN, S. and PORTER, R. R. (1967). 'Structure and biological activity of immunoglobulins.' *Advanc. Immunol.*, **4**, 287.

FAHEY, J. L. and BARTH, W. F. (1965). 'Heterologous and homologous skin-sensitizing activity of mouse $7S_{\gamma 1}$ and $7S_{\gamma 2}$ globulins.' *Nature (Lond.)*, **206**, 730.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964a). 'The immunoglobulins of mice. I. Four major classes of immunoglobulins: $7S_{\gamma 2}$-, $7S_{\gamma 1}$-, $_{\gamma 1A(\beta 2A)}$ and $18S_{\gamma 1M}$- globulins.' *J. exp. Med.*, **120**, 223.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964b). 'The immunoglobulins of mice. II. Two subclasses of mouse $7S_{\gamma 2}$-globulins: $_{\gamma 2a}$- and $_{\gamma 2b}$-globulins.' *J. exp. Med.*, **120**, 243.

FARR, R. S., GREY, H. M., DICKINSON, H. and ROSENSTEIN, D. H. (1963). 'Genetic influences on anti-BSA antibodies produced in mice.' *Fed. Proc.*, **22**, 269.

FINK, M. A. and QUINN, V. A. (1953). 'Antibody production in inbred strains of mice.' *J. Immunol.*, **70**, 61.

FUDENBERG, H. H. (1965). 'The immune globulins.' *Ann. Rev. Microbiol.*, **19**, 301.

HERZENBERG, L. A. and WARNER, N. L. (1968). *Genetic Control of Mouse Immunoglobulins.* (Ed. by B. Cinader). Thomas, Springfield, Illinois. (In press).

HERZENBERG, L. A., WARNER, N. L. and HERZENBERG, L. A. (1965). 'Immunoglobulin isoantigens (allotypes) in the mouse. I. Genetics and cross-reactions of the 7S γ2a-isoantigens controlled by alleles at the Ig-1 locus.' *J. exp. Med.*, **121**, 415.

HYSLOP, N. E., JR and MATHESON, D. N. (1967). 'Two functional types of macroglobulin antibody in the primary immune response of guinea pigs.' *Fed. Proc.*, **26**, 530.

JERNE, N. K. (1965). 'Studies on the primary response in mice.' *Symposium on the Molecular and Cellular Basis of Antibody Formation*, p. 459 (Ed. by J. Sterzl *et al.*). Publishing House of the Czechoslovak Academy of Sciences, Prague.

LENNOX, E. S. (1966). 'The genetics of the immune response.' *Proc. roy. Soc. B*, **166**, 222.

LEVINE, B. B., OJEDA, A. and BENACERRAF, B. (1963). 'Studies on artificial antigens. III. The genetic control of the immune response to hapten-poly-L-lysine conjugates in guinea pigs.' *J. exp. Med.*, **118**, 953.

MCDEVITT, H. D. and SELA, M. (1965). 'Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains.' *J. exp. Med.*, **122**, 517.

MAURER, P. H. (1964). 'Use of synthetic polymers of amino acids to study the basis of antigenicity.' *Progr. Allergy*, **8**, 1.

NOSSAL, G. J. V., SZENBERG, A., ADA, G. L. and AUSTIN, C. M. (1964). 'Single cell studies on 19S antibody production.' *J. exp. Med.*, **119**, 485.

NUSSENZWEIG, V. and BENACERRAF, B. (1967). 'Synthesis, structure and specificity of 7S guinea pig immunoglobulins.' *Nobel Symposia, Symposium on γ-Globulin* (Ed. by J. Killander), p. 223, Interscience, New York.

NUSSENZWEIG, R. S., MERRYMAN, C. and BENACERRAF, B. (1964). 'Electrophoretic separation and properties of mouse anti-hapten antibodies involved in PCA and passive hemolysis.' *J. exp. Med.*, **120**, 315.

OVARY, Z. (1964). 'Passive cutaneous anaphylaxis.' *Immunological Methods, C.I.O.M.S. Symposium*, p. 259 (Ed. by J. F. Ackroyd). Blackwell Scientific Publications, Oxford.

OVARY, Z. (1966). 'The structure of various immunoglobulins and their biological activities.' *Ann. N.Y. Acad. Sci.*, **129**, 776.

OVARY, Z. and BENACERRAF, B. (1963). 'Immunological specificity of the secondary response with dinitrophenylated proteins.' *Proc. Soc. exp. Biol. (N.Y.)*, **114**, 72.

OVARY, Z., BENACERRAF, B. and BLOCH, K. J. (1963). 'Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis.' *J. exp. Med.*, **117**, 951.

OVARY, Z., FAHEY, J. L. and BARTH, W. F. (1965). 'The immunoglobulins of mice. III. Skin sensitizing activity of mouse immunoglobulins.' *J. Immunol.*, **94**, 410.

RAPAPORT, F. T. and CHASE, R. M., JR (1965). 'The bacterial induction of homograft sensitivity. II. Effects of sensitization with staphylococci and other microorganisms.' *J. exp. Med.*, **122**, 733.

ROTHBERG, R. and TALMAGE, D. (1961). 'Circulating antibody and anaphylaxis in mice.' *J. Immunol.*, **86**, 302.

ROWLEY, D. and JENKIN, C. R. (1962). 'Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity.' *Nature (Lond.)*, **193**, 151.

SZENBERG, A., LIND, P. and CLARKE, K. (1965). 'IgG and IgM antibodies in fowl serum.' *Aust. J. exp. Biol. med. Sci.*, **43**, 451.

VAZ, N. M. and OVARY, Z. (1968). 'PCA and RPCA in mice with homologous and heterologous antibodies.' *J. Immunol.*, (In press).

VAZ, N. M. and PEIXOTO, J. M. (1963). 'Gelified suspensions of tri-calcium phosphate as adjuvants in the sensitization of mice.' *Ann. Acad. Bras. Ciencias*, **35**, 139.

VAZ, N. M., WARNER, N. L. and OVARY, Z. (1968). Manuscript in preparation.

WARNER, N. L., HERZENBERG, L. A. and GOLDSTEIN, G. (1966). 'Immunoglobulin isoantigens (allotypes) in the mouse. II. Allotypic analysis of the three γG2 myeloma proteins from (NZB $\times$ BALB/c)F_1 hybrids and of normal γG2 globulins.' *J. exp. Med.*, **123**, 707.

WHITE, R. G., JENKINS, G. C. and WILKINSON, P. C. (1963). 'The production of skin-sensitizing antibody in the guinea pig.' *Int. Arch. Allergy*, **22**, 156.