GENETIC CONTROL OF THE IMMUNE RESPONSE TO MYOGLOBINS

Both Low and High Responder T Cells Tolerant to the Other Major Histocompatibility Complex Help High but Not Low Responder B Cells

By Yoichi Kohno* and Jay A. Berzofsky

From the Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Several studies have indicated the important role of thymic environment, in which T cells differentiate and mature, in imposing the H-2 restriction on T cells (1). These findings were incorporated by Katz et al. (2) into the framework of his “adaptive differentiation” hypothesis. The H-2 restriction of maturing T cells was investigated primarily in experiments using bone marrow radiation chimeras with or without thymic grafts, both in T cell-mediated cytotoxicity (3, 4) and in T helper cell-dependent antibody responses (2, 5-11). Thymic environment during T cell maturation also affected immune response (Ir) gene function (10, 11). In studies set up in parallel in the same systems or in other systems, tolerance induced by neonatal tolerization, in contrast to bone marrow chimeras, was not usually found to unveil a T cell repertoire specific for antigen in association with the tolerated allogeneic H-2 antigens (7, 12), although exceptions were observed (13).

We have studied the mechanisms of regulation of the immune response to myoglobin (Mb), especially the cellular mechanisms of Ir gene control. We have found two H-2-linked genes controlling both the T cell proliferative response and the antibody response to Mb, mapping in different I subregions, I-A, and I-C, of the H-2 complex (14-16). In a recent study, we found that the Ir gene defect correlated with the inability of (high responder × low responder) F1 T cells to cooperate with low responder B cells, even in the presence of functional F1 antigen-presenting cells (APC) and even when the B cells could be demonstrated to be primed (by their ability to receive help from carrier-specific T cells). In all of these studies using high and low responder B cells and/or APC, the T cells had to be F1 hybrids to avoid allogeneic

* Present address: Department of Pediatrics, School of Medicine, Chiba University, Chiba, Japan.

1 Abbreviations used in this paper: APC, antigen-presenting cell; BSS, balanced salt solution; BUdR, bromodeoxyuridine; CFA, complete Freund's adjuvant; CML, cell mediated lympholysis; DTT, dithiothreitol; FACS, fluorescent-activated cell sorter; FCS, fetal calf serum; FyG, Fowl gamma globulin; Gp C', guinea pig complement; Ir, immune response; Mb, myoglobin; Mb-FyG, Mb-coupled FyG; MBI, methyl-4-mercaptobutyrimidate hydrochloride; MBS, m-maleimidobenzoyl-N-hydroxy-succinimide ester; MEM, Eagle's minimal essential medium; MHC, major histocompatibility complex; MLR, mixed-lymphocyte reaction; NaPi, phosphate buffer; PBS, phosphate-buffered saline; RaMB, rabbit anti-mouse brain-associated antigen; Rb C', rabbit complement; SAC, splenic glass-adherent cell; TdR, thymidine.

2 Kohno, Y., and J. A. Berzofsky. Genetic control of the immune response to myoglobin. Ir gene function in genetic restriction between T and B lymphocytes. Manuscript submitted for publication.
effects. Therefore, to explore directly the role of helper T cells in the response controlled by Ir genes, alloreactivity had to be eliminated. The method we elected to use was neonatal tolerization, partly to avoid the effects on the T cell repertoire previously observed in radiation chimeras, as noted above. Neonatal mice were injected intravenously with F1 spleen cells and the collaboration of T cells from tolerized mice with B cells from high responder, low responder, or F1 mice was studied.

Not only did we find that low responder mice had T cells competent to help the antibody response to Mb in vitro, but we found that this help was due to a repertoire in the tolerized low-responder mice specific for Mb in association with high responder alloantigens, i.e., a repertoire for antigen plus allo-major histocompatibility complex (MHC), which we did not expect to find in the neonatally tolerant mouse, in which the thymus is homozygous for the low responder H-2 haplotype. These observations are discussed in terms of the following questions: (a) Is the H-2 restriction of helper T cells exclusively determined by the thymic environment in which T cells differentiated and matured or also by the extrathymic environment? (b) Are there normally clones of T cells specific for Mb in the context of the H-2d high responder haplotype present in low responder mice, but masked by allogeneic effects? (c) What is the role of helper T cells in the genetic control of the response to Mb?

Materials and Methods

**Animals.** B10.D2/nSn, B10.BR/SgSn, B10.A(5R)/SgSn, C57BL/10 Sn, (B10.D2 × B10.BR) F1, and B6D2 F1 mice were obtained from The Jackson Laboratory, Bar Harbor, ME. The B10.HTT and (B10.D2 × C57BL/10) F1 mice were bred by us, the former from breeding pairs obtained from Dr. David H. Sachs of the National Institutes of Health (NIH). Mice were 8–16 wk of age at the first immunization.

**Antigens.** Sperm whale Mb was obtained from Accurate Chemical & Scientific Corp. (Westbury, NY). The major chromatographic component, IV, purified as described previously (14) by the method of Hapner et al. (17), was used throughout these studies. Fowl gamma globulin (chicken) (FTG) from United States Biochemical Corp. (Cleveland, OH) was used.

**Mb Coupling to FyG.** Preparation of Mb-coupled FyG (Mb-FTG) was performed by a modification of the method described by Schroer et al. (18). Two bifunctional reagents, methyl-4-mercaptobutyrimidate hydrochloride (MBI) and m-maleimido-benzoyl N-hydroxy-succinimide ester (MBS) (Pierce Chemical Co., Rockford, IL) were used to couple Mb to FyG; 2.4 mg of MBS in 0.12 ml of dimethylformamide (Pierce Chemical Co.) were added to 1/zmol of Mb in 0.8 ml of 0.1 M sodium phosphate buffer (NaPi), pH 7.1, and allowed to react for 1 h at 20°C with occasional stirring. The Mb-MBS solution was dialyzed against 0.05 M NaPi, pH 7.1, for 1.5 h. 100 nmol of FyG was dissolved in 0.14 ml of 0.05 M NaPi, pH 7.1, with 0.1 mM dithiothreitol (DTT) (Sigma Chemical Co., St. Louis, MO); 0.3 mg of MBI in 0.3 ml of 0.05 M NaPi, pH 7.1, with 0.1 mM DTT, was added to the FyG solution. The FyG and MBI solution was allowed to react for 1 h at 20°C with occasional stirring under argon gas to reduce oxidation. The MBI-FyG solution was dialyzed against 0.05 M NaPi, pH 7.1, with 0.01 mM DTT for 1.5 h under argon gas. After centrifugation of both MBS-Mb and MBI-FyG solutions, the MBI-FyG and MBS-Mb solutions were mixed and allowed to react for 3 h at 20°C under argon gas with occasional stirring. The Mb-FyG solution was kept in 0.05 M NaPi, pH 7.1, with 0.4 mM DTT, overnight to block residual active sites of maleimide on the MBS-Mb. The Mb-FyG was purified by gel filtration on Sephadex G75 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) to remove excess reactants. The sterilized Mb-FyG was stable and stored at 4°C in phosphate-buffered saline (PBS). The preparation of Mb-FyG used throughout these experiments was characterized as follows: the molar substitution of Mb to FyG was 1.5; the concentration of Mb was 0.53 mg/ml and that of FyG was 3.13 mg/ml. The concentrations specified in the cultures are those of the FyG moiety.

**Immunization Schedule.** Mice were immunized intraperitoneally with 150 μg of purified Mb
or with 200 μg of Mb-coupled FyG (Mb-FyG) in PBS emulsified 1:1 in complete Freund's adjuvant (H37Ra; Difco Laboratories, Detroit, MI) in total volume of 0.1 ml/animal. 3 wk after immunization, the mice were boosted intraperitoneally with 0.1 μg of purified Mb or Mb-FyG in PBS, and they were killed 1–2 wk later.

**Cell Culture.** The culture system, modified from that of Mishell and Dutton (19), was described in a previous paper (16). Briefly, 2.5 × 10⁶ spleen cells from immunized mice were cultured with 1 or 0.1 μg/ml of Mb or 0.01 or 0.001 μg/ml of Mb-FyG in 1.5 ml of RPMI 1640 medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Microbiological Associates, Walkersville, MD), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 5 × 10⁻⁵ M 2-mercaptoethanol in flat-bottomed wells (3524; Costar, Data Packaging, Cambridge, MA) for 10 d at 37°C, 6% CO₂ on a rocking platform. On the 4th d, 1 ml of supernatant was exchanged for fresh medium. On the 10th d, culture supernatants were harvested to measure the secreted antibodies.

**Preparation of Spleen Cells.** Spleen cells were prepared by gentle teasing of spleens, using forceps, in cold balanced salt solution (BSS, NIH Media Unit). The cell suspensions were treated with ammonium chloride lysing buffer to lyse the erythrocytes, and were washed with RPMI 1640 medium containing 5% FCS.

**Preparation of T Cells.** T cells were prepared by passage of spleen cells over nylon fiber columns (1 × 10⁸ cells/g of nylon) and collection of nylon-nonadherent eluate (20). Nonadherent cell populations were irradiated with 250 rad from a 137Cs source to eliminate memory B cells.

**Preparation of B Cells and Accessory Cells.** B cells and accessory cells were prepared by depleting splenic T cells by treatment with a rabbit antibody against mouse brain-associated antigen (RaMB) (Litton Bionetics, Kensington, MD) and guinea pig complement (Gp C').

**Preparation of Splenic Glass-adherent Cells (SAC).** The preparation of SAC was described previously (16).

**Sephadex G10 Passage.** Sephadex G10 (Pharmacia Fine Chemicals) passage of spleen cells was performed by a modification of the method described by Hodes et al. (21). Briefly, 3 × 10⁸ cells in 4 ml BSS containing 8% FCS (BSS-FCS) were added to a 30-ml Sephadex G10 column, incubated at 37°C for 45 min, and eluted with warmed BSS-FCS, collecting the first 20 ml of effluent. These effluent cells were resuspended in BSS-FCS and loaded on the second Sephadex G10 column. After incubation, the first 20 ml of effluent was collected and resuspended in culture medium.

**Radioimmunoassay for Antibody to Mb.** The assay of antibody to Mb in the culture supernatant was performed by a solid-phase radioimmunoassay technique as described elsewhere (16).

**Preparation of Neonatally Tolerized Mice.** The method of Billingham et al. (22) was used. The neonatal mice were rendered tolerant by the intravenous injection of ~2 × 10⁷ spleen cells from adult F₁ hybrid donor mice during the first 24 h after birth. Tolerance to H-2 alloantigens was assessed by responsiveness to these antigens in mixed-lymphocyte reaction (MLR) and cell-mediated lympholysis (CML). The resulting tolerized populations contained <5% F₁ cells by indirect immunofluorescence or 2-4% detectable by the fluorescence-activated cell sorter (FACS) using H-2-specific reagents. The indirect immunofluorescence staining was generously performed by Dr. Ronald E. Gress, NIH. The FACS studies were generously performed by Mrs. Susan O. Sharrow, NIH.

**Treatment of T Cells with Anti-H-2 Antibodies.** Nylon-fiber column-passed T cells were treated with antibodies to tolerated H-2 antigens and rabbit C' (Rb C') (Cedarlane low-tox complement, Accurate Chemical and Scientific Corp.). C57BL/10 T cells were incubated with a 1:60 dilution of anti-H-2d antibodies (4227:C57BL/10 anti-B10.D2, titer 1:128), B10.BR T cells were incubated with a 1:400 dilution of anti-H-2d [948:(C57BL/10 × A/J) F1 anti-B10.D2 absorbed with B10.BR, titer 1:1024], and B10.D2 T cells were treated with a 1:32 dilution of anti-H-2b (4241, B10.D2 anti-B10, titer 1:64), respectively, for 30 min at 37°C. After another incubation with Rb C’ for 30 min at 37°C, these cells were extensively washed out with RPMI 1640 supplemented with 5% FCS and cultured with Mb. The antisera were a generous gift of Dr. David H. Sachs, NIH.

**MLR.** 4 × 10⁶ spleen cells were cultured with 4 × 10⁶ stimulator cells, which were irradiated...
with 2,000 rad in 0.2 ml of Click's medium (NIH Media Unit) containing 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Gibco Laboratories), 5 × 10^{-6} M 2-mercaptoethanol (Microbiological Associates) and 0.8% fresh normal mouse serum. After 5 d, 0.5 μCi (methyl-3H) thymidine ([3H]TdR, New England Nuclear, Boston, MA; sp act 6.7 Ci/m mole) was added, and after 4 additional h at 37°C, the cells were harvested in an automated collecting device (Microbiological Associates). Proliferation was estimated by scintillation counting of the 3H incorporated into cellular DNA. All cultures were performed in flat-bottomed tissue culture plates (3596; Costar).

CML. Cytotoxic T lymphocytes were generated by suspending 4 × 10^6 responder cells in 2 ml of minimum essential medium (MEM) (Flow Laboratories, Inc., Rockville, MD) supplemented with FCS for 5 d in a 5% CO2 humidified air atmosphere with 3 × 10^6 stimulator cells irradiated with 2,000 rad (23). This number of stimulator cells was found to be more than sufficient to elicit maximal CML reactivity in all strain combinations tested. After harvest, the cells were assayed for their ability to lyse 51Cr-labeled 48-h concanavalin A blasts in a 4-h 51Cr release assay. Responses are expressed as mean percent specific lysis of triplicate determinations; percent specific lysis is calculated as: (cpm experimental − cpm medium control)/(cpm maximum release − cpm medium control). The CML was generously performed by Dr. Ronald E. Gress, NIH.

Results

Spleen Cells from Neonatally Tolerized Mice Were Unresponsive to Tolerized H-2 Alloantigens. To assess the tolerance of neonatally tolerized mice, their ability to respond to the tolerated alloantigens was assessed by means of MLR and CML. An example is shown in Table I. Spleen cells from B10.D2 (H-2^d), B10.A(5R) (H-2^e), and C57BL/10 (H-2^b) mice neonatally tolerized with B6D2 F1 (H-2^{b4d}) spleen cells showed extremely low alloreactivity to (B10.D2 × C57BL/10) F1 (H-2^{2b4b}) stimulator cells in an MLR. The same lack of MLR reactivity was demonstrable in other experiments using stimulator cells homozygous for the tolerated H-2 haplotype. The same cells did

| Tolerized with B6D2 F1 spleen cells | H-2 haplotype | Number of mice in pool | MLR stimulator | CML |
|-------------------------------------|---------------|-------------------------|----------------|-----|
| B10.D2§¶†§ | + H-2^d | 6 | 159 (1.7) | 5,439 (1.1) | B10.D2 F1 |
| B10.A(5R)§¶ | + H-2^e | 3 | 589 (1.3) | 5,731 (1.1) | B10 BR |
| C57BL/10§¶ | + H-2^d | 6 | 372 (1.2) | 7,279 (1.1) | B10.D2 |
| C57BL/10§** | + H-2^d | 3 | −39 (1.4) | 25,540 (1.1) | B10 BR |
| B10.D2 × C57BL/10†¶ | − − | 7 | −2,267 (1.1) | 27,038 (1.1) | ND |
| B10 HTT†¶ | − − | 1 | 16,600 (1.1) | 460 (1.2) | ND |
| C57BL/10** | − − | 1 | ND | ND | ND |
| B10.D2** | − − | 1 | ND | ND | B6D2 F1 |

* [3H]TdR incorporation mean ± cpm (geometric SEM). The control incorporation without stimulator (medium alone) for each responder population was: B10.D2, 62 cpm; B10.A(5R), 761 cpm; immune C57BL/10, 1,699 cpm; nonimmune C57BL/10, 1,736 cpm; (B10.D2 × C57BL/10) F1, 4,283 cpm; B10 HTT, 1,209 cpm.
† Percent of maximum 51Cr release (SEM) at effector/target ratio 40:1.
§ The cells used in this experiment were same source of cells used in the experiment of Fig. 2.
¶ Immunized with Mb.
¶¶ Not done.
** Nonimmune.
Tolerized Low Responder B10.BR or C57BL/10 T Cells Help F1 B Cells.

As shown in Fig. 1, tolerized B10.D2 or B10.BR T cells or (B10.D2 × B10.BR) F1 B cells were unresponsive to Mb by themselves. However, when T cells from B10.D2 were cocultured with B cells from F1, B10.D2 T cells helped F1 B cells quite well. Because B10.D2 mice were shown to be high responders to Mb in vivo or in vitro (14–16), the ability of B10.D2 T cells to help F1 B cells was reasonably expected. On the other hand, low responder B10.BR T cells tolerized to H-2^d alloantigens also could help F1 cells at birth.

In the present paper we do not experimentally distinguish between B cells and APC. However, in a subsequent paper, we show that these effects involve a genetic restriction in T cell-B cell interaction, whether or not they also involve a T cell-APC restriction.
B cells. Similar experiments were conducted with another strain combination, (B10.D2 × C57BL/10) F1 B cells and B10.D2, B10.A(5R), or C57BL/10 T cells, all tolerized to alloantigens on B6D2F1 spleen cell (Figs. 2, 3). C57BL/10 mice were tolerized to H-2d alloantigens, and B10.D2 mice were tolerized to H-2b alloantigens. Similar results were obtained, namely that tolerized higher responder B10.D2 T cells, as well as T cells from intermediate responder B10.A(5R) and low responder C57BL/10 mice, could help (B10.D2 × C57BL/10) F1 B cells. These data indicated that T cells

![Fig. 2](image)

Fig. 2. Mb-immune, not nonimmune, low responder tolerized T cells help FI B cells. B cells were obtained from Mb-immune (B10.D2 × C57BL/10) F1 spleen cells and T cells from Mb-immune B10.D2 or C57BL/10, or nonimmune C57BL/10 spleen cells from mice tolerized at birth with (B6D2) F1 spleen cells. The T cells were cultured with B cells at the ratio of 1:2 in the presence of Mb, 1 μg/ml (See legend to Fig. 1).

![Fig. 3](image)

Fig. 3. Low responder tolerized T cells pretreated with antibody to high responder H-2 alloantigens to eliminate any F1 T cells from the tolerization still help FI B cells. B cells were obtained from Mb-immune (B10.D2 × C57BL/10) F1 spleen cells and T cells from B10.D2, B10.A(5R), or C57BL/10 spleen cells neonatally tolerized with B6D2 F1 spleen cells. T cells derived from B10.D2 or C57BL/10 mice were pretreated with anti-H-2b or anti-H-2d antibody and Rb C+, respectively, to eliminate contaminating chimeric F1 spleen cells. The T cells were cultured with B cells at the ratio of 1:2 in the presence of Mb, 1 μg/ml (See legend to Fig. 1).
not only from high responder mice but also from intermediate or low responder mice can help F1 B cells in vitro.

At this stage, several possible artifacts must be considered that could provide trivial explanations for these observations: (a) helper activity of tolerized low responder T cells for F1 B cells might be due to an allogeneic effect, even in the absence of detectable MLR and CML reactivity; (b) the T cell help in the spleen of tolerized low responder mice might not be due to homozygous low responder T cells, but rather to F1 T cells that were derived from the donor cells used in neonatal tolerization. These possibilities were tested in the following experiments.

Evidence Against an Allogeneic Effect: Antigen-primed T Cells Are Required for Help. Mb-immune tolerized C57BL/10 T cells help (B10.D2 × C57BL/10) F1 B cells as noted above. However, nonimmune tolerized C57BL/10 T cells could not help F1 B cells in producing an anti-Mb response (Fig. 2). Because the allogeneic effect should have been the same in both cases, these data argue strongly against a positive allogeneic effect as the source of the help. However, one might argue that an allogeneic effect could enhance a weak response due to antigen-primed T cells. Therefore, we intentionally co-cultured F1 B cells with untolerized Mb-immune low responder T cells or untolerized nonimmune (normal) low responder T cells in the presence of Mb to test the degree of allogeneic help that could be observed in this combination. F1 B cells co-cultured with either normal low responder T cells or Mb immune (nontolerant) low responder T cells produced only a very low antibody response to Mb in the culture supernatants, and the response with immune T cells was no greater than that with nonimmune T cells (data not shown). Therefore, any allogeneic help produced by low responder T cells was very small and was not enhanced by antigen priming. Taken together, these experiments demonstrate that the response to Mb by F1 B cells with the help of tolerized low responder T cells was not due to an allogeneic effect. One additional piece of evidence against an allogeneic effect is presented below.

T Cells in the Tolerized Low Responder that Help F1 B Cells Were Not Derived from Donor F1 Cells. To study the existence of F1 spleen cells in the tolerized spleen cell populations, the phenotypes of spleen cells from tolerized mice were tested by indirect immunofluorescence or by an FACS using H-2-specific reagents. FACS and immunofluorescence staining were generously performed by Mrs. Susan O. Sharrow and by Dr. Ronald E. Gress, respectively. The spleen cells from neonatally tolerized mice at 10 wk of age were found to be contaminated with 2% F1 cells (limit of detectability) by indirect immunofluorescence staining and with 2-4% F1 cells by FACS analysis. However, no F1 cells could be detected in the thymus even by FACS analysis. This evidence for peripheral chimerization without thymic chimerization agrees completely with the more extensive studies of Streilein et al. (25) on this subject. The presence of these F1 cells may be important during antigen priming in vivo.

The existence of F1 cells in the spleen led to the concern that the helper activity attributed to low responder T cells was really due to F1 cells. Two types of evidence mitigate strongly against this possibility. (a) When Mb-primed F1 T cells were titrated into nonimmune T cells in various proportions, no help was observed at <10% primed F1 cells (data not shown). Because the contaminating F1 cells in the tolerized mice
were uniformly <5%, they would not have been sufficient to generate the help observed. (b) A more definitive experiment was to eliminate F1 cells from the low responder T cell population by treatment with an anti-H-2 alloantiserum and C'. As shown in Fig. 3, Mb immune-tolerized C57BL/10 T cells were pretreated with polyclonal antibodies to high responder H-2d alloantigens and Rb C' before culture. Even though chimeric F1 cells in the tolerized cell population were eliminated by this treatment, tolerized low responder C57BL/10 T cells could still help F1 B cells just as well as untreated T cells. The same results were found when tolerized B10.BR T cells were treated with anti-H-2Kd I-Ak antisera before co-culture with (B10.BR × B10.D2) F1 B cells. The help was not diminished by the treatment with alloantiserum plus C'. As a control, Mb-immune F1 T cells treated with the same antibody to H-2d alloantigens and Rb C' under the same conditions were lysed >98% and lost all helper function as well. Based on these data, as antibodies to H-2d alloantigens could eliminate functional F1 T cell activity (as well as kill >95% of F1 cells by dye exclusion), the T cells in tolerized low responder mice that helped F1 B cells were not derived from donor F1 T cells.

How Can Low Responder T Cells Interact with F1 B Cells?

Tolerized low responder T cells helped F1 B cells. However, it was not clear whether low responder T cells recognized F1 B cells via syngeneic low responder MHC antigens or high responder alloantigens. To elucidate the mechanism of the recognition of F1 B cells by low responder T cells, tolerized low responder T cells were co-cultured with B cells from F1 (H-2dxb or dk), high responder (H-2d), or low responder (H-2b or k) mice. The low responder mice used as the B cell source were immunized with Mb-FTG, because low responder B cells were possibly not primed with Mb when they were immunized with Mb alone. Low responder mice immunized with Mb-FyG instead of Mb produced a good response to Mb when they were cultured with Mb-FyG (Figs. 4–6). These data indicated B cells in low responder were primed with Mb. Tolerized low responder C57BL/10 T cells help (B10.D2 × C57BL/10) F1 and B10.D2 B cells but not syngeneic low responder C57BL/10 B cells (Fig. 4). As a control, the B10.D2 and C57BL/10 B cells from mice immunized with Mb-FyG both produced antibodies to Mb in the supernatants when cultured with syngeneic FyG-primed T cells in the presence of Mb-FyG (Fig. 4) (data for B10.D2 cultured with Mb-FyG not shown). The unlikely possibility of alloimmune help due to residual T cells in the B10.D2 B cell population was excluded by mixing the B10.D2 and B10 B cell populations. No response was seen in these mixtures without added T cells. Similarly, neonatally tolerized low responder B10.BR T cells helped high responder B10.D2 B cells but not syngeneic B10.BR B cells, both of which were immunized with Mb-FyG (Fig. 5).

High Responder T Cells Tolerized to Low Responder MHC Fail To Help Low Responder B Cells and APC.

It has been reported (26) that removal of alloreactivity by negative selection with bromodeoxyuridine and (BUDR) light unmasks a responder T cell population that can proliferate in response to antigen presented on low responder APC. We wanted to see whether higher responder B10.D2 T cells, neonatally tolerized to lower responder B10.BR (H-2b) alloantigens, would help B10.BR B cells and APC. The result (Fig. 6) was that such B10.D2 T cells did not help B10.BR B cells, even though the latter were adequately primed as demonstrated by their response to Mb-FyG. An additional result of this experiment was to lend further support to the conclusion above that the help observed was not due to positive alloimmune effects. In
Fig. 4. Low responder C57BL/10 tolerized T cells help F₁ and high responder B cells but not low responder B cells. B cells were obtained from Mb-immune (B10.D2 × C57BL/10) F₁, Mb-FyG immune B10.D2, or Mb-FyG immune C57BL/10 spleen cells. T cells were from Mb-immune tolerized C57BL/10 or control Mb-FyG immune normal C57BL/10 spleen cells. Mb-immune T cells were cultured with B cells at the ratio of 1:2 in the presence of Mb, 0.1 μg/ml (□). As a control, Mb-FyG immune C57BL/10 T cells were cultured with Mb-FyG-immune syngeneic B cells at the ratio of 1:2 in the presence of Mb-FyG, 0.001 μg/ml (■). To test for suppression, the C57BL/10 B cells were co-cultured with unseparated Mb-immune F₁ spleen cells at different ratios (last three bars).
Fig. 5. Low responder B10.BR neonatally tolerized T cells help high responder but not syngeneic low responder B cells. B cells were obtained from Mb-FyG immune B10.D2 or B10.BR spleen cells and T cells from Mb-immune tolerized B10.BR spleen cells. T cells were cultured with B cells at the ratio of 1:2 in the presence of Mb, 1 µg/ml (□). Mb-FyG immune B10.BR spleen cells treated with Gp C' alone were cultured with Mb-FyG, 0.01 µg/ml (■). (See legend to Fig. 1.)

Fig. 6. High responder tolerized T cells help F1 B cells but not low responder B cells. B cells were obtained from Mb-immune (B10.D2 X B10.BR) F1 or Mb-FyG immune B10.BR spleen cells and T cells from Mb-immune tolerized B10.D2 spleen cells. T cells were cultured with B cells at the ratio of 1:2 in the presence of Mb, 1 µg/ml (□). Mb-FyG-immune B10.BR spleen cells treated with Gp C' alone were cultured with Mb-FyG, 0.001 µg/ml (■). (See legend to Fig. 1.)
contrast to the tolerized low responder T cells, which help allogeneic high responder and F1 B cells and APC, but not syngeneic B cells and APC, the tolerized high responder T cells help syngeneic B cells and semiallogeneic F1 B cells, but not allogeneic low responder B cells and APC. Thus, the response depends on the Ir genotype of the B cell/APC source, not on its allogenicity relative to the T cell source. From all of these data together, we conclude that tolerized low responder T cells, like tolerized high responder T cells, can recognize Mb in the context of the high responder but not the low responder MHC antigens on the F1 B cells.

Low Responsiveness of Low Responder B Cells Is Not Due to Suppressor Cells in This Population. To test for the possibility that the failure of low responder B cells and APC to receive help was due to the presence of suppressor cells in the low-responder B cell population, we co-cultured these low responder B cells with unseparated, Mb-immune F1 spleen cells (last three bars of Fig. 4). No suppression was observed at any ratio of low responder B cells to F1 spleen cells. Therefore, the failure of low responder B cells to respond is not due to suppressor cells in their midst (unless the suppressor cells can act only on low responder B cells—a possibility that cannot be completely excluded, but which we think unlikely because such postulated suppressor cells cannot be detected when the same B cells are responding to FyG-specific help (Fig. 4, fourth column from right)). As another control for T cells in the B cell population, no allogeneic help could be demonstrated when these low responder B cells were mixed with Mb-primed B10.D2 B cells in the absence of exogenous helper T cells (data not shown).

Discussion

The data presented here indicated that clones of helper T cells specific for Mb exist in low responder mice and that these low responder T cells can collaborate with high responder B cells when alloreactivity is eliminated. At least two mechanistic questions arise. (a) How do these helper cells arise in the repertoire? (b) How are they primed to be specific for Mb in the context of alloantigen? The former, more difficult, question is addressed below. The latter question, we believe, is more straightforward. Because we can detect 2–4% chimerization by F1 cells in the spleens of the tolerized mice, we believe these may be sufficient during immunization in vivo for clones of T cells restricted to the high responder alloantigens (H-2^d) to be primed. Using a FACS, we consistently observed 2–4% F1 cells in the spleens of neonatally tolerized mice at 10–16 wk of age. In contrast, in most of the mice we could detect no F1 cells in the thymus (see below). Similarly, Streilein et al. (25) have reported detecting variable chimerization, from 0.2 to 4.0% F1 cells in the spleen, but none detectable (<0.1%) in the thymus.

It might be argued that the collaboration across H-2 barriers reported here could be due to an allogeneic effect (28–30), even though no alloreactivity could be detected by MLR or CML (Table 1 [31]). We can exclude this possibility as follows: nonimmune low responder tolerized T cells could not help F1 B cells, and immune high responder tolerized T cells could not help low responder B cells. If a positive allogeneic effect were the cause of our results, one or both of these controls should have been

---

5 Suppression was assessed only in a secondary response. Although suppressor cells have been reported which act only on a primary response (27), these could not account for our results, which all were obtained in secondary responses.
positive. Furthermore, under our culture conditions, little or no allogeneic help could be elicited even intentionally.

Another trivial explanation would be that the help is produced not by the low responder T cells themselves, but by high responder F1 T cells chimerizing the tolerized host. Even though chimerization was <5%, this was still a concern. The evidence against it is twofold: (a) the helper T cells from low-responder tolerized mice pretreated with antibodies to alloantigens and complement, to abolish contaminating chimeric F1 cells, could still help F1 B cells; (b) in these culture conditions, a ratio of T cells to B cells of <1:5 or :4 did not result in antibody production (16). Furthermore, if varying ratios of Mb-primed F1 T cells were mixed with unprimed F1 T cells and help for syngeneic F1 B cells assessed, 5% primed T cells were insufficient to produce help for a response to Mb. In other words, a contamination with <5% F1 T cells would not have been sufficient to produce the help observed. From these two different sorts of experiments, it is very unlikely that contaminating chimeric F1 T cells provided the help for F1 B cells.

Having ruled out these two artifactual explanations of our results, we conclude that the present results demonstrate that genotypic low responder (H-2k or H-1-2b) T cells neonatally tolerized to higher responder H-2a antigens can provide Mb-specific help for high responder H-2a B cells and APC. Two types of explanations are possible for the existence of these helper T cells specific for antigen in the context of allogeneic MHC antigens. (a) Such cells may be present normally in low responder mice but masked by allogeneic effects. When alloreactivity is removed, they are unmasked and can be detected. (b) The process of neonatal tolerization may actually expand the T cell repertoire at the same time that alloreactivity is eliminated.

The first type of explanation, that such cells are normally present but masked by allogeneic effects, can be broken down further into two possibilities. First, it is possible that the repertoire is normally present in all animals for most antigens in the context of most haplotypes. This concept would be consistent with several studies in which alloreactivity was acutely depleted (26, 32, 33). The second possibility is that the repertoire for reactivity across allogeneic barriers occurs only for certain combinations of antigen and foreign MHC, because these combinations cross-react with another antigen in association with self-MHC (i.e., Mb plus H-2d is recognized as a cross-reaction by T cells really specific for H-2b plus some other antigen [X]). This idea is consistent with the observations of Doherty and Bennink (34, 35). However, it has at least two problems. First, it would require that such an exceptional case apply to both H-2b and H-2a low responders, suggesting that it may not be so exceptional a situation. Second, it requires the implicit assumption that what is recognized is a neoantigenic determinant formed between H-2d and Mb, which can cross-react with another neoantigenic determinant formed between H-2b and antigen X or between H-2b and antigen Y. It is less compatible with mechanisms involving dual recognition of antigen plus MHC, or even less stringent “altered self” hypotheses (1).

The second type of explanation, namely that the process of neonatal tolerization actually expands the T cell repertoire, can also be divided into two subcategories—intrathymic and extrathymic mechanisms. First, it is possible that neonatal tolerization by injection of adult F1 spleen cells at birth alters the T cell repertoire by altering the thymic environment. This mechanism would be consistent with observations made in bone marrow chimeras (1, 5, 10, 11), except that the thymic component
bearing the foreign MHC antigens could not be the thymic epithelium, as has been suggested, but would have to be circulating cells, such as lymphocytes or macrophages of the donor, which could enter the host thymus, in agreement with the observations of Longo and Schwartz (36) in chimeras. However, Streilein et al. (25) have observed chimerization only in the periphery, not in the thymus, in mice neonatally tolerized by a procedure similar to ours. To test this question in our own mice, we examined tolerized animals by use of the FACS at the time of the culture experiments. Although most mice showed 2-4% peripheral chimerization with F1 cells in the spleen, only rare mice showed detectable F1 cells in the thymus. This result makes this mechanism less likely, but it remains a possibility, as we cannot exclude the presence of F1 cells in the thymus below our limits of detection, or during a short period after tolerization.

Second, if neonatal tolerization leads to an alteration of T cell repertoire without chimerization of the thymus, it is possible that the repertoire is developed extrathymically. This possibility is compatible with the observation of peripheral chimerization without thymic chimerization. Moreover, there is precedent for such a suggestion in the studies of cytotoxic T cells in thymus-engrafted nude mice by Zinkernagel et al. (37), Lake et al. (38), and Kruisbeek et al. (39). It is more difficult, however, to reconcile extrathymic repertoire development with the contraction of the repertoire in F1 → parent bone marrow chimeras despite peripheral F1 cells (1-6, 9, 10, 40, 41), or with the restriction to the thymic parental haplotype of helper cells from F1 nude mice engrafted with a parental thymus (42) and homozygous nude mice engrafted with an allogeneic thymus (43).

Thus, whether intrathymically or extrathymically, the act of neonatal tolerization could have altered the T cell repertoire of such tolerant mice to include clones of T cells capable of cooperating with high responder B cells and APC. The current study cannot distinguish these mechanisms from each other or from the possibility that the repertoire of T cells capable of cooperating with high responder B cells and APC may normally be present in the low responder mouse, but just masked by alloreactivity.

Although our results agree with those of Forman et al. (13) in the cytotoxic T cell response of neonatally tolerized mice, they appear to be somewhat discrepant with the results for neonatally tolerized mice in a virus-specific cytotoxic response (12, 40), and with those for the antibody response of cells acutely depleted of alloreactivity by filtration through an irradiated F1 host (44, 45). The H-2 K/D-restricted cytotoxic responses may not be directly comparable to the Ia-restricted antibody responses as these repertoires may be affected differently by neonatal tolerization (25, 46) (as well as in bone marrow chimeras [43]). In the case of the antibody response to sheep erythrocytes studied by Sprent and von Boehmer (44), the differences may be caused by differences in the method of tolerization. On the one hand, the acute depletion method may also remove some of the repertoire for exogenous antigen seen in the context of the tolerated Ia. On the other hand, long-term tolerance from birth may allow the emergence of a repertoire not unmasked by the short-term depletion of alloreactive cells immediately before experimental culture. Finally, neonatal tolerization may possibly alter the repertoire, as discussed above.

Besides the chimera studies and acute depletion studies mentioned above, there are a few other studies in the literature that suggest that low responder T cells can be elicited for a response to the antigen under Ir gene control if they are immunized or induced by the proper route. For instance, Pierce and Kapp (47) showed that helper
T cells specific for \( l\)-glutamic acid\(^{10} \)-\( l\)-alanine\(^{30} \)-\( l\)-tyrosine\(^{10} \) (GAT) on low responder APC could be elicited by immunizing F\(_1\) mice with antigen bound to those APC. Araneo et al. (48) found helper T cells for a lysozyme response in the popliteal lymph nodes of low responder mice early but not late in the course of the response. However, both the GAT and lysozyme responses involve active suppression in the low responder. Nevertheless, even for cases in which no suppression has been demonstrable as a mechanism of \( Ir \) gene control, such as responses to (\( T, G \))-A--L (49), T\(_6\)-A--L, (\( H, G \))-A--L (50), and IgG myeloma protein (51), the elicitation of helper T cells in low responders has been possible by various manipulations.

Finally, our observation that high responder T cells tolerant to low responder MHC antigens cannot help low responder B cells and APC appears to be in disagreement with the observation of Ishii et al. (26) that acute depletion of alloreactivity with BUdR and light allows responder T cells to proliferate in response to antigen presented on low responder APC. One difference may be that the secondary antibody response we are studying involves a T cell-B cell restriction as well as a possible T cell-APC restriction, in contrast to the proliferative response which involves only the latter. However, a second difference between our results lies in the methods of removal of alloreactivity. Treatment of alloreactive cells undergoing an MLR with BUdR results in uptake of the BUdR into the DNA of the dividing cells. Subsequent exposure to ultraviolet light produces cross-linking of the DNA and thus prevents further replication. Although these cells cannot proliferate again in a secondary MLR, they are not necessarily killed, and may still carry out functions not requiring replication, such as positive allogeneic effects which may enhance weak antigen-specific proliferative responses caused by residual high responder APC. Moreover, the T cell proliferative response may be more susceptible to such positive allogeneic effects than the secondary in vitro antibody response, which in our hands is rather insensitive to such effects.

It should also be pointed out that these results agree with some of the findings of Pierce et al. (52) but not others. These investigators studied allogeneic mixes of T cells and B cells in an \( Ir \) gene-controlled antibody response in a splenic fragment culture system. In our system, as in theirs, low responder T cells could collaborate with high responder B cells but not with low responder B cells. The only discrepancy between the two studies is that we do not find that high responder T cells, tolerant to low responder MHC, help low responder B cells. One difference was that their low responder B cells were specific for a hapten, not for the antigen under \( Ir \) gene control as in the present study. However, the difference also may be caused by the fact that in the splenic fragment system, no specific attempt was made to remove alloreactivity. Thus, the response observed could have been influenced by positive allogeneic effects, although controls were done to suggest otherwise. On the other hand, the hypothesis of Pierce et al. (52) predicts that specific tolerization of the high responder to low responder alloantigens would eliminate the repertoire they described for antigen in the context of low responder alloantigens. Thus, it can never be tested, by definition, in the absence of possible allogeneic effects (and for the same reason, is not actually incompatible with our results).

How do these results help us to pinpoint the \( Ir \) gene defect for the antibody response to Mb? Because low responder B cells can be primed to Mb by using Mb-FyG and produce anti-Mb in the presence of FyG-specific help, we know that the low responder has competent Mb-specific B cells. In this study, we also found competent Mb-specific
helper T cells in the low responder, which were not phenotypically different from those in the high responder, in that both helped only high responder or F1 B cells and APC, not low responder B cells and APC. Thus, the defect may be in neither the T cell nor the B cell alone, but in the interaction between low responder T cells and low responder B cells and APC, or may reflect selective priming of helper cells by APC during in vivo immunization, which is then mirrored by a T cell-B cell restriction in vitro. In a subsequent paper, we will demonstrate that at least one defect exists in T cell-B cell collaboration, independent of the source of APC.

Summary

We sought to examine the role of immune response (Ir) genes in helper T cells. To eliminate allogeneic effects, we used neonatally tolerized mice. The results bear not only on the mechanism of Ir genes, but also on the development of the T cell repertoire. B10.BR (H-2k) or C57BL/10 (H-2b) mice, which were low responders to myoglobin (Mb), were neonatally tolerized to high responder H-2a alloantigens, and B10.D2 mice, which were high responders to Mb, were neonatally tolerized to low responder H-2k or H-2b alloantigens. Spleen cells from these tolerized mice did not show any reactivity in mixed-lymphocyte reaction or cell-mediated lympholysis against alloantigens used in tolerization. Mb-immune F1 B cells were helped comparably by Mb-immune tolerized low or high responder T cells. Thus, low responder T cells functioned equivalently to high responder T cells. The failure of nonimmune T cells from tolerized low responder mice to help F1 B cells and antigen-presenting cells (APC) indicated that collaboration between B10.BR or C57BL/10 T cells and F1 B cells was not caused by a positive allogeneic effect. Spleen cells from tolerized mice were contaminated with 2-4% chimeric F1 cells, as judged by fluorescence-activated cell sorter analysis, and no F1 alloantigens were detectable in the thymus. However, removal of chimeric F1 T cells from the tolerized cell population by treatment with anti-H-2 and complement did not change the helper activity of tolerized low responder T cells. These data indicated that helper activity in the T cell population from low responder mice was not due to F1 cells. Also, the level of contamination was not sufficient to quantitatively account for the help. In examining the genetic restriction of these tolerized T cells, we found that T cells from tolerized low responder B10.BR or C57BL/10 mice helped F1 or high responder B10.D2 B cells and APC but not syngeneic B10.BR or C57BL/10 B cells and APC, which were immunized with Mb-coupled fowl gamma globulin instead of Mb to prime low responder B cells with Mb. On the other hand, high responder B10.D2 tolerized T cells helped syngeneic B10.D2 B cells but not allogeneic low responder B10.BR B cells.

These data indicated that clones of helper T cells specific for Mb exist in low responder mice, and these are not phenotypically different from those in high responder mice, in that both help high responder and F1 but not low responder B cells and APC. These data are discussed in terms of the mechanism for Ir gene control, and the mechanism of T cell repertoire development—whether intra- or extrathymically—in neonatally tolerized mice.

The authors thank Dr. Ronald E. Gress for performance of CML and indirect immunofluorescence staining, Mrs. Susan O. Sharrow for performance of fluorescence-activated cell sorting, and Dr. Richard Hodes, Dr. Alfred Singer, and Dr. David H. Sachs for helpful discussion and
critical review of the manuscript. We are grateful to Mr. Christopher Cox, Mrs. Linda Hargrove, and Ms. Carla Replogle for assistance in the preparation of the manuscript, and to Mrs. Freda Kraus for expert handling of the experimental animals.

Received for publication 12 April 1982 and in revised form 17 June 1982.

References

1. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. Adv. Immunol. 27:51.

2. Katz, D. H., B. J. Skidmore, L. R. Katz, and C. A. Bogowitz. 1978. Adaptive differentiation of murine lymphocytes. I. Both T and B lymphocytes differentiating in F1 → parental chimeras manifest preferential cooperative activity for partner lymphocytes derived from the same parental type corresponding to the chimeric host. J. Exp. Med. 148:727.

3. Bevan, M. J. 1977. In a radiation chimera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells. Nature (Lond.). 269:417.

4. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of “H-2 self-recognition” by T cells: evidence for dual recognition? J. Exp. Med. 147:882.

5. Bevan, M. J., and P. J. Fink. 1978. The influence of thymus H-2 antigens on the specificity of maturing killer and helper cells. Immunol. Rev. 42:3.

6. Sprent, J. 1978. Restricted helper function of F1 → parent bone marrow chimeras controlled by K-end of H-2 complex. J. Exp. Med. 147:1838.

7. Waldmann, H., H. Pope, L. Brent, and K. Bighouse. 1978. Influence of the major histocompatibility complex on lymphocyte interactions in antibody formation. Nature (Lond.). 274:166.

8. Waldmann, H., H. Pope, C. Bettles, and A. J. S. Davies. 1979. The influence of thymus on the development of MHC restrictions exhibited by T-helper cells. Nature (Lond.). 277:137.

9. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody responses. V. Helper T cell recognition of H-2 determinants on accessory cells but not B cells. J. Exp. Med. 149:1208.

10. Kapppler, J. W., and P. Marrack. 1978. The role of H-2 linked genes in helper T-cell function. IV. Importance of T-cell genotype and host environment in I-region and Ir gene expression. J. Exp. Med. 148:1510.

11. Hodes, R. J., K. S. Hathcock, and A. Singer. 1979. Cellular and genetic control of antibody responses. VI. Expression of Ir gene function by H-2a accessory cells but not H-2a T or B cells in responses to TNP-(T,G)-A--L. J. Immunol. 123:2823.

12. Zinkernagel, R. M., G. N. Callahan, J. W. Streilein, and J. Klein. 1977. Neonatally tolerant mice fail to react against virus-infected tolerated cells. Nature (Lond.). 266:837.

13. Forman, J., J. Klein, and J. W. Streilein. 1977. Spleen cells from animals neonatally tolerant to H-2Kb antigens recognize trinitrophenyl-modified H-2Kb spleen cells. Immuno-genetics. 5:561.

14. Berzofsky, J. A. 1978. Genetic control of the immune response to mammalian myoglobins in mice. I. More than one I-region gene in H-2 controls the antibody response. J. Immunol. 120:360.

15. Berzofsky, J. A., L. K. Richman, and D. J. Killion. 1979. Distinct H-2-linked Ir genes control both antibody and T cell responses to different determinants on the same antigen, myoglobin. Proc. Natl. Acad. Sci. U. S. A. 76:4046.

16. Kohno, Y., and J. A. Berzofsky. 1982. Genetic control of the immune response to myoglobin.
V. Antibody production in vitro is macrophage and T-cell dependent and is under control of two determinant-specific Ir-genes. J. Immunol. 128:2458.

17. Hapner, K. D., R. A. Bradshaw, C. R. Hartzell, and F. R. N. Gurd. 1968. Comparison of myoglobins from harbor seal, porpoise, and sperm whale. I. Preparation and characterization. J. Biol. Chem. 243:683.

18. Schroer, J. A., J. K. Inman, J. W. Thomas, and A. S. Rosenthal. 1979. H-2-linked Ir gene control of antibody responses to insulin. I. Anti-insulin plaque-forming cell primary responses. J. Immunol. 123:670.

19. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:423.

20. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:465.

21. Hodes, R. J., G. B. Ahmann, K. S. Hathcock, H. B. Dickler, and A. Singer. 1978. Cellular and genetic control of antibody responses in vitro. IV. Expression of la antigens on accessory cells required for responses to soluble antigens including a response under Ir gene control. J. Immunol. 121:1501.

22. Billingham, R. E., L. Brent, P. B. Medawar, and E. M. Sparrow. 1954. Quantitative studies on tissue transplantation immunity. I. The survival times of skin homografts exchanged between members of different inbred strains of mice. Proc. R. Soc. Lond. B Biol. Sci. 145:32.

23. Hodes, R. J., B. S. Handwerger, and W. D. Terry. 1974. Synergy between subpopulations of mouse spleen cells in the in vitro generation of cell-mediated cytotoxicity. Evidence for the involvement of a non-T cell. J. Exp. Med. 140:1646.

24. Sharrow, S. O., B. J. Mathieson, and A. Singer. 1981. Cell surface appearance of unexpected host MHC determinants on thymocytes from radiation bone marrow chimeras. J. Immunol. 126:1327.

25. Streilein, J. W., R. S. Gruchalla, P. Wood, and P. Strome. 1982. Active clonal deletion in neonatal H-2 tolerance. Ann. N.Y. Acad. Sci. In press.

26. Ishii, N., C. N. Baxevanis, Z. A. Nagy, and J. Klein. 1981. Responder T cells depleted of alloreactive cells react to antigen presented on allogeneic macrophages from nonresponder strains. J. Exp. Med. 154:978.

27. Sorensen, C. M., and C. W. Pierce. 1981. Haplotype-specific suppression of antibody responses in vitro. I. Generation of genetically restricted suppressor T cells by neonatal treatment with semiallogeneic spleen cells. J. Immunol. 126:1327.

28. Ordal, J. C., and C. F. Grumet. 1972. 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. J. Exp. Med. 136:1195.

29. Osborne, D. P., Jr., and D. H. Katz. 1972. The allogeneic effect in inbred mice. I. Experimental conditions for the enhancement of hapten-specific secondary antibody responses by the graft-versus-host reaction. J. Exp. Med. 136:439.

30. Katz, D. H., and D. P. Osborne, Jr. 1972. The allogeneic effect in inbred mice. II. Establishment of the cellular interactions required for enhancement of antibody production by the graft-versus-host reaction. J. Exp. Med. 136:455.

31. Streilein, J. W. 1979. Neonatal tolerance: towards an immunogenetic definition of self. Immunol. Rev. 46:125.

32. Thomas, D. W., and E. M. Shevach. 1977. Nature of the antigenic complex recognized by T lymphocytes: specific sensitization by antigens associated with allogeneic macrophages. Proc. Natl. Acad. Sci. U. S. A. 74:2104.

33. Heber-Katz, E., and D. B. Wilson. 1975. Collaboration of allogeneic T and B lymphocytes in the primary antibody response to sheep erythrocytes in vitro. J. Exp. Med. 142:926.
808 MYOGLOBIN LOW RESPONDER T HELP HIGH RESPONDER B CELLS

34. Doherty, P. C., and J. R. Bennink. 1979. Vaccinia-specific cytotoxic T-cell responses in the context of H-2 antigens not encountered in thymus may reflect aberrant recognition of a virus-H-2 complex. J. Exp. Med. 149:150.

35. Bennink, J. R., and P. C. Doherty. 1978. T-cell populations specifically depleted of alloreactive potential cannot be induced to lyse H-2-different virus-infected target cells. J. Exp. Med. 148:128.

36. Longo, D. L., and R. H. Schwartz. 1980. T cell specificity for H-2 and Ir gene phenotype correlates with the phenotype of thymic antigen-presenting cells. Nature (Lond.). 287:44.

37. Zinkernagel, R. M., A. Ahhage, E. Waterfield, B. Kindred, R. M. Welsh, G. Callahan, and P. Pincete. 1980. Restriction specificities, alloreactivity, and allotolerance expressed by T cells from nude mice reconstituted with H-2-compatible or -incompatible thymus grafts. J. Exp. Med. 151:376.

38. Lake, J. P., M. E. Andrew, C. W. Pierce, and T. J. Braciale. 1980. Sendai virus-specific, H-2-restricted cytotoxic T lymphocyte responses of nude mice grafted with allogeneic or semi-allogeneic thymus glands. J. Exp. Med. 152:1805.

39. Kruisbeek, A. M., S. O. Sharrow, B. J. Mathieson, and A. Singer. 1981. The H-2 phenotype of the thymus dictates the self-specificity expressed by thymic but not splenic cytotoxic T lymphocyte precursors in thymus-engrafted nude mice. J. Immunol. 127:2168.

40. Zinkernagel, R. M., G. N. Callahan, A. Altchage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus plus self-specific cytotoxic T cells: evidence for T help. J. Exp. Med. 157:897.

41. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. J. Exp. Med. 148:766.

42. Hedrick, S. M., and J. Watson. 1979. Genetic control of the immune response to collagen. II. Antibody responses produced in fetal liver restored radiation chimeras and thymus reconstituted F1 hybrid nude mice. J. Exp. Med. 150:646.

43. Singer, A., K. S. Hathcock, and R. J. Hodes. 1982. Self recognition in allogeneic thymic chimeras. Self recognition by T helper cells from thymus-engrafted nude mice is restricted to the thymic H-2 haplotype. J. Exp. Med. 155:339.

44. Sprent, J., and H. von Boehmer. 1976. Helper function of T cells depleted of alloantigen-reactive lymphocytes by filtration through irradiated F1 hybrid recipients. I. Failure to collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured in vivo. J. Exp. Med. 144:617.

45. Streilein, J. W., and R. S. Gruchalla. 1981. Analysis of neonatally induced tolerance of H-2 alloantigens. I. Adoptive transfer indicates that tolerance of class I and class II antigens is maintained by distinct mechanisms. Immunogenetics. 12:161.

46. Strehlein, J. W., and R. S. Gruchalla. 1981. Analysis of neonatally induced tolerance of H-2 alloantigens. II. Adoptive transfer indicates that tolerance of class I and class II antigens is maintained by distinct mechanisms. Immunogenetics. 12:161.

47. Pierce, C. W., and J. A. Kapp. 1978. Suppressor T-cell activity in responder × nonresponder (C57BL/10 × DBA/1) F1 spleen cells responsive to L-glutamic acid(L)-alanine(L)-tyrosine(L). J. Exp. Med. 148:1282.

48. Araneo, B. A., R. L. Yowell, and E. E. Sercarz. 1979. Ir gene defects may reflect a regulatory imbalance. I. Helper T cell activity revealed in a strain whose lack of response is controlled by suppression. J. Immunol. 123:961.

49. Howie, S., M. Feldmann, E. Mozes, and P. H. Maurer. 1977. In vitro studies on H-2 linked unresponsiveness. I. Normal helper cells to (T, G)-A-L and GAT in low and non-responder mice. Immunology. 32:291.
50. Stötter, H., A. Imm, M. Meyer-Delius, and E. Rüde. 1981. Specificity of H-2 linked Ir gene control in mice: demonstration of T helper cells recognizing branched synthetic polypeptides in low responder mice. J. Immunol. 127:8.

51. Janeway, C. A., Jr., W. E. Paul, T. P. Werblin, and R. Lieberman. 1976. IgG-specific helper activity of T lymphocytes from mice lacking the Ir-IgG gene. Immunogenetics. 3:393.

52. Pierce, S. K., N. R. Klinman, P. H. Maurer, and C. F. Merryman. 1980. Role of the major histocompatibility gene products in regulating the antibody response to dinitrophenylated poly (L-Glu30-L-Ala30-L-Phe)3. J. Exp. Med. 152:336.