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Analysis of Cell Transfer Studies in a Genetic Control of the Immune Response in Mice [1]

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I. Introduction

Genetic control of specific immune responsiveness has been reported for a number of different antigens [for review, see 6]. In two of these systems, cell transfer studies have shown that these genetic controls are an integral part of the specific immune response, but it has not yet been possible to unravel the mechanism of genetic control [6].

One particularly intriguing aspect of these controls is their relationship to histocompatibility antigens. Following the initial observation that genetic control of the immune response to branched synthetic polypeptide antigens in mice is due to a gene closely linked to, or identical with, the major histocompatibility *(H-2)* locus in the mouse [8], there have been several other reports linking genes controlling specific immune responses with genes coding for histocompatibility antigens. The immune response of inbred mice to wild type erythrocyte antigens is closely linked to the *H-3* and *H-6* loci in the fifth mouse linkage group [3]; the response of mice to the trinitrophenyl hapten is linked to the *H-2* locus [12]; the ability of inbred mice to respond to low doses of bovine γ-globulin and ovomucoid appears to be linked to the *H-2* locus [16]; and the ability of guinea pigs to respond to hapten poly-L-lysine conjugates has been shown to be closely linked to a major histocompatibility locus in the guinea pig [2].

From these results, it seems clear that the specific immune response to a wide variety of simple and complex antigens is under direct genetic control by genes which are expressed in immunocompetent cells, act as integral parts of the immune response, and in many cases are closely linked to, or identical with, histocompatibility antigens. We are thus faced with two related problems – the mechanism of this type of genetic control, and the role (if any) of histocompatibility antigens in this type of genetic control. The studies reported in this paper are part of a continuing analysis of the genetic control of specific immune responses in mice by means of cell transfer techniques.

II. Characteristics of the Genetic Control of Immune Response to Branched Synthetic Polypeptide Antigens in Inbred Mice

The antigens used in these studies are branched synthetic polypeptide antigens

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containing a backbone of poly-L-lysine with side chains of poly-D,L-alanine. On the tips of these side chains, there are a few further residues of tyrosine and glutamic acid [(T,G)-A--L], or histidine and glutamic acid [(H,G)-A--L], or phenylalanine and glutamic acid [(Phe,G)-A--L] [7]. These antigens elicit immune responses directed primarily against the terminal amino acid residues of each side chain, i.e., against tyrosine, glutamic acid and alanine in the case of (T,G)-A--L. Previous studies [cited in 6] have shown that the ability of inbred mice to respond to this type of antigen is a quantitative genetic trait in which responder strains have an approximately tenfold higher antibody response than low-responder (or non-responder) strains.

The major differences between responder and non-responder strains is due to a gene locus termed *Ir-1 (immune response-1)* which is closely linked or identical to the K (right-hand) part of the *H-2* (histocompatibility) locus in the ninth mouse linkage group. Several alleles exist allowing different antigen-specific responses to (T,G)-A--L, (H,G)-A--L and (Phe,G)-A--L. For example, all $H\text{-}2^b$ strains are high responders to (T,G)-A--L, low responders to (H,G)-A--L, and high responders to (Phe,G)-A--L. The response to these three antigens is well-correlated with *H-2* type among the inbred mouse strains. Non-inbred or wild mice have not yet been examined.

The exact position of the *Ir-1* locus with respect to the *H-2* locus is obviously a matter of considerable importance. Analysis of the immune response of several inbred strains carrying recombinant *H-2* alleles, derived from known crossovers between two different *H-2* alleles, has indicated that *Ir-1* lies in the middle of the *H-2* locus between the gene coding for *Ss* (Serum substance) and the K region of the *H-2* locus [6]. This result requires verification by genetic mapping studies, and a mapping study utilizing four loci in the ninth mouse linkage group is currently under way, but the final results are not yet available.

Cell transfer studies have shown that the *Ir-1* gene is expressed in immunocompetent cells and is an integral part of the immune response. It is possible to transfer responsiveness from responder strains into non-responder strains by the transfer of adult spleen cells, fetal liver cells, or highly purified peripheral blood lymphocytes. Attempts to transfer responsiveness with macrophages and with thoracic duct lymphocytes have so far been unsuccessful, although both of these latter experiments suffer from technical difficulties. There is considerable evidence [10] that the (Phe,G)-A--L high-responder allele of the *Ir-1* gene, when present, markedly affects the specificity of the antibodies produced. However, it is not clear whether this effect on specificity is mediated through an effect on antigen processing or antigen recognition, or on the structure of the antibody-combining site.

In all of the studies with these antigens in mice, primary immunization is carried out with antigen emulsified in complete Freund's adjuvant and secondary stimulation is carried out with aqueous antigen solutions. It should be pointed out that the major phenotypic difference between responder and non-responder animals appears in the secondary response to an injection of aqueous antigen. Prior to secondary stimulation, the antibody titers of the two strains are nearly equal and overlapping but, following secondary stimulation, the responder strain shows a prompt and sharp rise in antibody titer while the non-responder strain shows little change in titer.

In summary, the *Ir-1* locus alleles are autosomal dominants which recognize the amino acid composition of the antigen determinants; affect the specificities of the antibodies produced; are closely linked to,

or within the *H-2* locus; and are expressed in immunocompetent cells. In initial attempts to analyze the mechanism of gene action, it was found that *Ir-1* was not linked to the mouse *Ig-1* through *4* (immunoglobulin allotype) region [4], and was not due to an inability to synthesize specific antibody of a particular immunoglobulin class.

Further attempts to analyze the mechanism of gene action led to the finding that the difference between responder and non-responder animals could be abrogated by immunizing and boosting non-responder strains with (T,G)-A--L electrostatically complexed to methylated bovine serum albumin (BSA). This remarkable effect occurred only if both the primary and secondary immunizations were carried out with (T,G)-A--L complexed to methylated-BSA. This result implies that the non-responder strain has the genetic ability to produce anti-(T,G)-A--L, but is defective in its ability to recognize (T,G)-A--L as a foreign antigenic determinant unless it is attached to an immunogenic carrier. In the absence of other clues, this result suggests that the *Ir-1* gene affects the recognition of the antigen in some manner that may be related to histocompatibility antigens, but is *not* related to immunoglobulin structural genes. The latter statement is derived from the finding that *Ir-1* is not linked to the mouse immunoglobulin allotypes (located on the H-chain constant regions) and the recent finding of PRAHL *et al.* [11] that the variable and constant regions of rabbit heavy chains are linked. There are, however, several other possible mechanisms of gene action which require investigation.

III. Possible Mechanisms of Genetic Control of the Immune Response

The evidence presently available suggests that *Ir-1* acts in some type of lymphocyte [14]. The results of immunization with (T,G)-A--L complexed with methylated-BSA suggest that the defect in non-responder strains is a failure of recognition of (T,G)-A--L as a foreign antigen, rather than a genetic inability to produce specific anti-(T,G)-A--L antibody. The *Ir-1* locus (as well as several other genes controlling the immune response) is very closely linked to the *H-2* locus. Finally, the immune response to (T,G)-A--L is thymus-dependent [15]. These four observations, taken together with the other characteristics of the *Ir-1* gene already described, permit us to select certain possible mechanisms of gene action as more likely than others.

A. Genetic Control of Antigen Recognition at the Level of the Antigen-Reactive Cell

Almost all of the presently available experimental data fit with the hypothesis that the non-responder strains are deficient in thymus-derived antigen-reactive cells capable of recognizing (T,G)-A--L. (The only experimental finding which is at odds with this hypothesis is the finding of TYAN and MCDEVITT [14] that large numbers of responder thoracic duct lymphocytes fail to reconstitute thymectomized irradiated non-responders. Since it is possible that allogeneic inhibition, or a similar phenomenon, decreased the survival of the injected thoracic duct lymphocytes, this result is open to some technical criticism.) There are several possible ways in which the *Ir-1* gene might be related to histocompatibility antigens:

(1) The *Ir-1* gene effect might be due to a non-specific, positive effect of certain *H-2* antigen specificities on the cell membrane. These specificities might facilitate the interaction of cell receptors with particular antigenic determinants in a non-specific way.

(2) *Ir-1* could code for a separate non-*H-2*

membrane antigen, perhaps present in only certain cell types, such as thymocytes, in a manner similar to the thymus-leukemia antigen locus (TLa locus) [1] which is adjacent to the D region of the *H-2* locus.
(3) It is conceivable that the *Ir-1* effect is due to a new immunoglobulin class, restricted to cell membranes, and responsible for antigenic recognition. This suggestion is analogous to the postulated IgX recognition antibody already suggested by others. On the basis of the present findings, there is no particular reason to postulate this type of gene action, nor is there any way to exclude it.
(4) Since linkage of the genetic controls of the immune response with histocompatibility loci now appears to be a general phenomenon, the possibility that *Ir-1* gene effects are, in fact, due to a type of 'cross-tolerance' between self antigens and the immunizing antigen must be considered. This possibility will be discussed in more detail below.

B. Genetic Control of Variable Region Structure

The other major category of possible mechanisms of gene action is that the *Ir-1* gene represents genetic differences in the structure of antibody-combining sites which reflect genetic differences in immunoglobulin variable region genes. As already mentioned above, any direct inheritance of different types of heavy chain variable region genes seems to be excluded by the finding that the *Ir-1* locus is not linked to the mouse immunoglobulin allotype locus. This does not exclude a genetic control of light chain immunoglobulin variable region genes, but this does not at the present seem likely. On the other hand, a possible mechanism by which histocompatibility antigens might strongly influence the range and specificity of immunoglobulin variable regions generated by a particular individual or inbred strain has been postulated by JERNE [5].
If we can accept the four or five major possible mechanisms of gene action listed above as the most likely ones, it is then possible to design experiments which can differentiate at least some of these possibilities. In particular, there are two hypotheses which can be tested in the same experimental system, and which will be discussed separately.
The first hypothesis states that, if the *Ir-1* gene affects specificity of recognition by an antigen-reactive cell, then specific anti-(T,G)-A--L antibody in a stable responder-non-responder chimera should be of both responder and non-responder allotype. This hypothesis again derives from the finding that non-responder animals respond well when immunized with (T,G)-A--L complexed with methylated-BSA – a result which suggests that non-responders are capable of making anti-(T,G)-A--L antibody. This experiment would not differentiate a genetic effect on antibody specificity due to an indirect effect of *H-2* antigens on the sequences of V-region genes generated by a mechanism such as that postulated by JERNE. However, it would test direct control by *Ir-1* of immunoglobulin variable region structure. The most critical point of such an experiment is that any theory which localizes the *Ir-1* gene to the thymus-derived, antigen-reactive cell would require that the hypothesis in the form stated above be correct and that the specific anti-(T,G)-A--L antibody in a chimera be of both immunoglobulin allotypes. The possible exceptions to this statement would be: (i) a situation in which *H-2* antigens or *H-2* related antigens affect the recognition of foreign antigens at *both* the antigen-reactive cell and the antibody-producing cell level; and (ii) if it could be demonstrated that cooperation between thymus-derived antigen-reactive cells and bone marrow-derived antibody-producing cells of different *H-2* types does not occur.

The second hypothesis states that, if 'cross-tolerance' is a cause of genetic unresponsiveness, all mutually tolerant chimeras should be non-responders. This hypothesis requires testing because of the increasing number of genes controlling the immune response which are linked to histocompatibility antigens. The 'cross-tolerance' mechanism of gene action postulates that a strain which responds poorly to (T,G)-A--L does so because its own histocompatibility antigens cross-react with (T,G)-A--L and induce tolerance to the major antigenic determinants on this antigen. There is considerable experimental evidence against this hypothesis. First, responsiveness is a dominant trait in the F_1, although all the available evidence indicates that the F_1 possesses all the transplantation antigens of both parental strains. Second, when responder fetal liver is injected into an irradiated non-responder and allowed to mature, the resultant chimera is a responder. These two observations can be challenged: the first, on the grounds that there are as yet undetected recessive transplantation antigens, or alterations of transplantation antigens in the heterozygous state; and the second, on the grounds that the chimeras produced by the injection of responder fetal liver into irradiated non-responders are not mutually tolerant but are, in fact, undergoing a chronic graft-versus-host (GVH) reaction. The latter objection is susceptible to direct experimental test.

IV. The Analysis of Responder Chimeras Produced by the Injection of Fetal Liver Cells into Irradiated Recipients

In order to test the two hypotheses described above, it is necessary to produce stable radiation chimeras between homozygous responder and non-responder inbred strains. Responder F_1 into parental non-responder transfer systems cannot be utilized because responder F_1 mice produce anti-(T,G)-A--L antibody of both immunoglobulin allotypes, and because the use of F_1 cells does not permit a critical test of the hypothesis of 'cross-tolerance' as it is stated above. Since previous experiments [15] had shown that it was possible to produce responder chimeras by the injection of responder fetal liver cells into lethally irradiated non-responder recipients, *or* by the injection of non-responder fetal liver cells into lethally irradiated responder recipients, both types of chimeras were produced. Ten to 100 million CBA (non-responder) fetal liver cells were injected into lethally irradiated adult C57BL/10 (responder) mice or, alternatively, 10 to 100 million C57BL/10 fetal liver cells were injected into lethally irradiated adult CBA recipients. It was felt particularly desirable to utilize chimeras created by the injection of non-responder fetal liver cells into irradiated responder recipients, since this would presumably be the situation which would be most favorable for the detection of significant amounts of specific anti-(T,G)-A--L antibody of non-responder allotype. Large numbers of such stable radiation chimeras were produced, and 2½ to 5 months after cell transfer and irradiation, the surviving chimeras were immunized with (T,G)-A--L in complete Freund's adjuvant, followed by a second injection of (T,G)-A--L in aqueous solution, in the usual manner. Following immunization, the chimeric mice were tested for: (i) antibody response to (T,G)-A--L; (ii) quantitative immunoglobulin allotype levels or amounts of CBA and C57BL/10 γG_1- and γG_{2a}-globulin; (iii) the immunoglobulin allotype of specific anti-(T,G)-A--L antibody; (iv) the presence of CBA and C57BL/10 immunocompetent cells; and (v) the presence or absence of GVH-reactivity of CBA and C57BL/10 immunocompetent cells for *H-2* antigens of the opposite type.

Antibody response to (T,G)-A--L was determined by an antigen-binding assay [6], and quantitative immunoglobulin levels for γG_1 and γG_{2a} immunoglobulin were determined by inhibition of binding of labeled myeloma proteins of the respective allotypes by anti-allotype sera [for review, see reference 4].

It should be pointed out that, in an earlier publication [13], a similar study of the allotype of specific anti-(T,G)-A--L antibody in radiation chimeras was reported. However, subsequent studies revealed that the method of determining the allotype of specific anti-(T,G)-A--L antibody suffers from a serious methodological defect. For this reason, a new method of determining specific anti-(T,G)-A--L allotype was developed and subjected to critical testing. In this method, the highest dilution of chimeric anti-(T,G)-A--L antiserum which will still bind 50% of labeled anti-(T,G)-A--L in our standard antigen-binding assay is first incubated with an excess of mouse anti-allotype antiserum. The anti-allotype antiserum used was a pool containing antibody against γG_1 (*Ig-4* locus) and γG_{2a} (*Ig-1* locus) immunoglobulins of CBA or C57BL type.

Following incubation of anti-(T,G)-A--L antiserum with anti-allotype antiserum, the mixture was centrifuged and the supernatant was then removed and titered for anti-(T,G)-A--L antigen-binding capacity by the usual method. This method is reliable and reproducible, but it suffers from several defects. Because of difficulties in producing adequate amounts of antiserum against γG_{2b}-immunoglobulin allotype specificities, the method does not detect specific anti-(T,G)-A--L antibody in this immunoglobulin class. Second, the method is only practical with relatively high-titered antisera against (T,G)-A--L. With sera of lower titer, which must be tested at lower dilutions, large amounts of anti-allotype antiserum are required, and these antisera (of murine origin) interfere with the subsequent assay of anti-(T,G)-A--L antigen-binding capacity.

In studies using artificial mixtures of CBA and C57BL anti-(T,G)-A--L antisera, it was found that the lower limit of detectability of CBA anti-(T,G)-A--L antibody in such a mixture was 25%. As little as 10 to 20% of C57BL type anti-(T,G)-A--L antibody can be detected in artificial mixtures, reflecting the higher titers of mouse antisera against C57BL allotypes. This level of detectability for CBA type anti-(T,G)-A--L antibody is acceptable because it must be pointed out that chimeras created by the transfer of CBA fetal livers into irradiated CBA recipients make readily detectable amounts of anti-(T,G)-A--L antibody, and, for the purposes of this experiment, amounts of CBA antibody greater than 25% and preferably as high as 50 to 75% would be required for a critical test of the starting hypothesis.

Chimeras were tested for the presence of CBA and C57BL immunocompetent cells by injecting 15 to 30 million chimeric spleen and lymph node cells into 8-day-old $(C57BL \times BALB/c)F_1$ and 8-day-old $(CBA \times BALB/c)F_1$ recipients [12]. The relative spleen index was determined by comparing spleen weights per 10 g body weight of each recipient with uninjected, syngeneic-injected, and allogeneic-injected litter mate controls. The presence of graft-versus-host and host-versus-graft reactivity was determined by injecting 15 to 30 million chimeric spleen and lymph node cells into newborn CBA and C57BL recipients. Again, relative spleen indices were determined by comparing spleen weights per 10 g body weight of each recipient with those of uninjected, syngeneic-injected, and allogeneic-injected litter mate controls.

Table I presents a summary of the findings in 19 non-responder chimeras of both types. Immunoglobulin levels of CBA and C57BL type are reported as averages of the percentage of values obtained for γG_1-

and γG_{2a}-classes in control animals receiving immunizations with (T,G)-A--L in complete Freund's adjuvant and in saline similar to the chimeras. In general, radiation chimeras tend to have more immunoglobulins of the type derived from the fetal liver cell donor. In addition, it should be noted that the non-responder chimeras have relatively low levels of C57BL immunoglobulins; 26% of control in the case of CBA into C57BL/10 chimeras, and 51% in the case of C57BL/10 into CBA chimeras. In both types of chimeras, one or two animals had immunoglobulin levels near the controls and were still non-responders, but for the most part the non-responder chimeras tended to have levels of C57BL immunoglobulin that were considerably below the control levels. Most of the animals tested in the SIMSENON discriminant spleen assay had detectable C57BL cells.

Table II presents the results in responder chimeras. Several points emerge from this table:

(1) Responder chimeras, whether produced by the injection of CBA cells into C57BL recipients or C57BL cells into CBA recipients, almost uniformly have levels of C57BL immunoglobulins near control

Table I. Non-responder chimeras[1]

	CBA		C57BL/10	
	Ig	cells	Ig	cells
CBA →C57BL/10 (7 mice)	1–370% (av. 123%)	2 absent 5 n.t.	2–120% (av. 26%)	4 present 3 n.t.
C57BL/10 →CBA (12 mice)	1–10% (av. 3%)	2 present 3 absent 1 inc. 6 n.t.	1–165% (av. 51%)	9 present 3 absent

1 50% (T,G)-A–L-^{125}I binding titer < 1:250.

Table II. Responder chimeras[1]

	CBA		C57BL/10		Anti-(T,G)-A–L allotype
	Ig	cells	Ig	cells	
CBA →C57BL/10 (5 mice)	1–30% (av. 8%)	3 absent 2 inc.	55–200% (av. 80%)	2 present 1 absent 2 inc.	3 C57 2 titer too low
C57BL/10 →CBA (15 mice)	1–31% (av. 5%)	7 present 4 absent 4 inc.	25–216% (av. 85%)	12 present 3 n.t.	8 C57 7 titer too low

1 50% (T,G)-A–L-^{125}I binding titer < 1:250.

levels and very low levels of CBA immunoglobulins.

(2) In those animals with anti-(T,G)-A--L titers high enough for efficient determination of specific antibody allotype, all of the antibody that was detectable was of C57BL type and no antibody of CBA type was detectable.

(3) In general, analysis by the SIMONSEN discriminant spleen assay paralleled these results, but there were several exceptions in which animals contained readily detectable amounts of CBA or C57BL immunoglobulin but did not give positive results in the SIMONSEN discriminant spleen assay. This suggests either that these two assays are measuring different cell types, or that the SIMONSEN discriminant spleen assay is relatively insensitive. It is clear from these results that stable radiation chimeras produced by this method have a very decided tendency to evolve toward a predominance of immunoglobulin-producing cells of either CBA or C57BL type. Almost all of the animals studied had a very marked predominance of immunoglobulins from one of the two strains. This was, in general, true of the non-responder chimeras, which tended either to be predominantly CBA in immunoglobulin type, or to have predominantly C57BL immunoglobulins but in markedly reduced amount. This finding was even more marked in the responder chimeras, all of which had a marked predominance of C57BL immunoglobulins, and very low amounts of CBA immunoglobulins. Only two animals had values for CBA immunoglobulins above 10% of control levels. None of the other chimeras even approached a reasonable admixture of immunoglobulins of the two types.

These findings suggest that responsiveness requires the presence of a rather high proportion of C57BL immunocompetent cells. They also explain the basis for the responsiveness of chimeras created by the injection of CBA fetal liver cells into C57BL recipients. It is clear that animals of this type which are responder are able to respond on the basis of a nearly complete repopulation of their lymphoid tissue with C57BL host immunocompetent cells. Unfortunately, these results do not constitute a critical test of our first hypothesis, since all of the responder animals had a very marked preponderance of C57BL type immunoglobulins and there was, therefore, very little possibility of detecting CBA immunoglobulins in these animals. During the past year, we have tried several different methods for the production of stable chimeras with nearly equal admixtures of CBA and C57BL cells, but with very little success. It is possible that success might be obtained more readily in radiation chimeras produced in strains other than the C57BL, which is capable of mounting very violent histocompatibility reactions. Of the other available methods of producing stable chimeras, the most promising for the purposes of testing both of our hypotheses at the present seems to be the production of allophenic mice. Such experiments are currently under way.

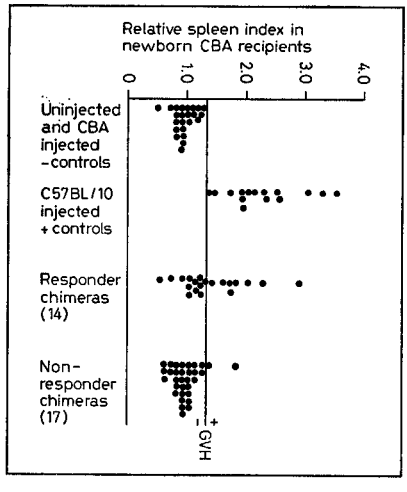

Figure 1. Relative spleen index in newborn CBA recipients injected with spleen cells from responder chimeras, non-responder chimeras, C57BL/10 positive controls and CBA negative controls.

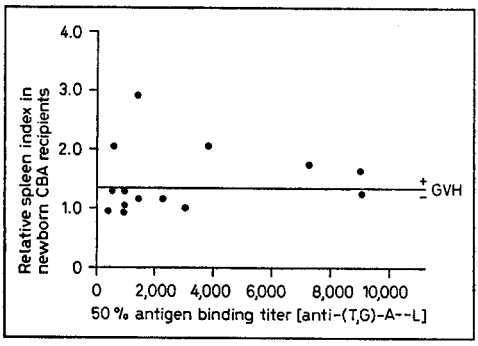

Figure 2. 50% antigen-binding anti-(T, G)-A--L titer in responder chimeras, versus relative spleen index in newborn CBA recipients injected with 20×10^6 spleen cells from these chimeras.

Figure 1 presents the relative spleen index in newborn CBA recipients injected with 15 to 30 million spleen and lymph node cells from CBA mice, C57BL mice, and responder and non-responder chimeras of both types. Cells from responder chimeras induced a GVH-reaction in newborn CBA in approximately half of the cases. Non-responder chimeras for the most part failed to induce any GVH-reactivity. These results indicate that, within the limits of sensitivity of this assay, numerous responder chimeras exist which lack any GVH-reactivity against CBA *H-2* antigens. The lower incidence of GVH-reactivity in non-responder chimeras may reflect a lower number of C57BL immunocompetent cells, as indicated by the immunoglobulin levels as well as a lower incidence of GVH-reactivity.

To further test the hypothesis that there is no correlation between the ability of a responder chimera to respond to (T,G)-A--L and the presence or absence of GVH-reactivity against CBA antigens, the relative spleen index in newborn CBA recipients was plotted against the 50% antigen-binding titer in the (T,G)-A--L antigen-binding assay. The results are presented in figure 2, which shows a complete lack of correlation between the presence or absence of GVH-reactivity and the relative titer of anti-(T,G)-A--L-binding activity. This confirms the conclusion that there is no relationship between the presence or absence of tolerance to CBA antigens and the ability to respond to (T,G)-A--L. Thus, within the limits of sensitivity of the GVH-assay, it would appear that responder chimeras exist in which the responder immunocompetent cells are completely tolerant of non-responder *H-2* antigens but are still capable of responding well to (T,G)-A--L. This evidence would exclude 'cross-tolerance' as a possible mechanism for a deficiency of thymus-derived antigen-reactive cells capable of recognizing (T,G)-A--L, or a possible deficiency of bone marrow-derived antibody-producing cells capable of producing antibody to (T,G)-A--L. It must be admitted that it is possible that minor degrees of histo-incompatibility could exist and not be detected by these experiments. This problem could be more critically tested in allophenic mice, which have been shown by MINTZ and SILVERS [9] to be completely tolerant of foreign *H-2* antigens as measured by permanent survival of allogeneic skin grafts.

V. Summary

Analysis of responder and non-responder chimeras produced by the injection of fetal liver cells into lethally irradiated adult recipients has shown that these chimeras usually have a marked predominance of immunoglobulins from one of the two strains, and that responder chimeras almost always have normal or near normal levels of responder immunoglobulins and very low levels of non-responder immunoglobulins. This suggests that responsiveness requires the presence of a large number of responder immunocompetent cells, and makes it difficult to test the hypothesis that the *Ir-1* gene in some way affects the recognition of antigen at the level of the thymus-derived antigen-reactive cell. On the other hand, analysis

of responder chimeras by the SIMONSEN discriminant spleen assay has shown no correlation between the ability to respond to (T,G)-A--L and the presence or absence of GVH-reactivity against CBA *H-2* antigens. Within the limits of this assay, this result excludes 'cross-tolerance' between *H-2* antigens and (T,G)-A--L as a possible mechanism of genetic control of the immune response.

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