GENETIC CONTROL OF THE IMMUNE RESPONSE TO STAPHYLOCOCCAL NUCLEASE

VII. Role of Non-H-2-Linked Genes in the Control of the Anti-Nuclease Antibody Response

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Staphylococcal nuclease is a naturally occurring protein that has been used in this laboratory over the past 5 yr as a model antigen for study of the genetic control of murine immune responses. Thus far, two sets of genes involved in the antibody response to nuclease have been defined: (a) an immune response gene (Ir gene) mapping to the I-B subregion of the H-2 complex (1), which determines the ability of an animal to generate an initial antibody response to nuclease determined 3 wk after immunization; strains of H-2 haplotypes a, k, d, and s are high responders, whereas strains of haplotypes b and q are low responders; and (b) genes coding for idiotypic determinants on the heavy chain variable (V\text{H}) region of anti-nuclease antibodies mapping close to the heavy chain (C\text{H}) allotype locus (2). These genes appear to be independent of H-2 as evidenced by absence of genetic linkage as well as by the expression of one idiotypic marker, the A/J anti-nuclease idiotype, in animals of the same allotype but different H-2 haplotype (3).

It has previously been observed that when antibody titers were measured 3 wk after an immunizing dose of nuclease in complete Freund's adjuvant, there was a significantly greater antibody response in B10.A (H-2\text{a}) animals than in B10 (H-2\text{b}) animals, allowing distinction of high vs. low response status. With hyperimmunization, however, these differences became progressively smaller, and the plateau values for antibody responses were virtually identical (4). Although the total amount of antibody produced in each strain was similar, the antibody populations differed with respect to the proportion directed against different antigenic determinants (5). In addition, although A/J and B10.A mice are both H-2\text{a} strains, A/J mice produced markedly greater levels of antibody to nuclease than did B10.A mice, even though the proportions directed at different regions of the molecule were similar (4). These results suggested that even though H-2-linked Ir gene(s) determined the antigenic sites to which antibodies could be made, there were possibly additional genetic elements that controlled the overall magnitude of the antibody response to nuclease.

In this report, we present further evidence for the contribution of non-H-2-linked genes to the magnitude of antibody responses to nuclease by analysis of the response of various congenic mice and crosses between them. The results presented here are consistent with the hypothesis that there is genetic control of the overall antibody response to an intact antigen independent of the H-2-linked and allotype-linked systems previously described.

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Materials and Methods

**Mice.** Mice of strains A/J, B10.A, A.BY, and B10 were purchased from The Jackson Laboratory, Bar Harbor, Maine. (A/J x B10.A)F~ animals and (A/J x B10.A) x B10.A backcross animals were produced in our own breeding colonies. All mice were male except for the backcross population, of which both males and females were used. Animals were immunized at 8-12 wk of age.

**Antigens.** Staphylococcal nuclease was purified according to published methods (6, 7). In one preparation used in these experiments, the predominant nuclease species had properties consistent with those described for pronuclease or nuclease B, a higher molecular weight form of nuclease containing 19 additional amino acids at the N-terminus (8).

**Immunizations.** Mice were immunized with 100 ng of nuclease in complete Freund’s adjuvant (CFA) and bled 3 wk later. Thereafter animals were bled and boosted with 10 ng of nuclease in saline at 7- or 10-day intervals. Peak antibody levels were achieved after the third or fourth booster immunization.

**Assay of Anti-Nuclease Antibodies.** The ability of antibodies to inactivate the enzymatic activity of nuclease was determined as previously described (1). Sera were assayed at multiple dilutions until a plateau value was obtained. Values are reported in terms of inactivating units per milliliter and represent the average of at least duplicate determinations.

**Determination of Allotype.** Allotype of individual backcross animals was determined by Ouchterlony diffusion analysis using specific antiallotype reagents produced by immunizing allotypically dissimilar animals with pertussis-antipertussis complexes by published methods (9).

**Determination of Idiotype.** The presence of the A/J idiotype was determined as described previously (2). In this assay, the ability of rat anti-idiotypic antisera to inhibit antibody-mediated inactivation was tested. A serum was considered to contain the idiotype if the anti-idiotype caused statistically significant inhibition of inactivation.

**Analysis of Antibody Specificity for Nuclease Fragments.** Binding of antibodies to fragments of nuclease was assayed as previously described (5), using polyethylene glycol (mol wt 6,000, 10% wt/wt final concentration) to precipitate antibodies plus bound 14C-labeled antigen, leaving free antigen in the supernate. The labeled fragments were prepared by carbamoylation of N-terminal amino groups with [14C] KCNO. The specific radioactivities of the products were 50 μCi/μmol for 14C-fragment (99-149) and 141 μCi/μmol for 14C-fragment (1-126). To assess the quantity of antibodies in serum that could bind to a given fragment, a complete binding curve was performed with increasing concentrations of labeled fragment and a constant 1:5 dilution of serum. The plateau value was taken as the concentration of antibody binding sites specific for that fragment. The same data were also plotted according to the method of Scatchard (10) to determine affinities.

Results

Previous studies (4, 5) indicated that antibody levels differed significantly among strains that were high responders to nuclease, with strains SJL (H-2*) and A/J (H-2b) both having much higher titers of antibody than B10.A (H-2b). On the basis of the comparison of H-2 identical strains A/J and B10.A, one could infer that non-H-2 genes present in the background of strain A/J permitted a much greater response to the antigen than could occur on the B10 background. From these data, however, it was not possible to determine the influence of non-H-2-linked genes on a low responding H-2 haplotype. To investigate this possible effect, the immune response of strain A.BY (H-2°) was determined and compared with a series of congenic strains.

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1 **Abbreviations used in this paper:** CFA, complete Freund’s adjuvant; CH, constant region of the immunoglobulin heavy chain; Ir, immune response; Vh, variable region of the immunoglobulin heavy chain.
Table I
Antibody Levels in Congenic Mouse Strains

| Strain | Number of animals examined | H-2 haplotype | Allotype | Units inactivated/milliliter ± SE |
|--------|---------------------------|---------------|----------|----------------------------------|
| A/J    | 8                         | a             | Ig-1e    | 175.0 ± 31.6                     |
| A.BY   | 10                        | b             | Ig-1e    | 4.6 ± 2.1                        |
| B10.A  | 9                         | a             | Ig-1b    | 27.3 ± 2.8                       |
| B10    | 7                         | b             | Ig-1b    | 3.9 ± 0.7                        |

Groups of animals of the strains listed were immunized with 100 μg of nuclease in CFA and bled at 3 wk. Antibody activity in terms of inactivating units per milliliter was determined as described in Materials and Methods. The arithmetic mean ± SE is reported for each strain.

Table I shows the results of initial antibody titers of strains A/J (H-2\textsuperscript{a}), A.BY (H-2\textsuperscript{b}), B10.A (H-2\textsuperscript{a}), and B10 (H-2\textsuperscript{b}) obtained 3 wk after immunization. It is clear that antibody activity present in antisera from A/J animals was significantly greater than that found in antisera from B10.A animals, but both were greater than that found in B10 or A.BY. In fact, both B10 and A.BY made barely detectable quantities of antibody. Thus, the increased antibody-producing capacity present in the A background and observed in the comparison of A/J and B10.A animals was not evident in the comparison of the initial response of the A.BY and B10 animals. These results indicated that in the initial antibody response to nuclease, non-H-2-linked gene(s) influenced the magnitude of a response that was dependent on the presence of H-2-linked genes. In the absence of H-2-linked \textit{Ir} genes that confer high responsiveness, the effect of non-H-2 genes on the initial response could not be measured.

Effect of Hyperimmunization. It has previously been shown that hyperimmunization of low responding B10 animals eventually leads to antibody levels equivalent to those found in B10.A animals (4). However only a single pair of congenic animals was examined, B10 and B10.A, making it difficult to generalize about this phenomenon. In the present study, animals from strain A.BY were hyperimmunized with nuclease according to the protocol previously used. As seen in Fig. 1, antibody levels in A.BY animals rose progressively to a plateau value of 1,200 U/ml, compared with a plateau value of 1,040 U/ml for sera from hyperimmune A/J animals and 120 U/ml for sera from hyperimmune B10.A animals. It thus appears that the overall magnitude of the antibody response to nuclease was controlled by non-H-2-associated rather than H-2-linked \textit{Ir} genes, and that after hyperimmunization it was possible for an initially "low responder" strain to make significantly greater quantities of antibody than an initially "high responder" strain.

Specificity of Antibodies from Strain A.BY. On the basis of previous work, one would predict that for strain A.BY, H-2-linked \textit{Ir} genes would control the relative proportion of antibodies to different antigenic determinants on the nuclease molecule whereas non-H-2-linked genes would control the quantity of such antibodies made (5). To test this prediction, antinuclease sera from A.BY mice were analyzed for their ability to bind to two fragments of nuclease, one comprising the N-terminal portion of the molecule from the 1st to 126th amino acid residue, fragment (1-126), and the other, the C-terminal portion of the
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FIG. 1. Effect of boosting on anti-nuclease antibody response of A.BY animals. Five A.BY animals were immunized with 100 µg of nuclease in CFA and bled 3 wk later. Thereafter, animals were boosted with 10 µg of nuclease in saline and bled at weekly intervals. Pooled sera were assayed as described in Materials and Methods. Peak responses of A/J and B10.A are illustrated for comparison.

TABLE II
Concentration of Antibodies to Subregions of Nuclease in Antisera to Native Nuclease

| Strain | Bleed | Concentration of antibody binding sites | Ratio anti-(99-149):anti-(1-126) |
|--------|-------|----------------------------------------|----------------------------------|
|        |       | Anti-(99-149)                           | Anti-(1-126)                     | anti-(99-149):anti-(1-126) |
|        | µM    |                                        |                                  |                             |
| A.BY   | 1     | <0.1                                   | 1.10 ± 0.19                      | <0.09                        |
| A.BY   | 5     | 2.55 ± 0.09                            | 17.20 ± 0.36                     | 0.15                         |
| A/J    | 1     | 2.21 ± 0.21                            | 5.44 ± 0.24                      | 0.41                         |
| A/J    | 5     | 5.75 ± 0.30                            | 12.70 ± 0.55                     | 0.45                         |

The concentration of antibodies binding to fragments of nuclease in various antisera was determined from plateau values of binding curves to 14C-labeled nuclease fragment. The values from the A/J fifth bleed have been previously reported (5).

molecule, nuclease fragment (99-149). Sera obtained 3 wk after immunization as well as after hyperimmunization were tested.2 Table II presents results obtained with A.BY sera, showing the concentration of binding sites present against the two fragments as well as the ratio of these concentrations. In the initial bleed, minimal activity could be detected against nuclease fragment (99-

2 In these experiments, the 3-wk sera of the A.BY strain came from animals that had been immunized with a nuclease preparation containing a large amount of a form of nuclease, termed nuclease B, which contains additional amino acid residues at the N-terminus (8). High responding animals produced virtually identical amounts of antinuclease antibodies when immunized with either type of nuclease. In the case of A/J animals the predominant idiotypes present and the patterns of response with respect to binding to fragments were also indistinguishable after immunization with either form of nuclease.
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whereas there was measurable binding detected against nuclease fragment (1-126). The ratio of antibody concentrations specific for the two fragments was similar to that found in the initial bleed of B10 animals (5). After hyperimmunization there was an increase in the concentration of binding directed against both fragments but with a predominance of antibody directed against fragment (1-126). The observed ratio of antibodies to the fragments, 0.15, is similar to that found with sera from hyperimmune B10 animals, 0.22, which also showed a preponderance of antibodies to fragment (1-126) (5). These results contrast with the ratio seen with hyperimmune A/J sera, 0.45, where the relative amount of antibodies to fragment (99-149) is threefold greater (cf. the B10.A sera which had four times the ratio of the B10 sera). These results are consistent with the notion that H-2-linked Ir genes are involved in relative response to different antigenic determinants on the same molecule and that non-H-2 genes control the overall amount of antibody made to all determinants.

Effect of Non-H-2-Linked Genes on Antibody Affinity. In a previous study, it was observed that there were differences in immune sera from strains A/J and B10.A with respect to the affinity of binding to fragment (1-126) (5). Although the overall concentration of antibodies reacting with this fragment was higher in immune sera from A/J than B10.A animals, the affinity of binding was 10-fold lower. To determine whether this difference represented a distinguishable feature of antibodies from B10 and A backgrounds, a similar analysis was performed using anti-nuclease antibodies from strains A.BY and B10. In Fig. 2 Scatchard plots are presented for binding to this fragment by sera from strains A/J, A.BY, B10, and B10.A. It is seen that there were consistent differences in such plots, and that similarities were seen in strains of the same background rather than of the same H-2 haplotype. Thus, strains A/J and A.BY showed nearly linear plots, whereas B10.A and B10 showed biphasic plots, in which the lower limb was nearly horizontal, preventing any assessment of the affinity of such binding. If only antibodies of significant affinity were compared (for strains of each H-2 haplotype), it was found that sera from the B10 background had antibodies of 7- to 10-fold higher affinity than those of a corresponding preparation from A background mice (Table III). Thus, non-H-2-linked genes affected the affinity of antibodies to antigenic determinants in fragment (1-126).

Scatchard analysis of the binding affinities of antibodies directed to fragment (99-149) indicated greater heterogeneity with respect to binding affinity than antibodies to fragment (1-126). Antisera from animals of both the A and B10 backgrounds showed similarities in terms of the range of binding affinities to fragment (99-149) represented (1.2 \times 10^6 - 72 \times 10^6 M^{-1} for sera from B10.A, 0.7 \times 10^6 - 10 \times 10^6 M^{-1} for A.BY, and 0.9 \times 10^6 - 30 \times 10^6 M^{-1} for A/J; sera from B10 animals had insufficient antibody for accurate measurement). Thus, the contribution of non-H-2-linked genes to determining antibody affinity for this antigenic determinant could not be assessed. It should be noted that in this type of analysis, it is possible to assign affinities only to antibodies present in highest concentration. It is thus possible that within the population of antinuclease antibodies produced by A/J mice, there were antibodies to fragment (1-126) of comparable affinity to those found in immune sera of B10.A mice. However, such antibodies, if present, comprised only a small percentage of the
FIG. 2. Scatchard analysis of binding of fragment (1-126) by hyperimmune anti-nuclease antibodies. Pooled sera from groups of 5-25 mice were used for all experiments. Total concentrations of binding sites for nuclease fragment (1-126) were 0.80 μM for B10.A, 1.2 μM for B10, 12.7 μM for A/J, and 17.2 μM for A.BY. All sera were utilized at a 1:5 dilution in phosphate-buffered saline as described in Materials and Methods. Data are plotted in terms of r/c vs. r where r is the fraction of antibody sites that are bound and c is the micromolar concentration of free antigen.

| Strain   | H-2 haplotype | Affinity (M⁻¹) | Ratio of affinities (B background to A background) |
|----------|---------------|----------------|--------------------------------------------------|
| B10      | b             | 4.5 × 10⁶      |                                                  |
| A.BY     | b             | 0.65 × 10⁶     | 7                                                |
| B10.A    | a             | 1.6 × 10⁶      |                                                  |
| A/J      | a             | 1.6 × 10⁶      | 10                                               |

The affinity of antibodies to fragment (1-126) of hyperimmune antisera from these strains was determined from the Scatchard plots presented in Fig. 1.

antibodies produced to fragment (1-126); this percentage must be much smaller than that found for such antibodies in B10.A sera.

Backcross Analysis of Non-H-2-Linked Genes. It was next of interest to determine the number and nature of non-H-2-linked genes controlling quantitative aspects of antibody production. As results presented thus far indicated that such non-H-2-linked genes did not alter H-2-linked aspects of genetic control but only modified their quantitative expression, it seemed reasonable to examine animals of the same H-2 type. Thus, A/J, B10.A, (B10.A × A/J)F₁, and a backcross to the low responding B10.A parent were examined. Mice were
immunized with 100 μg nuclease in CFA and antibody titers determined 3 wk later. The allotype of each animal was determined by Ouchterlony diffusion analysis. Because strains A/J (Ig-1e) and B10.A (Ig-1b) differ in terms of the predominant anti-nuclease idiotype expressed in immune sera, animals were also typed for the presence of the A/J antinuclease idiotype. Fig. 3 shows the distribution of antibody levels found in sera from various animals, with Ig-1b/Ig-1b and Ig-1b/Ig-1e backcross animals indicated separately. In Table IV antibody levels of F1 animals are compared to backcross animals grouped with respect to allotype. All the Ig-1b/Ig-1e heterozygotes bore the A/J idiotype, whereas there were seven apparent recombinants among the Ig-1b/Ig-1b homozygotes (11). This small group of idiotype-positive allotype homozygotes were analyzed separately.

Several points can be made with respect to these data. First, the response of F1 animals was equivalent to that of the parental A/J strain. This equivalence indicated that non-H-2-linked genes for high response were dominant, militating against the possibility of a dominant suppressive influence contributed by the B10.A parent.

Second, with respect to the distribution of antibody responses in the backcross population, slightly over half of the animals had antibody levels in the range expected for B10.A animals, whereas the remainder (not significantly different from the number for the first group) showed widely distributed levels ranging
TABLE IV
Antibody Levels in (B10.A × A/J) F1 and (B10.A × A/J) × B10.A Backcross Mice

| Group                        | A/J idioype | Number of animals | Units inactivated/milliter (mean ± SE) |
|------------------------------|-------------|-------------------|---------------------------------------|
| (B10.A × A/J) F1            | +           | 9                 | 168.0 ± 21.5                           |
| Backcross Ig-1b/Ig-1b*       | -           | 65                | 53.9 ± 5.4                             |
| Backcross Ig-1b/Ig-1e        | +           | 36                | 57.9 ± 10.1                            |
| Backcross Ig-1b/Ig-1d        | +           | 7                 | 59.5 ± 14.4                            |

(B10.A × A/J) F1 animals and offspring of the backcross (B10.A × A/J) × B10.A were immunized with nuclease and bled 3 wk later. Antibody levels were determined as described in Materials and Methods. Arithmetic mean ± SE was calculated for backcross animals grouped according to allotype as well as recombinant phenotype (Ig-1b/Ig-1b homozygotes demonstrating the A/J idioype).

* This group includes seven animals that expressed the A/J idioype and have been identified as putative recombinants. These seven animals are listed separately in line 4.

from 40 U/ml to greater than 200 U/ml, similar to the distribution of A/J or F1 levels. Because of the wide individual variation among animals of the same strain in the amount of antibody produced, it is difficult to divide the backcross population to allow an estimate of the number of genes involved. However, the results as described are not inconsistent with the operation of a single non-H-2-linked gene.

Third, there was no apparent influence of allotype or idioype on the amount of anti-nuclease antibody produced. This result indicates that the lower antibody production in B10.A animals was not due to a deficit of appropriate variable region sequences. In the presence of appropriate background genes, antibodies bearing Ig-1b constant region of the immunoglobulin heavy chain (C\text{H}) allotype were found in quantities equivalent to anti-nuclease antibodies in A/J animals. Similarly, the high response of A/J animals could not be attributed to the Ig-1e C\text{H} allotype or the A/J idioype, as backcross animals positive for these markers but producing very low antibody levels were identified. These results suggest the presence of genetic elements unrelated to the H-2 region or to variable region genes of the immunoglobulin heavy chain (V\text{H}) that modulate the overall level of antibodies to nuclease. Finally, there was no detectable effect of sex on antibody levels (data not shown), although the influence of X-linked control genes was not assessed in these studies because of the manner in which the backcross was designed.

Discussion

It is now well established that the immune response to both synthetic and natural antigens is under genetic control. Various patterns of such control have been identified, although the most intensively studied is control by H-2-linked Ir genes (12). However, there have been studies indicating (a) control by non-H-2-linked genes (13, 14), (b) interaction of two H-2-linked genes (15-17), (c) involvement of non-H-2 genes in an H-2-linked Ir gene controlled response
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(18), (d) control by genes linked to heavy chain allotype (19), (e) control by X-linked genes (20–22), and (f) multigenic control, particularly evident with complex antigens (23). The mechanisms by which these various forms of genetic control are exerted are as yet unknown.

In this communication, we have tried to assess the contribution of various genes in the control of the antibody response to staphylococcal nuclease. The influence of at least three genetic elements on the quantitative aspects of the antibody response can now be distinguished.

(a) H-2-linked Ir genes. The influence of such Ir genes can be demonstrated in at least two ways (4, 5). First, they determine the ability of an animal to generate an initial antibody response to nuclease. It appears that the recognition of an antigenic determinant present in the carboxy terminal fragment of nuclease, nuclease (99-149), underlies this initial response. Second, Ir genes determine the pattern of response with respect to the concentration of antibodies directed to different antigenic sites on nuclease. This effect of Ir genes has now been observed in congeneric animals of two different backgrounds, confirming the H-2 dependence of the effect and diminishing the possibility that genetic drift between the B10 and B10.A strains was responsible for previously observed differences (5). In addition, the pattern of response controlled by Ir genes can now be shown explicitly to be independent of immunoglobulin heavy chain structural genes as strains A/J and A.BY, which have been previously shown to bear the same idiotypes, differ in the proportion of antibodies they make to two antigenic determinants of nuclease.

(b) A gene (or genes) that determines the affinity of antibodies directed to one antigenic determinant on nuclease. Thus, antibodies from strains B10 and B10.A have a 7- to 10-fold higher affinity for nuclease fragment (1-126) than comparable antibodies from strains A.BY and A/J. Anti-nuclease antibodies from animals of A and B10 background have been shown to differ with respect to the predominant idiotypes expressed as determined by anti-idiotypic antisera. It is thus not unlikely that the differences in affinity of antibodies is a reflection of the different idiotypes expressed, suggesting that antibody affinity can be used as a VH region marker. However, only idiotype, and not affinity of antibodies, has been shown by formal backcross analysis to segregate with known allotypic markers; the affinity of antibodies from individual animals could not be assessed because the radioimmunoassay used in those studies required more antibody than can easily be obtained from a single animal.

(c) A gene (or genes) that regulates the overall magnitude of the antibody response to nuclease that is independent of the specificities of such antibodies as determined by H-2-linked Ir genes or the idiotypic determinants on such antibodies. It has been possible to recognize this type of control because of an unusual property of the immune response to nuclease. Thus, certain mouse strains categorized as low responders after an initial immunization will significantly augment this response after hyperimmunization. The antibodies found after such immunization are IgG (5). The mechanism of the rise in antibody after hyperimmunization is unclear and cannot yet be specifically ascribed to

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5 Berzofsky, J. A. Genetic control of the immune response to mammalian myoglobins in mice. I. More than one I-region gene in H-2 controls the antibody response. J. Immunol. In press.
known genes. The phenomenon, however, makes it possible to discern one of the apparent roles non-H-2 genes may play, that is, the regulation of overall level of response to nuclease. Thus, both in the case of B10 and B10.A, and A/J and A.BY, high and low responders on the same background reach the same peak level of anti-nuclease antibodies, with the relative proportions of antibodies to individual determinants differing similarly within each pair, with strains bearing the $H-2^a$ haplotype producing significantly more antibody to nuclease fragment (99-149) than the corresponding strain of the $H-2^b$ haplotype.

The existence of a control mechanism that regulates the total amount of antibody produced against an antigen can also be inferred from studies on idiotypic suppression by anti-idiotypic antibodies. Thus, in both the response to the streptococcal carbohydrate (24) and the arsonate haptenic determinant (25), administration of anti-idiotypic antisera to mice before immunization shifts the pattern of idiotypes expressed without altering the magnitude of the response. In the case of the arsonate system, the response observed after idiotypic suppression is characterized by the emergence of normally infrequently expressed or private idiotypes (26). Although these two experimental systems concern the modulation of the expression of different idiotypes to a single antigenic determinant, they imply control of the total level of antibody to an antigen irrespective of idiotype, which may be analogous to the control we have observed on total level of antibody to a single antigen molecule irrespective of fine specificity.

The nature of the sensing and control mechanism that can discriminate between various antibodies and regulate the amount directed against one antigen is unknown. One possible mechanism that could be postulated on the basis of the data presented here would involve negative feedback control of antibody production through the formation of antigen-antibody complexes. Thus, the feedback control would regulate the response to the whole antigen molecule, independent of the number of determinants recognized by the antibodies. Because it would require less high-affinity antibody than low-affinity antibody to complex a given amount of antigen, strains producing high-affinity antibody should produce less antibody than strains producing low-affinity antibody. This type of reciprocal relationship was in fact observed when comparing the antibody response of strains of the A and B10 background to determinants present on the nuclease fragments (1-126). However, as we have not yet shown in a backcross population that genes regulating antibody amount and affinity segregate reciprocally, this mechanism is purely speculative. It should be noted that in other studies investigating the possible interrelationship between antibody affinity and amount, a consistent pattern has not been observed (18, 27-30; see fn. 3).

Irrespective of the mechanism involved, feedback control of antibody levels and, by implication, of the proliferation and function of antibody-producing cells is of importance to the organism. Failure to modulate the proliferation of cells in response to antigen would lead to myeloma-like conditions after every antigenic exposure. Such control systems would set an upper limit on the extent to which such cells divide and grow. There may, however, be another aspect of this control that is also important, as suggested by the studies on idiotypic suppression as well as the results presented here. If exposure to an
antigen necessitates an antibody response to a genetically determined level, irrespective of the specificity of that response or the idiotypes present, then attempts at specific immunosuppression (e.g. by anti-idiotypic antisera) may lead only to an alteration of the pattern of the response without affecting magnitude. Thus, under conditions of suppression, (a) idiotypes that escape suppression may become predominant or (b) antibodies may be made to antigenic determinants that are not involved in the suppression but are not usually strongly immunogenic. The operation of control systems maintaining the overall level of antibody production to an antigen may become evident as attempts at such specific immune suppression become more widespread. It is hoped that continuing studies with model antigens like nuclease will permit further insight into these control mechanisms.

Summary

The role of non-H-2-linked genes in the control of the antibody response to staphylococcal nuclease has been investigated. 3 wk after immunization with nuclease in complete Freund's adjuvant, strain A/J (H-2a) mice produced significantly higher titers of antibody than strain B10.A (H-2a) mice, whereas mice of strains A.BY (H-2b) and B10 (H-2b) produced barely detectable titers. With hyperimmunization, A/J and A.BY mice reached the same peak levels for antibody titers, both severalfold higher than those reached by B10.A and B10 mice. Analysis of the specificity of antibodies by assessment of binding to two fragments of nuclease showed similarities between strains of the same H-2 haplotype. These results suggest that although H-2-linked genes determined initial responsiveness at 3 wk and the relative proportions of antibodies directed toward different antigenic determinants on the nuclease molecule, non-H-2-linked genes determined the overall magnitude of the hyperimmune response. Measurement of the affinity of the antibodies to the nuclease fragment (1-126) showed that strains B10 and B10.A produced antibodies with 7- to 10-fold higher affinity than comparable antibodies from strains A.BY and A/J. In a backcross of (B10.A × A/J) × B10.A, the level of antibody segregated independently of the Ig-1c Cμ allotype and the A/J anti-nuclease idiotypes. Thus, a gene(s) linked to neither H-2 nor heavy chain structural genes appears to control the aggregate response to antigenic determinants on the nuclease molecule independent of subspecificities of these antibodies or their idiotype.

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