Analysis of Anti-(T,G)-A--L Antibody in Tetraparental Mice

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THE production of a high-titered antibody response in mammals requires the interaction of at least two antigen-specific lymphoid cell types. It is quite likely that such a complex multicellular process is under regulation by one or more genes. Although the multideterminant nature of most antigens obscures the genetic controls which probably exist, there are now a number of antigens for which a genetic control over the immune response has been reported. For many, but not all, of the antigens the response is controlled by a single, dominant, autosomal gene linked to the major histocompatibility locus of the particular species.[1] One such immune response gene is the Ir-l gene in mice[1] which controls the humoral response to branched, multichain, synthetic polypeptides such as the copolymer of tyrosine, glutamic acid, alanine, and lysine known as (T,G)-A--L. The Ir-1 gene maps in the center of the murine histocompatibility complex (H-2) lying between the Ss-slp locus and the left-hand (H-2K) region.[2]

A large amount of data has been amassed which suggests that the Ir-1 gene is expressed in thymus-derived lymphocytes (T-cells).[1a] The experiments carried out thus far have not been able to differentiate directly between two possibilities: (1) whether Ir-1 is expressed exclusively in T cells, or (2) whether Ir-1 also is expressed in bone marrow-derived lymphocytes (B cells) as well as in T cells. The following study was undertaken to examine this question directly. Since high and low responders necessarily must differ at H-2 due to the linkage of Ir-1 to H-2, some method had to be found to allow T cells and B cells to coexist for a sufficient period of time to interact. Initially, radiation chimeras, produced by transfer of fetal liver into lethally irradiated adult animals were examined as a possible system.[3] These animals were not satisfactory since they were found to be largely nonchimeric in the lymphoid system. However, tetraparental (allophenic) mice provide an ideal environment for the required cell-cell interaction since the cells from the two input strains are operationally compatible and good mixes are produced with a reasonable frequency.

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MATERIALS AND METHODS

Mice

Experimental mice of both sexes were taken from strains maintained in our mouse colony: C3H/DiSn (H-$2^{k/k}$, Ir-$1^{low/low}$, $Ig^{a/a}$), CWB/13 Hz (H-$2^{b/b}$, Ir-$1^{high/high}$, $Ig^{b/b}$),[4] and C3H.SW/SnHz (H-$2^{b/b}$, Ir-$1^{high/high}$, $Ig^{a/a}$). The production of tetraparental mice has been detailed elsewhere.[5]

Antigens, Immunization Procedures, and Antibody Determinations

Previous publications from this laboratory have described immunization with (T,G)-A--L in complete Freund's adjuvant (CFA) or phosphate buffered isotonic saline (PBS); the collection of heparinized serum; and antibody determinations using rabbit anti-mouse gamma-globulin sera and radio-iodinated (T,G)-A--L in a modified Farr assay. Antibody titers are expressed as per cent antigen bound at a particular serum dilution.

Allotype Determinations for Total Serum IgG_{2a}

The inhibition of precipitation method of Herzenberg and Herzenberg was used.[6]

Preparation and Use of Antiallotype Columns

Cyanogen bromide-activated Sepharose was prepared according to the method of Cuatrecasus, Wilcheck, and Anfinsen.[7] The 50% saturated ammonium sulfate-precipitated globulin fraction from a polyvalent anti-Ig^a antiserum, containing antibodies against *a* alleles on IgG_1 ($Ig\text{-}4^a$), IgG_{2a} ($Ig\text{-}1^a$), and IgG_{2b} ($Ig\text{-}3^a$), was dissolved in 0.1 *M* $NaHCO_3$ (pH 9) and added to an equivalent amount of activated Sepharose.[6] At the completion of the binding reaction the coupled resin was washed with Ig^b normal mouse serum (NMS).

A 1.0-ml bed volume column was prewashed with 0.1 *M* HOAc buffer and then was equilibrated with a pH 7.6 tris-HCl buffer [0.05 *M* tris (*tris*-hydroxymethylaminomethane), 5% normal rabbit serum (NRS), 3% bovine serum albumin (BSA), and 5 × 10^{-5}% pentachlorophenol (PCP)]. A 10 μl aliquot of the serum to be fractionated was added to the column and elution was begun with the tris buffer; four 2 ml fractions were collected. These were the "passed" fractions containing Ig^b. Next, four 2 ml fractions were collected using a pH 3.1 buffer (0.1M HOAc, 1% NRS, and 5 × 10^{-5}% PCP in isotonic saline). These were the "acid-eluted" fractions containing Ig^a. The column was neutralized with PBS containing 1% NRS.

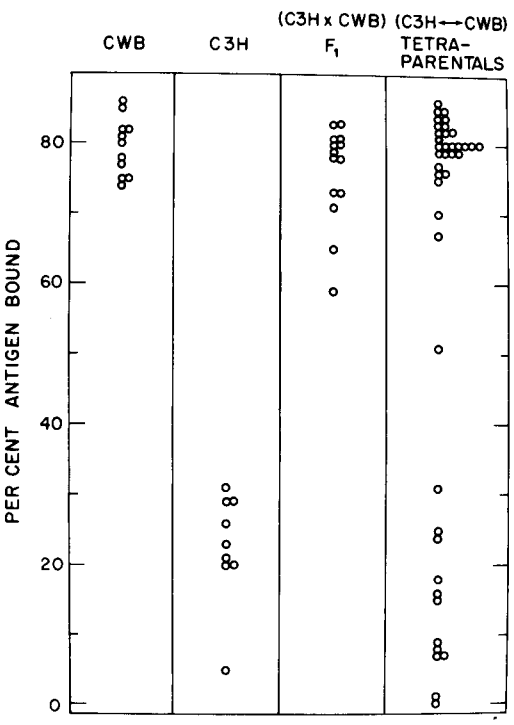

Fig. 1. Antibody response of tetraparental (C3H←→CWB), input strain (C3H, CWB), and F_1 (C3H × CWB) mice to immunization with (T,G)-A--L.

Antigen-binding Capacity (ABC) Determinations of Individual Columns Fractions

Individual fractions from the antiallotype column were diluted in 1/1000 NMS in twofold serial dilutions over a six- to eightfold range. The per cent antigen bound for each dilution was compared to a standard curve produced by an unfractionated 10 μl aliquot of the same serum. By this means a serum equivalent (in μl) was calculated for each fraction. The "passed" fractions were summed as were the "acid eluted" fractions. These sums were expressed as a per cent of the total recovered anti-(T,G)-A–L activity. In addition the overall recovery of antibody was calculated.

RESULTS

Forty-one C3H←→CWB tetraparental mice and suitable control animals were immunized with 10 μg (T,G)-A--L in CFA and boosted with an equivalent amount of antigen. The immune sera were examined at dilutions ranging from 1/50 to 1/12,500. The per cent antigen bound at a 1/500 dilution is given in Fig. 1.

Tetraparental mouse sera were examined for allotype distribution of the IgG_{2a} subclass (Ig-1 locus). Levels of $Ig\text{-}1^a$ and $Ig\text{-}1^b$ were determined quantitatively for each serum and then plotted against the (T,G)-A–L binding capacity of individual sera in Fig. 2. At both ends of the allotype distribution there are clusters of animals: Animals which have predominantly $Ig\text{-}1^b$ are high responders while animals which have predominantly $Ig\text{-}1^a$ are low. However, there are some animals (Nos. 404, 405, 410, and 423) which have significant amounts of $Ig\text{-}1^a$ but are still high responders.

Although the anti-Ig^a antiserum used in the preparation of the affinity chromatography columns had been characterized by precipitation methods,[6] it was necessary to show that, when bound to Sepharose, it

retained sufficient activity to separate quantitatively anti-(T,G)-A--L antibodies in the amounts required for the binding assay. For this reason artificial mixtures of high-titered antisera from CSW (Ig^a) and CWB (Ig^b) mice were separated on the column. The results presented in Fig. 3 show that the column gives nearly quantitative and specific separation over the entire mixture range from 0% to 100% Ig^a anti-(T,G)-A--L.

Eleven sera from selected mice were fractionated on the anti-*a* allotype column. The allotype distribution for the specific anti-(T,G)-A--L antibodies was determined and is given in Table 1. In addition, the Ig^a anti-(T,G)-A--L activity level in some of the sera was compared with an average value determined from the three highest-responding C3H (low responder) mice in the experiment. These results are also given in Table 1. Note that for higher responder animals the recovery of anti-(T,G)-A--L activity from the affinity chromatography column is essentially quantitative.

DISCUSSION

Tetraparental mice are formed by the fusion of two eight-cell cleavage-stage embryos; the resultant mosaic embryo is cultured, in vitro for 28 hr during which time embryogenesis proceeds to the late morula or early blastula stage. The embryo is then transplanted into a pseudopregnant foster mother; tetraparental mice are born 17 days later.

The strains (C3H and CWB) chosen to make the tetraparental mice are congenic except at two loci: the H-2 complex (including Ir-1) and the Immunoglobulin (Ig) locus. The Ir-1 difference causes CWB to be a high responder to (T,G)-A--L while C3H is a low responder. The immunoglobulin allotype difference permits the convenient identification of the anti-(T,G)-A--L antibodies produced by B cells.[8] The absences of differences at all other loci minimizes the chance of extraneous genetic effects and simplifies the timing of embryos during the production of the tetraparental mice.

Using congenic tetraparental mice as a model system we wished to examine directly two opposing hypotheses for Ir-1: (1) the Ir-1 gene is expressed only in T cells; (2) the Ir-1 gene is expressed in both B cells and T cells. If the Ir-1 gene is expressed only in T cells, then high responder T cells, upon antigenic stimulation, should be able to interact with both high and low responder B cells due to the unique environment of the tetraparental mouse. This would cause both types of B cells — high and low responder alike — to produce a high titered anti-(T,G)-A--L response. If, on the other hand, Ir-1 is expressed in both T cells and B cells, then high responder T cells could react only with high responder B cells because low responder B cells would

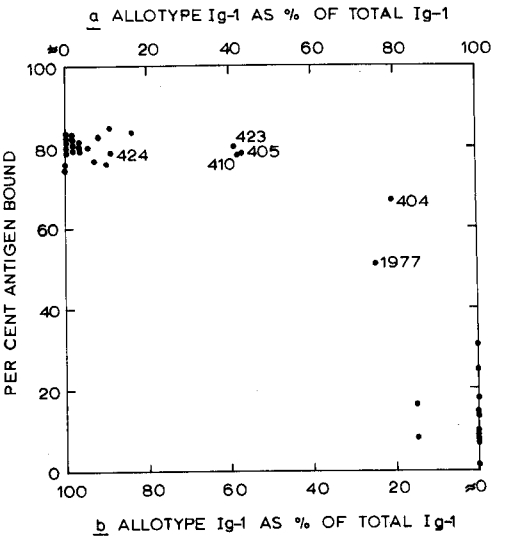

Fig. 2. Correlation of (T,G)-A--L binding capacity with the allotype distribution of total serum IgG_{2a} (Ig-1) for C3H⟷CWB tetraparental mice. Each point represents an individual animal. The numbers beside some of the points refer to tetraparental mouse numbers used in the text.

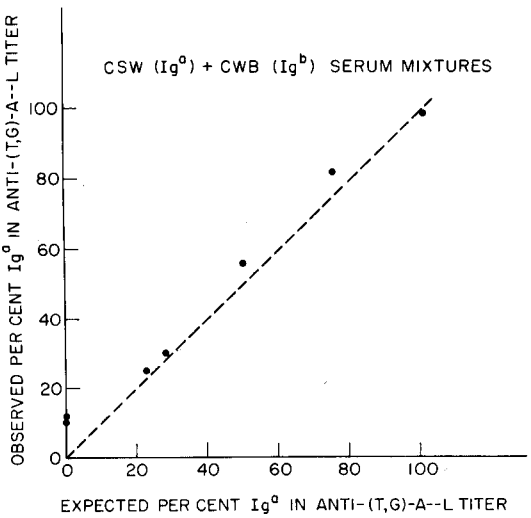

Fig. 3. Separation of artificial mixtures of CSW (Ig^a) and CWB (Ig^b) serum on the anti-Ig^a column. The dotted line represents the curve for perfect separation in all cases.

not be functional. This would lead to the production of anti-(T,G)-A--L antibodies which are exclusively of high responder allotype.

Examination of the allotype distribution of the specific anti-(T,G)-A--L antibodies, as given in Table 1, shows that high responder tetraparental mice tend to make predominantly Ig^b anti-(T,G)-A--L. However, in several cases (animals Nos. 424, 405, and 423) the Ig^a anti-(T,G)-A--L component was a significant part of the response, and in the case of animal No. 404 more Ig^a anti-(T,G)-A--L was produced than Ig^b. The anti-(T,G)-A--L response of the intermediate animal, No. 1977, which was about seven times higher than the C3H titer, was totally made up of the low responder allotype (Ig^a).

The two low responder tetraparental mice (Nos. 1980 and 421) produced approximately the same activity level of Ig^a anti-(T,G)-A--L as did the C3H low responders. (See Table 1.) The intermediate animal, No. 1977, produced about seven times the Ig^a anti-(T,G)-A--L antigen binding capacity (ABC) of a low responder. For those tetraparental mice with less than *ca.* 20% Ig^a anti-(T,G)-A--L activity, it was not possible to compare the Ig^a ABC with that of the C3H standard because the affinity chromatography column did not give totally quantitative separation for sera with less than 20% Ig^a. However, the high responder tetraparental mice (Nos. 424, 405, 423, and 404), for which the

Table 1. Allotype Distribution and Antigen Binding Capacity of Specific Anti-(T, G)-A--L Antibodies From C3H ⟷ CWB Tetraparental Mice*

Tetraparental Mouse (No.)	Anti-(T, G)-A--L Activity			Ratio of Iga ABC to Intact C3H ABC (All Iga)†	Per Cent (T, G)-A--L Bound at 1/500
	Per Cent Iga	Per Cent Igb	Per Cent Recovery		
410	12	88	88	—	79
407	14	86	95	—	80
402	15	85	99	—	70
414	19	81	101	—	77
424	27	73	100	82	79
405	33	67	96	75	79
423	36	64	96	31	80
404	64	36	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.

†ABC, antigen-binding capacity.

column separation was quantitative, produced considerably more anti-(T,G)-A--L activity than did the intact C3H animals. This occurred in spite of the fact that the Ig^a usually did not constitute the major portion of their response.

From these data we conclude the following: (1) In tetraparental mice B cells of C3H (low responder) origin produce more specific anti-(T,G)-A--L antibody than do low responder B cells in an intact C3H animal. (2) The low responder-type antibody constitutes a significant, and occasionally major, portion of the anti-(T,G)-A-L response in these animals. These data strongly support the hypothesis that the Ir-1 gene operates solely in T cells. This conclusion is valid barring nonspecific stimulation of low responder B cells,[9] which could arise from the residual graft-vs.-graft reaction, which may exist in tetraparental mice.[10] We are currently producing tetraparental mice from the strain C3H.Q ($H\text{-}2^{q/q}$, $Ir\text{-}1^{low/low}$, $Ig^{a/a}$) and CKB ($H\text{-}2^{k/k}$, $Ir\text{-}1^{low/low}$, and $Ig^{b/b}$) as control to test this possibility.

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