GENETIC CONTROL OF THE IMMUNE RESPONSE
TO STAPHYLOCOCCAL NUCLEASE
IV. H-2-linked Control of the Relative
Proportions of Antibodies
Produced to Different Determinants of Native Nuclease

BY JAY A. BERZOFSKY, ALAN N. SCHECHTER, GENE M. SHEARER, AND
DAVID H. SACHS

(From the Laboratory of Chemical Biology, National Institute of Arthritis, Metabolism, and
Digestive Diseases, and the Immunology Branch, National Cancer Institute, National Institutes of
Health, Bethesda, Maryland 20014)

Staphylococcal nuclease is a convenient model antigen for study of the genetics of the antibody response partly because of the large amount of information available about its chemistry and because of its relative simplicity among natural protein antigens. However, we have shown in the accompanying paper (1) that even for nuclease the interpretation of the genetic control of the immune response becomes complex after several immunizations. In contrast, we showed that for at least one peptide fragment of nuclease (99-149) as immunogen, the response pattern was the same as the initial response to the native protein but without the complexities which arose after repeated immunizations.

To explore further the complexities in the response to staphylococcal nuclease, and in particular the ability of the low responder C57BL/10 strain to increase in response with boosting to the level of antinuclease production of the congenic high responder B10.A, we have studied the specificities of antibodies which comprise the anti-whole nuclease sera, in these two strains as well as the A/J and SJL strains. Both initial and hyperimmune responses were examined. The question explored was whether low responders were uniformly low across the spectrum of specificities produced to the complex antigen, or whether they were selectively low responders to some specificities on the antigen molecule and not to others. We found the latter to be the case, at least for the C57BL/10 response to nuclease, indicating that H-2-linked Ir genes can control the antibody response to different determinants on the same antigen molecule separately from one another. One implication is the function of H-2-linked Ir genes at the level of the selection of specific B cells.

Materials and Methods

Mice. Mice were purchase from The Jackson Laboratory, Bar Harbor, Maine. Four strains were studied: high responders A/J (H-2*) and SJL/J (H-2*), intermediate responder B10.A/SgSn (H-2*), and low responder C57BL/10 Sn (H-2*). All mice were male except the SJL strain. All were 6-8-wk old at the start of each experiment.

Immunization Schedule. Mice were immunized i.p. with 100 μg of nuclease emulsified 1:1 in
complete Freund's adjuvant, and bled and boosted as previously described (1). Blood from 8-12 mice of each strain was pooled, and studies were all performed on pooled sera.

**Highly Purified Staphylococcal Nuclease.** Nuclease was purified by the method of Bohnert and Taniuchi (2). Because of the extraordinary sensitivity of immune systems to potential highly immunogenic albeit chemically minor contaminants in antigens, particular care was taken to insure the high purity of the nuclease used. The initial product had two faint bands on either side of the main band on polyacrylamide gel electrophoresis (7% acrylamide gels pH 9.5 run in Tris-(6 g/liter) glycine (29.9 g/liter) buffer at pH 8.4 at 4°C with reverse polarity). Therefore, the enzyme was further purified by phosphocellulose chromatography (41 mg applied to a 4-ml phosphocellulose column and eluted at room temperature with a linear gradient of ammonium acetate from 0.3 M, pH 5.7, to 1.0 M, pH 8.0. The resulting preparation (72% yield, sp act 2,400 U/mg) showed only the faint slower-moving band in addition to the main band on polyacrylamide gel electrophoresis. On sodium dodecyl sulfate gel electrophoresis (3) the molecular weight of the faint second band was estimated to be twice that of nuclease, consistent with its being a dimer as noted on gel filtration (2). This preparation showed only a single line on Ouchterlony double immunodiffusion against several goat antinuclease sera made to highly purified and partially purified nuclease.

**Assay of Antibodies to Nuclease by Inhibition of Enzymatic Activity.** The assay for antinuclease has been described previously (1). The ability to inhibit 1 U of nuclease activity is defined as 1 inhibition U and corresponds to 32.8 pmol of antibody binding sites.

**Radioimmunoassay for Antibodies Binding to Labeled Fragments of Nuclease.** To assess the specificities of antibody populations in antisera raised to native nuclease, binding to 14C-labeled fragments was measured by a method modified from that used to assay antibodies raised to fragments of nuclease described in the accompanying paper (1). This approach was feasible even though antibodies raised to native nuclease have been found to be specific for the native conformation of the molecule rather than the random conformations of the fragments, since binding of the fragments can be described by a model in which the fragments exist in a conformational equilibrium between random and native conformations (e.g., 0.02% native for fragment [99-149]) (4). Since the apparent affinities for fragments are thus lower by three-to-four orders of magnitude, only relatively high affinity anti-native nuclease antibodies can be measured by binding to fragments.\(^1\)

The radioimmunoassay protocol (1) was modified in that whole binding curves were done for each serum studied, and increasing concentrations of 14C-labeled fragments of nuclease (from 0.1 to 20 μM) were added first rather than serum. Dilutions of the labeled fragments were made in phosphate-buffered saline containing 1.6 mg/ml of goat gamma globulin fraction II (Miles Laboratories Inc., Elkhart, Ind. 82-572, lot 13) to avoid losses on vessel walls at high dilution. For each concentration of antigen, binding by preimmune serum was also measured and subtracted.

Concentrations of antibodies of a given specificity were obtained from the intercepts of Scatchard plots for binding to the appropriate fragments, or from plateau binding in saturation binding curves.

**Separation of IgM and IgG Classes of Immunoglobulin.** Approximately 1 ml of serum was passed over a previously calibrated 1.5 × 95-cm column of Sephadex G-200 in 0.15 M saline at room temperature at a flow rate of 6 ml/h. Fractions corresponding in size to IgM and IgG were pooled and concentrated by vacuum dialysis in a collodion bag against saline, and were assayed for binding activity.

**Results**

To understand which determinants of staphylococcal nuclease are involved in the antibody response to whole (native) nuclease by different strains of mice, we

\(^1\) In view of this important conformational effect, and since the antibodies remained active in the presence of polyethylene glycol before centrifugation to separate phases, it was important to show that polyethylene glycol did not increase the folding of the random conformation fragments. Polyethylene glycol at 12.5% wt:wt did not increase the helicity of fragment (99-149) assessed by circular dichroism, whereas this region of native nuclease has two of the three \(\alpha\)-helical segments of the molecule (5).
measured specificities of the mixtures of antibodies produced by immunization with nuclease.

**Binding to Fragment (99-149) by Hyperimmune Antinuclease Sera from High Responder A/J and SJL Mice.** Scatchard analysis of the binding of \(^{14}\)C-fragment (99-149) by antinuclease sera from these two highest responder strains (Fig. 1) shows almost superimposable binding curves for A/J and SJL sera. Thus, both the affinities and the concentrations of the mixture of different antibodies made to determinants in this subregion of nuclease are similar in the two strains. This result contrasts with the finding that the predominant idiotypes of the antibodies to nuclease made by A/J and SJL mice were distinct (6). However, the antibodies detected to this subregion represent only 10–20% of the total antinuclease antibodies, so the idiotypic antisera may be detecting other antibodies. Alternatively, comparable binding sites may not require identical variable regions.

**Binding to Fragment (99-149) by Initial and Hyperimmune Antinuclease Sera from Congenic C57BL/10 and B10.A Mice.** A similar analysis was used to compare the fraction of total antinuclease which bound to the fragment (99-149) in sera from the congenic C57BL/10 and B10.A strains (Fig. 2). Scatchard analysis of the data showed that a 2:5 dilution of the first-bleed serum from the B10.A mice, 3 wk after a single i.p. immunization with nuclease in complete Freund's adjuvant, had a 0.1–0.2 \(\mu\)M concentration of binding sites specific for
fragment (99-149). In contrast, no detectable binding to this fragment was observed for the corresponding serum from the C57BL/10 strain.

When the hyperimmune sera, after five immunizations with nuclease, were similarly compared for the two strains (Fig. 2, right panel), measurable binding to fragment (99-149) was detected for the C57BL/10 sera, but the concentration of sites specific for this region was threefold lower than that of the B10.A sera. The threefold difference was confirmed in the saturation curves for binding of fragment (99-149) by the same sera (not shown). Thus, although the initial low responder C57BL/10 appeared to reach the same level of response as the high responder B10.A after multiple boosts when only the overall concentration of antibodies to whole nuclease was measured (1), the concentration of antibodies specific for region (99-149) in these same sera never reached the same level as in the B10.A. With repeated immunizations, the distinction between high and low responder disappeared in an assay that measured only the overall response to the whole complex antigen, but remained in an assay that selectively measured antibodies to a more restricted region of the antigen.

This marked difference in response to nuclease when antibodies specific for the fragment (99-149) were assessed was reproducible in a completely separate group of mice immunized at a later time. By the third immunization, the C57BL/10 response to whole nuclease (about 88 inhibition U/ml serum) had actually surpassed that of the B10.A (about 50 inhibition U/ml serum), even
though the initial response was much lower for the C57BL/10 than for the B10.A. In contrast, no binding to the fragment (99-149) was detectable in the C57BL/10 third bleed serum, compared with about 1.5 μM antibody binding sites specific for this region in the B10.A serum (Fig. 3). Thus, even when the C57BL/10 response to nuclease had surpassed that of the B10.A animals, the production of antibodies to the 99-149 region by the C57BL/10 animals remained undetectable.

Binding to Fragment (1-126) by Initial and Hyperimmune Antinuclease Sera from Congenic C57BL/10 and B10.A Mice. In contrast to the binding to fragment (99-149), the concentration of antinuclease antibodies specific for determinants in the 1-126 region appeared to be about the same in the two strains. This equality held for both the initial antinuclease sera 3 wk after a single immunization in complete Freund's adjuvant (Fig. 4) and the hyperimmune antinuclease sera after five immunizations (curve not shown; see Table I). The concentrations of antibody binding sites was higher in all cases than the binding to fragment (99-149). This result is not surprising, since fragment (1-126) represents a much larger part of the nuclease molecule than fragment (99-149) and overlaps the latter from residues 99 to 126.

Relative Proportions of Antibodies Specific for Regions of Nuclease in Antiserum to Whole Nuclease. The concentrations of antibodies binding to different labeled fragments of nuclease, expressed as micromolar antibody binding sites in undiluted serum, are summarized in Table I. The right-hand column lists the ratio of antibodies specific for the two fragments of nuclease in the same sera, made to native nuclease.
H-2-LINKED CONTROL OF ANTINUCLEASE SPECIFICITIES

The most striking observation can be drawn from the data enclosed within the boxes in Table I. Whereas the C57BL/10 mice and B10.A mice made comparable amounts of antibodies specific for the 1-126 region, the C57BL/10 mice made far less antibody specific for the 99-149 region, so that the ratio of the two antibody subpopulations was markedly different for the two strains. This observation has been confirmed for a second group of mice (Fig. 3). Since the two strains are congenic, presumably differing only for the H-2 complex, one can conclude that genes in the H-2 complex can control the relative proportions of antibodies produced to two regions of a single antigen molecule.

A second observation is apparent from a comparison of the data for the B10.A and A/J strains (Table I) which share the same "high responder" H-2" haplotype. The large difference in total antinuclease between these two strains, which we have attributed to non-H-2-linked genes (1) was primarily in the relative concentrations of antibodies binding to the fragment (1-126), particularly in the initial response. This observation raises the possibility that some non-H-2-linked gene(s) influence the specificity for different determinants on the same antigen as well, rather than merely the overall magnitude of the response. In addition, the highest affinities of antibodies measurable in hyperimmune antinuclease sera of the two strains for fragment (1-126) differ by an order of magnitude (1.6 x 10^7 M^-1 for B10.A compared to 1.6 x 10^6 M^-1 for A/J), whereas the highest affinities for fragment (99-149) are similar in the two strains (about 2-3 x 10^7 M^-1). It is interesting that the higher responder has the lower affinity sera.

**Fig. 4.** Binding of C57BL/10 and B10.A primary antinuclease to 14C-fragment (1-126). Sera are those described in Fig. 2 (left panel), used at a 1:5 final dilution. (▲) C57BL/10; (△) B10.A.
Table I
Concentrations of Antibodies to Subregions of Nuclease in Antisera to Native Nuclease

| Bleed | Strain | Antibody binding sites, µM | Ratio       |
|-------|--------|-----------------------------|-------------|
|       |        | Total antinuclease | Anti-(99-149) | Anti-(1-126) | anti-(99-149)/ anti-(1-126) |
| 1°    | C57BL/10 | 0.38                      | 0.00 ± 0.01  | 0.61 ± 0.08  | 0.00 ± 0.02  |
|       | B10.A   | 1.35                      | 0.34 ± 0.08  | 0.81 ± 0.11  | 0.42 ± 0.11  |
|       | A/J     | 5.6                       | 0.58         | 4.95 ± 0.35  | 0.12         |
|       | SJL     | 2.2                       | 0.6          |              |             |
| 5°    | C57BL/10 | 3.9                       | 0.26 ± 0.03  | 1.2 ± 0.15   | 0.22 ± 0.04  |
|       | B10.A   | 3.7                       | 0.71 ± 0.03  | 0.80 ± 0.12  | 0.89 ± 0.14  |
|       | A/J     | 30.2                      | 5.75 ± 0.3   | 12.7 ± 0.55  | 0.45 ± 0.03  |
|       | SJL     | 28.8                      | 3.76 ± 0.7   |              |             |

Concentrations of antibodies specific for given regions were obtained as the mean concentration of bound antigen for the several points judged to be on the plateau of saturation binding curves similar to those in Fig. 3 and 4. The standard error of the mean for these plateau points was used as an estimate of the experimental uncertainty. All binding curves were carried out on pooled sera from 5 to 12 mice. Concentrations of total antinuclease were estimated from the enzyme inhibition assay (Materials and Methods).

The table also lists an estimate of the total concentration of antibodies to whole nuclease in these sera. However, since these values were obtained by a different method from those for antibodies binding to fragments (under different assay conditions, measuring antibodies of a different range of affinities and measuring only inactivating antibodies), the numbers may not be directly comparable. However, it is worth noting that the estimated total antinuclease is generally larger than the sum of the subpopulations binding to the two fragments, even though these subpopulations should overlap. A likely explanation is that since the affinity for the random conformation fragments should be about 10³-fold less than for the native structure (4), antibodies to nuclease with affinities for the native protein below about 10⁷ M⁻¹ will not be detected in binding to the fragments. Therefore, the observations made earlier from the data in this table apply only to the higher affinity 40–50% of the antibodies.

Test for Possible Anti-Random Fragment Antibodies in the B10.A Anti-Native Nuclease. To assess the possibility that some of the greater binding of B10.A antinuclease to fragment (99-149) relative to that of C57BL/10 antinuclease was due to the presence in the former of antibodies to the random conformation fragment which would not be expected to be made by the C57BL/10 animals (see accompanying paper) (1), a competition experiment was carried out...
Fig. 5. Competition of native nuclease against $^{14}$C-fragment (99-149) for binding to hyper-immune B10.A antinuclease. Serum was that described in Fig. 2 (right panel), used at a constant dilution of 1:5 (about $8 \times 10^{-7}$ M in binding sites). A constant final concentration of $2 \times 10^{-7}$ M $^{14}$C-fragment (99-149) was mixed with the increasing final concentrations of native nuclease shown on the abscissa before the addition of antiserum. The ordinate is bound/free labeled fragment.

(Fig. 5). The smooth curve obtained is suggestive of a reasonably homogeneous population of antibodies for which the lower limit of affinity$^2$ for native nuclease is about $10^6$ M$^{-1}$. These data allow us to rule out the presence of a significant concentration of anti-random-conformation-fragment antibodies by the following criteria: (a) The curve approaches B/F = 0 at high nuclease concentrations. If a significant fraction of the antibodies binding to $^{14}$C-fragment (99-149) were to random conformation determinants not present on native nuclease, the curve would be expected to plateau at some value above zero, as found by Curd et al. (b) No significant fraction of antibodies (i.e., <10%) show a transition corresponding to an affinity of less than $3 \times 10^5$ M$^{-1}$. If anti-random-conformation-fragment antibodies were present which bound to the $^{14}$C-fragment (99-149) with an affinity of $10^7$ (the highest affinity detected by Scatchard analysis of this same serum's binding to $^{14}$C-fragment [99-149], in Fig. 2), then according to the conformational equilibrium constant of about $3 \times 10^{-4}$ found for native nuclease by Furie et al. (8) using anti-random conformation antibodies, the apparent affinity of these antibodies for native nuclease would be only about $3 \times 10^3$ M$^{-1}$. Thus, these results rule out the production of anti-fragment antibodies by, for example, enzymatic digestion of

$^2$ We cannot determine affinities higher than about $10^6$ M$^{-1}$ in this system because of the concentrations of labeled fragment and antiserum necessary to obtain sufficient bound radioactivities to count. In addition, an exact assessment of affinity is limited by the fact that the anti-native nuclease serum contains antibodies to determinants on whole nuclease outside the region from residues 99 to 149. However, the total available nuclease on the abscissa represents an overestimation, since some of the nuclease is bound by these other antibodies irrelevant to the competition with fragment (99-149). Therefore, the lower limit of affinity may be several-fold higher than $10^6$ M$^{-1}$, the most conservative estimate.
the nuclease during antigen "processing," as an explanation of the difference in specificity of B10.A and C57BL/10 antinuclease.

The Class of Immunoglobulin Comprising the C57BL/10 Antinuclease Antibodies. To assess whether the C57BL/10 mice, whose overall antinuclease response after boosting had reached the same level as that of the B10.A mice, might have continued to manifest an Ir-gene defect by failure to switch from IgM to IgG production, we fractionated an aliquot of hyperimmune (fourth-bleed) C57BL/10 antinuclease on a Sephadex G-200 column into IgM and IgG fractions. After concentration by vacuum dialysis to approximately the original volume of serum, the IgG peak had about 70% of the original activity, whereas the IgM peak had no detectable activity in the nuclease inhibition assay. Thus, the hyperimmune C57BL/10 antinuclease response is mostly, or all, in the form of IgG.

Discussion
In the accompanying paper (1) we demonstrated a striking difference in the overall antibody response to nuclease between the A/J and B10.A strains, which share the same high-responder $H-2^a$ haplotype. The difference was attributed to non-$H-2$-linked genetic effects. The current results raise the question of whether the non-$H-2$-linked control has some specificity with regard to different determinants on nuclease because of the much greater contribution of anti-(1-126) than anti-(99-149) in accounting for the differences between these strains. In addition, the affinity of the lower responder B10.A antinuclease sera for fragment (1-126) is 10-fold higher than that of the higher responder A/J. In this regard the non-$H-2$-linked control may be different from that described for the response to poly-(L-Glu, L-Ala, L-Tyr) by Dorf et al. (9) in these same strains, in which an influence on the magnitude but not the specificity or affinity of the antibody response was demonstrated.

A more clear-cut comparison is the contrast of the low responder C57BL/10 strain ($H-2^b$) with the congenic high responder strain B10.A ($H-2^a$). In the accompanying paper it was seen that this distinction between high and low overall response to nuclease, defined for the initial antisera, disappears after the animals are immunized several times (1). The present results show that if one looks at only those antinuclease antibodies which react with the fragment (99-149), the distinction between the B10.A and C57BL/10 persists even after five immunizations. The C57BL/10 remains a poor responder to determinants in this region even though its overall response to nuclease is the same as that of the B10.A after boosting. The increase of the C57BL/10 response to nuclease to the levels of the B10.A thus represents an increase in antibodies with specificities for determinants outside the 99 to 149 region. The $H-2$-linked Ir-gene defect in the C57BL/10 appears to be specific for determinant(s) in the region from residues 99 to 149.

This conclusion is especially interesting in light of the observation in the accompanying paper (1) that when the fragments themselves are used as immunogens, it is the fragment (99-149) which appears to be under the same $H-2$-linked control as whole nuclease, and for which the C57BL/10 vs. B10.A difference appears to be all-or-none.

The observations also suggest that Ir-gene control may be more prevalent
than the number of antigens found under control to date would suggest, but that the complexity of the response to multi-determinant antigens can easily mask this control for many antigens.

Finally, a significant implication of this study is that in congenic strains which have the same repertoire of potential structural genes for immunoglobulin variable regions but presumably differ only at \( H-2 \), the \( H-2 \)-linked genes may control the relative antibody responses to different determinants on the same antigen molecule. Two of the possible alternative mechanisms which might explain the data have been ruled out:

(a) One possible explanation of the difference in specificities of C57BL/10 and B10.A antinuclease is that some of the nuclease immunogen is digested in vivo into random conformation fragments, among which the B10.A responds to the fragment (99-149) and the C57BL/10 does not (1). This digestion apparently does not occur in the goat, since immunization with native nuclease yields antibodies which cross-react with random conformation fragments with only 0.02% of the affinity for the native protein (4). The present study excludes digestion as the explanation of the differences observed in the mouse, since at least 90% of the antibodies reacting with \(^{14}\text{C}-\text{fragment} (99-149) in the B10.A hyperimmune antinuclease are indeed anti-native nuclease antibodies (Fig. 5).

(b) Another possible difference between the C57BL/10 and B10.A antibodies would be a failure of the low responder C57BL/10 to switch from IgM to IgG production, even though the overall magnitude of its response reaches that of the B10.A with boosting. We have also ruled out this possibility by fractionation of the C57BL/10 antibodies on Sephadex G-200. Most or all of the antinuclease activity was IgG. This has also been found true for the B10.A antinuclease serum 3 wk after a single immunization in complete Freund's adjuvant.

Therefore we conclude that in congenic strains of mice, \( H-2 \)-linked genes can control the antibody response to different determinants on the same immunogen molecule separately from one another. Since these strains have the same potential B-cell antibody production capabilities, at least in terms of variable-region structural genes, the \( H-2 \)-linked \( I_r \)-gene control would appear to be exerted at the level of selection of specific B cells. This result raises serious doubt about the notion that all that is necessary for an antibody response is the presence of T cells which can recognize a "carrier" determinant on the antigen molecule and through this present the antigen molecule to B cells specific for any "haptenic" determinant on the molecule (10). Since the C57BL/10 makes antibodies to the region of nuclease from residues 1 to 126, it must have T cells which can recognize a "carrier" determinant on nuclease. Then, since this strain should possess the same B-cell variable region potential as the B10.A, it should be capable of making antibodies to the 99 to 149 region. The fact that the C57BL/10 does not follow this behavior implies that the \( H-2 \)-linked \( I_r \)-gene control must also be exerted at a step in which specific B cells are selected.

There are two general classes of mechanism which might account for this behavior: (a) At the antigen level, there may be more than one "carrier" determinant on the same molecule such that each carrier determinant could function effectively with only some of the "haptenic" determinants on the molecule. Some suggestion that the hapten and carrier functions are located in the same part of the sequence of nuclease is given by the observations that the
response to the isolated fragment as immunogen appears to be under the same 
Ir-gene control as the response to whole nuclease (1), and it is this region to 
which antibody production is under H-2-linked control in the native nuclease 
molecule. Moreover, in a T-cell proliferation assay, the same region appears to 
be the important one in stimulation of the B10.A mice (R. H. Schwartz, J. A. 
Berzofsky, A. N. Schechter, and D. H. Sachs, manuscript in preparation). This 
closeness of carrier and hapten function also makes it unlikely that the mecha-
nism by which a carrier cannot service some of the haptenic determinants on the 
molecule is merely steric hindrance due to proximity of the haptenic determin-
ant to the site by which the antigen molecule is bound to the T-cell receptor. 
Thus, rather than to postulate several carrier regions servicing several nearby 
haptenic regions, it seems more reasonable to suggest that the same groups 
serve both functions for natural globular proteins.

(b) Alternatively, at the cellular level, to explain H-2 linked Ir-gene control in 
selection of B cells of certain specificities and not others, one would have to 
postulate some H-2-linked gene product which was clonally distributed among B 
cells along with the immunoglobulin variable regions, so that selection of these 
B cells on an H-2-linked basis within a single animal could occur. No H-2 gene 
product has yet been found to be clonally distributed within a population of B 
cells of a homozygous inbred strain. However, such is not impossible since the 
expression of Ia antigens may be affected differently during differentiation of 
different subpopulations of lymphocytes (e.g., I-J region Ia antigens on a 
subpopulation of T cells [11]). This H-2 gene product would not necessarily have 
be able to recognize antigen, i.e. be a second antigen-specific receptor on B 
cells, as long as it was clonally distributed with antigen receptors. It is conceiv-
able then that this product would be concerned with cooperative interaction 
between B cells and helper (or suppressor) T cells, but be distributed on B and T 
cells such that only certain B and T cells could interact with one another in a 
single animal. This restriction is distinct from requirements for cooperative 
interaction between T and B cells of different strains (12).

Bluestein et al. (13) observed a similar phenomenon in studying antibodies to 
poly-α-[L-glutamic acid (60%), L-alanine (30%), L-tyrosine (10%)] ("GAT") in 
guinea pigs. Both strain 2 and strain 13 guinea pigs responded to GAT, but 
strain 2 responded to GA (poly[L-Glu, L-Ala]), and not to GT (poly[L-Glu, L-
Tyr]), whereas strain 13 responded to GT and not GA. When antibodies to GAT 
were studied for binding to GA and GT, strain 2 but not strain 13 anti-GAT 
reacted with GA, consistent with the phenomenon described for nuclease here. 
However, both strain 2 and 13 anti-GAT reacted well with GT, so the conclusion 
regarding the GA result was hard to interpret. Moreover, since strain 2 and 13 
guinea pigs are not congenic, it was not possible to attribute the differences with 
certainty to major histocompatibility complex genes.

Similarly, Mozes et al. (14) showed that SJL and DBA/1 strains of mice 
responded to different determinants on poly(Phe,Glu)-poly-Pro--poly-Lys. How-
ever, these two strains of mice are not congenic, and the control of the response 
to the poly-Pro--poly-Lys determinant has been found to be non-H-2-linked (the 
so-called Ir-3 gene) (14).

The use of congenic strains in the current study reduces the possibility that 
the differences observed might not be linked to the major histocompatibility
complex. A potential question regarding the genetics might be the possibility of genetic drift in non-H-2 (e.g., immunoglobulin structural) genes between the B10.A and its ancestor C57BL/10. Genetic experiments are currently in progress to test this possibility.

The larger question, of whether this H-2-linked Ir-gene control of selection of specific B cells is exerted at the antigen level or the cellular level as discussed, awaits new experimental approaches.

Summary

The relative proportions of antibodies of different specificities within antisera raised to native staphylococcal nuclease have been studied in several strains of mice in which the antibody response has been shown to be under H-2-linked Ir-gene control. A method was developed in which binding to different radiolabeled fragments of nuclease was titrated against increasing fragment concentration until the binding capacity of the antiserum for that fragment was saturated. In comparing the low responder (H-2b) strain C57BL/10 with its congenic high responder counterpart B10.A (H-2a), it was found that the two strains made markedly and reproducibly different proportions of antibodies to different determinants on native nuclease. Since these two strains differ only at H-2, and therefore have identical immunoglobulin structural gene repertoires, we conclude that H-2-linked Ir genes can control the response to different determinants on the same antigen molecule independently of one another. This result suggests a possible role of H-2-linked genes in the selection of specific B cells.

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