

GENETIC CONTROL OF THE ANTIBODY RESPONSE TO POLY-L(TYR, GLU)-POLY-D,L-ALA--POLY-L-LYS IN C3H ↔ CWB TETRAPARENTAL MICE*

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In the past few years, a number of genes controlling specific immune responses have been discovered using (a) synthetic polypeptide antigens, (b) alloantigens and isoantigens, and (c) low doses of strongly immunogenic foreign proteins. Most, but not all of these genes have been found to be linked to the major histocompatibility complex of the species (1). This communication deals with one such gene, the immune response-1A (*Ir-1A*)¹ gene, which controls the ability of mice to produce a high titered antibody response to the synthetic, branched polypeptide poly-L(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] (2, 3). The *Ir-1A* gene has been found to map between *H-2K* and *Ss-Slp*, within the major murine histocompatibility complex, *H-2* (4, 5). Mice which are homozygous *H-2^{k/h}* produce low titers of specific anti-(T,G)-A--L antibody after immunization and are thus low responders to (T,G)-A--L. Mice which are *H-2^{b/b}* or *H-2^{k/b}* produce sera with up to 50-fold higher antigen-binding capacities than those produced by the *H-2^{k/h}* mice. *H-2^b* mice are thus high responders to (T,G)-A--L, and high response is dominant.

High responsiveness to (T,G)-A--L can be transferred from a high responder mouse to a lethally irradiated low responder mouse by transfer of spleen cells, fetal liver cells (4, 6, 7), or partially purified peripheral blood lymphocytes.² That is, the ability to produce a high titered anti-(T,G)-A--L response can be transferred by immunocompetent cells or precursors of immunocompetent cells. These results imply that the *Ir-1A* gene is expressed in cells of the immune system and is an intrinsic part of the immune response mechanism.

The *Ir-1A* gene(s) is specific for the antigenic determinant. Some inbred strains (e.g.,

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¹ *Abbreviations used in this paper:* ABC, antigen-binding capacity; C3H ↔ CWB, a tetraparental mouse between strain C3H/DiSn and strain CWB/13; GVH, graft-vs.-host; H, histocompatibility; (H,G)-A--L, poly-L(His, Gly)-poly-D,L-Ala--poly-L-Lys; Ig, immunoglobulin heavy-chain gene complex; *Ir-1A*, immune response-1A gene; PBS, phosphate-buffered saline; PLL, poly-L-Lysine; r, correlation coefficient; Sy.x, standard error of y around a point on the regression line; T cell, thymus-influenced lymphocyte; (T,G)-A--L, poly-L(Tyr, Glu)-poly-D,L-Ala--L-Lys.

² Tyan, M. L., and H. O. McDevitt. Unpublished data.

H-2^b mice) which are high responders to (T,G)-A--L are low responders to a related antigen, poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys [(H,G)-A--L]. In addition, the inbred strains carrying *H-2^k* are low responders to (T,G)-A--L, but high responders to (H,G)-A--L.

At least two antigen-specific cell types are required for production of a high titered antibody response. These are thymus-influenced lymphocytes (T cells) and bone marrow-derived lymphocytes (B cells) (8). In order to determine the cellular mechanism of action of the *Ir-1A* gene, it must be determined which cell type(s) of the immune response system expresses the genetically controlled difference in ability to respond.

Some evidence suggests that histocompatibility linked immune response genes, such as *Ir-1A*, are expressed in T cells. The most direct evidence comes from the work of Benacerraf and his colleagues on the response to poly-L-Lysine (PLL) in guinea pigs. PLL responder guinea pigs develop delayed hypersensitivity, a T-cell function (9), to the PLL antigen. PLL nonresponders do not develop delayed hypersensitivity to PLL (10). When responder and nonresponder guinea pigs are immunized with DNP-PLL, only responder animals produce detectable anti-DNP antibody. However, when responder and nonresponder guinea pigs are immunized with DNP-PLL which is electrostatically complexed to an immunologically recognizable foreign albumin, such as acetylated bovine serum albumin, then both responders and nonresponders produce high titers of anti-DNP antibody (11). Thus, it appears that PLL is not recognized as foreign in nonresponder guinea pigs and cannot act as carrier for a hapten (DNP) in these animals. These results strongly suggest that responder guinea pigs have T cells capable of recognizing and responding to PLL, while nonresponder guinea pigs lack such T cells.

It is known that T cells perform a helper function in production of humoral immunity (12). A genetic difference in T-cell function can, therefore, produce an apparent difference in B-cell function. Additional evidence suggests that the *Ir-1A* gene is selectively expressed in T cells and is not expressed in B cells. First, when high and low responder mice are immunized with (T,G)-A--L electrostatically complexed to methylated bovine serum albumin, both high and low responder strains of mice produce high titers of specific anti-(T,G)-A--L antibody (13). Thus, (T,G)-A--L appears to behave as a hapten in low responder mice. Second, when high and low responder mice are immunized with (T,G)-A--L in phosphate-buffered saline (PBS), both high and low responders first produce an equal, low titered IgM antibody response. After secondary challenge, however, the high responders shift to production of a high titered IgG response, while the low responders continue to produce a low titered IgM response and do not produce a second peak of response (14). The response status of the low responders can be altered by non-(T,G)-A--L-specific stimulation by T cells due to a graft-vs.-host (GVH) reaction. A GVH in the low responder causes both an increase in the (T,G)-A--L binding capacity produced and a shift to production of IgG antibody (15). These results suggest that high and low responder B cells may be functionally equivalent, but are normally under the influence of different sets of T cells. These results are consistent with expression of the *Ir-1A* gene in T cells and not in B cells.

In contrast, the limiting dilution studies of Mozes and Shearer suggest that in some combinations of mice and antigens, the *Ir-1A* gene is expressed in B cells, and not in T cells (16). In other strain and antigen combinations, however, the *Ir-1A* gene appears to be expressed in both T and B cells (17, 18). The reason for these differences is unknown.

In order to determine the cellular expression of the *Ir-1A* gene, we have performed an inductive cell interaction experiment using tetraparental (allophenic) mice. In these mice, T and B cells of high responder genotype and T and B cells of low

responder genotype are brought together in an operationally histocompatible milieu and allowed to interact. This cell interaction experiment is based on the hypothesis that if the *Ir-1A* gene is expressed in T cells but not in B cells, then both high and low responder B cells should be able to produce high titers of anti-(T,G)-A--L antibody under the influence of high responder cells. Thus, if the input mouse strains are chosen so that the antibody molecules produced by high and low responder B cells differ in heavy-chain allotype, then the high titered anti-(T,G)-A--L response produced by the chimeric mice should include antibodies of both allotypes. If, on the other hand, the *Ir-1A* gene is expressed in both T and B cells, then only high responder B cells should be able to produce high titers of anti-(T,G)-A--L antibody. In this case, only the allotype of the high responder input strain should be found in the high titered responses to (T,G)-A--L produced by the chimeric mice. These hypotheses are diagrammed for a simplistic T- and B-cell interaction model in Figs. 1 *a* and 1 *b*.

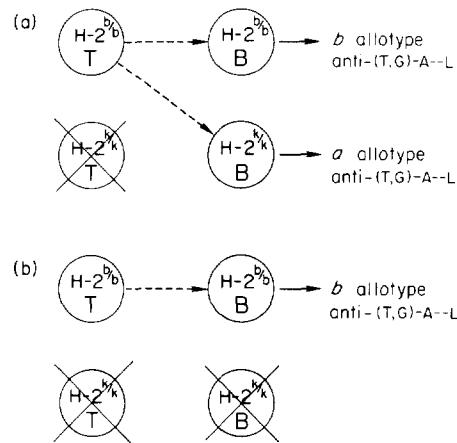


FIG. 1. Alternate models of *Ir-gene* action. Hypotheses for *Ir-1A* gene expression in terms of a simplistic T- and B-cell interaction model, (a) for *Ir-1A* expression in T cells only, and (b) for *Ir-1A* expression in both T and B cells. (T,G)-A--L-specific cells covered by an X are presumed to be missing or nonfunctional.

Tetraparental mice were made from congenic strains (C3H and CWB) which differ at the *H-2* complex, including *Ir-1A*, and at the *Ig* (Ig heavy-chain gene complex) locus. Several of the tetraparental mice possessed large numbers of cells of both input genotypes in their immune systems and were high responders to (T,G)-A--L. These tetraparentals produced high titers of (T,G)-A--L-specific low responder allotype antibody. It would thus appear that in tetraparental mice effective collaboration can occur across a histocompatibility disparity to permit B-cell stimulation and that both high and low responder B cells can respond equally.

Materials and Methods

Mice. Animals were obtained from the following inbred strains maintained at Stanford University: C3H/DiSn (*H-2^{k/k}*, *Ir-1A^{low/low}*, and *Ig^{a/a}*) and CWB/13 Hz (*H-2^{b/b}*, *Ir-1A^{high/high}*, and *Ig^{b/b}*) (19) (Table I). The method of production of tetraparental mice has been described in detail

TABLE I
Genetic Composition of C3H \leftrightarrow CWB Tetraparental Mice

| Genetic locus | Input strains | | Tetraparental mice C3H \leftrightarrow CWB |
|-----------------------------|---------------|-----------|---|
| | C3H | CWB | |
| <i>H-2</i> | k/k | b/b | k/k + b/b |
| <i>Ir-1A</i> for (T,G)-A--L | low/low | high/high | low/low + high/high |
| <i>Ig</i> | a/a | b/b | a/a + b/b |

elsewhere (20, 21). Briefly, each tetraparental mouse was constructed by aggregation of two 8-16 cell embryos, one of high responder genotype (CWB) and one of low responder genotype (C3H) to form a chimeric blastocyst. Several chimeric embryos were then transplanted into the uterus of a pseudopregnant recipient to complete development. The tetraparental mice (C3H \leftrightarrow CWB) thus produced were mosaic for cells of the two input strain genotypes (Fig. 2).

Antigens, Immunization Procedures, and Antibody Determinations. The synthetic, branched polypeptide antigen (T,G)-A--L lot no. 509, 220,000 mol wt, was kindly provided by Dr. Michael Sela, Department of Chemical Immunology, Weizmann Institute for Science, Rehovot, Israel. Mice were immunized at 2-3 mo of age with 10 μ g of (T,G)-A--L 509 in complete Freund's adjuvant (2 mg *Mycobacterium tuberculosis/ml*), boosted 3 wk later with 10 μ g of (T,G)-A--L 509 in PBS and bled from the tail 10 days after the boost.

Antigen-binding activity was determined using rabbit antimouse γ -globulin sera and radioiodinated (T,G)-A--L 509 in a modified Farr assay (4). All dilutions were made in 1% bovine serum albumin in PBS. In a standard assay, 2.5 ng of 125 I-labeled (T,G)-A--L in 50 μ l was mixed with 25 μ l of a dilution of the experimental serum and incubated at 37°C for 1 hr. Then 50 μ l of a dilution of rabbit antimouse γ -globulin which gave maximum precipitation of the mouse γ -globulin was added and incubation at 37°C was continued an additional 2 hr. The titration tubes were spun for 15 min at 10,000 *g* and 50- μ l aliquots of the supernates were sampled and counted on a Nuclear Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Antibody titers are expressed as the percent of labeled antigen bound by a particular serum dilution.

Quantitation of Total Serum a and b allotype IgG₁ and IgG_{2a}. The milligrams per milliliter of a and b allotype IgG₁ and IgG_{2a} were determined for the total serum of each unimmunized tetraparental mouse using the inhibition of precipitation method of Herzenberg and Herzenberg (22). Briefly, a dilution of the test serum was added to a known concentration of 125 I-labeled myeloma protein. Then a known dilution of anti-allotype serum, which had been made class specific by absorption, was added with rapid mixing. The reaction was incubated 2-3 h at 37°C, then overnight at 4°C. The next morning the tubes were spun, sampled, and counted as described above. The inhibition of precipitation of the radioiodinated myeloma protein was converted to milligrams per

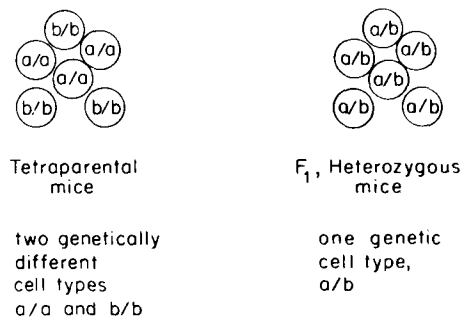


FIG. 2. Genetic composition of tetraparental mice compared to genetic composition of F₁ mice. Tetraparental mice are a mosaic of cells of the two input strain genotypes.

milliliter by interpolation on a standard curve produced by adding known concentrations of unlabeled myeloma protein to the assay system.

Allotype composition is expressed as the mean of percent *a* allotype in the two subclasses IgG₁ and IgG_{2a}:

$$\text{mean percent } a = \frac{1}{2} \left(\frac{\text{mg/ml } a \text{ IgG}_1}{\text{mg/ml } (a + b) \text{ IgG}_1} + \frac{\text{mg/ml } a \text{ IgG}_{2a}}{\text{mg/ml } (a + b) \text{ IgG}_{2a}} \right)$$

Quantitation of a and b Allotypes in the Specific Anti-(T,G)-A--L Response. The immune sera were separated into *a* and *b* allotype fractions by passage through an anti-*a* allotype affinity chromatography column prepared by binding polyvalent anti-*a* allotype serum to cyanogen bromide-activated Sepharose-4B. The *b* allotype antibodies passed through the column as it was washed with the buffer (pH 7.6) used for binding; the *a* allotype molecules were then released by washing the column with 0.1 M acetic acid buffer (pH 3.1). The *a* and *b* allotype fractions were then assayed for anti-(T,G)-A--L activity in a modified Farr assay similar to that described above. The (T,G)-A--L binding capacity of the eluted fractions was compared to a standard curve and an undiluted serum equivalent was thus obtained for both the *a* and *b* allotype fractions. These serum equivalents were expressed as a volume percent of the total serum recovered from the column. Recoveries of anti-(T,G)-A--L activity were usually quantitative. Under the conditions used for these columns C3H/DiSn nonresponder mice did not have a detectable titer. This method, including standardization of the columns with known serum mixes, is described in detail in a separate communication.³

Results

Total Serum Allotype Mix. The distribution of input cell types into embryonic and extraembryonic tissues in the tetraparental blastocyst appears to occur randomly so that the population of cells in a tetraparental mouse can range from 100% high responder parental type, through intermediate percents of high and low responders, to 100% low responder parental type. In addition, the mix of cells may vary from one tissue to another within an animal (23). In order to estimate the contribution of cells of the two input strains to the total B-cell population of each C3H ↔ CWB tetraparental mouse, the milligrams per milliliter of *a* and *b* allotype IgG₁ (*Ig-4*) and IgG_{2a} (*Ig-1*) were determined for the total serum of each unimmunized mouse, and the relative amounts of *a* and *b* allotypes were expressed as the mean of the allotype percentages in the two subclasses. (See Materials and Methods and Fig. 3). If the total nonimmune serum of a tetraparental mouse contains only *a* allotype IgG₁ and IgG_{2a}, then the B cells are presumed to be all of C3H (low responder) genotype. If the total serum contains only *b* allotype, then the B cells are presumed to be all of the CWB (high responder) genotype. When the total serum contains both *a* and *b* allotypes, then the immune system contains both C3H and CWB genotype B cells, and the relative amounts of *a* and *b* allotypes presumably approximate the relative numbers of C3H and CWB B cells.

39 C3H ↔ CWB mice have been analyzed. Of these, 23 are apparently nonchimeric in their immune systems. That is, 15 of the tetraparentals do not have significant amounts of *a* allotype IgG₁ or IgG_{2a}. An additional eight of the tetraparentals do not have significant amounts of *b* allotype IgG₁ or IgG_{2a}.

³Freed, J. H., and L. A. Herzenberg. 1974. Separation of murine allotype mixtures by affinity chromatography on sepharose. Manuscript submitted for publication.

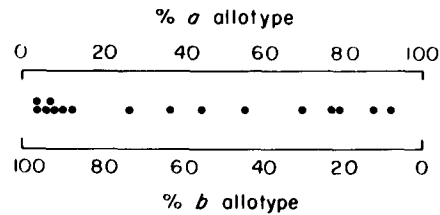


FIG. 3. Total serum allotype distribution of the 16 known chimeric C3H \leftrightarrow CWB tetraparental mice. Mean percent Ig^a and Ig^b in the total serum of the unimmunized tetraparental mice was calculated from the quantities of *a* and *b* allotype IgG_{2a} and IgG₁, as described in the Materials and Methods.

However, it should be pointed out that the 23 apparent nonchimeric mice may have a small undetectable degree of chimerism for IgG₁ and IgG_{2a} and/or may be chimeric for other classes of Ig. The remaining 16 mice have detectable amounts of both *a* and *b* allotype Ig in at least one of the two subclasses, IgG₁ and IgG_{2a}, and are thus chimeras. The range of chimerism present in this population is shown in Fig. 3. The tetraparental mice cover the entire range of possible allotype mixes, and therefore presumably cover the entire range of B-cell mixes (See below). Gornish et al. (24) have shown, using a translocation chromosome marker, that in individual tetraparental mice the proportions of cells from the two input strains in the thymus, bone marrow, and spleen correlate well with each other. Thus, the above estimates of B-cell mixtures are probably also good estimates of the T-cell mixtures in the tetraparental mice.

Total Response to (T,G)-A--L. The 39 C3H \leftrightarrow CWB tetraparental mice and parental strain mice were immunized with 10 μ g (T,G)-A--L 509 in complete Freund's adjuvant, given a second injection of 10 μ g of antigen in PBS, and bled 10 days after the boost. Serial dilution antigen-binding curves for the secondary immune sera from the C3H and CWB control animals are shown in Fig. 4. The CWB, high responder, titers range from 74 to 86% antigen bound at a 1/500 serum dilution. The C3H, low responder, titers range from 5 to 26% antigen bound at 1/500 dilution, and there is a greater than 50-fold difference between C3H and CWB in the serum dilution required to produce 50% antigen binding. The CWB strain is among the highest responding of *H-2^b* high responder strains.

The serum dilution curves for the 39 C3H \leftrightarrow CWB tetraparental mice are shown in Fig. 5. With one exception, these mice fall into distinct high and low responder groups. The low responder group ranges from 1 to 31% antigen bound at a 1/500 serum dilution. The high responder group ranges from 67 to 86% antigen bound at a 1/500 dilution. The one intermediate animal produces 51% antigen binding.

For comparison in a single figure, the percentages of antigen bound by 1/500 serum dilutions of C3H, CWB, and (C3H \times CWB)_{F₁} control animals and C3H \leftrightarrow CWB tetraparental mice are replotted in Fig. 6. The high responder group of tetraparental mice correlates well with the CWB and (C3H \times CWB)_{F₁}, high responder, animals. The low responder group of tetraparental mice correlates well with C3H, low responder, animals. Thus, the level of humoral response to (T,G)-A--L produced by the tetraparental mice is similar to the level of response produced by the normal control mice.

ANTIBODY RESPONSE IN TETRAPARENTAL MICE

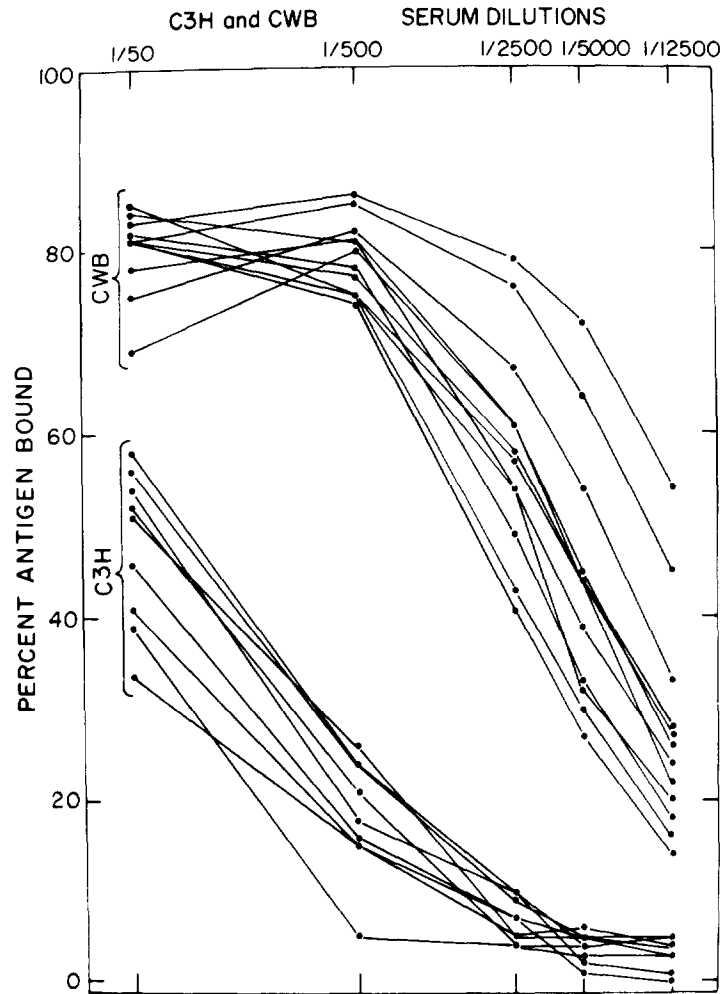


FIG. 4. Anti-(T,G)-A--L antibody responses of C3H (low responder) and CWB (high responder) mice. C3H and CWB mice were immunized and boosted with $10 \mu\text{g}$ (T,G)-A--L 509 as described in the Materials and Methods. "Percent antigen bound" is the percent of 2.5 ng of ^{125}I -labeled (T,G)-A--L 509 precipitated by $25 \mu\text{l}$ of the experimental serum dilution in the standard antigen-binding assay (4).

Anti-(T,G)-A--L Response Related to the Total Serum Allotype Mixture. Fig. 7 shows the correlation between the level of anti-(T,G)-A--L response produced by each animal and the genetic composition of the animal's immune system. Among the 16 chimeric C3H \leftrightarrow CWB mice, animals which tend toward more *b* allotype in their total serum produce high titered, responder level, anti-(T,G)-A--L responses. Animals with very little *b* allotype in their total serum produce only low titered anti-(T,G)-A--L responses. Among the 23 apparently nonchimeric tetraparentals, the 15 with only *b* (CWB, high responder) allotype detectable are all high responders. The eight animals with only *a* (C3H, low responder) allotype detectable are low responders. Thus, there is a striking correlation between the total serum allotype mixture (reflecting the

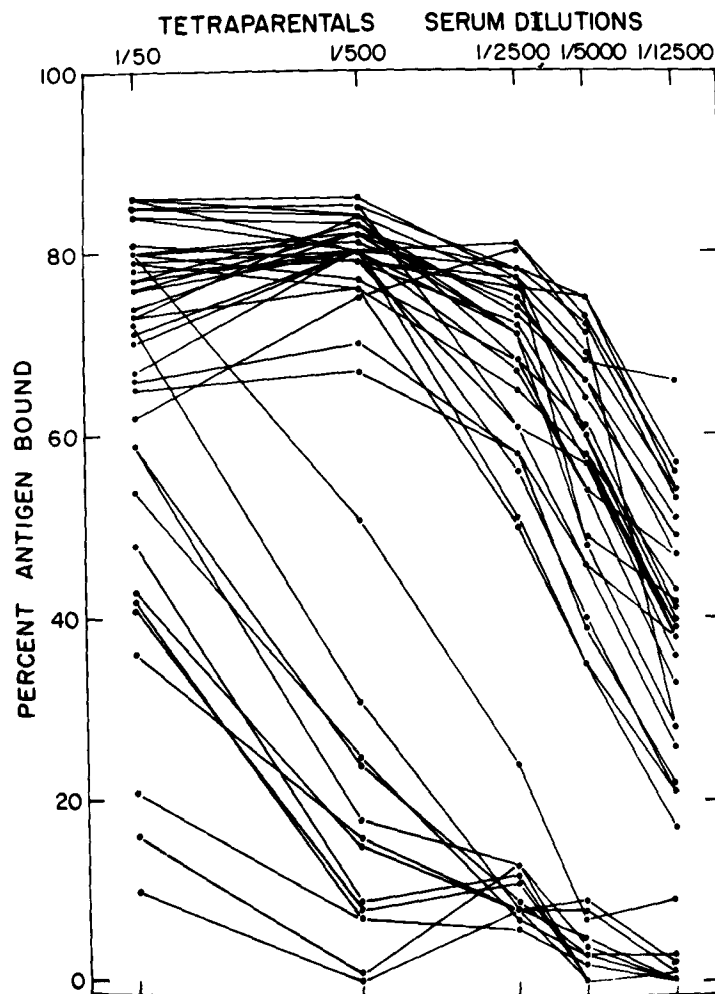


FIG. 5. Anti-(T,G)-A--L antibody responses of C3H ↔ CWB tetraparental mice. The 39 C3H ↔ CWB tetraparental mice were immunized and boosted with 10 μ g (T,G)-A--L 509 as described in the Materials and Methods. Percent antigen bound is the percent of 2.5 ng of 125 I-labeled (T,G)-A--L 509 precipitated by 25 μ l of the experimental serum dilution in the standard antigen-binding assay (4).

genetic composition of the T- and B-cell populations) and the high or low responder status of the anti-(T,G)-A--L responses of the tetraparental mice.

Allotype Composition of the Specific Anti-(T,G)-A--L Response in Tetraparental Mice. There are several responder tetraparental mice with significant amounts of low responder (*a*) allotype Ig in their total serum (Fig. 7). These animals presumably have a large number of low responder genotype T and B cells in their immune system and thus are the best candidates for determination of the allotype composition of a specific, high titered, anti-(T,G)-A--L response.

According to the rationale for the tetraparental mouse experiment, if the *Ir-1A* gene is expressed in T cells, but not in B cells, then the responder tetraparental mice which are mosaic in their immune system, i.e., which have both *a* and *b*

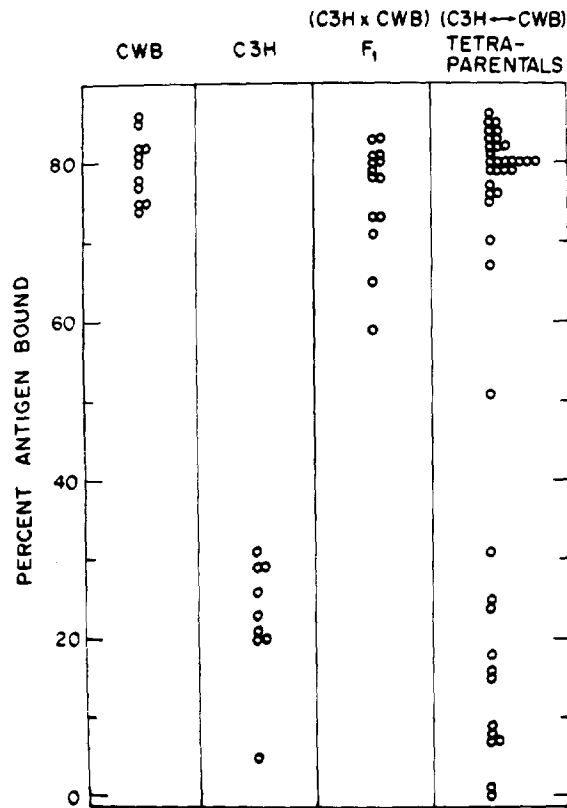


FIG. 6. Comparison of Anti-(T,G)-A--L antibody responses of C3H, CWB, (C3H × CWB)F₁, and C3H ↔ CWB tetraparental mice. Percent antigen bound is the percent of 2.5 ng of ¹²⁵I-labeled (T,G)-A--L 509 precipitated in the standard antigen-binding assay (4) by 25 μl of a 1/500 dilution of the experimental serum (see Figs. 4 and 5).

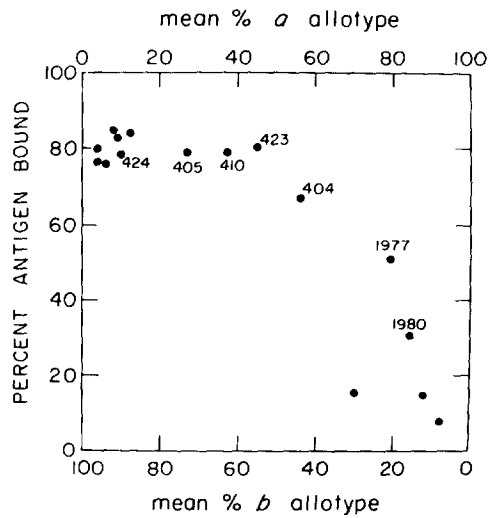


FIG. 7. Anti-(T,G)-A--L antibody response versus total serum allotype mixture for C3H ↔ CWB tetraparental mice. Each point represents 1 of the 16 proven chimeric C3H ↔ CWB tetraparental mice. See Figs. 3 and 6 for explanation of abscissa and ordinate, respectively.

allotypes in their total serum, should produce large amounts of anti-(T,G)-A--L antibodies of both *a* and *b* allotypes (Fig. 1 *a*). If, on the other hand, the *Ir-1A* gene is expressed in both T and B cells, then the B cells of the nonresponder would not recognize (T,G)-A--L and would not be able to produce a high titered anti-(T,G)-A--L response even in the presence of responder type cells. Thus, the high titered anti-(T,G)-A--L responses of tetraparental mice would contain only responder (*b*) allotype antibody (Fig. 1 *b*).

The allotype mixture of the specific anti-(T,G)-A--L response of several of the tetraparental mice was determined using an anti-*a* allotype affinity chromatography column. The method and standardization of the column have been presented in a separate communication.³ 11 sera from selected C3H ↔ CWB tetraparental mice, both high and low responders, were fractionated on the anti-*a* allotype column. The distribution of the anti-(T,G)-A--L response between *a* and *b* allotype fractions is shown in Table II. One of the animals studied, no. 1980, produced the highest titered specific antiserum of the low responder group of tetraparental mice. Only very low levels of antigen-binding capacity were detectable in the unbound (*b* allotype) fraction after this antiserum was fractionated on the anti-*a* allotype affinity chromatography column. The detectable titer appeared to be mainly in the *a* (low responder) allotype fraction. A second animal, no. 1977, produced an intermediate response to (T,G)-A--L. The total response was significantly higher than that of low responder strain

TABLE II
*Allotype Distribution and ABC of Specific Anti-(T,G)-A--L Antibodies from C3H ↔ CWB Tetraparental Mice**

| Tetra- parental mouse | Anti-(T,G)-A--L Activity | | | Ratio of <i>a</i> allotype ABC to intact C3H ABC (all Ig ^a) | (T,G)-A--L Bound at /‰ |
|-----------------------------|--------------------------|---------------------|------------------------|---|------------------------------|
| | % <i>a</i> allotype‡ | % <i>b</i> allotype | Average % recovery§ | | |
| | | | | | % |
| 410 | 11, 13 | 98, 87 | 88 | — | 79 |
| 407 | 14, 15 | 86, 85 | 88 | — | 80 |
| 402 | 15, 10 | 85, 90 | 99 | — | 70 |
| 414 | 17, 12 | 83, 88 | 107 | — | 77 |
| 424 | 23, 31 | 77, 69 | 100 | 82 | 79 |
| 405 | 30, 35 | 70, 65 | 96 | 75 | 79 |
| 423 | 27, 34, 47 | 73, 66, 53 | 96 | 31 | 80 |
| 404 | 68, 60 | 32, 40 | 105 | 23 | 67 |
| 1977 | 100 | 0 | 76 | 6.5 | 51 |
| 1980 | 90 | 10 | 80 | 2.3 | 31 |
| 421 | ≈100 | ≈0 | ≈67 | ≈0.6 | 25 |

* Determined by fractionation of the immune whole serum on an anti-Ig^a column followed by titration of Ig^a and Ig^b fractions with (T,G)-A--L.

‡ % *a* allotype, percent of the total anti-(T,G)-A--L ABC which is found in the *a* allotype fraction, each value representing a separate determination.

§ Average % recovery, percent of original ABC detected after separation on the anti-allotype column.

animals, and the response appeared to be entirely of the *a* (low responder) allotype. Eight of the animals listed in Table II were members of the high responder group of tetraparental mice. Four of these mice (nos. 424, 405, 523, and 404) produced significant levels of *a* (low responder) allotype anti-(T,G)-A--L. (Values less than 20% *a* allotype were not accepted as significant because the affinity chromatography column did not give completely quantitative separation below this point.³ One of the mice, no. 404, showed greater antigen-binding activity (antigen-binding capacity, ABC) in the *a* (low responder) allotype fraction than the *b* (high responder) allotype fraction.

The activity of the *a* allotype anti-(T,G)-A--L antibodies in 8 of the 11 C3H ↔ CWB tetraparental sera was compared to an average value determined from the three highest responding of the C3H (low responder) control mice (Fig. 4). As shown in Table II, the low responder tetraparental mice produced approximately the same activity level of *a* allotype anti-(T,G)-A--L as did the C3H low responders. The high responder tetraparental mice, on the other hand, showed considerably higher *a* allotype anti-(T,G)-A--L activity than did the normal C3H low responders. There was a 23- to 82-fold difference between the calculated dilution of *a* allotype anti-(T,G)-A--L tetraparental serum required to produce 50% antigen binding and the C3H, low responder, serum dilution required to produce 50% antigen binding.⁴ Thus, it appears that B cells of both high and low responder genotype can produce large amounts of anti-(T,G)-A--L antibody, under the conditions present in high responder ↔ low responder tetraparental mice.

Allotype Mix in the Specific Response Related to Total Serum Allotype Mix. The correlation between the percent of *a* allotype in the total serum and the percent of *a* allotype in the specific anti-(T,G)-A--L response, is shown in Fig. 8 for the 11 C3H ↔ CWB tetraparental mice listed in Table II. Using all of the data points except animals no. 402 and no. 421, which are nonchimeric, a line was fitted by least squares regression analysis. This line, denoted in Fig. 8 by short dashes, has a slope of 0.98 and a y intercept of 4.4%. The standard error of y around a point on the regression line ($S_{y,x}$) is 14% and the correlation coefficient (*r*) is 0.90.

Similar results are obtained when the data points from only the chimeric, high responder tetraparentals are used. The best-fit-line, $y = 0.62x + 12\%$, is designated in Fig. 8 by a solid line. This line is truncated at the point where extrapolation begins because mice which have higher percentages of *a* allotype in their total serum are low responders to (T,G)-A--L, and thus different (i.e., low responder) conditions pertain. The standard error, $S_{y,x}$, for the data from high responder tetraparentals was 12% and the correlation coefficient, *r*, is 0.71. Thus, there is a high degree of correlation between the allotype mixture found in the total nonimmune serum of a C3H ↔ CWB tetraparental mouse and the allotype mixture in the specific anti-(T,G)-A--L response subsequently produced by that mouse.

⁴For mice nos. 410, 407, 402, and 414 no value could be calculated since it was impossible to accurately determine the level of an allotype anti-(T,C)-A--L antibodies in these mice.

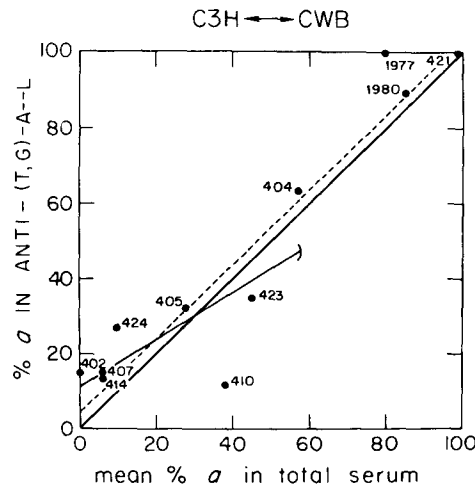


FIG. 8. Correlation of percent *a* allotype in specific antibody with percent *a* allotype in total serum. [—], line predicted if there is perfect correlation. [---], line fit by least squares analysis using all data points except 402 and 421. $y = .98x + 4.4\%$, $Sy.x = 14$, and $r = .90$. [—], line fit by least squares analysis using only points from chimeric, high responder tetraparental mice. Line is truncated at the point where extrapolation begins. $y = .62x + 12\%$, $Sy.x = 12\%$, $r = .71$.

Discussion

This paper gives results from an inductive cell interaction experiment in C3H ↔ CWB tetraparental mice in which the cellular expression of the *Ir-1A* gene was examined. The C3H and CWB strains used to construct the tetraparental mice were congenic on the C3H/DiSn genetic background, and differed genetically for *I_g* allotype and for the *H-2* complex, including the *Ir-1A* gene. The composition of the lymphoid system of each tetraparental mouse was estimated by quantitatively determining the percentage of *a* and *b* allotypes of *IgG₁* and *IgG_{2a}* in the total serum of immunologically virgin animals. The allotype composition of this serum presumably represents a random sampling of the total B-cell population. The percentages of T cells of the two input strains were not measured directly. However, the ratio of T cells is probably similar to that estimated for B cells. This conclusion rests on the experiments of Gornish et al. (24), who showed that the percentage of cells derived from an input embryo carrying a homozygous T6/T6 translocation marker was nearly constant among the various lymphoid compartments in a tetraparental mouse.

The studies presented here demonstrate that the total serum allotype mixture in tetraparental mice accurately reflects the composition of the cell population(s) which controls the humoral immune response to (T,G)-A--L. Those animals which had high percentages of *b* (high responder) *Ig* allotype in their total nonimmune serum subsequently produce high titers of anti-(T,G)-A--L antibody. Those mice with low percentages of *b* *Ig* allotype produce low titered responses. This confirms a previous finding that *Ir-1A* control is a property of

cells of the immune system (4, 6, 7). It does not, however, distinguish between T and B cells as the site of *Ir-1A* expression.

11 tetraparental mice were tested for the allotype composition of their anti-(T,G)-A--L responses. In the serum of those mice which were high responders to (T,G)-A--L, large proportions of both *b* (high responder strain) and *a* (low responder strain) specific antibodies were found. These percentages showed a high degree of correlation (r , 0.71) with the percentages of *b* and *a* allotypes in the total sera of the nonimmunized tetraparental mice. If the stimulation of high responder B cells had been more efficient than the stimulation of low responder B cells in the tetraparental mouse, the proportion of *b* allotype in the specific response should have been on the average greater than the proportion of *b* allotype in the total unimmune serum. Conversely, if the stimulation mode for low responder B cells had been more efficient, a larger amount of *a* allotype should have been found in the specific response compared to the total serum. Thus, it would appear that in tetraparental mice, the high and low responder B cells are equivalent and are stimulated by similar, if not identical, pathways to produce high-titered anti-(T,G)-A--L responses.

It is unlikely that high or low responder T or B cells are phenotypically converted to the opposite response type by maturation in the presence of cells of the opposite *H-2 (Ir)* type, since radiation chimeras, produced by transfer of bone marrow or fetal liver, respond as predicted by the genotype of the transferred lymphoid precursors (reference 6 and footnote 2).

The close correlation of total serum and specific antibody allotype mixtures suggests that cell fusion also is not the explanation for the *a* allotype responses. It is apparent from the wide range of total serum allotype mixtures found in the tetraparental population that the tetraparental immune systems do not consist totally of high responder-low responder fused cells. If the *a* allotype response was produced only by fused cells, whereas the *b* allotype response was produced by both fused and normal cells, then there should have been on the average, less *a* allotype in the specific response than in the total serum. This was not the case. Therefore, the *a* allotype response appears to be the normal product of low responder B cells.

It has been shown by Ordal and Grumet (15) that induction of a GVH reaction against host histocompatibility antigens in a normal low responder mouse, at the time of (T,G)-A--L immunization, can cause the low responder mouse to produce a low to moderate titer of anti-(T,G)-A--L antibody, i.e. an "allogeneic effect," (25) which can increase the antibody response of the low responder strain. Wegmann et al. (26) have found that immunocompetent cells from tetraparental mice can react in tissue culture against cells of the two input strains, but that they are blocked from responding by specific blocking factors present in the tetraparental serum. Meo et al. (27), in contrast, found tetraparental cells to be classically tolerant of parental *H-2* types. Preliminary results⁵ with tetraparental mice of another strain combination, in which there can be no allogeneic effect against the low responder B cells, show that these animals produce equally high titers of both high and low responder strain Ig allotype. In addition, tetraparental mice produced between two histoincompatible low responder strains are all low responders to

⁵ Bechtol et al. Manuscript in preparation.

(T,G)-A--L.⁵ If an allogeneic effect were the cause of low responder B-cell stimulation some of these mice should have produced high responses. Thus, the response of low responder B cells in tetraparental mice does not appear to be due to an allogeneic effect against these cells.

Experiments by Shevach et al. (28) and by Shevach and Rosenthal (29) have suggested that there is probably an association on the lymphocyte surface between major histocompatibility (H) antigens and the products of H-linked specific *Ir* genes. In F_1 animals, the *H* and *Ir* alleles which are *cis* in the genome appear to be contiguous on the cell surface. The reason for this purported association is not known. However, manifestations of this association are seen in the phenomena of anti-H serum blocking of an immune response (29) and in the apparent requirement for H compatibility in some cell interaction systems (29, 30). In contrast, this preferential *cis* association does not appear to play a critical role in other cell interaction systems, e.g. the present tetraparental mouse experiments, the *in vitro* experiments of Benacerraf et al. (31), and the limiting dilution studies of Shearer et al. (17, 18). This disparity may be due (a) to differences in antigens used, (b) to the degree and/or types of differences between the *H* and *Ia* types in the pairs studied, and/or (c) to specific aspects of the cell interaction model systems employed.

At this point, we cannot rule out the possibility that high responder B cells are responsible for stimulation of both high and low responder B cells. However, experiments to test this possibility are in progress.

Summary

In order to further delineate the mechanisms underlying genetic unresponsiveness, tetraparental mice were constructed from immune response-1A gene high responder and low responder parental genotypes, then were immunized with poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys ((T,G)-A--L). An analysis of the total serum allotype mixture and of the antigen-binding capacity of the separated allotypes demonstrated that in the milieu of a tetraparental mouse, both high and low responder B cells could be stimulated equally to produce identical high titered anti-(T,G)-A--L responses. Furthermore, these studies show that effective stimulation could occur across a histocompatibility disparity.

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