THE ROLE OF H-2-LINKED GENES IN HELPER T-CELL FUNCTION.

III. Expression of Immune Response Genes for Trinitrophenyl Conjugates of Poly-L(Tyr,Glu)-Poly-d,L-Ala--Poly-L-Lys in B Cells and Macrophages*

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For some years there has been intense interest in the problem of cellular expression of immune response (Ir) genes. Initial experiments suggested that T cells alone were responsible for Ir manifestations since, for example, mice which were low responders to poly-L(Tyr,Glu)-poly-d,L-Ala--poly-L-Lys [(TG)-A--L] could make antibodies to this antigen coupled to methylated bovine serum albumin (1) and guinea pigs nonresponsive to the dinitrophenyl (DNP) hapten coupled to poly-L Lys could make antibodies to DNP coupled to other carriers (2).

The idea of T-cell expression of Ir genes was strengthened by later experiments which showed that T cells from high responder strains could divide in response to the appropriate antigen, whereas T cells from low responder strains could not (3-5). In an elegant experiment, Bechtol et al. (6) showed that low responder B cells could make antibody to (TG)-A--L in tetraparental low responder ↔ high responder mice. This result again suggested that Ir genes were not expressed in B cells. The role of macrophages (Møs), however, was not studied in any of these experiments.

More recently, however, Ir genes have been found to be associated with B cells and/or Møs. In the guinea pig, F₁, low responder × high responder T cells proliferated when cultured with high responder Møs pulsed with antigen, but not with low responder Møs (7, 8). Similarly, Katz et al. (9) have shown in the mouse that F₁, high responder × low responder T cells would help antibody responses of B cells from high responder mice, but not low responder mice to DNP-poly-L-Glu, L-Lys, L-Tyr. Our work has shown that the Ir gene controlling cross-reaction between sheep erythrocytes (SRBC) and burro erythrocytes (BRBC) at the helper T-cell level is expressed at least by the B cell in vitro (10, 11).

The work of several laboratories (12-18) has suggested that at least three types of low responder animals can exist. The first type, exemplified by the response of mice of the $\text{H}^-$
2\(^{nd}\) haplotype to (TG)-A--L, is unable to respond because it has no functional B cells for this antigen, even though helper T cells are present (13-15). The second, exemplified by the response of mice of the \(H-2^{d}\) haplotype to (TG)-A--L, is unable to respond because it lacks helper T cells even though B cells responsive to this antigen are present (12). Finally, animals may lack both T cells and B cells responsive to a particular antigen, exemplified by the response of some \(H-2^{d}\) mice to (TG)-A--L and to poly(Phe,Glu)-poly-I-ala-poly-L-Lys (16-18).

In almost all cases studied, response or lack of response to a particular antigen maps in or close to the major histocompatibility complex (MHC) of the species in question (15, 19, 20). This is true even when separate genetic controls for B and T cells have been shown to exist, though there may be some non-MHC-encoded influence on at least B-cell responsiveness (18, 20).

Given the continuing controversy over the expression of Ir genes in T cells, B cells, or M\(\phi\)s, we decided to dissect the response in vitro, where purified cell populations can more easily be separated and titrated together than in vivo. Direct plaque-forming cell responses to trinitrophenylated (TNP) (TG)-A--L in mouse spleen cell cultures were studied. These proved to have the same strain distribution of response as IgG responses to (TG)-A--L in vivo (19). When high responder \(\times\) low responder F\(_1\) cells were titrated with various combinations of B cells and M\(\phi\)s of either the parental or F\(_1\) \(H-2^{d}\) type, high responsiveness required the presence of at least high responder B cells, and, in the one case studied, high responder M\(\phi\)s in the cultures, indicating the expression of Ir genes in both B cells and M\(\phi\)s.

**Materials and Methods**

**Mice.** B10.A \(\times\) C57BL10/Sn (B10) F\(_1\), B10.M \(\times\) B10 F\(_1\), B10.M \(\times\) B10.A F\(_1\), B10.M, B10.A (4R), and CBA/J \(\times\) C3H SW/Sn F\(_1\) were bred in our vivarium. Breeding mice for the B10.M, B10.A(4R), C3H.SW/Sn and B10.S strains were kindly provided us by Doctors M. Cherry and J. Stimpfling. All other mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

**Cultures.** Mouse spleen and lymph node cells were cultured by the methods of Mishell and Dutton (21), with some modifications (11).

**Antigens.** Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem, San Diego, Calif., and after dissolving in saline, was centrifuged at 78,000 g for 2 h (22). The pellet was then redissolved in saline and sterilized before storage. (TG)-A--L was purchased from Miles Laboratories Inc., Elkhart Ind. During these experiments two different lots were used (numbers MC3 and MC6), both of which had been previously tested by Miles and shown to give high titres of antiserum in responder, C3H.SW, and low titres of antiserum in low responder, C3H/HsJ or C3H/ DiSn, mice. The two lots proved indistinguishable in our hands.

TNP\(_{nr}\)-KLH, assuming a mol wt of \(8 \times 10^6\) for KLH, and TNP-\(\beta\)-galactosidase (TNP\(_{nr}\)-GZ) were prepared by the method of Rittenberg and Pratt (23). TNP-(TG)-A--L was prepared similarly with the following exceptions. 50 mg of (TG)-A--L and 7 mg of trinitrobenzene sulfonic acid were mixed in 3 ml of cacodylate buffer (pH 6.9). After 30 sec, the reaction was stopped by the addition of excess glycyglycine. Samples having 2.6 and 3.2 TNP substitutions/100,000 daltons (TG)-A--L were used in these experiments. \(^{125}\)I-TNP-(TG)-A--L was prepared by standard methods (24). 5 mg of TNP-(TG)-A--L was dissolved in 0.2 ml saline to which 0.1 ml 0.1 M sodium borate, pH 7.8, was added. To this, 7.5 \(\mu\)l \(^{125}\)I-labeled sodium iodide was added. The mixture was vortexed and stood at room temperature for 1 min. 0.5 ml of 4 \(\mu\)M iodine chloride was then added, and the mixture was dialyzed extensively against saline followed by balanced salt solution (BSS).

TNP-\(E.\) coli lipopolysaccharide (TNP-LPS) was prepared as described previously (25).

**Immunizations.** Mice were immunized to yield (TG)-A--L-specific T cells by injection of 100 \(\mu\)g (TG)-A--L in 40 \(\mu\)l complete Freund's adjuvant (CFA) in the base of the tail (26, 27). 7 days later, the periaortic and inguinal lymph nodes were removed and used as a source of T cells. The
spleens of mice injected i.p. with 20–50 μg of KLH in CFA were used as a source of KLH-primed T cells. The spleens of mice injected i.p. 7 days previously with 1 μg TNP-LPS were used in most cases as a source of TNP-primed B cells (28). Such cells were primed in C3H/HeJ and C3H.SW/Sn mice by i.p. injection of 100 μg TNP-GZ in CFA 4–8 wk before use. In vitro immunization of cultures with TNP-KLH was by addition of 0.1 μg/ml TNP-KLH or as described below.

Antigen-Pulsed Mφs. Mφs were pulsed with antigen by modifications of the method of Pierce et al. (29). Briefly, the peritoneal cavities of normal mice were washed with ice-cold BSS. These washings were then centrifuged, and the cells were resuspended to 2 x 10^7/ml in ice-cold BSS. The relevant antigen, TNP-(TG)-A--L or TNP-KLH, was added to a final concentration of 100 μg/ml, and the mixture was incubated on ice for 1 h. The cells were then washed exhaustively with ice-cold BSS and counted before use in vitro. Experiments in which Mφs were pulsed with ^3H-TNP-(TG)-A--L showed that 10^6 Mφs bound ~150 ng of antigen. Of this, ~80% of the antigen was released during overnight culture. This was true for Mφs from both high responder and low responder strains. Although peritoneal washings clearly contain a heterogeneous population of cells, including lymphocytes and Mφs (30), the antigen-presenting cells will be referred to as Mφs throughout the rest of the paper since in our hands the functional cells have the following properties, all of which are characteristic of Mφs. They adhere to nylon fiber and Sephadex G-10. They are present in plastic adherent cells, >99% of which phagocytose latex particles. They are irradiation- and anti-T serum-plus-complement-resistant. Recent studies by others have, however, suggested that Mφs themselves may be heterogeneous, both in the Ia antigens they bear (31, 32), and in their biological and biochemical properties (32, 33). Yamashita and Shevach (32) have reported that it is the Ia-positive subpopulation of Mφs which is most efficient at antigen presentation in their experiments. We have not characterized the active subpopulation in our experiments.

T and B Cells. (TG)-A--L-specific T cells were prepared from the periaortic and inguinal lymph nodes of immunized mice (26, 27), KLH-specific T cells were isolated from the spleens of KLH-immunized mice. In both cases, the cells were passed through nylon fiber columns (11, 34, 35) to remove B cells, Mφs, and other nylon fiber-adherent cells before use in vitro. B cells were isolated from spleen cell suspensions from TNP-primed mice by treatment of the cells with anti-T serum and complement (36). In some experiments it was also necessary to remove Mφs from the B-cell populations. In these cases the B cells were Mφ-depleted by passage through Sephadex G-10 columns (37) before treatment with anti-T serum and complement.

Direct Plaque-Forming Cell (PFC) Assay. After 4 days of culture, two or three identical culture wells were pooled and assayed in duplicate for direct anti-TNP PFC using the slide modification (21) of the Jerne hemolytic plaque assay. Parallel determinations were made using TNP-horse erythrocytes (TNP-HRBC) and HRBC, and the difference was recorded as the number of anti-TNP PFC. For these assays, lightly conjugated TNP-HRBC were prepared by the method of Rittenberg and Pratt (23), as modified by Kettman and Dutton (38). HRBC from a single animal were obtained from the Colorado Serum Co., Denver, Colo.

Assay of Helper T-Cell Activity. Helper T-cell activity was titrated as previously described (28). Culture wells were set up containing 3 x 10^6 TNP-primed B cells with or without Mφs from the appropriate strain of mouse. For TNP-(TG)-A--L responses, 10^5–2 x 10^6 TNP-(TG)-A--L-pulsed Mφs were added to each culture. For TNP-KLH responses, either 10^5–2 x 10^6 TNP-KLH-pulsed Mφs were added to each culture, or the culture medium was supplemented with TNP-KLH to a final concentration of 0.1 μg/ml. Varying numbers of T cells primed to the appropriate antigen were then added to the cultures. A plot of anti-TNP PFC/culture vs. the number of helper cells added yielded a titration with an initially linear slope. The least squares line was fitted to the initial points, and the slope of the line was taken as the activity of the helper population (Fig. 1).

Results

Conditions for in Vitro Anti-TNP-(TG)-A--L Responses. Conditions were established under which we could observe anti-TNP-(TG)-A--L responses in vitro. In preliminary experiments we found that the conditions which we had previously used to study anti-TNP-KLH responses (28) were not sufficient to generate in vitro anti-TNP-(TG)-A--L responses. A number of alterations were required, as described below.
Fig. 1. Titration of (TG)-A--L-specific and KLH-specific helper T cells. Three B6AF1 mice were primed with (TG)-A--L, and two with KLH. Seven days later, T cells were prepared from these mice and titrated for their helper activity in anti-TNP-(TG)-A--L responses (©) and anti-TNP-KLH responses (O), respectively, as described in Materials and Methods. The number of anti-TNP PFC/culture on day 4 is plotted vs. the number of (TG)-A--L-primed or KLH-primed T cells/culture. The initial slope ± SE of each titration line is also shown.

T cells isolated from the spleens of mice immunized i.p. with (TG)-A--L in CFA were not effective as helper cells in this response. This problem was solved by using T cells isolated from the periaortic and inguinal lymph nodes of mice immunized in the base of the tail with (TG)-A--L in CFA as described in Materials and Methods, and by others (26, 27). Such preparations were rich in (TG)-A--L-specific helper T cells.

We were unsuccessful in obtaining responses to TNP-(TG)-A--L when the antigen was added in soluble form to our cultures. This has been a common result when using soluble, relatively small antigens in our laboratory (39). This problem was solved by adding the antigen to cultures bound to peritoneal Mφs (Materials and Methods).

As had been our previous experience with TNP-protein antigens in vitro (28), a vigorous anti-TNP-(TG)-A--L response in vitro required the use of TNP-primed B cells. These were prepared from the spleens of mice primed either with TNP-LPS or, in the case of mice of the C3H background, with TNP-GZ in CFA.

After these conditions had been satisfied, good anti-TNP-(TG)-A--L responses occurred in our cultures. For example, in Fig. 1 the anti-TNP-(TG)-A--L response of C57BL/6 × A/J (B6AF1) B cells and Mφs is plotted as a function of the number of (TG)-A--L-primed B6AF1 T cells added to the cultures. Anti-TNP PFC numbers rose linearly as the numbers of (TG)-A--L-primed T cells in cultures were increased. In a control experiment, these same B cells and Mφs were shown to respond well to TNP-KLH in the presence of increasing numbers of KLH-primed B6AF1 T cells. Other experiments, not shown here, demonstrated that (TG)-A--L priming of the T cells was required, and that TNP-
### Fig. 2. Strain distribution of response to TNP-(TG)-A--L.

T cells from different strains of mice were titrated for their helper activity in anti-TNP-(TG)-A--L responses as described in Materials and Methods and in Fig. 1. Shown here are the results of 38 separate determinations in which each strain was tested at least three times. For each titration, the number of anti-TNP PFC/culture was plotted vs. the number of T cells added, and the initial slopes were determined by the least squares method. Slopes for all determinations with a given strain were averaged and are shown ± SEM.

(TG)-A--L had to be present in the cultures for anti-TNP-(TG)-A--L responses to occur.

**Strain Distribution of in Vitro Responses to TNP-(TG)-A--L.** The ability of cells from different strains of mice to respond to TNP-(TG)-A--L was measured by titrating the ability of (TG)-A--L-primed T cells from each strain to stimulate the direct PFC response to TNP-(TG)-A--L of TNP-primed B cells and Mφs from the same strain. As a control, KLH-primed T cells were also prepared in each strain and tested for their ability to stimulate an anti-TNP-KLH response in the same B cell and Mφ population. The averaged results of a number of experiments on the strains so far tested are shown in Fig. 2. All strains responded well to TNP-KLH (results not shown). By contrast, B10, B10.D2/nSn, and C3H.SW mice all responded well to TNP-(TG)-A--L, but B10.A, B10.S, B10.M, and C3H/He mice responded poorly, if at all. Of the F₁ mice tested, B10.A × B10 F₁, B6AF₁, and B10.M × B10 F₁ all gave large responses, whereas B10.M × B10.A F₁ mice did not respond. These results are in agreement with the reports of in vivo IgG responses to (TG)-A--L (19) and with some of the published reports of in vivo IgM responses to (TG)-A--L (20, 40, 41), and in vitro IgM responses to TNP-(TG)-A--L (42), with exceptions discussed later.

| STRAIN | H-2 | HELPER ACTIVITY (ANTI-TNP-PFC/CULTURE/10⁶ T CELLS) |
|--------|-----|-----------------------------------------------|
| B10    | b   | ![Graph](image1) |
| B10.D2 | d   | ![Graph](image2) |
| B10.A  | a   | ![Graph](image3) |
| B10.S  | s   | ![Graph](image4) |
| B10.M  | f   | ![Graph](image5) |
| C3H.SW | b   | ![Graph](image6) |
| C3H/He | k   | ![Graph](image7) |
| C57BL/6 x A F₁ | b/a | ![Graph](image8) |
| B10.A x B10 F₁ | a/b | ![Graph](image9) |
| B10.M x B10 F₁ | f/b | ![Graph](image10) |
| B10.M x B10.A F₁ | f/a | ![Graph](image11) |
TABLE I
Mapping of Ir Gene(s) for Anti-TNP-(TG)-A--L Responses in Vitro

| Strain       | H-2 subregions* | Anti-TNP-(TG)-A--L response (PFC/10^6 T cells)† |
|--------------|------------------|-----------------------------------------------|
|              | K    | I-A  | I-B  | I-J  | I-E  | I-C  | S    | G    | D    |          |
| B10.A(5R)    | b    | b    | b    | k    | k    | d    | d    | d    | d    | 685 ± 351 |
| B10.A(4R)    | k    | k    | b    | b    | b    | b    | b    | b    | d    | 8 ± 7     |
| B10.A(2R)    | k    | k    | k    | k    | k    | d    | d    | b    | d    | 4 ± 5     |
| B10.A        | k    | k    | k    | k    | k    | d    | d    | b    | d    | 8 ± 6§    |
| B10.D2       | d    | d    | d    | d    | d    | d    | d    | d    | d    | 385 ± 246§ |
| B10          | b    | b    | b    | b    | b    | b    | b    | b    | b    | 228 ± 88§ |

* Haplotype data from references 50–52.

† Average ± SEM of between 3 and 6 separate determinations.

§ Data from Fig. 1.

Experiments were performed in B10 congenic mice with recombinant H-2 haplotypes to allow preliminary mapping of the Ir gene(s) controlling the in vitro direct PFC response to TNP-(TG)-A--L. As shown in Table I, B10.A(5R) mice responded well to the antigen, but B10.A(4R) and B10.A(2R) responded poorly, suggesting that the gene(s) controlling this phenomenon map in the K, I-A end of the H-2 complex. This location for genes controlling in vivo and in vitro responses to (TG)-A--L has already been well established by others (15, 19, 42).

Expression of the Ir Gene(s) in B Cells. Having shown that the in vitro response to TNP-(TG)-A--L was under the control of Ir gene(s), we wished to determine which cell type(s) were expressing the gene(s) in vitro: T cells, B cells, or Mφs. Since these cell types cannot be taken from different unrelated mice and mixed in vitro without generating mixed lymphocyte reactions and complicating allogeneic effects, we designed these experiments along the lines we have previously described (10, 11, 43, 44). Thus, (TG)-A--L-primed T cells were obtained from F1 mice, the cross between high responder and low responder parents. These T cells were then titrated for their ability to stimulate anti-TNP-(TG)-A--L responses of B cells and Mφs obtained from congenic mice identical at H-2 with either the high responder or low responder parent. TNP-(TG)-A--L was added to the cultures bound to either high responder or low responder Mφs. F1 T cell and congenic B cell and Mφ donors were also selected such that no anti-Mls activity (45) would be obtained. This protocol permitted cultures to be set up with cells from mice differing at H-2, and it eliminated undesirable allogeneic effects since the F1 T cells were incapable of recognizing the H-2 or Mls antigens of the B cells and Mφs. We hoped that this protocol would determine whether high responder F1 T cells were sufficient for a high anti-TNP-(TG)-A--L response, or whether high responder B cells and/or Mφs were also required.

Three examples of this type of experiment are shown in Fig. 3. The results in Fig. 3 show that when (TG)-A--L-immunized B6AF1 (H-2b × H-2a) T cells were titrated for their ability to help anti-TNP-(TG)-A--L responses of B10 (H-2b, high responder) or B10.A(2R) (H-2a, low responder) cells, high responses were obtained with B10 cells and low responses with B10.A(2R) cells, irrespective of the type of Mφ bearing antigen in the cultures. Identical experiments
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Fig. 3. Expression of Ir genes in B cells. F1, high responder × low responder mice were primed with (TG)-A-L. Their T cells were subsequently titrated for helper activity in antitNP-(TG)-A-L responses of B cells and Mφs from high or low responder mice congenic with the parents of F1 at H-2. TNP-(TG)-A-L was added to cultures bound to high or low responder Mφs. Slopes ± SE of titrations for different T-cell, B-cell and Mφ, and antigen-bound Mφ preparations are shown, obtained in representative experiments. Each experiment was performed three times. Results are shown for three different strain combinations. (a) B6AF1 T cells, B10 A(2R) low responder, and B10 high responder B cells and Mφs; (b) CBA × C3H.SW FI T cells, C3H/He low responder, and C3H.SW high responder B cells and Mφs; (c) B10.M × B10 F1 T cells, B10.M low responder, and B10 high responder B cells and Mφs.

were performed with similar results using CBA/J × C3H.SW (H-2^k × H-2^b) F1 (TG)-A-L-primed T cells and B cells and Mφs from C3H/HeJ (H-2^k, low responder) or C3H.SW (H-2^b, high responder) mice (Fig. 3b). Another set of experiments was performed with B10.M × B10 F1 (H-2' × H-2^b) (TG)-A-L-primed T cells and B cells and Mφs from B10.M (H-2', low responder) or B10 (H-2^b, high responder) mice, again with qualitatively similar results (Fig. 3c). It should be noted, however, that the anti-TNP-(TG)-A-L responses of B10 B cells and Mφs, when stimulated by B10.M × B10 F1, T cells, were the highest of all the strain combinations tested, and that the responses of B10.M B cells and Mφs, when stimulated by the same T cells, were much smaller than these, but appreciable by comparison with other strain combinations. Each of these experiments has been performed three times with similar results.

Thus, in the three cases examined, the presence of high responder F1, T cells was not a sufficient condition for high response. In each case, cultures also had to contain high responder B cells for good anti-TNP-(TG)-A-L responses to occur, indicating the expression of Ir genes in B cells. We were tempted to conclude from our experiments that the Ir-type of the antigen-presenting Mφ
was irrelevant in the response. Some subsequent control experiments using $^{125}$I-
TNP-(TG)-A--L, however, indicated that this conclusion was not justified. In
these experiments both high and low responder Mφs were shown to take up
approximately the same amount of TNP-(TG)-A--L during our pulsing proce-
dure. More importantly, both types released $\approx$80% of this bound antigen within
24 h of culture. Thus, in the experiments shown in Fig. 3, there was a possibility
of antigen-transfer from the original antigen-bearing Mφs to those introduced
with the B-cell preparation. The high responses obtained when F₁ T cells were
cultured with high responder B cells and Mφs and antigen-pulsed low responder
Mφs might have been due to antigen-transfer to the high responder Mφs. The
problem of Ir-gene expression in Mφs in our cultures was, therefore, still
unanswered. This issue was addressed in a further set of experiments described
in the following section.

Two types of controls were performed for these mixing experiments. First, F₁
(TG)-A--L-primed T cells were titrated into cultures containing splenic B cells
and Mφs from one strain, and peritoneal Mφs from the other strain, in the
absence of antigen. TNP PFC/culture/$10^{6}$ T cells were always <2 under such
circumstances, suggesting that no nonspecific stimulation of anti-TNP PFC
responses was resulting from the mixing of peritoneal cells from one strain with
B cells and Mφs from the other, in the presence of T cells. In other control
experiments, low responder B cells were shown to respond to TNP coupled to
unrelated antigens by testing their response to TNP-KLH bound to high
responder or low responder Mφs in the presence of F₁, KLH-primed T cells
(results not shown).

Expression of Ir Gene(s) in B Cells and Mφ. To discover whether Ir genes
were being expressed in Mφs as well as B cells in our cultures, splenic B cells
had to be depleted of Mφs by passage over Sephadex G-10 columns. Such a
maneuver prevented possible antigen transfer from the Mφs on which it was
added to cultures to Mφs in our splenic B-cell populations. Thus B10.A or B10 B
cells were depleted of Mφs, TNP-(TG)-A--L was added to cultures bound to
either B10 or B10.A Mφs, and B6AF₁ (TG)-A--L-primed T cells were titrated
into the cultures. The results of a typical experiment of three are shown in Fig.
4a. As in the previous experiments, B10.A B cells did not respond to TNP-(TG)-
A--L in the presence of B6AF₁, (TG)-A--L-primed T cells whether the antigen
was added to cultures bound to B10.A or B10 Mφs. B10 B cells responded to
TNP-(TG)-A--L in the presence of helper T cells if the antigen was added to
cultures on the surface of B10 Mφs but, unlike the previous experiment, not if
antigen was added on the surface of B10.A Mφs. These results suggested that Ir
genes were being expressed in vitro by both B cells and Mφs. Again, in control
experiments both the B10 and B10.A Mφs and B cells were shown to be
functional in anti-TNP-KLH responses in the presence of KLH-primed F₁, T
cells.

These results could also be explained by a theory in which Ir genes are
expressed only in B cells or Mφs in vitro, but H-2 compatibility is required for
good B cell-Mφ cooperation. Thus, Ir genes might be expressed in Mφs only, for
example, but B10 Mφs would be unable to cooperate with B10.A B cells because
the two are histoincompatible. To eliminate this explanation, experiments were
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Ir genes expressed by B cells and macrophages

(a) B6AF1 mice were primed with (TG)-A--L and their T cells were subsequently titrated for helper activity in anti-TNP-(TG)-A--L responses of MΦ-depleted B cells from B10.A, low responder, or B10, high responder animals. Antigen was added to cultures bound to B10.A or B10 MΦs. The initial slopes ± SE obtained in a representative experiment of 3 are shown. (b) As in (a), except that MΦ-depleted B cells were obtained from B10.A and B6AF1 animals and antigen-pulsed MΦ were obtained from B10.A and B10 × B10.A F1 animals.

![Graph](image)

Fig. 4. Expression of Ir genes in B cells and MΦs. (a) B6AF1, mice were primed with (TG)-A--L and their T cells were subsequently titrated for helper activity in anti-TNP-(TG)-A--L responses of MΦ-depleted B cells from B10.A, low responder, or B10, high responder animals. Antigen was added to cultures bound to B10.A or B10 MΦs. The initial slopes ± SE obtained in a representative experiment of 3 are shown. (b) As in (a), except that MΦ-depleted B cells were obtained from B10.A and B6AF1 animals and antigen-pulsed MΦ were obtained from B10.A and B10 × B10.A F1 animals.

Discussion

The problem of cellular expression of Ir genes in antibody responses seems to be particularly difficult to solve, since at one time or another B cells, MΦs, and T cells have all been implicated. Proliferation experiments in guinea pigs and mice, for example, have shown that at least MΦs, and possibly also T cells, may express the genes (5, 7, 8). Other experiments have shown that B cells may also be involved (9-11). The problem is not simplified if the experiments considered are confined to those involving only one antigen, (TG)-A--L. McDevitt et al. (4), Bechtol et al. (6) have shown that H-2k-bearing B cells in H-2k ↔ H-2b
tetraparental mice are not defective in their ability to respond to this antigen, although T cells and/or Mφs from mice of the H-2x haplotype are apparently nonresponsive (3). On the other hand, the experiments of Munro and Taussig (12), Lichtenberg et al. (14), Erb and Feldmann (13), and Howie and Feldmann (46) suggest that H-2x B cells are defective and H-2x T cells are functional in anti-(TG)-A--L responses.

According to Munro and Taussig (12), Lichtenberg et al. (14), Mozes et al. (16), and Munro et al. (18), however, in other strains of mice the defect in response to (TG)-A--L may lie in either the T cells alone (B10.M, A.CA, H-2x; A.SW, H-2x), or in the T cells and B cells (S.JL: H-2x). The role of the Mφ was not studied in these experiments, but it has been suggested by others that so-called T-cell-defective mice did in fact contain deficient Mφs.

Since it is very difficult to deplete mice of Mφs, it seemed reasonable to us to examine the possible expression of Ir genes for (TG)-A--L by B cells and Mφ in vitro. Thus, we established conditions in vitro under which antibody responses to TNP coupled to (TG)-A--L could be obtained. Our direct PFC responses were secondary since both B cells and T cells were primed to antigen. When the response of cells from different strains of mice to TNP-(TG)-A--L was measured, the pattern of response was as predicted from the in vivo distribution of IgG responses to (TG)-A--L (19) and a recently published survey of IgM primary responses to TNP-(TG)-A--L in vitro (42). The one exception to this was our observation that B10.D2 mice were high responders to TNP-(TG)-A--L, whereas in vivo results predict that they should be intermediate. There is some controversy in the literature over Ir restriction of IgM responses to (TG)-A--L (20, 40-42). Results seem to depend on the antigen and conditions used. Our system, however, detects Ir-controlled differences in direct PFC responses.

We were somewhat surprised to find that mice of the H-2a and H-2x haplotypes were low responders to TNP-(TG)-A--L, since the experiments of Howie and Feldmann (46) show that they should be high responders. Singer et al. (42), who also show that H-2a and H-2x mice are nonresponders to TNP-(TG)-A--L, have suggested that this anomalous result of Howie and Feldmann (46) results from their methods of preparing antigen. We were also disappointed to note that B10.M × B10.A F1 mice were low responders to TNP-(TG)-A--L in our hands. Munro and Taussig (12) and Munro et al. (18) originally showed that gene complementation occurred in this F1 combination, a result which is very intriguing. In subsequent experiments, McDevitt (41) and Munro and Taussig (47) have been unable to repeat the original finding, in agreement with our result.

We concluded, however, that our in vitro IgM secondary responses were measuring the presence of an Ir gene(s) with a strain distribution identical to that identified by others in vivo and in vitro. Moreover, this gene(s) mapped in the same part of the H-2 complex as gene(s) controlling other (TG)-A--L responses, viz K, I-A.

Having established this, we set up experiments along the lines originally published by Katz et al. (9) and Shevach and Rosenthal (7), and more recently ourselves (10, 11). (TG)-A--L primed T cells were obtained from F1 mice, the cross between high and low responder parents. These T cells were titrated for their ability to help anti-TNP-(TG)-A--L responses of B cells and Mφs of either
parental H-2 type. Three different strain combinations were studied. In every case, low responder B cells (B10.A(2R), H-2b; C3H/HeJ, H-2k; B10.M, H-2b) were unable to respond in the presence of active T cells and Møs of the high responder type. We concluded from these experiments that Ir genes were being expressed at least by B cells in all three low responder strains studied here.

We wished to test for the expression of Ir genes by Møs in antibody responses. To do this, Møs were removed from our B-cell populations to prevent antigen transfer, and F1 cells were titrated into cultures containing B10.A (H-2b, low responder) or B10 (H-2b, high responder) B cells. Antigen was added on B10.A or B10 Møs. B10.A B cells did not respond to TNP-(TG)-A--L, regardless of the Mø type in vitro. B10 B cells responded only when B10 Møs were added. Using F1 Møs, we were able to show that the lack of response of B10.A B cells was not due to H-2 differences between the B cells and Møs.

We therefore concluded that B10.A mice were low responders to TNP-(TG)-A--L because they lacked both functional B cells and functional Møs for this antigen. Of the other strains studied, C3H/HeJ (H-2k), B10.A(2R), and B10.M (H-2b) lacked at least functional B cells. Our finding that B10.M B cells are nonfunctional is in direct contradiction to the results of Munro and Taussig (12), who suggest that B10.M mice should contain functional B cells and nonresponsive T cells for (TG)-A--L. This is a contradiction we are at present unable to resolve except by pointing out that the conditions of our experiments are vastly different.

Although the antigen presenting cells in our cultures have been identified as Møs by several criteria (Materials and Methods), we have not characterized the subpopulation of Møs which are active in our experiments. Since it has been shown by other investigators that it is the Ia-positive subpopulation of Møs which are most active in antigen presentation (32), our future experiments will be designed to test whether it is the Ia-positive Møs which differ in their ability to present TNP-(TG)-A--L between low responder and high responder strains of mice.

None of the experiments described in this paper examine the question of Ir gene expression in T cells. Thus, all the low responder strains we have tested may also contain nonfunctional T cells for (TG)-A--L responses. Our future experiments will be designed to tackle this question.

The results which we present here are consistent with our previous findings (11) that the Ir gene which controls the ability of helper T cells to respond to a cross-reacting determinant on SRBC and BRBC is expressed at least at the level of the B cell.

A number of models have been proposed to explain how MHC-linked Ir genes can control the activity of helper T cells even though they are expressed in B cells and Mø (12, 48, 49). At present, we favor the associative or dual recognition hypothesis in which the specificity of helper T cells is determined by the simultaneous recognition of antigen and Ir-gene products on either the Mø or B-cell surface (49). Thus, B cells and Møs in high responder strains would possess, and in low responder strains would lack the appropriate I-region encoded molecule which could be recognized in association with the antigen. The attraction of this model is that it is consistent with a large body of evidence.
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in the literature (13, 29, 48, 49), including work from this laboratory (11, 43, 44) concerning the interaction of helper T cells with antigen.

Summary

Using lymph node T cells from poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(TG)-A--L]-primed animals and B cells from animals primed with trinitrophenylated (TNP) protein or lipopolysaccharide, we have obtained anti-TNP-(TG)-A--L direct plaque-forming responses in vitro. Response to this antigen was shown to be controlled by the H-2 haplotype of the animal studied. The strain distribution of in vitro response was very similar to that previously reported by others for in vivo secondary IgG responses to (TG)-A--L.

We investigated the cell types expressing the Ir gene(s) for (TG)-A--L in our cultures. F1, high responder × low responder mice were primed with (TG)-A--L. Their T cells were active in stimulating anti-TNP-(TG)-A--L responses of high responder but not low responder B cells and macrophages (MΦ), even though both preparations of B cells and MΦ were obtained from mice congenic at H-2 with one of the parents of the F1. For three low responder strains tested, of the H-2\(^{\alpha}\), H-2\(^{\beta}\), and H-2\(^{\gamma}\) haplotypes, the anti-TNP-(TG)-A--L response of low responder B cells and MΦs in the presence of high responder, F1 T cells could not be improved by the addition of high responder, antigen-bearing MΦs to the cultures. In one strain of the H-2\(^{\alpha}\) haplotype, it was shown that neither the B cells nor MΦs could be functional in anti-TNP-(TG)-A--L responses. Our results therefore suggested the Ir genes for anti-TNP-(TG)-A--L responses were expressed at least in B cells in all the low responder strains we studied, and, in mice of the H-2\(^{\alpha}\) haplotype, in MΦs too.

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