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# Strain Specificity of Monodisperse Gamma-Globulin Appearance After Immunization of Inbred Mice[1] (37913)

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Normal rabbits respond to certain bacterial antigens by developing high-titer, monodisperse gamma-globulin antibody subpopulations (1–7),[4] especially after thymectomy, appendectomy, and irradiation (2). Streptococcal cell wall and C-polysaccharide antigens elicit monodisperse gamma-globulin (MDG) antibodies of great molecular homogeneity, and this host response seems to be under genetic control (1, 3, 6, 8). This report concerns observations which suggest that genetic factors control the appearance of monodisperse immunoglobulin response to immunization of inbred mice with bacterial antigens.

*Materials and Methods. Experimental animals.* Male and female mice derived from inbred lines in this laboratory, originally obtained from Jackson Laboratories, Bar Harbor, ME, were used in these studies. The strains employed were A/J, CBA, C57BL/6, DBA/2, (C57BL/6 × A/J)$F_1$, (C57BL/6 × CBA)$F_1$, (DBA × CBA)$F_1$, and (DBA × C57BL/6)$F_1$.

*Antigens. Brucella abortus* (No. 34504) and *Salmonella typhi* H (No. 34501) antigens were purchased from the Sylvana Company, Millburn, NJ. *H. Pertussis* vaccine (USP) was obtained from Parke Davis, Inc., Detroit, MI.

*X-irradiation.* Mice were exposed to 350–400 R of total-body x-irradiation at 2–3 weeks of age in the same manner as described previously (9).

*Immunization procedures and collection of sera.* Immunization was begun at 10 weeks of age. Each antigen was given intraperitoneally (0.05 ml, 1:10 dilution) every 2 weeks thereafter, totaling ten injections over a 20-week period. The blood samples were removed from the retro-orbital plexus 7 days after each immunizing injection, using a sterile Pasteur pipet.

*Electrophoresis.* Agar gel electrophoresis and immunoelectrophoresis were performed by methods described previously (2) The goat antimouse serum protein serum was purchased from Melpar Biological Products, Falls Church, VA.

*Absorption and elution of antityphoid bacilli antibodies. S. typhi* organisms contained in the immunizing vaccine were washed twice with phosphate-buffered saline and packed to 0.2 ml by centrifugation at 2,000*g* for 20 min. The packed antigen was mixed with 0.2 ml MDG-containing serum; the mixture was incubated for 1 hr at 37°, then for an additional 12 hr at 4°.

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Following incubation, the mixture was centrifuged, the supernatant serum collected, and the absorption repeated once more. Pellets obtained from both absorption procedures were pooled and washed twice with cold phosphate-buffered saline prior to the elution procedure.

Elution was accomplished by adding buffered saline adjusted to pH 2.5–3.0. The mixture was stirred at 37° for 90 min and centrifuged; the supernatant fluid was then removed, concentrated by pressure dialysis against phosphate-buffered saline, and examined by agar gel electrophoresis.

*Results. Occurrence of monodisperse immunoglobulins in the serum of mice receiving bacterial vaccine.* Fifty to sixty percent of mice which had been irradiated at 2 weeks of age or not, and thereafter given multiple injections of *S. typhi* vaccine, developed electrophoretically monodisperse bands in the gamma-globulin region in

TABLE I. Occurrence of Monodisperse Gamma-Globulins in Mouse Serum After Irradiation and Immunization with Bacterial Antigens.

| Strain | Antigen used | No. developing MDG/No. in group: Individual experiments | No. developing MDG/No. in group: Combined experiments |
|---|---|---|---|
| A. Irradiated, immunized groups[a] | | | |
| C57BL/6 | *H. pertussis* | 0/1, 2/7, 1/6 | 3/14 |
| | *S. typhi* | 1/1, 0/1, 3/5, 3/6 | 7/13 |
| CBA | *H. pertussis* | 0/6 | 0/6 |
| | *S. typhi* | 0/10 | 0/10 |
| A/J | *S. typhi* | 0/10 | 0/10 |
| C57BL/6 × A/J | *H. pertussis* | 1/9 | 1/9 |
| | *S. typhi* | 3/5 | 3/5 |
| | *B. abortus* | 2/9 | 2/9 |
| C57BL/6 × CBA | *H. pertussis* | 0/2, 2/10, 1/5, 2/6, 2/5 | 6/27 |
| | *S. typhi* | 6/7, 2/3, 3/4, 0/4 | 11/18 |
| CBA × DBA/2 | *H. pertussis* | 1/6, 3/3 | 4/9 |
| | *S. typhi* | 3/4, 2/2 | 5/6 |
| B. Nonirradiated, immunized groups[b] | | | |
| C57BL/6 | *S. typhi* | 1/3 | 1/3 |
| C57BL/6 × CBA | *S. typhi* | 2/5 | 2/5 |
| C57BL/6 × DBA/2 | *S. typhi* | 3/5 | 3/5 |
| CBA × A/J | *S. typhi* | 1/5 | 1/5 |
| C. Nonirradiated, nonimmunized groups[c] | | | |
| C57BL/6 | | 0/10 | 0/10 |
| C57BL/6 × A/J | | 0/10 | 0/10 |
| D. Irradiated, nonimmunized groups[d] | | | |
| DBA/2 | | 2/10 | 2/10 |

[a] In Group A the indicated strains were irradiated (350–400 R) at 2 weeks of age, then injected intraperitoneally with 0.05 ml, 1:10 dilution, of the indicated bacterial vaccine every 2 weeks up to 20 weeks. The appearance of one or more monodisperse gamma-globulin bands in serum, taken 7 days after any injection, is recorded. Those developing no bands after 10 injections were considered negatives.

[b] These experiments were conducted identically to Group A but not irradiated.

[c] These experiments were conducted identically to Group A, but the animals were neither irradiated nor immunized.

[d] These experiments were conducted identically to Group A, and the animals were irradiated but not immunized.

TABLE II. Appearance of Monodisperse Globulins in Mouse Serum: Summary of Individual Experiments.[a]

| Group | | No. in group | No. developing MDG | % Positive |
|---|---|---|---|---|
| A. By experimental protocol | | | | |
| 1. Irradiation plus immunization | | | | |
| *H. pertussis* | | 66 | 14 | 21 |
| *S. typhi* | | 62 | 27 | 44 |
| *B. abortus* | | 10 | 2 | 20 |
| 2. Irradiation alone | | 10 | 2 | 20 |
| 3. Immunization alone | | | | |
| *S. typhi* | | 18 | 7 | 39 |
| B. By strain of mouse and H-2 allele, all antigens | | | | |
| C57BL/6 | $H\text{-}2^b$ | 30 | 11 | 37 |
| A/J | $H\text{-}2^a$ | 10 | 0 | 0 |
| CBA | $H\text{-}2^k$ | 17 | 0 | 0 |
| C57BL/6 × A/J | $H\text{-}2^{b\times a}$ | 63 | 6 | 10 |
| C57BL/6 × CBA | $H\text{-}2^{b\times k}$ | 61 | 20 | 33 |
| C57BL/6 × DBA/2 | $H\text{-}2^{b\times d}$ | 5 | 3 | — |
| A/J × CBA | $H\text{-}2^{a\times k}$ | 5 | 1 | — |
| DBA/2 × CBA | $H\text{-}2^{d\times k}$ | 15 | 9 | 60 |

[a] Data taken from experiments shown in Table I.

their serum. These bands appeared quite similar to those induced previously in rabbits (6). The incidence of such bands was nonuniform as to strain, interval of appearance, and vaccine used, as shown by the data from all experiments summarized in Tables I and II and Fig. 1.

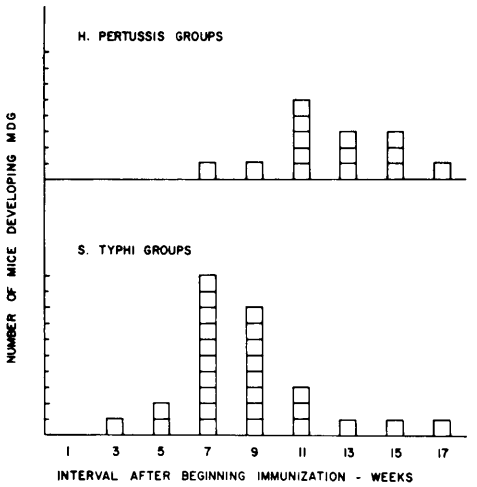


FIG. 1. The number of mice of all strains which had been irradiated and received either *H. pertussis* or *S. typhi* vaccine intraperitoneally, by interval at which MDG were first found in the serum.

The strain of mouse immunized was the most significant variable; A/J and CBA strains failed to develop MDG, while C57BL/6 mice developed a high incidence of bands. Moreover, $F_1$ hybrids having either C57BL/6 or DBA/2 parents yielded the highest incidence of bands; the (DBA × CBA) combination had the highest incidence of MDG of groups given *S. typhi*. Unfortunately, these experiments were performed prior to the elucidation of the *Ir-1* gene locus in mice and the responder concept. Data critical to linking the occurrence of MDG more precisely to the *H-2* locus were not obtained.

Of the three vaccines employed, *S. typhi* gave the highest incidence of MDG. No attempts were made to give quantitatively equivalent doses of each vaccine in terms of numbers of organisms, so these differences could be quantitative rather than qualitative. However, the interval at which MDG appeared after beginning the injections was significantly different for the two vaccines, as shown in Fig. 1. About 80% of the animals which developed MDG in

their serum did so after 4 or 5 immunizing injections, or 8–10 weeks in the case of *S. typhi*. After 12–14 weeks, 70% of the *H. pertussis*-injected mice had shown MDG. Neither the number nor the intensity of the MDG appeared related to the number of immunizing injections, however. Irradiation was not a significant factor in MDG formation, for control groups which were not irradiated but which were injected with *S. typhi* by the same schedule developed the same incidence of MDG as those which were irradiated.

*Characterization of MDG.* Figure 2 illustrates the variety of patterns observed in agar gel electrophoresis. One, two, or, in some instances, three discrete bands appeared in the injected groups. The MDG varied in intensity from being faintly discernible in the diffuse gamma-globulin background to discrete and intensely staining bands resembling those which occur in the sera of mice having myeloma tumors. Immunoelectrophoretic characterization of the MDG-containing sera, using both rabbit and goat anti-mouse immunoglobulin reagents, confirmed the discrete nature of the bands. By both methods the rare bands appearing in the sera of noninjected animals (illustrated in Fig. 2, group C) were uniformly faint and diffuse in comparison with most MDG in the immunized groups. The MDG bands were uniformly sharpest and most intense soon after initial appearance; on repeated bleeding they diffused and became indistinguishable from the gamma-globulin background.

Evidence that the MDG represents specific antibody was obtained in four of the sera examined, in that the discrete bands were absorbed completely by the immunizing antigen. When the absorbed material was eluted at low pH, the eluate had the same mobility characteristics as those in the

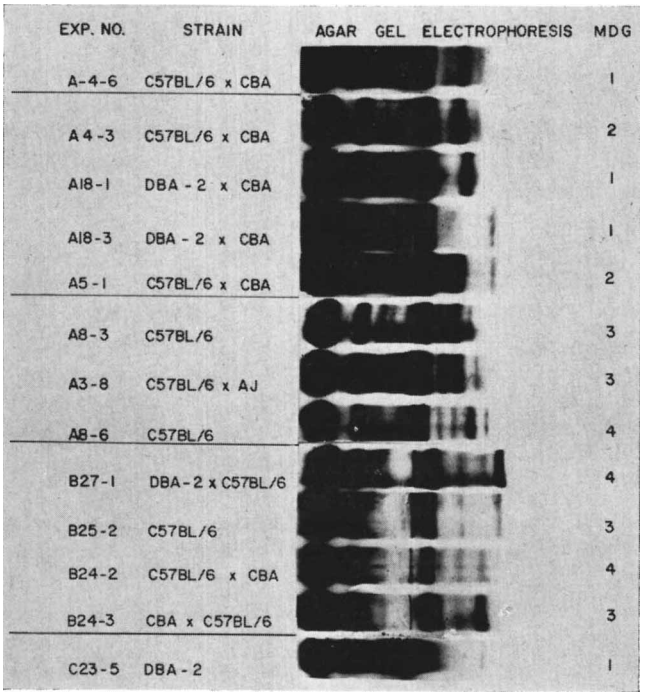


FIG. 2. Agar gel electrophoresis patterns illustrating the varieties of MDG appearing in the sera of mice. Groups A and B: patterns from animals which had been irradiated and immunized with *S. typhi;* group B shows sera from 4 animals which developed bands 7 days after three injections of vaccine. The serum from the CBA × C57BL/6 animals in Expt 27.1 is the same one used to illustrate the absorption experiments in Fig. 3. Group C illustrates the occasional band which appeared in animals irradiated but not immunized.

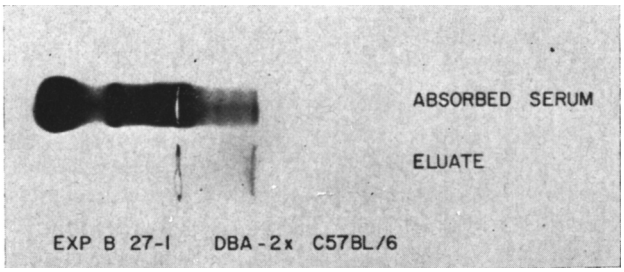


FIG. 3. Agar gel electrophoresis pattern illustrating the removal of MDG bands from serum by absorption twice with *S. typhi* organisms. The electrophoretic migration of the eluted material is identical to that of the original band. The serum is the same as illustrated in Fig. 2, group B.

unabsorbed serum (Fig. 3). More complete characterization of such MDG bands in terms of antibody activity, immunoglobulin class, and molecular homogeneity was not performed.

*Discussion.* These data demonstrate that certain strains of mice respond to intensive immunization with bacterial antigens by synthesizing immunoglobulins which often show a remarkable degree of electrophoretic homogeneity.

The incidence of the MDG was related most clearly to the antigen used for immunization and the strain of mouse examined. In C57BL/6 or $F_1$ hybrids having either C57BL/6 or DBA/2 parents, MDG was elicited in over 60% of those injected with *S. typhi* vaccine. The incidence of MDG in these strains was not higher in animals which had been sublethally irradiated prior to beginning the immunization process; irradiation was, therefore, clearly not critical to the induction of MDG. Evidence is presented that some of these bands represent antibody specific to the immunizing antigen, but further work is needed to establish this point more firmly.

Several investigators (1, 3, 6) have reported that relatively monodisperse antibody could be elicited in rabbits by immunization with group-specific streptococcal antigens. No pretreatment of the rabbit was apparently required to elicit this phenomenon. Antibody induced in these animals migrated as a sharp narrow band on zone electrophoresis, was homogeneous with regard to class, allotype, specificity, and light-chain class, and contained identical amino acids in the first three *N*-terminal light-chain positions. In order to induce the rabbit to form homogeneous antibodies to other bacterial antigens, however, thymectomy and/or appendectomy combined with sublethal x-irradiation were required. In animals so treated, monodisperse antibody protein appeared in the sera after multiple antigenic stimulation and had characteristics quite similar to those reported here for the mouse. X-irradiation of the mice in these experiments was not required, however, to elicit MDG responses to either *H. pertussis* or *S. typhi* immunization.

Invocation of MDG required three or more immunizing injections. Repeated immunizations after the appearance of homogeneous antibody resulted, however, in a decrease, the eventual disappearance of the MDG bands, and the final development of a broad immunoglobulin band. Pure single-determinant antigens were not used here, and the initial MDG might represent responses to a single set of antigenic determinants on the bacterial antigen and, thus, homogeneous antibody. As other determinants evoked antibody responses, the combined characteristics of antibody evoked would be expected to present a more diffuse pattern of gamma-globulins. Perhaps the necessity for thymectomy and appendectomy in the rabbit may be related to a reduction in the numbers of antigen-reactive cells and, thus, to increased possibility that a homogeneous response could be observed.

It is of considerable theoretical interest that not all strains of mice gave rise to MDG when repeatedly injected with bac-

terial antigens. A/J and CBA mice were found to be distinctly different from DBA/2 and C57BL/6 strains and hybrids with respect to a propensity to form MDG. When immunized with *S. typhi,* A/J and CBA mice responded with high levels of anti-*S. typhi* agglutinins, but monodisperse bands did not appear. C57BL/6 and DBA/2 may be high-MDG strains, and A/J and CBA may be low-MDG strains. More critical tests of this hypothesis should be made in appropriate congenic lines and backcrosses, since it suggests an additional mechanism whereby genetic control over the immune response might be exerted in mice.

*Summary.* Immunization of various inbred lines of young mice led to the appearance of monodisperse bands of gamma-globulin having antibody activity in the serum. Whereas the antibody titers achieved did not differ significantly, the proportion which developed these bands was as high as 60% in DBA/2 or C57BL/6 and $F_1$ hybrids having these as one parent. This activity did not occur in A/J or CBA strains. These studies suggest an additional way in which genetic control might be exerted over the quantity of the immune response.

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