ANTIBODY RESPONSE OF C3H ↔ (CKB × CWB)F_1 TETRAPARENTAL MICE TO POLY-L(TYR,GLU)-POLY-D,L-ALA--POLY-L-LYS IMMUNIZATION*

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A number of antigen-specific genetic controls of the immune response, found in several species including mouse, rat, guinea pig, and man, are today under intensive study. Most of the genetic controls of the immune response which have been detected are linked to the major histocompatibility complex of the species. The effect of these immune response, *Ir*, genes has been detected after immunization with *(a)* synthetic polypeptide antigens *(b)* allo- and isoantigens, and *(c)* very low doses of strongly immunogenic foreign proteins (1). This communication deals with one of the *Ir* genes in the mouse, the *Ir-1A* gene, which exerts an effect on the antibody response to the synthetic branched polypeptide poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] (2, 3).[1] The *Ir-1A* gene maps between the *K* and *Ss-Slp* loci within the murine major histocompatibility complex, *H-2* (4, 5). In the inbred strains of mice used in this study, the *Ir-1A* allele determining low response to (T,G)-A--L is associated with the *H-2* haplotypes *k* and *q*, while high responsiveness to (T,G)-A--L is associated with the $H\text{-}2^b$ haplotype. F_1 $(H\text{-}2^{k/b})$ mice also produce relatively high, although somewhat more variable, responses. Thus, high response is semidominant.

The *Ir-1A*-controlled immune response phenotypes can be transferred by injection of spleen cells, fetal liver cells (4, 6, 7), or partially purified peripheral blood lymphocytes into lethally irradiated recipient mice, irrespective of the *Ir-1A* genotype of the recipient. The reconstituted animals respond to immunization with (T,G)-A--L as predicted by the genotype of the cells transferred. Thus, the *Ir-1A* genetic control is apparently expressed

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[1] *Abbreviations used in this paper:* ABC, antigen-binding capacity; BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; C3H ↔ (CKB × CWB)F_1, tetraparental mouse constructed from a C3H embryo and a (CKB × CWB)F_1 embryo; DNP-OM, hen ovomucoid substituted with an average of 4.6 2,4-dinitrophenyl groups per molecule; GAT^{10}, synthetic amino acid polymer, composed of glutamic $acid^{60}$-$alanine^{30}$-$tyrosine^{10}$; GLPhe, synthetic amino acid polymer, composed of glutamic $acid^{58}$ $alanine^{38}$ $phenylalanine^{4}$; GvH, graft-versus-host; Ig, gamma globulin; *Ig*, locus of heavy chain constant region of immunoglobulins; *Ir-1A* gene, immune response 1A gene controlling response to (T,G)-A--L; *K*, left-hand major transplantation locus of the *H-2* complex; PBS, phosphate-buffered saline; PLL, poly-L-lysine; RAMP, rabbit antimouse gamma globulin sera; *Ss-Slp*, locus of serum substance, sex-limited protein; (T,G)-A--L, synthetic branched polypeptide, poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys.

in cells of the immune system and is an intrinsic aspect of the immune response mechanism.

At least two major classes of antigen-specific cells are required for production of a high-titered antibody response: the thymus-dependent lymphocyte (T cell) and the bone marrow-derived lymphocyte (B cell). Several lines of evidence suggest that at least some *Ir* genes are expressed in T cells. Kantor et al. have shown that guinea pigs nonresponsive to poly-L-lysine (PLL) manifest no delayed-type hypersensitivity to PLL (8) and produce an antihapten antibody response only when the hapten-PLL conjugate is electrostatically complexed to an immunologically recognizable foreign albumin, acetylated bovine serum albumin (BSA). PLL-responder guinea pigs, on the other hand, produce both delayed hypersensitivity to PLL and antihapten antibody when immunized with hapten-PLL alone (9). These results suggest that nonresponder guinea pigs lack functional PLL-specific T cells, while responder guinea pigs possess them.

In mice, *Ir-1A* low responders to (T,G)-A--L can produce a high-titered response after immunization with (T,G)-A--L electrostatically complexed with methylated BSA (10). In addition, the response of an *Ir-1A* low responder can be altered toward high responder type by induction of a graft-versus-host (GvH) reaction (allogeneic effect) in the mouse at the time of immunization (11). Both of these experiments are ostensibly additions of T-cell function to the low responder. Conversely, genetically high responder mice can be converted to phenotypic low responders by depletion of T cells through thymectomy followed by lethal irradiation and bone marrow reconstitution. The response pattern of *Ir-1A* low responder mice is not altered by this T-cell deprivation (12). Thus, in the *Ir-1A* system low responder mice appear to be deficient in the T-cell-dependent, (T,G)-A--L-specific helper function. These results also suggest that the *Ir-1A* gene is not expressed in the ability of B cells to respond, since the B cells do respond to (T,G)-A--L in high responder fashion when provided with appropriate helper stimulation.

In a previous paper (13) it was shown that, in tetraparental mice, both high and low responder genotype B cells can respond equally to antigenic stimulation with (T,G)-A--L and can produce equally high titers of specific antibody. The experiments reported in the present communication are designed to further explore the stimulation of low responder-genotype B cells in tetraparental mice.

First, tetraparental mice were constructed using a genetic combination which precludes a classic GvH reaction leading to allogeneic stimulation of low responder B cells. Second, tetraparental mice were constructed between two histoincompatible low responder strains to determine whether there exists in tetraparental mice a histoincompatibility reaction which is capable of stimulating B-cell responses. Finally, intact normal mice were immunized with two antigens simultaneously, one to which they were high responders and one to which they were low responders, to test the possibility of nonspecific stimulation of the low responder response. The responses to (T,G)-A--L produced by the mice in each of these three sets of experiments are consistent with stimulation of low responder B cells in tetraparental mice by a normal response mechanism. It thus appears, that, in tetraparental mice, B cells of both high and low responder genotype can be stimulated and respond equally.

Materials and Methods

Mice. Animals were obtained from the following inbred lines maintained at Stanford: C3H/DiSn ($H\text{-}2^{k/k}$, $Ir\text{-}1A^{\text{low/low for (T,G)-A--L}}$, $Ig^{a/a}$); C3H·SW (abbreviated CSW; $H\text{-}2^{b/b}$, $Ir\text{-}1A^{\text{high/high}}$, $Ig^{a/a}$); congenic with C3H/DiSn but with the $H\text{-}2^{b}$ complex of Swiss Webster origin (14); CWB/13Hz (*H-*

TABLE I
Genetic Composition of Tetraparental Mice

Mouse strain and tetraparental	Genetic loci		
	H-2	*Ir-1A* for (T,G)-A--L	*Ig*
C3H	*k/k*	low/low	*a/a*
(CKB × CWB)F_1	*k/b*	low/high	*b/b*
C3H ↔ F_1	*k/k* + *k/b*	low/low + low/high	*a/a* + *b/b*
C3H·Q	*q/q*	low/low	*a/a*
CKB	*k/k*	low/low	*b/b*
C3H·Q ↔ CKB	*q/q* + *k/k*	low/low + low/low	*a/a* + *b/b*

$2^{b/b}$, $Ir\text{-}1A^{high/high}$, $Ig^{b/b}$) congenic with CSW but possessing the Ig^b complex from C57BL/10SnHz (15); CKB ($H\text{-}2^{k/k}$, $Ir\text{-}1A^{low/low}$, $Ig^{b/b}$). The CKB line was derived from the F_2 generation of a cross between C3H/DiSn and CWB/13Hz. Those F_2's which were $H\text{-}2^{k/k}$, $Ig^{b/b}$ provided the CKB-strain ancestors. The derived strain was tested by reciprocal skin grafting for recombination within the *H-2* complex. The C3H·Q strain ($H\text{-}2^{q/q}$, $Ir\text{-}1A^{low/low}$, $Ig^{a/a}$) is congenic with C3H/HeJ, but has the $H\text{-}2^q$ complex of STOLI/Lw (14).

Tetraparental mice were produced from two genetic combinations: *(a)* a C3H/DiSn embryo and a (CKB × CWB/13Hz)F_1 embryo (Table I), and *(b)* a CKB embryo with a C3H·Q embryo (Table I). The method of tetraparental mouse production has been presented in detail elsewhere (16–18). Briefly, each tetraparental mouse was constructed by aggregation of two 8–16 cell embryos, in pairs as listed above, to form a chimeric blastocyst (See Fig. 2 in 18). Several chimeric embryos were then transplanted into the uterus of a pseudopregnant recipient to complete development. Some of the tetraparental mice thus produced were mosaic for cells of the two input genotypes. That is, they contained cells of both input genotypes (See Fig. 2 of 13).

Antigens, Immunization Procedures, and Antibody Determination. (T,G)-A--L 509 (mol wt 232,000; residue molar ratio Tyr:Glu:Ala:Lys of 2.1:4.1:19.6:1.0; 100 poly-D,L Ala sidechains per molecule on average) was a gift of Dr. Michael Sela, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel. (T,G)-A--L 509 was synthesized by the polymerization of *N*-carboxy alpha-amino acid anhydrides (19). BSA fraction V was purchased from Armour Pharmaceutical Company. Bovine gamma globulin (BGG) fraction II was purchased from Miles Laboratories Inc., Elkhart, Ind. Hen ovomucoid (OM) was purchased from Worthington Biochemical Corp., Freehold, N. J., and was substituted with an average of 4.6 dinitrophenyl groups per molecule (DPM-OM) by Dr. John H. Freed.

Immunizing doses were 10 μg for (T,G)-A--L, 100 μg for BGG, and 1 μg for DNP-OM. Mice were immunized at 2–3 mo of age with antigen emulsified in complete Freund's adjuvant (CFA, 2 mg *Mycobacterium tuberculosis*/ml), boosted 3 wk later with the same dose of antigen in phosphate-buffered saline (PBS), and bled from the tail 10 days after the boost.

Antigen binding capacity (ABC) was determined using rabbit antimouse gamma globulin sera (RAMP) and radioiodinated (T,G)-A--L 509 or DNP_{12}-BSA in a modified Farr assay (4). All dilutions were made in 1% BSA in PBS; the diluent also contained 1/1,000 normal mouse serum when dilutions of experimental serum were greater than 1/1,000. In a standard assay, 50 μl of ^{125}I-(T,G)-A--L (0.01 μg/ml) or ^{125}I-DNP_{12}-BSA (0.02 μg/ml) was mixed with 25 μl of a dilution of the experimental serum and incubated at 37°C for 1 h. Then 50 μl of a dilution of RAMP, which gave maximum precipitation of the mouse gamma globulin, was added and incubation at 37°C was continued for an additional 2 h. The titration tubes were spun for 15 min at 10,000 *g*, and 50-μl 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 IgG_1 and IgG_{2a}. The milligrams per milliliter of *a* and *b* allotype IgG_1 and IgG_{2a} were determined for the total serum of individual unimmunized tetraparental mice using the inhibition of precipitation method of Herzenberg and Herzenberg (20). Briefly, a dilution of the test serum was added to a known concentration of ^{125}I-myeloma

protein. Then a known dilution of antiallotype 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 milliliter by interpolation on a standard curve produced by adding known concentrations of unlabeled myeloma protein to the assay system. Purified myeloma proteins and class-specific antiallotype sera were the kind gift of Dr. Leonard Herzenberg.

Allotype composition is expressed as the mean of percent *a* allotype in the two subclasses IgG_1 and IgG_{2a}:

$$\text{mean percent } a = \tfrac{1}{2}\left(\frac{\text{mg/ml } a\ IgG_1}{\text{mg/ml } (a + b)\ IgG_1} + \frac{\text{mg/ml } a\ IgG_{2a}}{\text{mg/ml } (a + b)\ IgG_{2a}}\right) \times 100.$$

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, as described previously (21). The *b* allotype antibodies were not bound to the column and were eluted with the buffer (pH 7.6) used for binding. The *a* allotype molecules; which were bound to the column, were then eluted by washing the column with 0.1 M acetic acid buffer (pH 3.1) which was collected into an equal volume of neutralizing buffer (21). The *a* and *b* allotype fractions were then assayed for anti-(T,G)-A--L antibody 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 for the same serum, 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 antibody activity were usually quantitative. The column was standardized using known allotype mixtures. The percentages of *a* allotype in anti-(T,G)-A--L responses are corrected values obtained by using a standard curve where known percentages of *a* (based on ABC) in an input mixture of *a* and *b* allotype sera were plotted versus the percent of recovered ABC which was in the *a* allotype fraction after chromatography. The correction curves for each of the columns used in these experiments are shown in Fig. 1. Experimental values for the tetraparental mice were interpolated on the standard curves to give corrected values for the percentages of *a* allotype in the anti-(T,G)-A--L response. Under the conditions used for these columns, sera from C3H/DiSn low responder mice did not have a detectable titer.

Results

C3H ↔ (CKB × CWB)F_1 Tetraparental Mice. Tetraparental mice were constructed in order to bring high and low responder T and B cells together in an operationally histocompatible milieu. The rationale for this inductive cell-interaction experiment is shown in terms of a simple helper function and responding B-cell diagram in Fig. 2 a and b. As has been reported previously (13), in tetraparental mice constructed between C3H (low responders which produce *a* allotype immunoglobulin) and CWB (congenic high responders which produce *b* allotype immunoglobulin), high ABC of specific anti-(T,G)-A--L was found in the immunoglobulin (Ig) of both *a* and *b* allotypes.

To rule out the possibility that low responder B-cell stimulation in the tetraparental mice was due to an allogeneic effect against the low responder B cells, new tetraparental mice were constructed under genetic conditions where no such allogeneic stimulation should occur. Katz (22) has shown that the allogeneic enhancement of a specific antibody response occurs only when the histoincompatibility reaction is directed against the responding B cells. Therefore, the input embryos were chosen as follows to have only a hemizygous *H-2 (Ir-1A)* difference: The low responder embryo was C3H ($H\text{-}2^{k/k}$, $Ir\text{-}1A^{low/low}$, $Ig^{a/a}$) as in previous experiments. The high responder embryo was an F_1 between

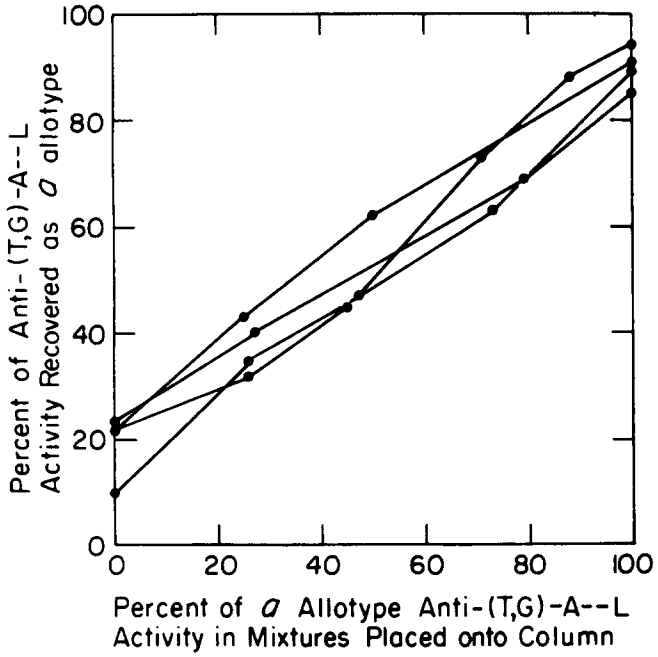

FIG. 1. Separation of standard mixtures on anti-*a* allotype columns. The percent of anti-(T,G)-A--L activity which was due to antibodies of *a* allotype was calculated on the basis of the relative anti-(T,G)-A--L titers of the CSW (*a* allotype) and CWB (*b* allotype) sera used in constructing the mixtures.

CKB ($H\text{-}2^{k/k}$, $Ir\text{-}1A^{low/low}$, $Ig^{b/b}$) and CWB ($H\text{-}2^{b/b}$, $Ir\text{-}1A^{high/high}$, $Ig^{b/b}$). Thus, the high-responder embryo was $H\text{-}2^{k/b}$, $Ir\text{-}1A^{low/high}$, $Ig^{b/b}$ (Table I). The response of the low responder ($H\text{-}2^{k/k}$) B cells is not subject to graft-vs.-graft attack, as the high responder cells, $H\text{-}2^{k/b}$, share the whole of the $H\text{-}2^{k}$ complex including *Ir-1A* with the low responder cells. In the C3H ↔ F_1 tetraparental mice there is a complete Ig-allotype difference between the low and high responder embryos, *a*/*a* and *b*/*b*, respectively, so that allotype analysis of the total serum and of the anti-(T,G)-A--L antibody response can be conducted in a manner analogous to that used for the C3H ↔ CWB tetraparental mice.

Total Serum Allotype Composition. During aggregation of embryos and formation of the tetraparental mouse, the cells of the two input genotypes become distributed through the embryos and extra-embryonic membranes in an apparently random manner. Only a small number of the cells of the total blastocyst contribute to formation of the final embryo (23). Thus, the total compositions of the tetraparental mice at birth can vary widely, from 100% of one parental type (e.g., C3H) to 50–50% for both parental genotypes, to 100% of the other parental type (e.g., (CKB × CWB)F_1). Additionally, the genetic mixture of cells in different tissues and organs within an individual animal can differ. To estimate the genetic mix of the B-cell population, and presumably of the total immune system, of each of the C3H ↔ F_1 ($Ig^{a/a} \leftrightarrow Ig^{b/b}$) tetraparental mice, the milligrams per milliliter of *a* and *b* allotype IgG_1 (*Ig-4*) and IgG_{2a} (*Ig-1*) were determined for the total serum taken just before immunization at 2–3 mo

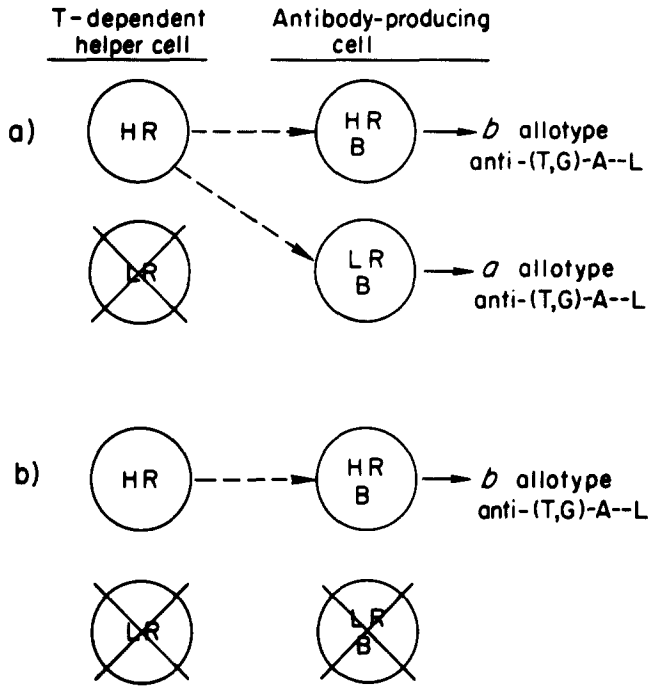

FIG. 2. Model for *Ir*-gene action. (a) *Ir-1A* expression at the level of helper function only. (b) *Ir-1A* expression in both helper function and ability of B cells to respond to help. (T,G)-A--L-specific cells covered by an X are presumed to be missing or nonfunctional. HR, high-responder genotype; $H\text{-}2^{b/b}$ or $H\text{-}2^{k/b}$. LR, low-responder genotype; $H\text{-}2^{k/k}$.

of age. The total milligram per milliliter of Ig is not constant from mouse to mouse. Therefore, for comparison of members of the tetraparental population one with another, the total serum allotype mixture is reported as the mean percent *a* allotype in the two subclasses IgG_1 and IgG_{2a} (see Materials and Methods).

In the population of 22 C3H ↔ (CKB × CWB) F_1 tetraparental mice analyzed, the complete range of possible total serum allotype mixtures was detected. Five sera contained *a* allotype Ig, but no *b* allotype Ig detectable above background in either the IgG_1 or IgG_{2a} subclass. Tetraparental mice with unimmune sera of such composition presumably contain predominantly or solely C3H (low responder), *a*-genotype B cells. Four unimmune sera from tetraparental mice contained *b* allotype Ig, but no detectable *a* allotype Ig in either the IgG_1 or IgG_{2a} subclass. Mice with such sera were presumably of predominant or complete (CKB × CWB)F_1 (high responder), *b* genotype in their B-cell population. The remaining 13 tetraparental sera contained both *a* and *b* allotype Ig, indicating that the mice producing these unimmune sera contained both C3H and (CKB × CWB)F_1 B cells. Fig. 3 shows the mean percentage of *a* and *b* allotypes in the serum from these 13 tetraparental mice. This distribution of allotype presumably reflects the B-cell genetic composition of each mouse. The B-cell composition in turn is an estimate of the composition of the total immune system. Gornish et al. (24) have shown, using the T6 chromosome marker, that in

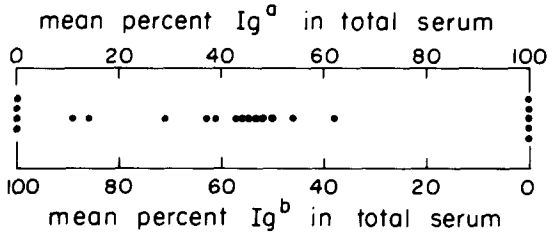

FIG. 3. Total serum allotype mixtures for the 22 C3H ↔ (CKB × CWB)F_1 tetraparental mice. The mean percent of Ig^a and Ig^b in the serum of unimmunized tetraparental mice was calculated from the quantitation of *a* and *b* allotype IgG_{2a} and IgG_1 as described in Materials and Methods.

tetraparental mice there is a close correlation in the proportions of mitotic spreads of the two input types in bone marrow, spleen, and thymus. This correlation, however, is not absolute as shown by Ford et al. (25). The variation from identity of compositions among the various lymphoid compartments of a given tetraparental mouse may account for the unexpected high or low responses produced by some of the tetraparental mice (see below).

Total Response to (T,G)-A--L Immunization. CKB and (C3H × CWB)F_1 control mice as well as the 22 C3H ↔ (CKB × CWB)F_1 tetraparental mice were immunized at 2–3 mo of age with 10 μg of (T,G)-A--L 509 in CFA. They were boosted 3 wk later with 10 μg of (T,G)-A--L 509 in PBS and bled 10 days after the boost. The immune sera were titered with ^{125}I-(T,G)-A--L in a modified Farr assay. Approximately a 17-fold difference was found between CKB, low responder mice and F_1, high responder mice in the serum dilution required to produce 50% antigen binding (Fig. 4). This is in contrast to the clear-cut, 50-fold difference between homozygous, CWB high responders and C3H low responders seen in a previous study (13). The lower responses of F_1 mice predict that *(a)* the population of responses produced by C3H ↔ F_1 tetraparental mice will be somewhat lower than the responses produced by the C3H ↔ CWB tetraparental, and *(b)* the population of responses may not fall into distinct high and low groups, but form a continuum of responses. Both of these predictions are borne out by the serial-serum-dilution antigen-binding curves for the 22 C3H ↔ F_1 tetraparental mice, as shown in Fig. 5.

Anti-(T,G)-A--L Response Related to the Total Serum Allotype Mixture. Fig. 6 shows the total serum allotype mixtures of the unimmunized C3H ↔ (CKB × CWB)F_1 tetraparental mice related to the anti-(T,G)-A--L response subsequently produced by each of these mice. Four mice were apparently all *b* (high responder) allotype in their unimmune serum, and these mice subsequently produced high titered responses. Nine mice were detectably chimeric in their immune system before immunization and subsequently produced high titered responses. These mice are candidates for analysis of the responding capability of genetically low responder B cells in a high responder milieu (see below).

Four chimeric C3H ↔ (CKB × CWB)F_1 tetraparental mice produced low titered responses to (T,G)-A--L. Thus, chimerism in itself does not appear to be sufficient to cause production of a high titered response in the tetraparental mice.

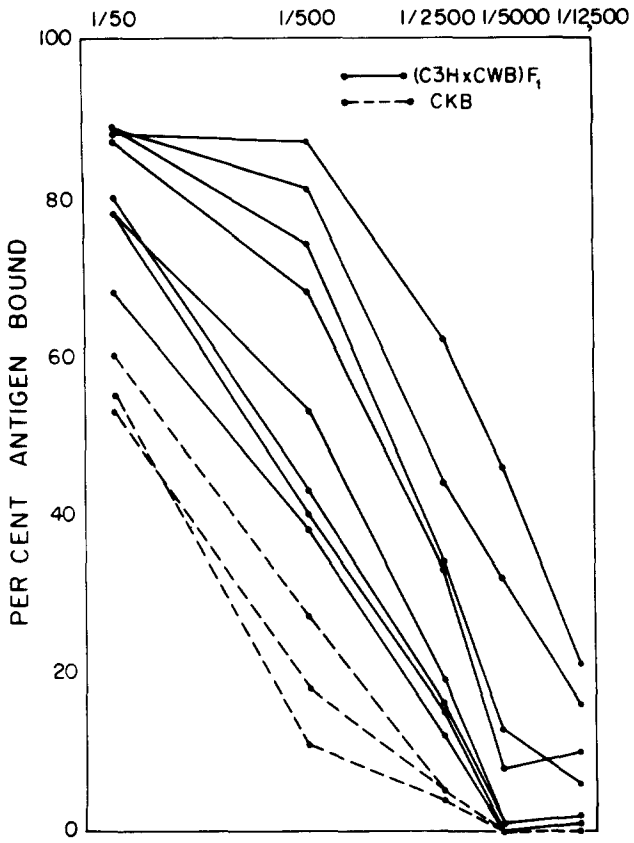

FIG. 4. Anti-(T,G)-A--L antibody responses of CKB and (C3H × CWB)F_1 control mice. The mice were immunized and boosted with 10 μg (T,G)-A--L 509 as described in Materials and Methods. The abscissa is the serum dilution. The ordinate is the percent of 2.5 ng of ^{125}I-(T,G)-A--L 509 precipitated by 25 μl of the serum dilution in the standard antigen-binding assay (4). Each curve represents an individual mouse.

Five mice were apparently all *a* (low responder) allotype in their unimmune serum. Four of these produced low titered responses. The fifth produced a high response. Although normal intact low responder mice do very rarely produce high responses, the reason for such a response is not clear here. As this is the single occurrence of this type in all of the tetraparental mice analyzed to date, it is difficult to speculate as to its cause. It may be due *(a)* to infection or *(b)* to chance variation in cell populations within the animal, so that the mouse had a significant population of high responder genotype T cells specific for (T,G)-A--L while having a low percentage of *b* allotype (high responder genotype) B cells (24, 25). The cause of events in this particular mouse must for the moment remain in question.

Allotype Composition of the Specific Anti-(T,G)-A--L Response. The allotype composition of the specific anti-(T,G)-A--L responses produced by the nine chimeric tetraparental mice which produced high titered responses was determined using an anti-*a* allotype affinity chromatography column, as previously described (21). The distribution of the total anti-(T,G)-A--L ABC between the *a*

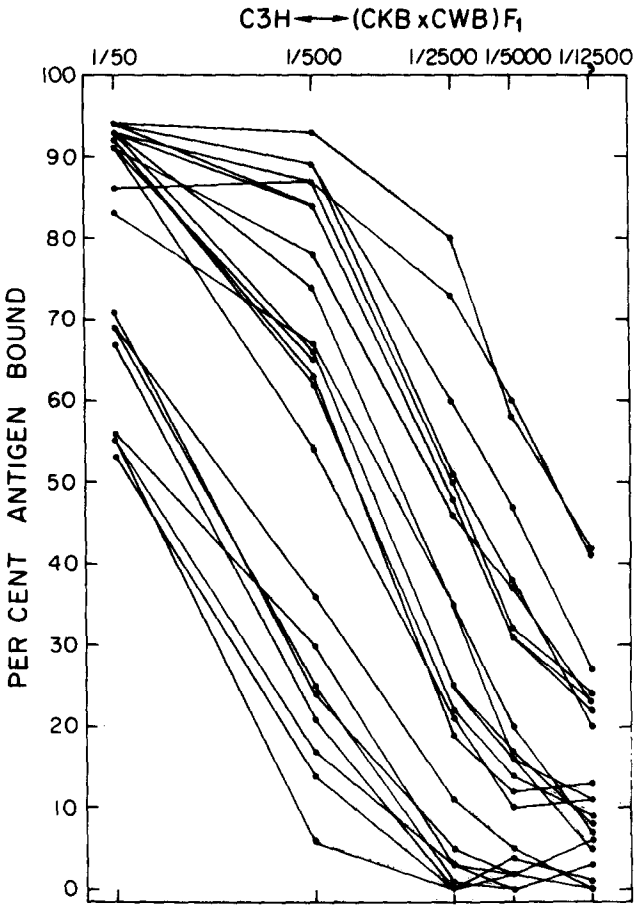

FIG. 5. Anti-(T,G)-A--L antibody responses of C3H ↔ (CKB × CWB)F_1 tetraparental mice. The 22 C3H ↔ F_1 tetraparental mice were immunized and boosted with 10 μg (T,G)-A--L 509 as described in Materials and Methods. The abscissa is the serum dilution. The ordinate is the percent of 2.5 ng of ^{125}I-(T,G)-A--L 509 precipitated by 25 μl of the serum dilution in the standard antigen-binding assay (4). Each curve represents an individual tetraparental mouse.

(low responder) and *b* (high responder) allotype fractions is shown in Table II. Six of the tetraparental mice produced significant *a* (low responder) allotype responses: 20, 45, 49, 55, 56, and 62% of their total antibody response were of the *a* allotype. The ABC's of the individual *a* allotype fractions were, respectively, 8, 4, 16, 20, 4, and 22 times the ABC of the average of three C3H (*a* allotype) low responder sera. Thus, four of the C3H ↔ (CKB × CWB)F_1 tetraparental mice have produced definite high responder level responses in their *a* (low responder) allotype fractions.

The sera of four (C3H × CWB)F_1 mice were also separated in a similar manner. Only three of the four F_1 mice analyzed produced significant ABC in their *a* allotype fractions. These were approximately 28, 40, and 52% of the total responses (Table II). The one F_1 mouse which failed to produce detectable *a* allotype ABC contained significant percentages of both *a* and *b* allotype Ig in its

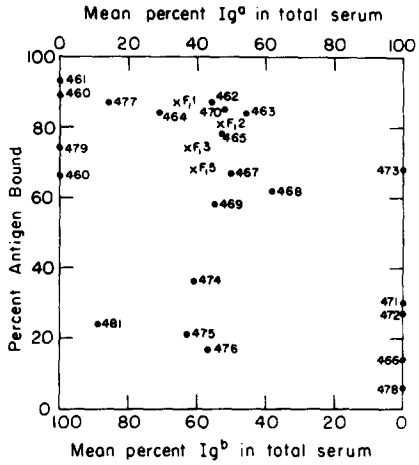

FIG. 6. Anti-(T,G)-A--L response related to total serum allotype mixture for 22 C3H ↔ (CKB × CWB)F_1 tetraparental mice and four (C3H × CWB)F_1 mice. Percent antigen bound by 1/500 serum dilution (see Figs. 4 and 5). Mean percent *a* allotype in total unimmune serum is described in Fig. 3.

TABLE II

*Allotype Distribution and ABC of Specific Anti-(T,G)-A--L Antibodies from C3H ↔ (CKB × CWB)F_1 Tetraparental Mice**

Tetraparental mouse	Anti-(T,G)-A--L activity		Ratio of *a* allotype ABC to intact C3H ABC (all Ig^a)	(T,G)-A--L bound at 1/500
	a allotype‡	Average recovery§		
	%			%
477	0	89	–	87
462	0;2;16	96	–	87
469	6	70‖	–	53
470	20	86	8	89
467	45	30‖	4	65
463	39;59	98	16	84
465	55	93	20	78
468	56	84	4	62
464	60;65	91	22	84
(C3H × CWB)F_1				
F_1 (no. 1)	0;1	114	–	87
F_1 (no. 5)	13;43	96	–	68
F_1 (no. 3)	35;45	101	–	74
F_1 (no. 2)	37;66	65	–	81

* 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.

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

§ Average percent recovery, percent of original ABC detected after separation on the antiallotype column.

‖ Low recovery from low titered sera.

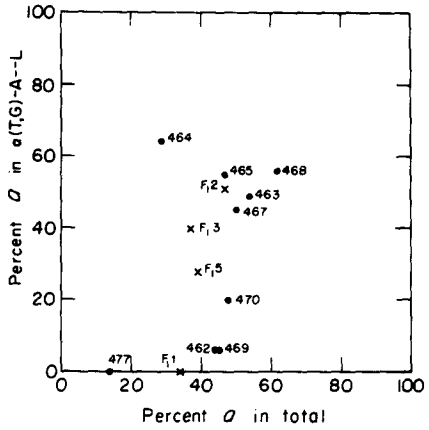

FIG. 7. Allotype composition of specific anti-(T,G)-A--L response related to total serum allotype mixture for C3H ↔ (CKB × CWB)F_1 tetraparental mice and (C3H × CWB)F_1 mice. For descriptions of abscissa and ordinate see Fig. 6 and Table II, respectively.

unimmune serum and was found by Ouchterlony test with anti-Ig^a and anti-Ig^b to have large amounts of both *a* and *b* allotype Ig in the total immune serum.

Allotype Composition of Specific Anti-(T,G)-A--L Responses Related to Total Serum Allotype Mixture. In Fig. 7 the percent of *a* allotype in the unimmune serum of the nine chimeric, high responding C3H ↔ (CKB × CWB)F_1 tetraparental mice is related to the percent of *a* allotype in the anti-(T,G)-A--L response each mouse subsequently produced. Seven of the nine tetraparental mice were approximately 50% *a* allotype in their unimmune total serum. They subsequently produced from 6 to 56% of the anti-(T,G)-A--L response in their *a* allotype fraction.

Four (C3H × CWB)F_1 [$(Ig^a \times Ig^b)F_1$] mice were also analyzed. They also contained approximately 50% *a* allotype in their total unimmune serum and produced responses that were from 0 to 50% *a* allotype. In the B cells of the F_1 mice the Ig^a or Ig^b allotype is expressed as a result of allelic exclusion, and all cells are of the $H\text{-}2^{k/b}$ genotype. If allelic exclusion occurs in the expression of any part of the *H-2* complex, including *Ir*, this would presumably occur at random with respect to the unlinked *Ig* alleles. Thus, the (C3H × CWB)F_1 mice test the potential for production of Ig^a and Ig^b in a high titered anti-(T,G)-A--L response irrespective of any possible influences of the *H-2* complex on cellular interactions. The allotype distribution of the responses of these four mice provides a basis for evaluation of the allotype mixtures produced by responding B-cell populations in the tetraparental mice, where the two *Ig* allotypes are necessarily expressed in cells of different *H-2 (Ir)* genotype (i.e., $H\text{-}2^{k/k}$ and $H\text{-}2^{k/b}$). In the tetraparental mice the effects of *Ir* and *H-2* are tested.

As shown in Fig. 7, the C3H ↔ (CKB × CWB)F_1 tetraparental mice produced high titered anti-(T,G)-A--L responses with the same distribution of *b* allotype antibody (*Ir-1A* high responder cells) and *a* allotype antibody (*Ir-1A* low responder cells) as was produced by the population of F_1 mice, each heterozygous for *b* and *a* allotypes. The percent *a* in the specific responses of both F_1 and tetraparental mice varied from an almost perfect representation of the allotype

mixture in the total immune serum to zero *a* in the specific response. This variation in percent *a* in the specific responses of animals, each approximately 50–50 in the allotype mix of its total unimmune serum, may represent variation due to random error in sampling from a small population of (T,G)-A--L-specific B cells. The tendency toward less than 50% *a* allotype in the specific responses of these mice may be due to the small sampling size or may reflect the additional *Ig* allotype-linked difference in ability to respond to (T,G)-A--L, which has been described by Grumet (personal communication). Grumet has found that normal immunized *a* allotype mice produce slightly lower responses than do *b* allotype mice of the same *H-2* type. Preliminary studies using congenic strains suggest that this difference in response is linked to the *Ig* locus. Since the variation patterns are the same for both F_1 and tetraparental mice, the pattern itself does not appear to be a function of the tetraparental milieu or *H-2 (Ir-1A)*, but is due to the equal response of both *Ir-1A* high and low responder-genotype B cells under genetic conditions where there should be no allogeneic effect against the low responder B cells.

Low Responder ↔ Low Responder Tetraparental Mice. To further test the potentiating effect of any possible histoincompatibility reaction (allogeneic effect) on the specific response of tetraparental mice to (T,G)-A--L, tetraparental mice were constructed between two histoincompatible low responder strains. If an allogeneic effect were present in tetraparental mice and causing low responder cells to produce a high titered response, then some of these low responder ↔ low responder tetraparental mice should produce elevated responses. The two input strains used were both of C3H origin. C3H·Q is $H\text{-}2^{q/q}$, $Ir\text{-}1A^{low/low}$, $Ig^{a/a}$, and CKB is $H\text{-}2^{k/k}$, $Ir\text{-}1A^{low/low}$, $Ig^{b/b}$ (Table I). $H\text{-}2^{q}$ and $H\text{-}2^{k}$ are known to differ in the *K*, *I*, and *D* regions of the *H-2* complex, and they are the strains used by Ordal and Grumet (11) to show that a GvH reaction can cause an increased response to (T,G)-A--L in low responder mice. C3H·Q and CKB also differ in Ig allotype.

Of the 11 C3H·Q ↔ CKB tetraparental mice which were constructed, 4 were shown to be chimeric by quantitation of *a* and *b* allotype IgG_1 and IgG_{2a}. They were 93, 55, 12, and 9% *a* in their unimmune sera and produced anti-(T,G)-A--L sera with 4, 0, 16, and 2% ABC at a 1/500 dilution. Seven C3H·Q ↔ CKB tetraparental mice were apparently nonchimeric, with two mice apparently all *b* and five mice all *a*. All C3H·Q ↔ CKB tetraparental mice produce clearly low responses to (T,G)-A--L (Fig. 8). In the previous studies of high responder ↔ low responder tetraparental mice with homozygous *H-2* difference, C3H ↔ C57 (18) and C3H ↔ CWB (13), 8 of the 13 high responding chimeras tested produced significant *a* allotype responses to (T,G)-A--L. (There were a total of 17 chimeric mice in these two studies.) In the present study, none of the four chimeric low responder ↔ low responder tetraparental mice produced responses that would have been detectable after separation on the antiallotype column. These results suggest that even in tetraparental mice with a complete histocompatibility difference between input strains there is insufficient histoincompatibility reaction to cause an increase in the immune response to (T,G)-A--L.

Indirect B-Cell Stimulation. The possibility of indirect stimulation of low responder, (T,G)-A--L-specific B cells by an adjacent, ongoing high response was

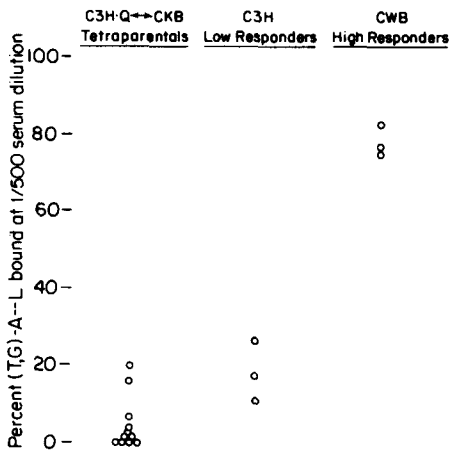

FIG. 8. Anti-(T,G)-A--L responses of 11 C3H·Q ↔ CKB tetraparental mice and CWB, high-responder, and C3H, low-responder mice. Points represent the percent of 2.5 ng of ^{125}I-(T,G)-A--L bound by 1/500 dilutions of sera from individual mice.

TABLE III

Lack of Nonspecific Effect on Anti-(T,G)-A--L Response of Genetic Low Responder Mice

Group	Mouse strain	Immunization*		^{125}I-(T,G)-A--L bound by 1/50 serum dilution	
		1°	2°	Average (range)	No. of mice
				%	
A	CKB	(T,G)-A--L	(T,G)-A--L	22 (18–25)	4
B	CKB	(T,G)-A--L	BGG + (T,G)-A--L	28 (12–32)	10
C	CKB	BGG + (T,G)-A--L	BGG + (T,G)-A--L	20 (14–25)	6
D	CKB	CFA	(T,G)-A--L	36 (30–42)	4
E	CKB	BGG	(T,G)-A--L	37 (29–41)	4
F	CKB	BGG	BGG + (T,G)-A--L	30 (25–38)	4
				Percent antigen bound by 1/1,250 dilution	
G	CWB	(T,G)-A--L	(T,G)-A--L	74 (55–95)	4

* Immunizations were with antigen in CFA or with CFA alone for the primary immunization. When two antigens were administered, each was emulsified with adjuvant separately. Both antigens were injected intraperitoneally, one immediately after the other. All secondary immunizations were as described above, but with antigens in PBS. Immunizing doses were 10 μg (T,G)-A--L 509 and 100 μg BGG. Mice were bled at 10 days after secondary immunization, and these sera were titered as described in Materials and Methods.

tested in one of its aspects as shown in Table III. CKB mice (low responders) were immunized and boosted with (T,G)-A--L alone or with (T,G)-A--L plus 100 μg BGG. CKB mice are high responders to this dose of BGG. (26, B. Deak, personal communication). As seen in groups A–C in Table III, the titer of the secondary, low response to (T,G)-A--L was unaffected by the simultaneous high

TABLE IV
Lack of Nonspecific Effect of Anti-(T,G)-A--L Response of Genetic High Responder Mice

Group	Mouse strain	Immunization*		^{125}I-DNP-BSA bound by 1/50 serum dilution	
		1°	2°	Average (range)	No. of mice
				%	
A	CWB	DNP-OM	–	3 (0–10)	4
B	CWB	(T,G)-A--L + DNP-OM	(T,G)-A--L	0 (0–1)	4
C	CWB	DNP-OM	DNP-OM	4 (0–9)	4
D	CWB	(T,G)-A--L + DNP-OM	(T,G)-A--L + DNP-OM	1 (0–2)	4
E	CWB	CFA	(T,G)-A--L + DNP-OM	0 (0–2)	4
F	CWB	(T,G)-A--L	(T,G)-A--L + DNP-OM	0 (0–1)	3
G	CKB	DNP-OM	DNP-OM	79 (77–81)	3

* Primary immunizations were with antigens in CFA or with CFA alone, as listed. In those cases where two antigens were administered, each was emulsified with adjuvant separately. Both antigens were then injected intraperitoneally one immediately after the other. The total volume of adjuvant administered remained constant. Secondary immunizations were as described above, but with antigens in PBS. Immunizing doses were 1 μg DNP-OM and 10 μg (T,G)-A--L 509. Mice were bled at 10 days after secondary immunization, and these sera were titered as described in Materials and Methods.

response to BGG. The normal response of the high responder strain CWB is shown in group G.

A similar situation to the above is seen in groups D–F. A response to BGG did not alter the titer of the primary response to (T,G)-A--L in the low responders. The responses to (T,G)-A--L found in groups D–F are slightly higher than those of groups A–C and are consistent with a 10-day primary response. These two results militate against the high response of low responder B cells in the tetraparental mouse being due to a nonspecific stimulation by the adjacent high responder reaction.

The effect of a high response to (T,G)-A--L on a concomitant low responder response was also tested (Table IV). CWB mice are low responders to low doses (1 μg) of DNP-OM (27). CWB were immunized and boosted with 1 μg DNP-OM alone or with 1 μg DNP-OM plus 10 μg (T,G)-A--L. The CWB response to DNP-OM was undetectable using the CFA immunization regimen previously employed for (T,G)-A--L immunization of the tetraparental mice. Simultaneous immunization with DNP-OM and (T,G)-A--L did not cause an increase in the anti-DNP response of CWB mice to a detectable level (groups B, D, and F, Table IV). This result suggests that production of a high titered anti-(T,G)-A--L response does not nonspecifically induce a large increase in concomitant low responder antibody responses.

Discussion

In this paper we report the results of several inductive cell interaction experiments conducted to test whether the specific anti-(T,G)-A--L responses produced by low responder-genotype cells in tetraparental mice were due to *(a)*

histoincompatibility reaction (allogeneic effect [22]) or *(b)* indirect B-cell stimulation (i.e., by adjacent reactions). Tetraparental mice were constructed under genetic conditions where there should be no allogeneic effect against the low responder cells. The high responder input embryo was a (CKB × CWB)F_1 [*H-2*$^{k/b}$, *Ir-1A*$^{low/high}$, *Ig*$^{b/b}$]. This F_1 embryo shared the whole of the *H-2* complex, including *Ir-1A*, with the low responder input embryo, C3H [*H-2*$^{k/k}$, *Ir-1A*$^{low/low}$, *Ig*$^{a/a}$]. There was a complete Ig allotype difference between the F_1 and C3H cells so that analysis of the immune system and the source of specific antibodies could be made in the same manner as in previous studies (13).

The unimmune sera of the 22 C3H ↔ (CKB × CWB)F_1 tetraparental mice were tested for Ig allotype composition. Nine mice were not detectably chimeric in their immune system by this criterion. Five appeared to be all *a* (low responder strain) allotype, and four appeared to be all *b* (high responder strain) allotype. The remaining 13 tetraparental mice (59% of the population) contained detectable amounts of both *a* and *b* allotype Ig. Eight of these (36% of the population) were approximately 50–50 allotype mixtures in their total serum.

In a previous study (13) a different type of tetraparental mice, the C3H ↔ CWB [(*H-2*$^{k/k}$, *Ir-1A*$^{low/low}$, *Ig*$^{a/a}$) ↔ (*H-2*$^{b/b}$, *Ir-1A*$^{high/high}$, *Ig*$^{b/b}$)] mice were less frequently chimeric in their immune system. 16 of 39, or 41%, of these were detectably chimeric, and only two mice, or 5%, of the population were approximately 50–50 in allotype composition in their unimmune serum. Lower frequencies of chimerism were also seen in the C3H·Q ↔ CKB population reported on here. 4 of the 11 mice, or 36%, were detectably chimeric in their total serum Ig, and only one mouse, or 9%, was approximately 50–50 in *a* and *b* allotypes. These differences in degrees of chimerism suggest that C3H and (CKB × CWB)F_1 cells are more nearly equal than those with homozygous *H-2* differences in their ability to contribute to the immune system and/or the whole tetraparental mouse. Three of the mouse strains involved in these experiments, C3H, CWB, and CKB, are congenic on the C3H/DiSn genetic background, while the C3H·Q strain was derived from C3H/HeJ. The apparently greater inequalities between cells of C3H and CWB genotype and between cells of C3H·Q and CKB genotype may be due to the homozygous difference for *H-2* and *H-2*-linked genes and/or to homozygous differences for residual or accumulated allelic pairs between the members of each pair. This latter explanation seems rather unlikely since it would require similar changes in two unrelated strains.

The 22 C3H ↔ (CKB × CWB)F_1 tetraparental mice produced responses to (T,G)-A--L immunization which covered the complete range of responses of CKB and (C3H × CWB)F_1 normal immunized mice. The four C3H ↔ (CKB × CWB)F_1 mice which were apparently all *b* (high responder) allotype in their unimmune serum produced high titered responses to (T,G)-A--L immunization. Of the 5 C3H ↔ (CKB × CWB)F_1 tetraparental mice which were apparently all *a* (low responder) allotype in their unimmune serum, four produced low titered responses to (T,G)-A--L, and one produced a high titered response (see Results). Nine of the C3H ↔ (CKB × CWB)F_1 mice which were detectably chimeric in their unimmune serum produced high titered responses to (T,G)-A--L. These mice were analyzed for the allotype composition of their specific response (see below). The remaining four chimeric mice produced low titered responses.

A similar observation was made with C3H ↔ C57 tetraparental mice (18),

where one chimeric tetraparental mouse with a large amount of *b* allotype and three of the apparently all-*b* tetraparental mice produced low titered responses. In contrast, among the C3H ↔ CWB tetraparental mice (13) only animals with 30% *b* allotypes or less in their total unimmune serum produced low titered responses. All other chimeric C3H ↔ CWB mice were high responders. The presence of high *b*, chimeric low responders in the C3H ↔ C57 and C3H ↔ (CKB × CWB)F_1 tetraparental mouse populations and not in the C3H ↔ CWB population is most likely due to the lower overall level of response produced by normal immunized populations of the high responder input embryos C57 and (CKB × CWB)F_1 compared to CWB. Thus, if the high responder embryo alone would have produced a relatively lower response when intact, this component could be insufficient, as only a fraction of the total immune system, to induce a high titered response.

The existence of low responding chimeras shows that chimerism as such is not sufficient to induce a high titered response. This conclusion is borne out by the consistently low responses of four C3H·Q ↔ CKB, low responder ↔ low responder chimeric tetraparental mice. In this case, the two input strains have a complete difference at *H-2*, but are both *Ir-1A* low responders to (T,G)-A--L. Thus, in tetraparental mice constructed from embryos with either partial or complete histocompatibility *(H-2)* differences, there does not appear to be sufficient histoincompatibility reaction to cause an increase in the immune response to (T,G)-A--L. That is, the total high response appears to be a direct result of the normal functioning of *Ir-1A* high responder cells present.

25 of the 35 chimeric, low responder ↔ high responder tetraparental mice in the three populations studied produced high titered responses to (T,G)-A--L. C. M. Warner (personal communication) found that most low responder ↔ high responder tetraparental mice chimeric for coat color were high responders to another synthetic amino acid polymer, glutamic acid^{58}-lysine^{38}-phenylalanine^{4} (GLPhe). In contrast, Warner et al. (28, and personal communication) found that most tetraparental mice were nonresponders to a third antigen, glutamic acid^{60}-alanine^{30}-tyrosine^{10} (GAT^{10}). The presence of specific suppressor T cells in *Ir-GAT* low responder mice but not in *Ir-GAT* high responder mice has recently been demonstrated (29). Such a suppressor mechanism could explain the frequent low responses of tetraparental mice to GAT immunization (Warner, personal communication). Specific suppressor T cells have not yet been reported in the case of low responsiveness to either GLPhe or (T,G)-A--L. If in fact suppression is not the mechanism of low responsiveness to GLPhe and (T,G)-A--L in the strains used to construct tetraparental mice, this would be consistent with the overall high responses produced by low responder ↔ high responder tetraparental mice immunized with either of these two antigens. The data on tetraparental mice suggest a basic difference in induction of an immune response between *Ir-GAT*^{10} on the one hand and *Ir-GLPhe* and *Ir-1A* for (T,G)-A--L on the other.

To determine the specific composition of the anti-(T,G)-A--L response produced by C3H ↔ (CKB × CWB)F_1 tetraparental mice, the immune sera of the nine chimeric, high responding tetraparental mice were separated on an anti-*a* allotype affinity chromatography column. Six of the tetraparental mice pro-

duced significant *a* (low responder strain) allotype responses. These represented 20, 45, 49, 55, 56, and 62% of their total anti-(T,G)-A--L ABC's. The ABC's of the *a*-allotype fractions were 9, 4, 16, 20, 4, and 22 times the ABC of the average of three C3H (*a* allotype) low responder sera. Thus, four of the C3H ↔ (CKB × CWB)F_1 tetraparental mice produced high responder level responses in their *a* (low responder strain) allotype fractions.

All told, the specific responses of a total of 21 out of 25 high responding, chimeric tetraparental mice in three studies have been analyzed for allotype composition. 12 of the 21 mice were found to have specific binding activity of the *a* allotype which was significantly greater than the responses of low responder mice.

The immune sera of four (C3H × CWB)F_1 [$(Ig^a \times Ig^b)F_1$] mice were similarly separated into allotype fractions. Only three of the four mice produced significant anti-(T,G)-A--L ABC in their *a* allotype fractions. These were approximately 28, 40, and 52% of the total ABC's.

The percent of *a* in the specific responses was compared to the total serum allotype mixtures of the mice. Seven of the nine tetraparental mice and all four of the F_1 mice were approximately 50% *a* allotype in their unimmune serum. They produced specific responses to (T,G)-A--L which for the tetraparental mice were 6–56% *a* (low responder strain) allotype and for the $(Ig^a \times Ig^b)F_1$ mice were 0–51% *a* allotype.

The Ig^a or Ig^b allotype is expressed in a given B cell of a (C3H × CWB)F_1 mouse as a result of allelic exclusion. All cells are of $H\text{-}2^{k/b}$ $(Ir\text{-}1A^{low/high})$ genotype, and expression of this genotype is presumably unaffected by the allelic expression at the unliked *Ig* locus. Thus, the F_1 mice define the pattern of Ig allelic mixtures which would result from stimulation of a system in the absence of possible effects of the *H-2* complex on cellular interactions. The distributions of allotype mixtures in the specific antibodies were similar in the F_1 and the highly-chimeric tetraparental mouse populations.

Thus, the B cells producing *a* and *b* allotype are stimulated and respond in the same manner whether *(a)* derived by Ig allelic exclusion among otherwise identical cells and stimulated in an F_1 mouse or *(b)* derived through construction of the animal from genetically dissimilar cells and stimulated in a stable chimeric tetraparental mouse.

It has been shown previously that determinants must be on the same molecule or complex in order to cooperate in a carrier-hapten manner in induction of humoral immune response (30). To test the possibility of nonspecific stimulation of an *Ir-1*-controlled low response by a simultaneous *Ir-1*-controlled high response, two sets of experiments were performed. In one set, low responders to (T,G)-A--L were immunized with (T,G)-A--L alone or simultaneously with (T,G)-A--L and a dose of BGG to which they are high responders. In both cases the responses to (T,G)-A--L were the same. In the reverse experiment low responders to DNP-OM were immunized with DNP-OM alone or with both DNP-OM and (T,G)-A--L, to which they are high responders. Here, the anti-DNP response was not increased to a detectable level. These results show that the high and low responses to (T,G)-A--L can neither provide nor receive nonspecific stimulation in conjunction with other simultaneous low or high

responses. This militates against such stimulation of low responder B cells in tetraparental mice.

The C3H ↔ (CKB × CWB)F_1 tetraparental mice produced high titered responses which had the same distribution of specific *a* allotype antibodies (*Ir-1A* low responder embryo) and *b* allotype antibodies (*Ir-1A* high responder embryos) as did the responses of (C3H × CWB)F_1, $Ig^{a/b}$ heterozygous mice. Thus, the *Ir-1A* high and low responder-genotype B cells of tetraparental mice, under conditions where there should be no allogeneic effect against the low responder-genotype cells, are both responding in equal fashion to helper stimulation.

The results of the studies in tetraparental mice are in direct contrast to recent findings from two other laboratories: *(a)* Taussig et al. (31) have reported that an antigen-specific factor capable of replacing the need for T cells, and putatively derived from educated T cells, can be obtained from either high or low responder cells. Regardless of the derivation, the factor, when transferred with antigen and bone marrow cells into irradiated recipients, can mediate the stimulation only of high responder, but not of low responder, genotype B cells. Although this would suggest an *Ir* gene action at the B-cell level, it should be noted that only IgM antibody can be obtained under the experimental conditions employed, whereas the major responder-nonresponder difference in the present experiments is in the secondary IgG response (10, 12); *(b)* Katz et al. (32, 33) have demonstrated, both in vivo and in vitro, that the antigen-specific stimulation of homozygous B cells mediated by homozygous allogeneic T cells can occur only when allelic differences do not exist in the *I* region of the *H-2* complex. Furthermore, when an antigen under *Ir* gene control is used, nonresponder B cells are not able to effectively interact with (nonresponder × responder) F_1 T cells, again suggesting either *Ir* gene action at the level of the B cell or *I* region haplotype-specific interaction between T and B cells.

The (T,G)-A--L antigen, and the congenic mice of the *H-2* haplotypes used in the tetraparental mouse experiments have been tested in this laboratory, following the protocol of Katz et al. (32); the results are similar to those reported by Katz et al. in that only high responder genotype B cells can collaborate in vivo with F_1 T cells (J. Press, personal communication). Thus, the disparity in results must arise from differences in the experimental protocols used. In the tetraparental system, chimerism has been established during embryogenesis and is long term; the interacting cells are therefore presumably mutually tolerant and have been immunized and boosted in situ. In contrast, the test system of Katz et al. employs an acute adoptive transfer system where the B cells and T cells are immunized in separate environments and then required to interact in a secondary host.

Several recent findings support the results of the tetraparental experiments indicating that effective B-cell–T-cell interaction can occur across homozygous *H-2* differences. Heber-Katz and Wilson (34) have demonstrated in an in vitro Mishell-Dutton system, that when rat allogeneic T-cell populations are specifically depleted of alloreactive T cells, such T-cell populations can effectively interact across homozygous *AgB* differences with B cells to generate a primary IgM antibody response. Additional confirmation of the tetraparental result has come from the studies of von Boehmer et al. (35) where long term stable

chimeras were constructed by transferring T-cell-depleted bone marrow from two *H-2*-different parental mouse strains into a lethally irradiated F_1 host. Such stable chimeras are immunized 3–4 mo after reconstitution, then the B cells and T cells of each *H-2* type are separated, recombined in allogeneic combinations, and challenged with antigen. Under these conditions, a secondary IgG response to SRBC can be obtained from the interaction of chimeric but allogeneic B and T cells.

It is clear that there are striking similarities between the construction of bone marrow chimeras and of tetraparental mice. Several mechanisms can be postulated to explain the results obtained in the tetraparental-chimera systems: *(a)* Low responder-genotype B cells are fully competent, and high responder-genotype T cells are capable of interaction with them, but are blocked or suppressed from doing so under certain experimental conditions. *(b)* Low responder-genotype B cells are competent, and in stable chimeric situations, low responder-genotype T cells become competent to interact in high responder fashion with low responder B cells. *(c)* Low responder B cells are competent, and high responder genotype T cells acquire the ability to interact with low responder genotype B cells in the tetraparental milieu. *(d)* In stable chimeras, the low responder-genotype B cell is altered to become competent to respond to stimulation. In the first three mechanisms, the site of *Ir* gene action in this system would be in a cell type other than the B cell, possibly in the T cell. The second, third, and fourth possibilities would necessitate some form of information exchange, perhaps by exchange of genetic information or cell surface molecules. The possibility that some form of information exchange takes place in both the tetraparental mouse and bone marrow chimera, either by exchange of genetic information or by exchange of cell surface molecules, cannot as yet be excluded. However, in two tetraparental rabbit chimeras using Ig allotype markers, Munro and Gardner (personal communication) have found no information exchange.

Alternatively, the ability of chimeric or tetraparental B and T cells to interact effectively may reflect a specificity of antigen recognition determined at the time of primary immunization by the specific combination of antigen and cell surface molecules (e.g., *H-2, I*) in a manner analogous to the specificity of cytotoxic T cells induced by virus-infected allogeneic cells (36, 37). It is not clear whether T cells intrinsically have the ability to recognize such antigen-cell surface complexes, or whether the recognition capacity of T cells is altered as a function of the in situ immunization process. Experiments are in progress to resolve the above issues.

Summary

To test whether the antigen-specific stimulation of low responder-genotype B cells in tetraparental mice is due to a histoincompatibility reaction (allogeneic effect) against these B cells, tetraparental mice were constructed *(a)* between an *Ir-1A* low responder to the antigen poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys. [(T,G)-A--L] and an *Ir-1A* F_1 high responder and *(b)* between two histoincompatible *Ir-1A* low responders. In the first case the F_1 high responder embryo shares the whole of the *H-2* complex, including *Ir*, with the low responder embryo.

Under these genetic conditions there should be no allogeneic effect against the low responder B cells. Nevertheless, high titers of specific anti-(T,G)-A--L antibody of the Ig allotype of both of the input embryos were produced. In the histoincompatible combination between two low responders, if there were sufficient histoincompatibility reaction in tetraparental mice to cause an increase in anti-(T,G)-A--L response, some of these mice would have been expected to produce increased responses. All of these tetraparental mice produced low responses. In addition, normal high and low responder-genotype mice were immunized simultaneously with an antigen to which they were low responders and an antigen to which they were high responders. There was no evidence that a high response to (T,G)-A--L could nonspecifically stimulate a simultaneous, unrelated low response, or that simultaneous but unrelated high response could nonspecifically stimulate a low response to (T,G)-A--L. In conclusion, it was not possible to demonstrate an enhancing effect of any potential histoincompatibility reaction on the low response of cells to (T,G)-A--L. Under genetic conditions where the potential for such a histoincompatibility reaction should not exist, both high and low responder genotype B cells respond to stimulation equally.

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