GENETIC CONTROL OF IMMUNE RESPONSE TO MYOGLOBIN

Ir Gene Function in Genetic Restriction between T and B Lymphocytes

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Over the past decade, hypotheses regarding the site of Ir gene action have changed considerably (1, 2). The original, widely held concept (3) that Ir genes were expressed in T cells gave way to the belief that they were expressed in antigen-presenting cells (APC) of the macrophage-monocyte-dendritic cell lineage, or at the very least, in T cell-APC interactions. The B cell, which actually produces the antibodies, was given a relatively minor role. Some authors (4) presented evidence that the B cell played no role at all, that everything could be explained solely on the basis of T cell interaction with the APC. Others (5, 6) presented evidence that the B cell played a role parallel to that of the APC in that T cells were genetically restricted to interact with high but not low responder B cells as well as APC. This controversy was at least partially resolved by the discovery (7, 8) that T cell help for Lyb5+ B cells was genetically restricted, whereas that for Lyb5- B cells was not. These and related studies led to the hypothesis that, what the T cell saw first on the APC (a certain combination of antigen and Ia), it must see reproduced on the B cell to mediate help (9, 10). Most of these studies involved synthetic repeating polypeptide antigens, such as (T,G)-A--L.

We have been studying the genetic control of the immune response to a natural globular protein antigen, sperm whale myoglobin (Mb), and have found that the in vivo antibody response (11), the in vitro T cell response (12), and the in vitro antibody response (13) are all controlled by at least two Ir genes mapping in distinct subregions, I-A and I-C, of the H-2 complex. These genetically separable genes control responses to chemically distinct determinants on the same antigen molecule (12, 13). In the case of T cell proliferation, we found that the selection of which antigenic determinant was stimulatory for (high responder × low responder)F1 T cells depended on the Ir genes of the strain from which the APC were obtained (14). Thus, the Ir gene restriction of T cell-APC interaction led to what had been described by Rosenthal et al. (3) as “determinant selection.”

However, we wished to explore the cellular interactions involved in regulating antibody production rather than T cell proliferation. To do so, we recently developed (13) an in vitro culture system in which we can measure soluble Mb-specific antibody

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1 Abbreviations used in this paper: APC, antigen-presenting cell; CFA, complete Freund’s adjuvant; FCS, fetal calf serum; FyG, fowl gamma globulin; Ir, immune response; Mb, sperm whale myoglobin; Mb-FyG, myoglobin coupled fowl gamma globulin; PBS, phosphate-buffered saline; RaMB, rabbit anti-mouse brain-associated antigen; SAC, splenic glass adherent cell.
secreted into the culture supernatant fluid and can determine its fine specificity for different fragments of myoglobin. The response is dependent on both T cells and APC. In the previous paper (15), we explored the role of the T cell in this response by using neonatally tolerant mice to avoid allogeneic effects (16). We found that low responder mice, just like high responder mice, had helper T cells that could cooperate with F1 or high responder B cells and APC but not low responder B cells and APC. Because high and low responder T cells could not be functionally distinguished, the present study was carried out to examine the role of the B cell and APC in this response. In this study, the B cells were specific for the antigen under Ir gene control, myoglobin, rather than for a hapten, as in most of the earlier studies. Low responder B cells were found to be competent to produce anti-Mb antibodies but unable to receive help from Mb-specific T cells regardless of the source of APC. (We have not been able to demonstrate a similar APC restriction in this secondary response, although we cannot prove that one does not exist.) Thus, although low responder T cells and B cells both appear competent to respond to Mb, the Ir gene defect expresses itself in the interaction between the two. The results are discussed in terms of mechanisms for Ir gene action.

Materials and Methods

Animals. B10.D2/nSn, B10.BR/SgSn, C57BL/10Sn (also denoted B10), (B10.BR × B10.D2)F1, and B6D2F1 mice were obtained from The Jackson Laboratories, Bar Harbor, ME. Mice were 8–16 wk of age at the first immunization. (B10 × B10.D2)F1 mice were bred by us. (CBA/N × DBA/2)F1 and (DBA/2 × CBA/N)F1 mice were a gift of Dr. John T. Kung and Dr. William E. Paul, National Institute of Allergy and Infectious Disease.

Antigens. Mb was obtained from the Accurate Chemical and Scientific Co., Hicksville, NY. The major chromatographic component, IV, purified as described previously (11), was used throughout these studies. Fowl gamma globulin (chicken) (FyG) from United States Biochemical Corp., Cleveland, Ohio, was used throughout these experiments.

Preparation of Mb-coupled FyG (Mb-FyG). Preparation of Mb-FyG was performed by the method described by Schroer et al. (17). Our method of preparation of Mb-FyG was described in detail in the previous paper (15). The molar substitution of Mb to FyG was 1.5:1. Concentrations specified in the cultures are those of the FyG moiety.

Immunization Schedule. All mice were immunized intraperitoneally with 150 µg of purified Mb or with 200 µg of Mb-FyG in PBS emulsified 1:1 in complete Freund's adjuvant (CFA) (H37Ra; Difco Laboratories, Detroit, MI) in total volume of 0.1 ml per animal. B6 → B6D2F1 (donor → recipient) chimeric mice were immunized intraperitoneally with 150 µg of Mb in CFA and injected intravenously with 4 × 10⁷ hybrid F1 unirradiated APC (normal spleen glass adherent cells; SAC). 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 by cervical dislocation 1–2 wk later.

Cell Culture. A culture system modified by that of Mishell-Dutton (18) was described in a previous paper (13). 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 supplemented with 10% heat-inactivated fetal calf serum (FCS) (Microbiological Associates, Walkervillle, 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 secreted antibody.

Preparation of T Cells. T cells were prepared by passage of spleen cells over nylon-fiber column and collection of the nylon-nonadherent eluate (19). 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 pretreatment with a rabbit antibody against mouse brain-associated antigen (RaMB) (Litton Bionetics, Kensington, MD) and guinea pig complement (Cedar Lane low-tox complement, Accurate Chemical and Scientific Corp.), as described (13).

**Preparation of Splenic Glass Adherent Cells (SAC).** The preparation of SAC was described previously (13).

**Sephadex-G10 Passage.** Sephadex G10 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) passage of spleen cells performed by a modification of the method described by Hodes et al. (20) was described previously (13, 15).

**Radioimmunoassay for Antibodies to Mb.** The assay of antibody to Mb in the culture supernatants was described elsewhere (13).

**Chimeras.** Recipient mice were irradiated with 950 rad from an x-ray source and reconstituted 4–6 h later with 1–2 X 10⁷ bone marrow cells that had been pretreated with RaMB and complement. Chimeras are designated as bone marrow donor → irradiated recipient. The animals used in this experiment were typed for H-2 using H-2-specific antisera (kindly supplied by Dr. David Sachs, National Institutes of Health, Bethesda, MD) and complement. In all animals tested results indicated that splenocytes were of donor origin with no detectable cells of recipient haplotype by dye exclusion. Dr. Richard J. Hodes and Dr. Pat Dwyer, National Institutes of Health, generously provided these chimeric mice.

Before use in culture, to remove any F1 cells that might be present, derived either from the host or from the F1 SAC inoculum during immunization, spleen cells from chimeric mice were incubated with a 1:60 dilution of anti-H-2<sup>d</sup> antibodies (4227: C57BL/10 anti-B10.D2, titer 1:128) for 30 min at 37°C. After another incubation with rabbit complement (Cedarlane low-tox complement, Accurate Chemical and Scientific Corp.) for 30 min at 37°C, cells were extensively washed with RPMI 1640 supplemented with 5% FCS and were cultured with Mb. The antiserum used was a generous gift of Dr. David H. Sachs, National Institutes of Health.

**Results**

**Low Responder Mice Immunized with Mb-FyG Produced Antibody to Mb.** To study cellular interactions in low responder mice, we first had to ascertain whether low responder B cells could produce anti-Mb under any circumstances. When low responder mice of haplotype H-2<sup>b</sup> or H-2<sup>k</sup>, were immunized with Mb, they could not produce antibody to Mb in vivo (11) or in vitro (13). However, if low responder mice were immunized with Mb-FyG, their spleen cells, cultured with Mb-FyG, produced a considerable amount of antibody to Mb in the culture supernatants (Fig. 1). To

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**Fig. 1.** Low responder mice immunized with Mb-FyG showed a response to Mb. Spleen cells from mice immunized with Mb or Mb-FyG were cultured with Mb, 0.1 µg/ml, or Mb-FyG, 0.01 µg/ml, respectively. The antibody concentrations determined by solid-phase radioimmunoassay are expressed as mean ± SEM. □, cultured with Mb, 0.1 µg/ml; ■, cultured with Mb-FyG, 0.01 µg/ml.
Fla. 2. FyG-primed T cells can help Mb-primed B cells in the presence of Mb-FyG. Mb-primed B cells were prepared by depletion of T cells by pretreatment with RaMB and complement. FyG-primed T cells were prepared by passage of spleen cells over a nylon-fiber column and collection of nonadherent cells. Nonadherent cells were irradiated with 250 rad from $^{100}$Cs source to eliminate memory B cells. Mb-primed B cells cocultured with FyG-primed T cells in the presence of Mb-FyG, 0.01 μg/ml, or a mixture of Mb 0.1 μg/ml and FyG 0.01 μg/ml. The antibody concentrations are expressed as mean Δ cpm ± SEM, from a solid-phase radioimmunoassay. *

study the cellular mechanism of this responsiveness to Mb-FyG. Mb-immunized high responder $H-2^d$ or F1 B cells were cultured with syngeneic FyG-immunized T cells. FyG-immunized T cells help Mb-immunized B cells in the presence of Mb-FyG (Fig. 2). However, when Mb-immunized B cells were cultured with FyG-immunized T cells in the presence of a mixture of free Mb and free FyG, FyG-immunized T cells could not help Mb-immunized B cells (Fig. 2). Therefore, the covalent complex of Mb and FyG is necessary to obtain this carrier effect. In the case of the low responder, unseparated spleen cells immune to Mb-FyG produced anti-Mb only when Mb-FyG was present in the culture, not when free Mb alone was present (see control bars 2 and 9 in Fig. 3). These data indicated that Mb-immunized B cells in the low responder were helped by FyG-immunized T cells but not Mb-immunized T cells. Based on these data, we conclude that clones of B cells specific for Mb exist in the low responder and that coupling Mb to a carrier such as FyG can overcome the barrier of the genetic control of responsiveness to Mb.

The fine specificities of antibodies in the culture supernatants stimulated with Mb-FyG were studied, and low responder mice immunized with Mb-FyG produced
antibodies specific for both fragment (1–55) and fragment (132–153) (data not shown). However, we do not yet know whether or not the B cell precursor frequencies are the same in the high and low responder.

*(High Responder × Low Responder)*F1 Mb-immunized T Cells Help High Responder B Cells But Not Low Responder B Cells. We next asked whether these Mb-immune low responder B cells could be activated by Mb-specific help rather than carrier (F'ld3)-specific help. Mb-immunized (B10.D2 × B10.BR)F1 (H-2d × H-2k) T cells help Mb-FyG-immunized high responder B10.D2 B cells but not Mb-FyG-immunized low responder B10.BR B cells (Fig. 3). In control cultures, Mb-FyG-immunized B10.BR spleen cells produced antibody to Mb in the presence of Mb-FyG, and Mb-FyG-immunized B10.BR B cells cultured with Mb-FyG-immunized B10.BR T cells in the presence of Mb-FyG made antibody to Mb (Fig. 3). These control data indicated that B10.BR B cells were competent and primed well with Mb. Thus, Mb-immunized F1 helper T cells collaborate with the high responder B10.D2 B cell and APC population but not the low responder B10.BR B cell and APC population. To know whether the failure of F1 T cells to help low responder B cells was due to the B cell itself or to a requirement for high responder APC, F1 APC were added to culture of F1 T cells and
B10.BR B cells at the level of 20% of the cultured cells (Fig. 3). Whereas the same number of F1 APC could reconstitute the response of F1 spleen cells depleted of APC by passage over Sephadex G10 (Fig. 4), addition of F1 APC did not enhance the response of F1 T cells and B10.BR B cells. This experiment was repeated at several ratios of F1 SAC to F1 T cells and B10.BR B cells, and at no ratio was a response by B10.BR B cells observed. From these results, we conclude that at least one manifestation of the Ir gene control of the response to Mb is an inability of otherwise competent low responder B cells to receive Mb-specific help from otherwise competent F1 T cells.

*T Cell-B Cell Ir Gene Restriction in a Low Responder → (High Responder × Low Responder)\textsubscript{F1} Bone Marrow Chimera.* The existence of a T cell-B cell restriction resulting in low responsiveness was also demonstrated in bone marrow chimeras, in experiments in which the possibility of negative allogeneic effects due to residual T cells in the low responder B cell population could be completely excluded. Chimeras were made by reconstituting lethally irradiated (low responder × high responder)\textsubscript{F1} B6D2\textsubscript{F1} mice with T-depleted bone marrow from low responder B6 mice. Several months later, these mice were shown to have no detectable residual F1 lymphoid cells in the spleen, but only donor B6 cells that had repopulated the mouse. A number of groups have shown that such low responder T cells, which have matured in an F1 thymus, are tolerant to high responder alloantigens and can also cooperate with high responder B cells and APC in an antigen-specific response (2, 21). To be sure that F1 as well as low

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responder APC were present in vivo, these mice were injected intravenously with 40 × 10^6 F1 SAC at the time of immunization. If the only genetic restriction were between T cells and APC, we would have expected the chimeric spleen cells so immunized to respond to Mb in culture. However, they did not respond, either alone or when additional high responder (B10.D2) or F1 APC were added (Fig. 5). Only when F1 B cells (as well as APC) were added was a response seen. Thus, the chimeric low responder B6 T cells could provide Mb-specific help for B6D2F1 B cells, but they could not provide such help for syngeneic chimeric B6 B cells even in the presence of F1 APC. Therefore, genetic restriction in the Mb-specific interaction between competent chimeric low responder T cells and syngeneic low responder B cells resulted in nonresponsiveness.

Failure to Demonstrate Genetic Restriction for T Cell-Antigen-presenting Cell Interaction in Secondary In Vitro Response. From the results of Fig. 3, we knew that there was an Ir genetic restriction in the interaction between T cells and B cells, independent of the source of antigen-presenting cells. However, these experiments did not address the question of whether there was also a genetic restriction on the interaction between T cells and antigen-presenting cells in the same cultures. To explore this issue, we depleted Mb-immunized (high responder × low responder)F1 spleen cells of antigen-presenting cells by two sequential passages over Sephadex G10. The remaining population of Mb-primed T cells and B cells did not secrete anti-Mb in response to soluble Mb in the culture in the absence of added APC (Fig. 6). When these cultures were reconstituted with splenic glass-adherent cells (SAC) that had been pulsed with...
antigen by preincubation overnight with 10 μg/ml Mb, either high responder or low responder SAC restored the response (Fig. 6). The same was true when unpulsed SAC and soluble Mb were used in the culture, but even under the most stringent condition of pulsed low responder SAC and no soluble Mb, no genetic restriction at this level could be demonstrated (Fig. 6). It is difficult to deplete spleen cells of APC sufficiently to prevent an in vitro secondary response without also depleting B cells. Because we were able to deplete sufficiently to prevent any response to soluble antigen without added APC, the depletion must have been rather stringent. Nevertheless, it is impossible to be sure that every F1 APC was removed. Therefore, we cannot conclude that no genetic restriction exists for any APC function in this secondary response, only that it is very difficult to demonstrate one. In contrast, the ease of demonstrating an Ir genetic restriction on T cell-B cell interaction leads us to conclude that the major manifestation of Ir gene control during these secondary responses in cultures is a genetic restriction on T cell-B cell, not T cell-APC interaction.

Lyb-5+ B Cells Participate in the Response to Mb. CBA/N mice and F1 male mice, which are hemizygous for the CBA/N X chromosome, carry an X-linked recessive defect that is reflected in part in an absence of a subclass of mature B cells (22). B cells can be separated into two subpopulations based on their expression of the differentiation antigen, Lyb-5. B cells expressing the Lyb-5 determinant are a late-appearing subpopulation in normal mice and are absent in mutant CBA/N mice (22). Recently, the requirement was demonstrated (7, 8) for T helper cell recognition

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Fro. 7. Lyb-5− B cells can participate in the response to Mb. Spleen cells from B10.D2, B10.BR, (CBA/N × DBA/2)F1 male (Lyb-5−) or (DBA/2 × CBA/N)F1 male (Lyb-5− and Lyb-5+) mice immunized with Mb were cultured with Mb 0.1 μg/ml. The antibody concentrations are expressed as mean ± SEM.

of the MHC determinants expressed on B cells for activation of the Lyb-5-negative B cell subpopulation but not for activation of the Lyb-5-positive B cell subpopulation in the primary response and secondary response to TNP-KLH. In view of the MHC genetic restriction in T-B cell interaction that we just demonstrated to be at least partially responsible for the $I^r$ gene control of the response to Mb, we examined the possible participation of Lyb-5-negative B cells in the tertiary in vitro antibody response to Mb. To do so, we compared the response of spleen cells from (CBA/N × DBA/2)F1 male mice containing only Lyb-5− B cells with that of spleen cells from reciprocal (DBA/2 × CBA/N)F1 male mice containing both Lyb-5+ and Lyb-5− B cells. (CBA/N × DBA/2)F1 male mice produced the same amount of antibodies specific for Mb as (DBA/2 × CBA/N)F1 or high responder B10.D2 mice and much higher than low responder B10.BR mice (Fig. 7). These observations indicated that the Lyb-5-negative B cell subpopulation can participate in the response to Mb and probably constitutes a major portion of the response because no increment was observed when both subpopulations were present.

Discussion

In this communication, we attempted to elucidate the role of cellular interactions in regulating the immune response to Mb. To study the interaction between T cells and B cells, helper T cells from F1 hybrid mice and B cells from parental mice were cocultured. The primary observation was that F1 T cells cooperated only with high responder, not with low responder, B cells, even when both populations of B cells
were competent to produce anti-Mb with appropriate help, and even in the presence of adequate F1 APC. This result was also observed in chimeras and may be correlated with the function of Lyb-5-negative B cells demonstrated in this response. It was difficult to demonstrate any similar restriction on the interaction between T cells and APC in this secondary response.

In this in vitro tertiary response, both T cells and B cells must be primed in vivo (13). Therefore, we had to be certain whether B cells from low responders were primed well with Mb in vivo or not. Mb-coupled FyG was used to prime B cells in low responder mice by taking advantage of the carrier effect provided by FyG (23–25). The results indicated that clones of B cells specific for Mb exist in low responder mice, and these B cells can be primed in vivo and induced to secrete anti-Mb in vitro by taking advantage of carrier (FyG)-specific helper T cells, even though they do not function with syngeneic or F1 Mb-specific T cells. (The function of helper T cells specific for Mb in low responder strains was examined in the previous paper [15].)

We do not believe that the failure of Mb-specific F1 T cells to help low responder B cells is due to a negative allogeneic effect (26) or to the existence of suppressor cells in the B cell population in low responder mice. Although the B cells used in our studies appeared to be adequately depleted of T cells, to rule out these possibilities, low responder B cells were cultured with unseparated F1 spleen cells. Any suppressive effect by low responder B cells or by residual T cells contaminating the B cell population could not be observed (15). Also, the same results were obtained with B cells from tolerant B6 → B6D2F1 chimeras. Therefore, there is no evidence to suggest that suppression in the low responder B cell preparation is responsible for the failure of help by F1 helper T cells for low responder B cells. We have not excluded the possibility of a haplotype-specific T suppressor cell in the F1 T cell population that suppresses the response of low but not high responder B cells and APC, as has been observed in the response to the terpolymer of glutamic acid 6°-alanine3°-tyrosine1° (GAT) (27). However, in contrast to the GAT system, we could not detect suppression by Mb-primed low responder spleen cells of the response of syngeneic Mb-FyG-primed spleen cells to Mb-FyG in culture (H. Kawamura and J. A. Berzofsky, unpublished observation). Therefore, there is no reason to suspect such suppression is responsible for the genetic restriction observed.

Our experiments complement those of several previous studies (5, 6, 28) in which B cells were primed with the hapten tri- or dinitrophenyl (TNP or DNP) to which the response is not genetically controlled, and T cells were primed with a synthetic polypeptide carrier to which the response is controlled by the relevant Ir genes. In the current study, B cells were primed with Mb, to which the response is controlled by Ir genes, and T cells were primed with either Mb or the immunogeneic carrier FyG. Therefore, if Ir genes functioned in B cells, we could expect to detect their activity more readily. The current study extends these earlier results (5, 6, 28) to show that low responder B cells specific for the antigen under Ir gene control can be primed in vivo and can be stimulated to secrete anti-Mb when helped by syngeneic carrier (FyG)-specific T cells. However, they are not helped by Mb-specific T cells (from either syngeneic or F1 hybrid mice). Either this result could be due to the failure of Mb-specific B cells to receive help from Mb-specific T cells, or it could be due to a failure to prime helper T cells specific for Mb in the context of low responder Ia antigens. Comparing our results with the earlier results using hapten-specific B cells...
that should not be directly affected by the *Ir* gene function, we might conclude that this phenomenon is independent of B cell specificity, and, thus, that the latter explanation seems more likely. However, given our observation of T cell-B cell restriction in the absence of demonstrable T cell-APC restriction, we must emphasize that this T cell-B cell genetic restriction may be a fundamental *Ir* restriction in its own right, not just a mirror of T cell priming. Thus, whether the B cells are specific for the antigen under *Ir* gene control or for a hapten attached to that antigen, B cells bearing only low responder Ia antigens may be unable to receive help from T cells specific for the antigen under *Ir* gene control. The latter explanation, however, is still subject to two interpretations: either the T cells that would be specific for Mb in the context of low responder Ia are missing from the repertoire, or they are potentially present but cannot interact with antigen on low responder APC or B cells because of a defect in antigen presentation by the low responder Ia. This latter dichotomy of interpretations remains unresolved.

In a response not controlled by *Ir* genes, Sprent (29) reported that F1 T cells activated in mice of one parental strain collaborated well with B cells of this strain but poorly with B cells of the opposite strain. On the other hand, Singer et al. (30) showed that each parent-specific F1 T cell subpopulation was triggered only by one parent's accessory cells (APC); but once triggered, each parent-specific F1 T cell subpopulation was capable of activating either parent's B cells in vitro in a primary anti-hapten PFC response to TNP-keyhole limpet hemocyanin (KLH). The latter results suggested that there exists a genetic restriction in the cellular interaction between T cells and APC, not between T cells and B cells. One explanation for the apparent discrepancy regarding the requirement for T cells to recognize the MHC determinants expressed on B cells rather than just on APC was proposed by Singer et al. (7) and Asano et al. (8). These investigators showed that ontogenically distinct subpopulations of B cells have distinct cellular activation requirements and that activation by helper T cells of Lyb-5- B cells is MHC restricted, whereas activation of Lyb-5+ B cells is not genetically restricted in the primary or secondary response to TNP-KLH in vitro. A different, but in some ways analogous, result was reported by Andersson et al. (31), namely, that small resting B cells required MHC-restricted T cell help, whereas B cell blasts could respond to less specific signals. Thus, one may conclude in general that the type of B cell participating in the response may determine whether one observes genetic restrictions in T-B interactions or not.

To test for the participation of Lyb-5- B cells in the *Ir*-gene controlled in vitro response to Mb under the conditions we use, we took advantage of the CBA/N X-linked genetic trait that results in the absence of Lyb-5+ B cells in these mice or the male progeny of CBA/N mothers. Spleen cells from (CBA/N2 × DBA/2J)F1 male mice, containing only Lyb-5- B cells, showed just as high a response to Mb as spleen cells from phenotypically normal reciprocal (DBA/2J × CBA/N2)F1 male mice (i.e., from DBA/2 mothers) containing both Lyb-5- and Lyb-5+ B cells. This result indicated that Lyb-5- B cells also participated in the response to Mb under our conditions of immunization and culture, and the genetic restriction between T cells and B cells reported in this communication is reasonably assumed to be due to the participation of Lyb-5- B cells. Therefore, these data demonstrate, in analogy with the results for genetic restriction in responses not under *Ir* gene control (7, 8), that *Ir* gene defects are manifested in T cell-B cell as well as T cell-APC interactions in
Fig. 8. Genetic restriction in the cellular interaction between T and B lymphocytes: a site of Ir gene action. Competent Mb-specific helper T cells in low responder mice (tolerant to high responder H-2 and Ia antigens) were demonstrated by their ability to help high responder Mb-specific B cells (15). Competent Mb-specific B cells in the low responder were demonstrated by their ability to make anti-Mb in the presence of carrier (FyG)-specific syngeneic helper T cells and Mb-FyG. However, a manifestation of the Ir gene defect is a failure of the competent low responder Mb-specific B cells to be helped by Mb-specific T cells. See text for details.

responses in which Lyb-5− B cells are the major participants.

It should be noted that we, like Yamashita and Shevach (6), could not detect T cell-APC restriction in the in vitro culture, even though both we and they readily detected T-B restriction. When we tested F1 T cells and B cells (depleted of APC by two passages over Sephadex G10) and splenic adherent cells derived from high responder or low responder mice, low responder APC reconstituted the response as well as did high responder APC (Fig. 6). In both their case and ours, residual F1 APC are very difficult to exclude, so it is impossible to say that there was no restriction for antigen presentation by adherent cells in the in vitro secondary response, only that we could not detect it as readily as we could detect T cell-B cell restriction. Nevertheless, neither of these results excludes the possibility that such T cell-APC genetic restriction is critical during antigen priming in vivo and, therefore, accounts for the failure to prime helper T cells restricted to interact with the low responder B cells. In fact, one interpretation of our results is that a T cell-APC genetically restricted interaction is essential during in vivo priming, at which time T cells specific for antigen in the context of high responder Ia proliferate. Having once proliferated as much as necessary during in vivo priming and boosting, these helper T cells may no longer require a genetically restricted interaction with APC in the in vitro tertiary response, so that the only genetically restricted interaction required in the culture is between T cells and B cells. This T-B restriction may mirror the T-APC restriction during priming (9, 10) but, also, it may be viewed as a fundamental restriction of equal importance to that during priming.

Our findings are summarized schematically in Fig. 8. In the previous paper (15), we showed that low responder mice can produce competent, Mb-specific helper T

2 The possibility that the helper T cells need no longer proliferate to function in the in vitro tertiary response is supported by the observation that their activity in vitro is resistant to low-dose irradiation (250–500 rad), although this dose might not be sufficient to prevent all proliferation of helper T cells, even though it does prevent proliferation and secretion by B cells.
cells, but only ones competent to interact with high responder or F1 B cells and APC. In the present paper, we found that low responder mice have B cells competent to produce anti-Mb antibodies when they receive the appropriate help. However, the help must be specific for a carrier such as FyG, not for Mb itself. Thus, although both the helper T cell compartment and the B cell compartment in the low responder have functional Mb-specific cells, the low responder or even (high responder × low responder)F1 Mb-specific T cells cannot help low responder Mb-specific B cells, regardless of the source of APC. It is in this T cell-B cell interaction that the Ir gene "defect" lies, at least at the effector stage of the response. Of course, a defect in a cellular interaction must reflect the properties of one or both cells involved. Because the low responder B cells are competent to make anti-Mb and because similar results have been found for hapten-specific B cells (see above), it seems likely that the failure to interact reflects the specificity of the helper T cell. The competence of the low responder Mb-specific T cells is for helping high responder B cells, not low responder B cells. As noted above, this specificity may be imposed at the time of in vivo priming, but ultimately may be due either to an absence from the T cell repertoire of cells capable of recognizing Mb in association with low responder Ia antigens or to a defect in some molecular interaction between Mb and low responder Ia on the APC and/or on the B cell itself. In the latter case, especially in view of the failure of low responder B cells to receive help from high responder, low responder, or F1 Mb-specific T cells (this report and ref. 15), the Ir gene defect behaves operationally as a B cell defect. This last dilemma is the subject of current investigation.

Finally, we suggest that if this Ir gene phenomenon is a fundamental property of the Ia on the B cell in the same way that it involves the Ia on the APC (9), the B cell should be able to mediate determination selection in the same way the APC has been shown to do in the case of T cell proliferative responses (3, 14). That is, the Ia on the B cell should limit the epitope fine specificity of antigen-specific T cells that can help that B cell. We are currently testing that hypothesis.

Summary

We studied the genetic restrictions on the interaction between T cells, B cells, and antigen-presenting cells (APC) involved in the H-2-linked Ir gene control of the in vitro secondary antibody response to sperm whale myoglobin (Mb) in mice. The B cells in this study were specific for Mb itself, rather than for a hapten unrelated to the Ir gene control, as in many previous studies. Low responder mice immunized in vivo with Mb bound to an immunogenic carrier, fowl gamma globulin (FyG), produced B cells competent to secrete anti-Mb antibodies in vitro if they received FyG-specific T cell help. However, (high-responder × low responder)F1 T cells from Mb-immune mice did not help these primed low responder (H-2 b or H-2 k) B cells in vitro, even in the presence of various numbers of F1 APC that were demonstrated to be competent to reconstitute the response of spleen cells depleted of APC. Similar results were obtained with B6 → B6D2F1 radiation bone marrow chimeras. Genotypic low responder (H-2 b) T cells from these mice helped Mb-primed B6D2F1 B cells plus APC, but did not help syngeneic chimeric H-2 b B cells, even in the presence of F1 APC. In contrast, we could not detect any Ir restriction on APC function during these in vitro secondary responses. Moreover, in the preceding paper, we found that low responder mice neonatally tolerized to high responder H-2 had competent Mb-specific helper T
cells capable of helping high responder but not low responder B cells and APC. Therefore, although functional Mb-specific T cells and B cells both exist in low responder mice, the \textit{Ir} gene defect is a manifestation of the failure of syngeneic collaboration between these two cell types. This genetic restriction on the interaction between T cells and B cells is consistent with the additional new finding that Lyb-5-negative B cells are a major participant in this in vitro secondary response because it is this Lyb-5-negative subpopulation of B cells that have recently been shown to require genetically restricted help. The \textit{Ir} gene defect behaves operationally as a failure of low responder B cells to receive help from any source of Mb-specific T cells, either high responder, low responder, or F1. The possible additional role of T cell-APC interactions, either during primary immunization in vivo or in the secondary culture, is discussed.

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