GENETIC CONTROL OF THE IMMUNE RESPONSE

THE EFFECT OF GRAFT-*VERSUS*-HOST REACTION ON THE ANTIBODY RESPONSE TO POLY-L(TYR,GLU)-POLY-D,L-ALA--POLY-L-LYS IN NONRESPONDER MICE*

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The immune response of mice to the branched multichain synthetic polypeptide poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L][1] is under the control of a codominant gene, *Ir-1*, which maps near the middle of the major histocompatibility (*H-2*) complex (1). $H\text{-}2^{b/b}$ mice given a primary challenge of (T,G)-A--L in adjuvant and a secondary challenge of (T,G)-A--L in saline produce large amounts of anti-(T,G)-A--L antibody. In contrast, $H\text{-}2^{k/k}$ mice so immunized produce markedly lower amounts (2). Responses to many other antigens have also been shown to be controlled by immune response genes linked to *H-2* (3). Histocompatibility-linked immune responses have been demonstrated in other species as well. In guinea pigs, the ability to respond to poly-L-lysine (PLL) or haptens bound to PLL is linked to the guinea pig major histocompatibility locus (4). Nonresponder strain guinea pigs can be made to produce large amounts of anti-dinitrophenyl (DNP)-PLL antibodies, if they are challenged with DNP-PLL electrostatically coupled to foreign albumin carriers (5). Nonresponder animals first made tolerant to bovine serum albumin (BSA) and then challenged with DNP-PLL-BSA are unable to synthesize anti-DNP-PLL antibody. This indicates that nonresponder animals are capable of making anti-hapten antibody only when the hapten is attached to an immunologically recognizable carrier (6). These data can be interpreted as suggesting that the PLL gene regulates

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[1] *Abbreviations used in this paper:* B cell, bone marrow-derived antibody-producing cell; BGG, bovine gamma globulin; BSA, bovine serum albumin; DNP, dinitrophenyl; DNP-D-GL, DNP conjugate of the copolymer of D-glutamic acid and D-lysine; FCS, fetal calf serum; GVH, graft-*versus*-host; MBSA, methylated BSA; 2-Me, 2-mercaptoethanol; MEM, minimum essential medium; OVA, ovalbumin; PBS, phosphate-buffered saline; PLL, poly-L-lysine; T cell, thymus-derived lymphocyte; (T,G)-A--L, poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys.

antigen recognition, rather than the ability to synthesize a particular antibody specificity.

In analogous studies in mice, nonresponder mice immunized with (T,G)-A--L electrostatically complexed to the carrier methylated bovine serum albumin (MBSA) in adjuvant will produce anti-(T,G)-A--L antibody in amounts equal to responder mice immunized with (T,G)-A--L or MBSA-(T,G)-A--L in adjuvant (2). Hence nonresponders have the capacity to make the anti-(T,G)-A--L antibody, provided (T,G)-A--L is attached to a recognizable carrier. These data fit the hypothesis that the *Ir-1* gene is not expressed in the bone marrow-derived antibody-producing cell, or B cell, but instead is involved in antigen recognition.

Further evidence as to the nature of the *Ir-1* gene effect can be found if one uses an immunizing regimen of (T,G)-A--L in saline only. Here $H\text{-}2^{b/b}$ and $H\text{-}2^{k/k}$ mice produce an approximately equal IgM anti-(T,G)-A--L antibody response after primary challenge with (T,G)-A--L; however, upon secondary antigen challenge, $H\text{-}2^{b/b}$ mice will shift from IgM to IgG antibody production, while $H\text{-}2^{k/k}$ mice will neither shift nor produce another peak of IgM anti-(T,G)-A--L antibody (7). This suggests that the *Ir-1* gene affects the shift from IgM to IgG production. This could involve an actual switching of IgM-producing cells to IgG production, or could involve activation of a population of antibody-producing precursor cells committed to producing IgG. There are currently no data to distinguish between these two possible mechanisms.

It has also been demonstrated that thymectomized $H\text{-}2^{b/b}$ mice are functional nonresponders, that is, they have an intact IgM response after primary challenge with (T,G)-A--L in saline, but they will not shift from IgM to IgG production after secondary challenge with (T,G)-A--L in saline (8). Since thymectomy converts a responder into a phenotypic nonresponder, it appears likely that the *Ir-1* gene is expressed at the level of the thymus-derived antigen-reactive lymphocyte, or T cell.

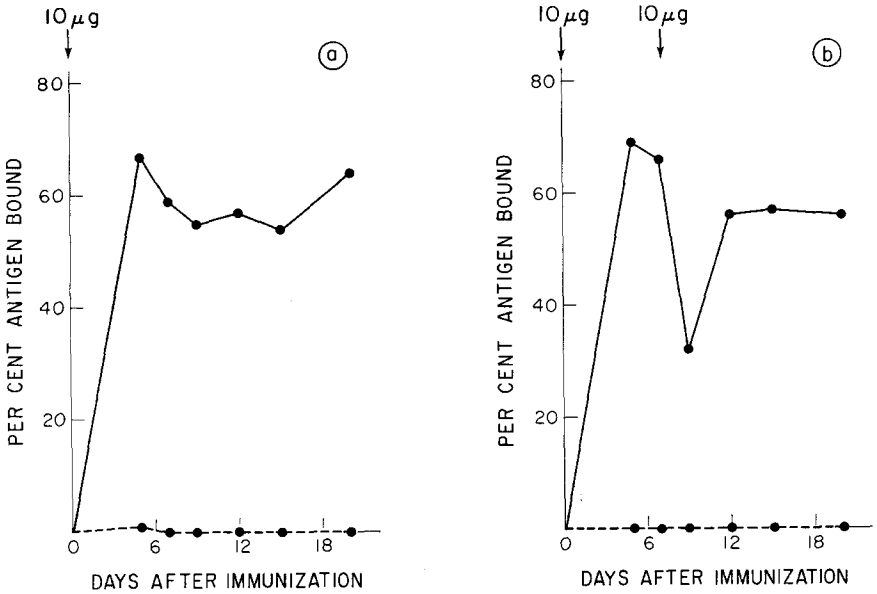
From the preceding data two hypotheses can be made. First, in a responder, challenge with (T,G)-A--L activates T cells, which then influence B cells, causing a shift from IgM to IgG anti-(T,G)-A--L antibody production. In a nonresponder no such T cell activation occurs, and consequently this shift does not occur. If one assumes the effect of activated T cells on B cells to be nonspecific, a second hypothesis can be made, namely, that activation of T cells in nonresponder mice by a process other than challenge with (T,G)-A--L may cause the shift from IgM to IgG production. One method of inducing T cell activation is by means of a graft-*versus*-host (GVH) reaction (9). GVH reactions have been used in certain systems to substitute for carrier-reactive T cells in the generation of a secondary antibody response. This has been accomplished in guinea pigs through the transfer into DNP-ovalbumin (OVA)-primed recipients of allogeneic or semiallogeneic cells (10, 11). In mice an allogeneic cell transfer has been used to activate a clone of anti-DNP antibody-producing cells (12).

The present report describes the results of inducing a GVH reaction in (T,G)-A--L nonresponder mice at the time of challenge with aqueous (T,G)-A--L. Consistent with the stated hypotheses, GVH induction was associated with the production of IgG anti-(T,G)-A--L antibody in the absence of the responder *Ir-1* allele.

Materials and Methods

Mice.—C3H.Q mice were kindly provided by Dr. Donald C. Shreffler (Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, Mich.). C3H/DiSn and (C3H.Q × C3H/DiSn)F_1 mice were bred at Stanford. The C3H.Q strain is congenic with the C3H/HeJ strain, C3H.Q being $H\text{-}2^{q/q}$ and C3H/HeJ being $H\text{-}2^{k/k}$. The C3H/HeJ and C3H/DiSn strains are closely related, coming originally from the same ancestor line.

Antigens, Immunization Procedures, and Antibody Determinations.—(T,G)-A--L is a branched, multichain, synthetic polypeptide which has been described previously (13, 14). (T,G)-A--L 52 (mol wt 180,000) was diluted in phosphate-buffered saline (PBS) to a concen-



FIGS. 1 *a* and 1 *b*. Total (——) and 2-mercaptoethanol-resistant (MeR) (-----) antibody response of C3H/DiSn ($H\text{-}2^{k/k}$) mice to (*a*) primary (day 0) challenge with 10 μg (T,G)-A--L intraperitoneally and (*b*) primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of five mice.

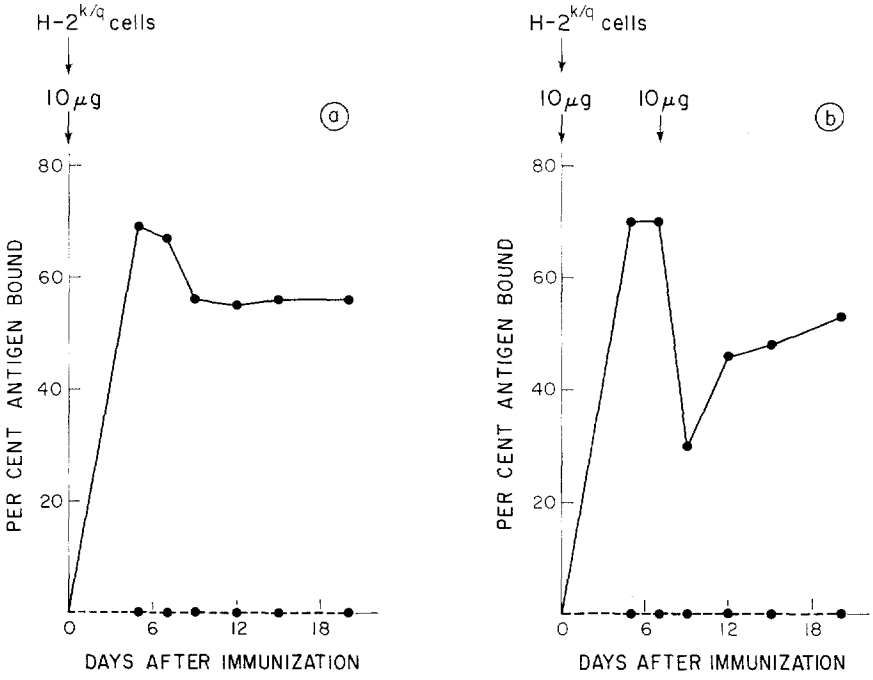
tration of 170 μg/ml; for all immunizations, 0.06 ml (10 μg) was injected intraperitoneally. Mice were bled from the tail or from the retroorbital sinus to obtain plasma for antibody determinations. Equal samples of heparinized plasma from each individual mouse in a group were pooled and stored frozen until assayed by titration.

Antibody was assayed at a plasma dilution of 1/25 in BSA by using a modified Farr assay as described earlier (7). ^{125}I-labeled (T,G)-A--L 52 (0.008 μg/ml) and ^{125}I-labeled (T,G)-A--L 509 (0.01 g/ml) were used interchangeably as the labeled antigens. Titers of 2-mercaptoethanol (2-Me)-resistant (IgG) antibody were determined by incubating plasma pools with equal volumes of 0.1 M 2-Me at 37°C for 60 min and then diluting the mixture with BSA for titration.

Preparation of Cells.—Spleen and inguinal lymph node cells were teased through a wire mesh screen into a solution of minimal essential medium without $NaHCO_3$ (MEM) + 5% fetal calf

serum (FCS). The MEM and FCS were obtained from Grand Island Biological Co., Grand Island, N.Y. The MEM was made up in deionized water and to it was added Na_2PO_4 and $MgCl_2$, each to a final concentration of 0.001 M.

Cells were dispersed with a Pasteur pipette and washed three times in MEM + 5% FCS, and then injected into the tail vein in a volume of 0.4 ml. Concentrations of cell suspensions used for injection were determined by suspending a 5 μl sample in 20 ml of filtered 37% formaldehyde-saline solution to which 0.1 mg saponin had been added, and then counting in a Model B Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) with a 100 μ aperture.



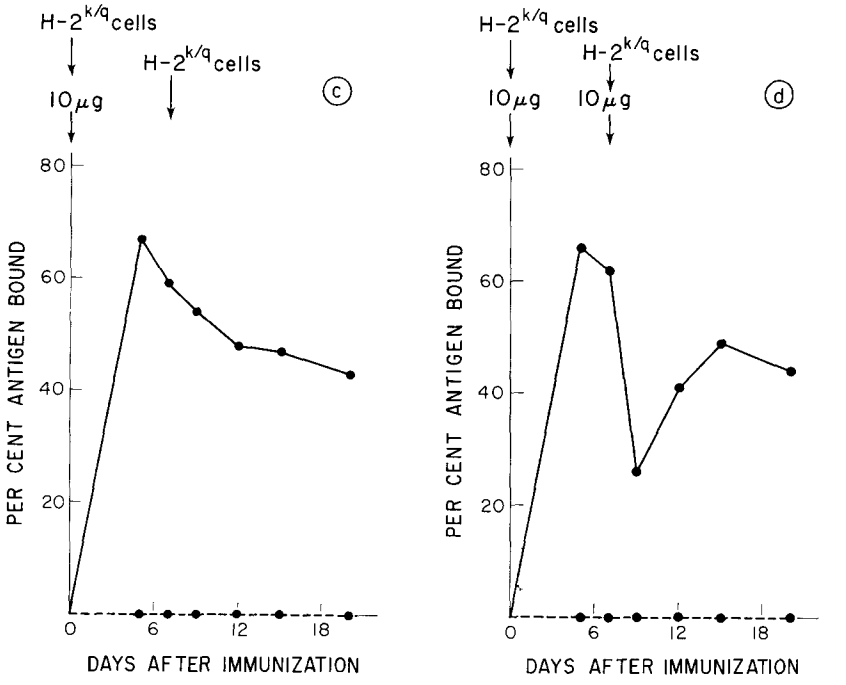
FIGS. 2 *a* and 2 *b*. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 (H-$2^{k/q}$) mice given 88 × 10^6 H-$2^{k/q}$ lymphoid cells intravenously on day 0 and (*a*) primary (day 0) challenge with 10 μg (T,G)-A--L intraperitoneally and (*b*) primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of five mice.

RESULTS

Figs. 1 *a* and 1 *b* show the total antibody and 2-Me-resistant (IgG) antibody made against (T,G)-A--L in C3H/DiSn (H-$2^{k/k}$) mice after primary and secondary antigen challenge. In both cases the antibody produced was entirely IgM. After secondary antigen challenge, there was no shift to IgG production, as would occur in responder C3H.SW (H-$2^{b/b}$) mice (7); rather, there was merely a transient dip in the IgM titer. Similar curves were obtained for C3H.Q and (C3H.Q × C3H/DiSn)F_1 mice challenged once or twice with

(T,G)-A--L; i.e. the response was limited to the production of IgM antibody, and no shift to IgG antibody occurred after secondary challenge.

Figs. 2 *a–2 d* show the response of F_1 mice after the injection of syngeneic (*H-2*$^{k/q}$) lymphoid cells and challenge with (T,G)-A--L. The results are virtually identical with the results for the C3H/DiSn (*H-2*$^{k/k}$) mice (given antigen but no syngeneic cells) in Figs. 1 *a* and 1 *b*. Again only IgM antibody was pro-



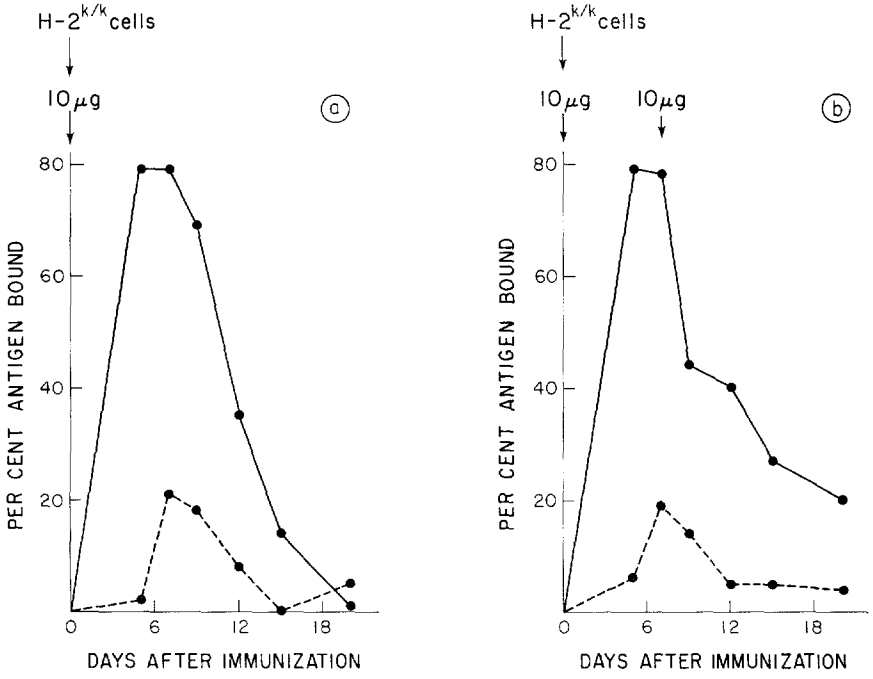
FIGS. 2 *c* and 2 *d*. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 (*H-2*$^{k/q}$) mice given 130 × 10^6 *H-2*$^{k/q}$ lymphoid cells intravenously on day 0 and 142 × 10^6 *H-2*$^{k/q}$ lymphoid cells intravenously on day 7 and (*c*) primary (day 0) challenge with 10 μg (T,G)-A--L intraperitoneally and (*d*) primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of three to five mice.

duced and there was no shift to IgG antibody production after secondary challenge.

In contrast to the effect of the transfer of syngeneic (*H-2*$^{k/q}$) cells into F_1 mice, Figs. 3 *a–3 d* show the response in F_1 mice to challenge with (T,G)-A--L when given at the same time as the transfer of parental (*H-2*$^{k/k}$) cells. Figs. 3 a and 3 *b* show groups of mice receiving 72 × 10^6 *H-2*$^{k/k}$ lymphoid cells at the same time as primary challenge with (T,G)-A--L. Both groups had higher peaks of total antibody than the F_1 mice receiving F_1 cells; moreover, both

groups produced detectable IgG anti-(T,G)-A--L antibody (reaching a peak of about 20% on day 7). As shown in Fig. 3 *b*, a second challenge with (T,G)-A--L had little effect; in particular, it did not elicit increased IgG anti-(T,G)-A--L antibody production.

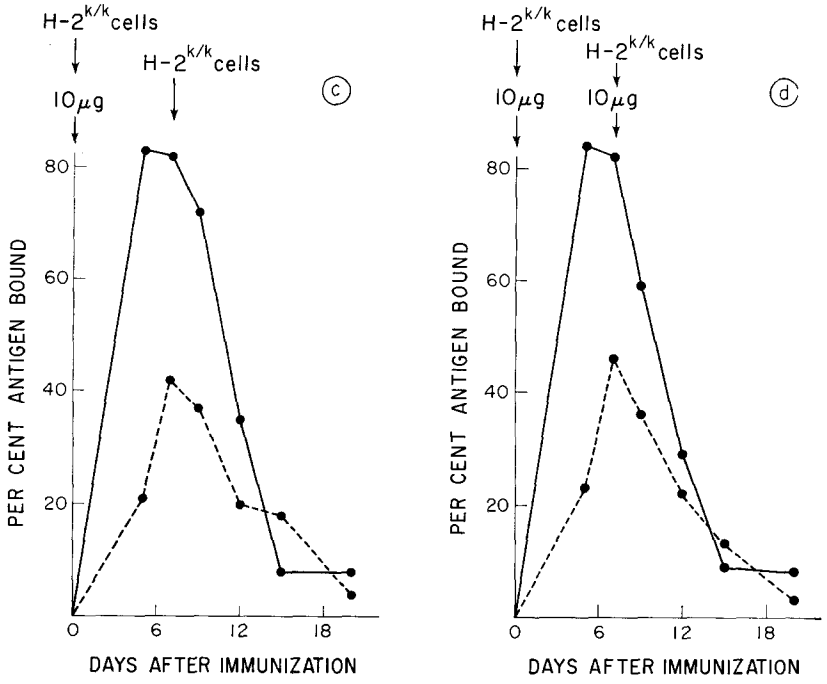
Figs. 3 *c* and 3 *d* demonstrate the effect of the transfer of a larger number of parental cells into the F_1 at the same time as primary challenge with (T,G)-



FIGS. 3 *a* and 3 *b*. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 ($H\text{-}2^{k/q}$) mice given 72 × 10^6 $H\text{-}2^{k/k}$ lymphoid cells intravenously on day 0 and (*a*) primary (day 0) challenge with 10 g (T,G)-A--L intraperitoneally and (*b*) primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of four or five mice.

A--L. Here 150 × 10^6 cells were given on day 0, about twice as many as given to those groups shown in Figs. 3 *a* and 3 *b*. The result once again was an increased total antibody titer when compared with the F_1 controls in Figs. 2 *a*–2 *d*. In addition, the amount of IgG anti-(T,G)-A--L antibody produced was considerably greater than in the groups shown in Figs. 3 *a* and 3 *b*, which received fewer cells. Again the highest IgG antibody titers (about 45%) were found on day 7, and the transfer of more parental cells on day 7 with or without secondary antigen challenge did not stimulate further production of IgG anti-(T,G)-A--L antibody. It is of interest to note that in all of the F_1 groups receiving parental cells the levels of both total and 2-Me-resistant antibody

fell off rather quickly after reaching their peak and by day 20 were quite low in most cases. It is also important to note here that the IgG antibody produced was being made after primary challenge with (T,G)-A--L; this is in contrast to normal responder mice, in which 10 μg (T,G)-A--L does not elicit IgG antibody production until after secondary challenge (7).



FIGS. 3 *c* and 3 *d*. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 ($H\text{-}2^{k/q}$) mice given 150 × 10^6 $H\text{-}2^{k/k}$ lymphoid cells intravenously on day 0 and 208 × 10^6 $H\text{-}2^{k/k}$ lymphoid cells intravenously on day 7 and (*c*) primary (day 0) challenge with 10 μg (T,G)-A--L intraperitoneally and (*d*) primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of three to five mice.

The timing between the transfer of parental cells and the challenge with antigen is quite critical. This is demonstrated in Fig. 4. Here parental ($H\text{-}2^{k/k}$) cells were given to F_1 recipients 7 days before antigen challenge, as well as with both primary and secondary antigen challenges. Not only was there no IgG antibody produced, but also total (IgM) antibody titers were markedly reduced.

Fig. 5 shows the effect of the transfer into F_1 recipients of parental ($H\text{-}2^{k/k}$) cells which have been previously sensitized to $H\text{-}2^q$ specificities. Cell transfers were given with both the primary and secondary challenges with (T,G)-A--L. As can be seen, no IgG anti-(T,G)-A--L antibody was made, and again total (IgM) antibody production was sharply reduced.

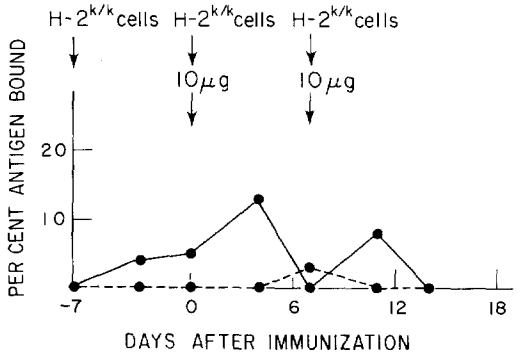


FIG. 4. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 ($H\text{-}2^{k/q}$) mice given $H\text{-}2^{k/k}$ lymphoid cells intravenously on day −7 (100 × 10^6 cells), day 0 (140 × 10^6 cells), and day 7 (230 × 10^6 cells) and primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of five mice.

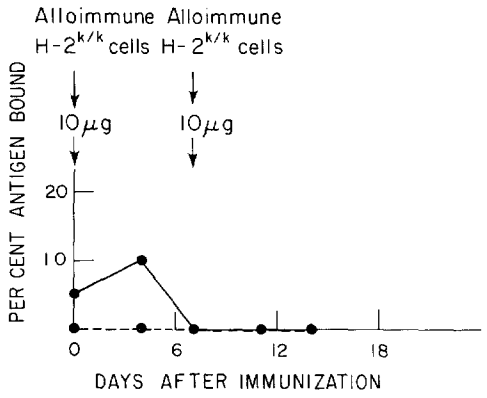


FIG. 5. Total (——) and MeR (-----) antibody response of (C3H.Q × C3H/DiSn)F_1 ($H\text{-}2^{k/q}$) mice given alloimmune (primed to $H\text{-}2^q$ specificities) $H\text{-}2^{k/k}$ lymphoid cells intravenously on day 0 (200 × 10^6 cells) and day 7 (160 × 10^6 cells) and primary (day 0) and secondary (day 7) challenge with 10 μg (T,G)-A--L intraperitoneally. Each point represents a plasma pool of five mice. (The alloimmune cells were prepared as follows: 100 × 10^6 C3H.Q ($H\text{-}2^{q/q}$) spleen cells were injected intraperitoneally into C3H/DiSn ($H\text{-}2^{k/k}$) recipients. 1 wk later these recipients were sacrificed and their spleen cells [now alloimmune] used for injection into the F_1 recipients.)

DISCUSSION

A large amount of data is available to support the concept that at least two cell types participate in the generation of immune responses to most antigens (15). One of these is the bone marrow-derived precursor of the antibody-producing cell, or B cell. The other cell type, which does not appear to secrete antibody, is the thymus-derived lymphocyte, or T cell. It has been

suggested that, in general, T cells react with a carrier moiety on the antigen involved in a given immune response, while B cells react with the hapten moiety (15, 16). This view is given support by findings in mice and guinea pigs which show that genetic nonresponders to specific antigens can be made to produce anti-hapten antibody if the antigen involved is coupled to a recognizable carrier (2, 5). The concept of carrier-reactive T cells and hapten-reactive B cells can be invoked to explain the results found in mice challenged with aqueous (T,G)-A--L. Specifically, it appears that the production of IgM anti-(T,G)-A--L antibody by B cells is T cell independent, but that the production of IgG anti-(T,G)-A--L antibody by B cells requires T cell recognition of the antigen (8). Presumably, in responders there exists a population of T cells which can recognize a carrier moiety on (T,G)-A--L and become activated (and perhaps proliferate); the activated T cells may then interact with B cells [which recognize haptenic determinants on (T,G)-A--L] via cell-to-cell contact or perhaps via a humoral factor (or perhaps both), and thereby induce the B cells to produce IgG antibody. Nonresponders, on the other hand, are apparently deficient in (T,G)-A--L-reactive T cells, and hence never shift to IgG anti-(T,G)-A--L antibody production.

Given that nonresponders lack (T,G)-A--L-reactive T cells, it can be postulated that activation of nonresponder T cells by a means other than (T,G)-A--L may be possible, and furthermore, that these activated T cells may be able to cause the IgM to IgG shift. In order to test this possibility, a GVH reaction was employed as a method of T cell activation (9). Congenic $H\text{-}2^{k/k}$, $H\text{-}2^{q/q}$, and $H\text{-}2^{k/q}$ mice [all nonresponders to (T,G)-A--L] were used. GVH reactions were induced in $H\text{-}2^{k/q}$ recipients by injecting parental ($H\text{-}2^{k/k}$) lymphoid cells. The recipients were challenged with (T,G)-A--L at the time of cell transfer, and this resulted in the production of IgG anti-(T,G)-A--L antibody.

This production of IgG anti-(T,G)-A--L antibody was found to be critically dependent upon the timing and severity of the GVH reaction induced. For instance, the transfer of parental ($H\text{-}2^{k/k}$) cells 1 wk before administration of antigen did not elicit IgG antibody production; indeed, the amount of IgM antibody produced was sharply reduced. Also, when parental cells were given at the same time as (T,G)-A--L, the transfer of a larger number of cells elicited a higher titer of IgG anti-(T,G)-A--L antibody. However, if the GVH reaction was too severe, such as in the transfer of $H\text{-}2^{k/k}$ cells from mice primed to $H\text{-}2^{q/q}$ cells, there was no IgG antibody production and again total (i.e., IgM) antibody production was diminished. These results suggest that T cell activation must occur at the same time as B cell exposure to (T,G)-A--L, and that a greater degree of T cell activation exerts a greater ("helper") influence on B cells, as measured by the production of IgG anti-(T,G)-A--L antibody. Too severe a GVH reaction, however, may have a negative ("killer") effect on B cells, reducing antibody production and preventing the IgM to IgG shift.

GVH reactions have been employed in other systems to stimulate the production of antibody against certain antigens. In guinea pigs, the transfer of strain 2 lymphoid cells into DNP-OVA-primed strain 13 recipients stimulates the production of both anti-DNP and anti-OVA antibodies, without further antigen challenge (10). Moreover, these recipients are capable of generating a marked secondary anti-DNP response when challenged with DNP-bovine gamma globulin (BGG) (10), or even with a DNP conjugate of the copolymer of D-glutamic acid and D-lysine (DNP-D-GL) which is normally tolerigenic in guinea pigs (17). These data suggest that this "allogeneic effect" has removed the requirement for carrier-specific T cells. Similar results are obtained in a system where parental (strain 2) lymphoid cells are injected into (2 × 13)F_1 recipients (11).

In irradiated CBA mice, the transfer of allogeneic (AKR) cells together with an anti-DNP antibody-forming clone of CBA lymphocytes will elicit in that clone an anti-DNP response after challenge with DNP-OVA (12). If syngeneic cells are used instead of allogeneic cells in the transfer, no such anti-DNP response occurs, suggesting that the allogeneic cells serve as a replacement for carrier-primed T cells. Consistent with this hypothesis, it was also found that allogeneic cells added to cells from the clone pretreated with anti-θ and complement restore the production of anti-DNP antibody after challenge with DNP-BGG (12).

It therefore appears that the requirement for host T cells in certain immune responses can be circumvented by the use of allogeneic or semiallogeneic cells which are capable of recognizing host histocompatibility antigens. In nonresponder mice immunized with (T,G)-A--L, we have shown that a GVH reaction can induce the shift from IgM to IgG anti-(T,G)-A--L antibody production. It is of interest to note that this shift occurs without a second challenge with 10 μg (T,G)-A--L. This differs from the kinetics of antibody production in responders, where primary challenge with 10 μg (T,G)-A--L will not elicit IgG anti-(T,G)-A--L antibody production, but primary challenge with 100 μg (T,G)-A--L will. It can be postulated that in responder mice a primary challenge with 10 μg (T,G)-A--L elicits the proliferation of T cells which, when activated upon secondary challenge with 10 μg (T,G)-A--L, are present in sufficient quantity to influence (hapten-reactive) B cell antibody production (i.e. cause the IgM to IgG shift). In this model, 100 μg (T,G)-A--L into a responder would act as both a primary and a secondary challenge, that is, enough antigen would still remain after the proliferation of T cells to activate these cells. Similarly, the induction of a GVH reaction in a nonresponder may activate (by recognition of histocompatibility differences) a large enough number of T cells to enable the shift to IgG production to occur after primary challenge with (T,G)-A--L.

From these data, it is impossible to say whether or not activated parental T cells stimulate the same B cells that activate them, or different ones, or if parental T cells instead activate host T cells in some manner. It is even possible

to postulate that the production of IgG anti-(T,G)-A--L antibody is due to some nonspecific effect of the GVH reaction, such as an alteration in the "processing" of antigen by macrophages, which somehow "triggers" B cells. Assuming an effect of parental T cells on B cells, it is not known if these donor T cells can stimulate only host B cells, which appear foreign, or whether donor B cells can be stimulated as well. Both donor $H\text{-}2^{k/k}$ B cells and host $H\text{-}2^{k/q}$ B cells may be making IgG anti-(T,G)-A--L antibody. The nature of the T cell interaction with B cells is also unknown. Direct cell-to-cell contact may be required between activated T cells and B cells exposed to antigen, and/or T cells may release a humoral factor which could influence nearby B cells. T cells in GVH reactions are known to release a number of nonantibody mediators (18), and it is possible that one of these factors may stimulate (T,G)-A--L-reactive B cells. Regardless of the mechanism of the T and B cell interaction, the data presented here are consistent with the model that T cell activation, whether induced by antigen (in a responder) or a GVH reaction (in a nonresponder) is required for the shift from IgM to IgG anti-(T,G)-A--L antibody production.

SUMMARY

The transfer of parental ($H\text{-}2^{k/k}$) nonresponder lymphoid cells into heterozygous ($H\text{-}2^{k/q}$) nonresponder recipients at the time of primary challenge with aqueous poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] elicited the production of both IgM and IgG anti-(T,G)-A--L antibody. Normally, the production of IgG anti-(T,G)-A--L antibody is restricted to strains possessing the responder *Ir-1* allele. The timing and intensity of the graft-*versus*-host (GVH) reaction required for this effect were found to be critical. Injection of $H\text{-}2^{k/k}$ cells into $H\text{-}2^{k/q}$ recipients 1 wk before antigen challenge did not elicit IgG anti-(T,G)-A--L antibody production, and markedly suppressed IgM anti-(T,G)-A--L antibody production. The transfer of alloimmune ($H\text{-}2^{q}$-primed) $H\text{-}2^{k/k}$ cells at the time of antigen challenge was also associated with no IgG and little IgM anti-(T,G)-A--L antibody production. These data are consistent with the model that nonresponder thymus-derived lymphocytes (T cells) activated in a GVH reaction can substitute for (T,G)-A--L-reactive T cells to induce a shift from IgM to IgG anti-(T,G)-A--L antibody production.

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