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Genetic Control of the Immune Response to (T,G)-A--L in C3H ↔ C57 Tetraparental Mice[1]

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When 15 C3H↔C57 tetraparental (allophenic) mice were analyzed for coat color, hemoglobin, and immunoglobulin allotype, all but two were shown to be chimeric. These 15 tetraparental mice were immunized with the synthetic polypeptide (T,G)-A--L, and the origin of the (T,G)-A--L-specific antibody produced was determined by using genetic markers (allotypes) on the immunoglobulin heavy chain constant region. Five tetraparental mice were high responders to (T,G)-A--L and had significant amounts of *a* (low responder) allotype antibody in their total serum. Three of these mice had significant amounts of anti-(T,G)-A--L antibody of the *a* (low responder) allotype. The antigen binding capacities of the *a* allotype fractions of these three were 4–5 times higher than the antigen binding capacities of immunized C3H (low responder) control mice. These results are compatible with the hypothesis that the inability of low-responder mice to produce significant amounts of anti-(T,G)-A--L antibody is a function of *Ir-1A* gene expression at the level of T cells.

INTRODUCTION

The ability to make a specific immune response to particular antigenic determinants is under dominant antigen-specific control (1). A large number of such genetic controls have been discovered, and many of them are linked to the species' major histocompatibility locus. One such gene is immune response-1A (*Ir-1A*), which controls the specific immune response of mice to the branched synthetic polypeptide poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] (2).

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Linkage studies have shown that the *Ir-1A* gene is linked to and maps near the center of the gene region which controls the major murine histocompatibility (H-2) antigens (3).

Previous analyses have demonstrated that *Ir-1A* is expressed in cells of the immune system. It has been shown that the ability to produce a high antibody response can be transferred from a mouse of a high-responder strain to a lethally irradiated mouse of a low-responder strain by transfer of immunocompetent cells or precursors of immunocompetent cells (4–6). At least two *antigen-specific* cell types are required for production of a high titered antibody response: (a) bone marrow-derived lymphocytes (B cells), which are the precursors of antibody producing cells, and (b) thymus-influenced lymphocytes (T cells) which perform a "carrier" or "helper" function via antigen presentation to B cells and enhancement of antibody production (7). Macrophages are required in *in vitro* immune responses, but at least in the mouse they do not appear to express any histocompatibility-linked functional differences (8). The histocompatibility-linked response genes, including *Ir-1A*, affect T cell functions such as delayed hypersensitivity and graft rejection (9). Thus, these genes certainly influence T cell expression. Some evidence suggests that *Ir-1A* is expressed solely in T cells, (i.e., it is not expressed in B cells). For example, if mice from high- and low-responder strains are immunized with (T,G)-A--L that is electrostatically complexed to methylated bovine serum albumin (MBSA), all of the mice produce a high titered antibody response to (T,G)-A--L (10). Thus, it appears that if a determinant which can be recognized by T cells is added to the (T,G)-A--L molecule then both high- and low-responder strains can respond to (T,G)-A--L with the production of high titers of specific antibody.

A similar situation has been described for the PLL gene in guinea pigs where complexing with acetylated serum albumin results in production of anti-DNP-PLL antibody. In addition, Schlossman and Williamson (11) have shown that the anti-DNP-PLL antibody produced by nonresponder guinea pigs has the same isoelectric focusing characteristics as the antibody produced by responder animals. This indicates that high and low responders have the same genetic information for antibody structure, and presumably B-cell receptors, but nevertheless differ in their ability to recognize antigens such as (T,G)-A--L and DNP-PLL (dinitrophenyl polylysine).

In the present report, we have used tetraparental (allophenic, chimeric) mice to test the ability of high- and low-responder B cells to respond to (T,G)-A--L under the influence of high-responder immunocompetent cells. For this purpose, tetraparental mice were constructed from C3H ($H\text{-}2^{k/k}$,$Ir\text{-}1A^{low/low}$, $Ig^{a/a}$) and C57 ($H\text{-}2^{b/b}$,$Ir\text{-}1A^{high/high}$,$Ig^{b/b}$) strains. In tetraparental mice, T and B cells of high-responder genotype and T and B cells of low-responder genotype can coexist in an operationally histocompatible environment. According to the hypothesis (Fig. 1) on which this experiment is based, if the *Ir-1A* gene is expressed in T cells only, then high- and low-responder B cells should be functionally equivalent in their ability to produce large amounts of anti-(T,G)-A--L antibody. Both high- and low-responder B cells should be able to produce large amounts of anti-(T,G)-A--L antibody under the influence of high-responder T cells. The high- and low-responder input strains for our tetraparental mice differ for antibody allotype (Ig^a vs Ig^b). Thus, if the *Ir-1A* gene is expressed in T cells only, the anti-(T,G)-A--L anti-

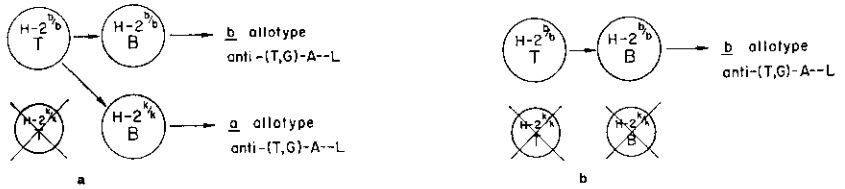

FIG. 1. Hypothesis for cell interaction and antibody production in high-responder ↔ low-responder tetraparental mice. (a) Expression of *Ir-1A* in T cells only; (b) expression of *Ir-1A* in both T and B cells. $H\text{-}2^{b/b}$ mice are *Ir-1A* high responders to (T,G)-A--L; the high-responder strain used produces *b* allotype immunoglobulin. $H\text{-}2^{k/k}$ mice are *Ir-1A* low responders to (T,G)-A--L, the low-responder strain used produces *a* allotype immunoglobulin. × indicates an absent or nonfunctional cell.

bodies produced by the tetraparental mice should include antibodies of both *a* and *b* allotypes. The proportions of the two allotypes in the specific response should, on the average, be approximately the same as the allotype mix in the total serum, i.e., the B cells responding to (T,G)-A--L should be a random sampling of the total B cell population.

In contrast, if the *Ir-1A* gene is expressed in both T and B cells, then the B cells of the low responder should not be able to recognize and respond to (T,G)-A--L under normal stimulation by responder-type T cells. In this case the high titered anti-(T,G)-A--L response of the tetraparental mice should be only from responder B cells and only of responder allotype.

The results of these studies suggest that B cells of low-responder origin can produce significant titers of anti-(T,G)-A--L antibody under the influence of immunocompetent cells of high-responder genotype. The amount of low-responder allotype anti-(T,G)-A--L produced in these tetraparental mice is higher than that found in intact low-responder animals, although it is not completely in the responder range. However, with the reservations presented in the discussion section, these results support the hypothesis that the *Ir-1A* gene is expressed solely in T cells.

MATERIALS AND METHODS

Tetraparental Mice

The tetraparental mice for these experiments were made from C3H/HeJ and C57BL/10Sn parental strains, which were originally obtained from the Jackson Laboratories, Bar Harbor, ME and are now maintained at the Biological Laboratories at Harvard University. These two strains were chosen because they differ

TABLE 1

GENETIC COMPOSITION OF C3H, C57, AND C3H ↔ C57 TETRAPARENTAL MICE

	C3H	C57	C3H ↔ C57
H-2	*k/k*	*b/b*	*k/k* + *b/b*
Ir-1A[1]	low/low	high/high	low/low + high/high
Ig	*a/a*	*b/b*	*a/a* + *b/b*

[1] For (T,G)-A--L.

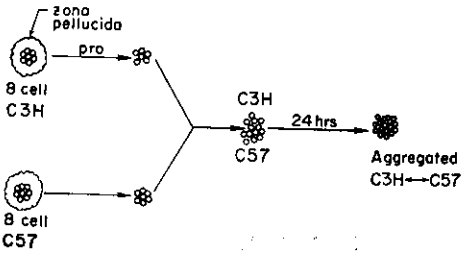

FIG. 2. Method of production of tetraparental mice.

at the major murine histocompatibility complex, *H-2*, *Ir-1A*, and immunoglobulin allotype, *Ig*, as shown in Table 1.

For the production of tetraparental mice (Fig. 2), 8- to 16-cell embryos of C3H and C57 strains were treated briefly (4–7 min) with an 0.5% pronase solution to remove their zonae pellucidae (12). The zona-free embryos were then paired, one C3H with one C57. The embryos of each pair were left touching each other, with each embryo pair in a separate microdroplet of Brinster's embryo culture medium under heavy paraffin oil. The embryo pairs were cultured at 37°C under a 5% CO_2-in-air atmosphere for approximately 24 hr. During this time they formed a single ball of cells and continued to develop to the late morula or early blastocyst stage. Several (1–8) aggregated embryos were transplanted to the uterus of a pseudopregnant parous Swiss female that had been mated to a vasectomized male. The young tetraparental mice were born 17 days later. Complete materials and methods for tetraparental mouse production are available from Dr. Wegmann on request.

Coat Color and Hemoglobin Determination

The proportions of C3H/HeJ and C57BL/10Sn hemoglobin in each tetraparental mouse were estimated by a gel electrophoresis method as previously described (13). The method for estimation of percentages of agouti (C3H/HeJ) and black (C57BL/10Sn) in the coats of the tetraparental mice has also been described elsewhere (13). Both hemoglobin and coat color mixes were determined when the tetraparental mice were approximately 1 mo old.

Antigen, Immunization, and Antigen Binding Activity

A schematic representation of the synthetic branched polypeptide poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] is shown in Fig. 3. (T,G)-A--L 509 (MW, 232,000) was kindly provided by Dr. M. Sela, Weizmann Institute of Science, Rehovot, Israel. The residue molar ratio is T:G:A:L= 2:4:19:1. At 2–6 mo of age, mice were injected in the hind foot pads with 10 μg of (T,G)-A--L 509 in complete Freund's adjuvant (containing 2 mg/ml Mycobacterium tuberculosis). The mice were injected again in the hind foot pads 3 wk later with 10 μg of (T,G)-A--L 509 in phosphate-buffered saline and were bled from the tail 10 days after this injection.

Immune sera were tested in a modified Farr assay (4) by using rabbit anti-mouse gammaglobulin sera and ^{125}I-(T,G)-A--L 509. In a standard assay, 2.5 ng of

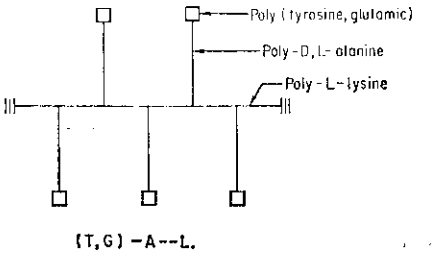

FIG. 3. Schematic diagram of the structure of (T,G)-A--L [from McDevitt and Sela (2)].

^{125}I-(T,G)-A--L was mixed with 25 μl of a particular dilution of the immune serum. These were incubated for 1 hr at 37°C. Then 50 μl of an appropriate dilution of rabbit anti-mouse gammaglobulin serum was added, incubation was continued another 2 hr, the tubes were spun for 15 min at 10,000*g*, and the supernatant was sampled and counted in a Nuclear Chicago gamma ray counter. Antibody titers are expressed as the percent of labeled antigen precipitated by a particular serum dilution.

Quantitation of Total Serum a *and* b *Allotype* IgG_{2a}

The mg/ml of *a* and *b* allotype IgG_{2a} in the total serum of each of the tetraparental mice was determined by the inhibition of the precipitation assay of Herzenberg and Herzenberg (14). In a representative *a* allotype IgG_{2a} assay, 5-μliter aliquots of various dilutions of the serum to be tested were added to inhibit precipitation of a constant concentration of ^{125}I-*a* allotype IgG_{2a} with a constant concentration of IgG_{2a}-specific anti-*a* allotype serum. The mg/ml of immunoglobulin varies from mouse to mouse. Therefore, the amounts of *a* and *b* allotype are listed as their percentage contribution to the total serum IgG_{2a}.

Quantitation of a *and* b *Allotype Antibodies in the Specific Anti-(T,G)-A--L Response*

The separation of immune sera into *a* and *b* allotype fractions has been described elsewhere (15). Briefly, an aliquot of immune serum was passed over the anti-*a* allotype affinity chromatography column consisting of anti-*a* allotype serum attached to cyanogen bromide-activated sepharose. The unbound fractions, eluted at pH 7.6 were designated the *b* allotype fractions. The bound, *a* allotype, fractions were eluted at pH 3.1. All fractions were titered for antigen binding activity as described above, except that 0.5 ng of ^{125}I-(T,G)-A--L was used and all dilutions were made in 1/1000 normal mouse serum. The total antigen binding activity in all *a* fractions and all *b* fractions was calculated along with the total percent recovery. The acid treatment had no detectable effect on the bound antibodies since approximately 100% of the anti-(T,G)-A--L titer put onto the column was recovered in the *a* + *b* eluate. The column was standardized by using known allotype mixtures, as shown in Table 2. The percentages of *a* allotype in anti-(T,G)-A--L responses are corrected values obtained by using a standard curve where the known percentage of *a* in an input serum (e.g., 10% *a*, 90% *b*) was plotted versus the percentage *a* recovered after chromatography. Experimental values for the tetraparental mice

TABLE 2

SEPARATION OF STANDARD MIXTURES ON ANTI-*a* ALLOTYPE COLUMN

Serum or mixture (vol %)	Percent of Anti-(T,G)-A--L activity recovered as *a* allotype
C3H·SW/Hz [1] (*a* allotype)	94
C57BL/10Sn [1] (*b* allotype)	7
90% C57BL/10Sn [2] 10% C3H·SW/Hz [2]	14
50% C57BL/10Sn [2] 50% C3H·SW/Hz [2]	60

[1] High responder.
[2] Sera of equal titer.

were interpolated on the standard curve to give corrected values for the percentages of *a* allotype in the anti-(T,G)-A--L response.

H-2 Type of Anti-(T,G)-A--L Plaque Forming Cells

Spleen and lymph node cells were tested by using a modified Jerne plaquing method developed by F. C. Grumet (manuscript in preparation). Immunized mice were given a tertiary injection of 10 μg (T,G)-A--L 509 in saline 5 days before plaquing. The target lawn consisted of sheep red blood cells (SRBC) that had been coated with (T,G)-A--L by using the chromic chloride conjugation method (16).

TABLE 3

COAT COLOR, HEMOGLOBIN, AND IMMUNOGLOBULIN ALLOTYPE

C3H ↔ C57	% C3H coat color	% C3H hemoglobin	% *a* allotype IgG_{2a}	% Antigen bound by 1/500 serum dilution
1	90	56	0	88
2	50	7	0	59
3	100	100	100	3
4	100	100	100	12
5	20	11	0	12
6	1	<5	0	21
7	60	40	0	26
8	99	93	100	13
9	95	67	21	66
10	95	52	7	54
11	30	22	3	30
12	95	76	18	75
13	100	18	100	31
14	95	43	22	53
15	60	58	34	60

Aliquots of spleen or lymph node cells were pretreated with anti-$H\text{-}2^k$, anti-$H\text{-}2^b$, or normal mouse serum and complement. The number of plaques produced by the cells remaining after each of these treatments was determined and reported as plaques per 10^6 untreated cells.

RESULTS AND DISCUSSION

Chimerism in the Individual Tetraparental Mice

The contribution to each of the tetraparental mice of coat color, hemoglobin, and immunoglobulin allotype markers from the two input strains is shown in Table 3. In each case the percent that is of C3H genotype (Table 1) is listed. All but two of

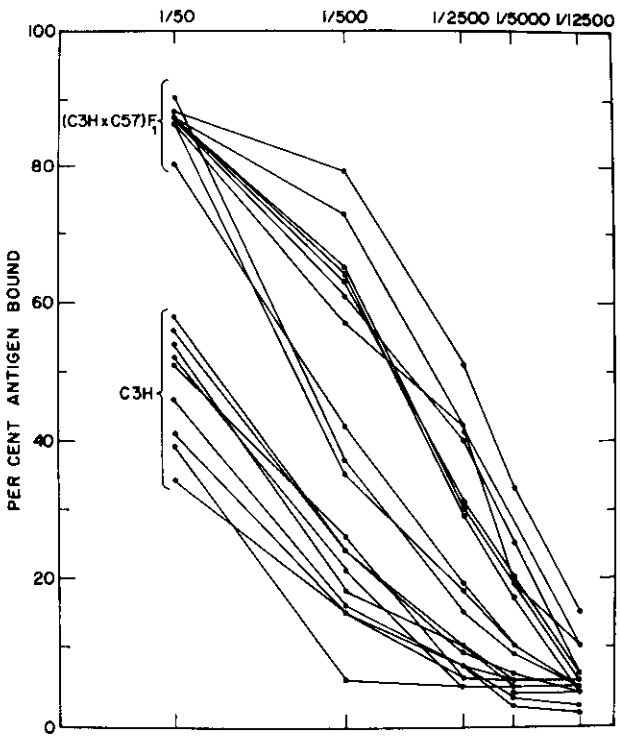

FIG. 4. Anti-(T,G)-A--L antibody responses of C3H and (C3H×C57)F_1 mice; serial serum dilution curves (horizontal axis, serum dilution). Percent antigen bound equals the percent of 2.5 ng of ^{125}I-labeled (T,G)-A--L precipitated by 25 μl of the experimental serum dilution in the standard antigen binding assay.

the animals (Nos. 3 and 4) can be shown to be chimeric by using these three markers.

Among the 13 animals that are chimeric, the mixes in coat color, hemoglobin, and immunoglobin allotype do not correlate well with each other. Moreover, there is a striking difference in the average percent of C3H in these three cell populations. Immunoglobulin has a lower average percent of C3H component than does hemoglobin, which shows less C3H component than the coat color. There are several possible explanations for this observation. First, it may be an artifact due to the

inherent difficulties in estimating the various mixes. Second, there may be a selection at ontogeny for the C57 component in the erythropoetic and lymphopoetic systems and/or for C3H cells in the hair follicles.

Third, the three systems may have equal probabilities of being 50–50 mixes at ontogeny and may later come under progressive selection. The coat color pattern does not change grossly with time (13) and in this experiment represents the earliest sampled of the three systems. The average percent C3H type in this system is 68%. Hemoglobin was sampled at 1 mo of age (average percent C3H component, 42%), and immunoglobulin allotype was sampled at 3–7 mo of age by using the immune serum (average percent C3H component, 16%). There is evidence from

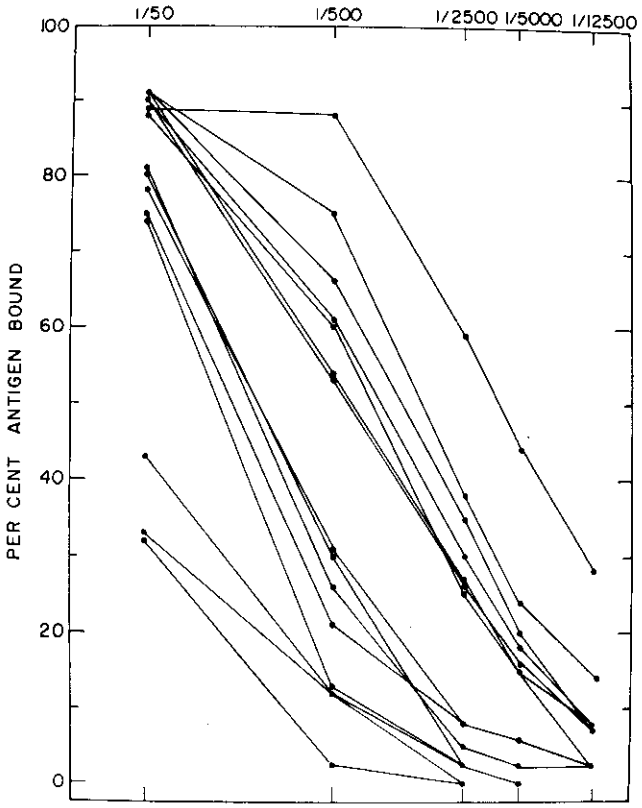

FIG. 5. Anti-(T,G)-A--L antibody response of C3H↔C57 tetraparental mice; serial serum dilution curves (horizontal axis, serum dilution). Percent antigen bound equals the percent of 2.5 ng of ^{125}I-labeled (T,G)-A--L precipitated by 25 μl of the experimental serum dilution in the standard antigen binding assay.

chimeric cattle (17) and from tetraparental mice (13) that the hemoglobin mix in chimeric animals can shift with time. It is possible that the immunoglobulin allotype mixture can undergo a similar shift (13). The basis for such a shift toward either input genotype is not known. It may be due to some form of immunologic rejection or may be inherent in the physiology of the cells of the two different genotypes. In addition, immunization with (T,G)-A--L could have contributed to the apparent shift by selectively expanding the high-responder B cell population.

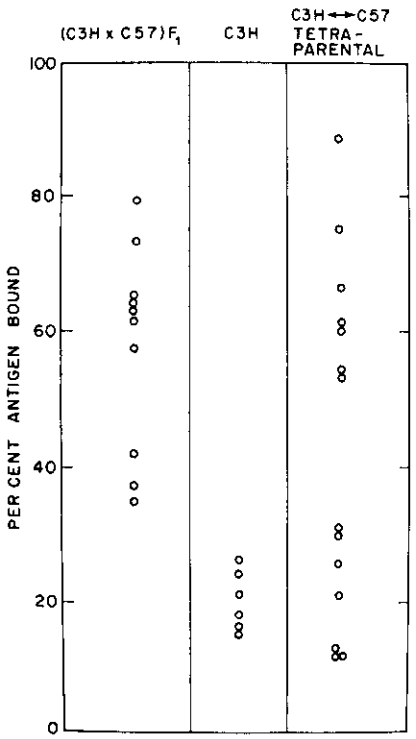

FIG. 6. Comparison of anti-(T,G)-A--L antibody responses of C3H, (C3H×C57)F_1, and C3H ↔ C57 tetraparental mice. (See Figs. 4 and 5.)

Cell–Cell Cooperation Immune Response Experiment

The tetraparental mice were constructed from an *Ir-1A* low-responder and an *Ir-1A* high-responder to (T,G)-A--L, so that T and B cells of low-responder genotype and T and B cells of high-responder genotype could interact in an operationally histocompatible environment. According to the hypothesis on which this experiment is based (Fig. 1), if the *Ir-1A* gene is expressed in T cells only, then the B cells of both high and low responders should be able to produce a high titered anti-(T,G)-A--L antibody response, and the antibody allotypes of both input strains should be represented in the specific anti-(T,G)-A--L response. However, if the *Ir-1A* gene is expressed in both T and B cells, then only the high-responder B cells should be able to produce a high titered anti-(T,G)-A--L response and only the allotype of the high-responder input strain should be represented in the specific response.

Total Serum Anti-(T,G)-A--L Response

C3H and (C3H × C57)F_1 control mice and the 15 C3H↔C57 tetraparental mice were immunized with 10 μg of (T,G)-A--L 509, and the immune sera were titered as described in the section on materials and methods. The serial-dilution antigen-binding curves for the immune sera of the C3H and F_1 mice are shown in Fig. 4. The C3H (low-responder) mice range from 5% to 26% antigen bound at a 1/500

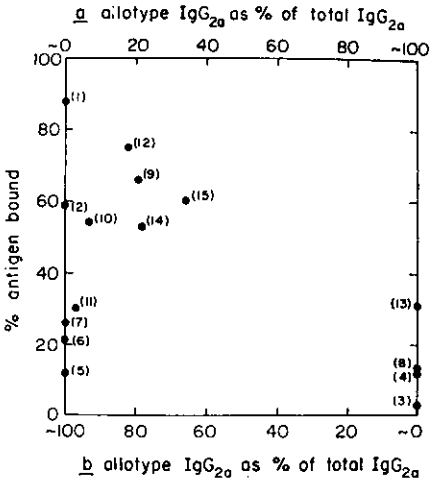

FIG. 7. Response related to total serum allotype mix. The percent antigen is bound by a 1/500 serum dilution (see Fig. 5). The C3H (low responder) input strain produces the *a* allotype antibody. The C57 (high responder) input strain produces the *b* allotype antibody. Numbers in parentheses are the numbers of the individual tetraparental mice.

serum dilution. The F_1 (high-responder) mice produce a much higher anti-(T,G)-A--L response, which ranges from 37% to 79% antigen bound at a 1/500 serum dilution. There is approximately a 20-fold difference between C3H and F_1 mice in the serum concentration required to produce 50% antigen binding.

The responses of the 15 C3H↔C57 tetraparental mice are shown in Fig. 5. They cover the entire range of responses from the lowest of the low-responder control animals to the highest of the high-responder control animals. For comparison of these titers with those of the control mice, the percent antigen bound by 1/500 serum dilutions in Figs. 4 and 5 are replotted together in Fig. 6. It can be seen that the levels of the total responses produced by the tetraparental mice are similar to those of the normal immune mice.

Anti-(T,G)-A--L Response Related to Total Serum Allotype Mix

In order to determine why a tetraparental mouse is a high or a low responder, it is necessary to know whether the genetic makeup of the animal's total immune system is predominantly that of a high or low responder. The mix of allotypes in the total serum immunoglobulin (Table 3) provides an estimate of the input strain mix in the total B cell population. This, in turn, gives an estimate of the total T cell population (18).

In Fig. 7 the total serum allotype mixes are plotted versus the antibody titers at the 1/500 serum dilution for the 15 C3H↔C57 tetraparental mice. As expected, there are high-responder mice that have large amounts of *b* (high-responder) allotype in their total serum and low-responder mice with large amounts of *a* (low-responder) allotype. However, in addition, there are several tetraparental mice with very high percentages of *b* (high-responder) allotype in their total serum which nevertheless produce very low anti-(T,G)-A--L responses. These exceptions (1) may reflect an incorrect estimate of T cells (which control the level of response),

owing to independent shifting of the T and B cell population over time, or (2) may be due to the previously observed highly variable expression of the *Ir-1A*^{high} allele on the C57 genetic background (19). The unexpected group with low responses in animals with predominantly high-responder total serum allotype is not found in tetraparental mice of another strain combination which does not involve the C57 genotype (Bechtol, Freed, Herzenberg, McDevitt; in preparation).

H-2 Type of Anti-(T,G)-A--L Plaque Forming Cells

Among the 15 C3H↔C57 tetraparental mice, there are several that are high responders to (T,G)-A--L and that have significant amounts of *a* (low responder) allotype in their total serum. One of these mice, No. 12 (18% *a* in its total serum), was tested for the *H-2* type of its anti-(T,G)-A--L plaque forming cells. The target lawn consisted of (T,G)-A--L-coated sheep red blood cells. Cell preparations were aliquoted and treated with normal mouse serum, or anti-*H-2*^{k}, or anti-*H-2*^{b}, and complement before plaquing. The anti-(T,G)-A--L plaque forming cells of this animal were found to be almost entirely *H-2*^{b}, high-responder, cells (Table 4). That is, very few or none of the plaque forming cells were of *H-2*^{k}, low-responder, origin. As will be seen in the next section, this result is consistent with the allotype distribution in the specific response produced by No. 12. Normally no plaque forming cells are detectable in immunized C3H, low-responder, mice.

Allotype Composition of the Specific Anti-(T,G)-A--L Response

The five chimeric high-responder tetraparental mice were tested for the allotype distribution of their specific anti-(T,G)-A--L response. Their immune sera were separated into *a* and *b* allotype fractions, as described in the section on materials and methods, and the fractions were titered for antigen binding activity. The values

TABLE 4

H-2 TYPE OF PLAQUE FORMING CELLS

Tetraparental or Control Mouse	Cells	Antiserum Pretreatment	PFC [1]/10^6 cells
Tp No. 12	Spleen	NMS [2]	105
		anti-*H-2*^{k}	98
		anti-*H-2*^{b}	5
	Lymph node	NMS	134
		anti-*H-2*^{k}	121
		anti-*H-2*^{b}	16
(C3H × C57)F_1	Spleen	NMS	100
		anti-*H-2*^{k}	1
		anti-*H-2*^{b}	2
	Lymph node	NMS	300
		anti-*H-2*^{k}	0
		anti-*H-2*^{b}	1

[1] Plaque forming cells. Results are reported as number of plaque forming cells per 10^6 untreated cells.

[2] Normal mouse serum.

for the percent of specific antigen binding activity in the two allotype fractions, as reported in Table 5, are corrected for known deviation from ideal separation (see Table 2). Thus, the values 14%, 19%, and 19% *a* (low-responder) allotype in the specific anti-(T,G)-A--L responses of three of the tetraparental mice are significantly above the background level of the assay. That is, three of the C3H↔C57 tetraparental mice produced detectable amounts of *a* (low-responder) allotype anti-(T,G)-A--L in a high titered specific response. A normal immune C3H serum does not show a detectable titer after the dilution required during the column separation process. The antigen binding capacities of the *a* allotype fractions of these three sera are, respectively, 4.6, 3.8, and 5.0 times the antigen binding capacity of the highest responding of the normal immunized C3H (low-responder) control animals (Table 5). Therefore, animals Nos. 9, 10, and 14 appear to have produced *a* allotype portions of their responses that have greater antigen binding activity than would normally be produced by C3H (low-responder) animals. This is the result expected, according to our hypothesis (Fig. 1a), if the *Ir-1A* gene is expressed only in T cells.

Four of the five tetraparental mice in Table 5 showed a lower percent of *a* allotype in their specific anti-(T,G)-A--L response than in their total serum mix. The fifth showed more *a* in its specific response than in the total serum. This deviation from overall correlation of total serum and specific allotype percentages may be due (a) to the fact that the specific response represents a more recent sampling of the shifting B cell population than does the total serum immunoglobulin, (b) to a less efficient cooperation between C3H B cells and C57 T cells than between T and B cells, both of which are the same (C57) genotype (20), or (c) to the small number of animals sampled. The correlation between percentage of *a* in the specific response and in the total serum was much closer in using congenic mice on a C3H background (Bechtol, Freed, Herzenberg, McDevitt; in preparation).

Significance of the Response of Tetraparental Mice

There seems to be little doubt that the *Ir-1A* gene is expressed in T cells. It may code for the T cell antigen-specific receptor itself or for a surface molecule that inter-

TABLE 5

ALLOTYPE COMPOSITION OF THE SPECIFIC ANTI-(T,G)-A--L RESPONSE

Tetraparental mouse No.	% of anti-(T,G)-A--L activity recovered as *a* allotype (Corrected Values [1])	Total serum *a* allotype IgG_{2a} as % of total IgG_{2a}	% antigen bound at 1/500	*a* ABC relative to C3H [2]
9	14	21	66	4.6
10	19	7	54	3.8
12	0	18	75	0
14	19	22	53	5.0
15	6	34	60	2.5

[1] Values are corrected for nonspecific binding of *b* allotype globulin. Corrected values are based on the standard serum mixtures shown in Table 2.

[2] Antigen Binding Capacity (ABC) of the *a* allotype fraction relative to the ABC of the highest of the low-responder (C3H) control mice.

acts with the receptor. Limiting dilution experiments suggest in some strain pairs that the *Ir-1A* gene is expressed in both T and B cells (21, 22) and in other strain pairs that the *Ir-1A* gene is expressed in B cells (23). However, Schlossman and Williamson (11) have shown, using isoelectric focusing, that in guinea pigs the major DNP-PLL specific clones of PLL responders and nonresponders may be identical.[6] Thus, by analogy, one might expect that the *Ir-1A* gene would not be expressed at the level of antibody specificity in B cells. Katz *et al.* (24) have recently shown in an adoptive transfer system that the *Ir* gene(s) or a gene closely linked to it is involved in T and B cell interaction. Whether this effect on interaction plays a detectable role in the *H-2* combination used in our experiments and/or in the more optimal physiological conditions of a tetraparental mouse is presently under investigation.

The experiments of Wegmann *et al.* (25) and of Phillips and Wegman (26) suggest that the cells of tetaparental mice may be immunologically reactive against each other and may be blocked from reacting by serum enhancing factors. Katz (27) and Ordal and Grumet (28) have shown that a histoincompatibility reaction can cause B cells to produce a more vigorous response than they otherwise would (allogeneic effect). However, this effect seems unlikely as an explanation for the *a* allotype responses of the tetraparental mice, since the mice produce responses of comparable magnitudes to those of normal immunized mice. In order to determine more precisely whether there is an allogeneic effect on the response of low-responder B cells to (T,G)-A--L in tetraparental mice, it is necessary to remove the possibility of this effect. It is possible to do this by (a) using a hemizygous difference in which the low responder is homozygous $H\text{-}2^{k/k}$ and the high responder is a heterozygous ($H\text{-}2^{k/b}$) and (b) using a homozygous low-responder difference, $H\text{-}2^{k/k}$ *and* $H\text{-}2^{q/q}$. These experiments are in progress.

We have shown that some cell type of the high responder can cause the B cells of the low responder to react to (T,G)-A--L. Two cell types besides B cells participate in the formation of an antibody response. They are T cells and macrophages. T cells are antigen specific and appear to express the *Ir* genes (8). Thus the high-responder T cells appear the most likely candidate for the cell type which is stimulating the B cells of low-responder genotype to produce a high titered anti-(T,G)-A--L response. These results, taken with the reservations discussed above, are compatible with the hypothesis that the *Ir-1A* gene is expressed only in T cells.

Note added in proof: *Ir-1* has been redesignated *Ir-1A*; see Shreffler, D. C., David, C. S., Götze, D., Klein, J., McDevitt, H. O., and Sachs, D. M., *Immunogenetics* **1** (in press), 1974.

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[6] *Note added in proof*: I. Melchers, K. Rajewsky, and D. C. Shreffler (*Eur. J. Immunol.*, in press) have shown by using isoelectric focusing (IEF) that both low- and high-responder mice to LDH_B (*Ir-1B*) produce antibody responses of similar limited degrees of heterogeneity and that a few of the IEF bands appear repeatedly in individual sera, irrespective of the *H-2* allele of the donor.

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