Brief Definitive Report

B LYMPHOCYTE IMMUNE RESPONSE GENE PHENOTYPE IS GENETICALLY DETERMINED*

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Experiments with radiation-induced bone marrow chimeras have demonstrated that the T cell repertoire for self-major histocompatibility complex (MHC) gene products and antigen is profoundly affected by the developmental milieu (1–7). Stem cells from (A × B)F_1 mice that develop in a parent A animal become adult T cells restricted to recognizing antigen in association with parent A MHC products. No cooperative interactions with parent B cells can be detected. In addition, when parent A stem cells mature to adult T cells in (A × B)F_1 animals, they develop the capacity to interact with parent B and F_1-specific MHC products. Furthermore, parent A cells that develop in a parent B host recognize antigen only in association with parent B MHC products. No cooperative interactions with parent A cells are detectable. Thus, it appears that the genetic restrictions expressed by adult T cells are acquired during their passage through the host thymus.

The interaction of T and B cells under certain circumstances has been found to be MHC restricted (8). The issue concerning what role the developmental milieu plays in determining the subsequent cooperative interactions of B cells with T cells is still controversial. Katz and co-workers (6) used a hapten carrier-specific adoptive transfer system in which A/J, BALB/c, or CAF_1 carrier-primed T cells were transferred together with B cells derived from F_1 → parent or parent → F_1 radiation chimeras that were previously primed to the hapten. It was shown that the B cells from CAF_1 → A/J chimeras cooperate preferentially with T cells from A/J but not with T cells from BALB/c mice. Conversely, CAF_1 → BALB/c chimeric B cells cooperate well only with BALB/c T cells and not with A/J T cells. In this series of experiments, no allogeneic effects were observed. It was concluded that host environmental milieu directs the differentiation pathways of donor B cells. Sprent and Bruce (9) addressed the same question by first depleting CBA T cells of alloreactivity to C57BL/6 (B6) determinants (denoted CBA_{-B6}) and examined the capacity of such T cells to cooperate with B cells from a variety of sources. They found that primed CBA_{-B6} cells were restricted to cooperative activity with primed CBA B cells only, and such cells could not interact with primed B6 B cells in an anti-sheep erythrocyte response. However, primed CBA_{-B6} T cells could cooperate equally well with primed B cells from F_1 → B6 or F_1 → CBA chimeras. If the maturation environment had an effect

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on the B cell's self-recognition capacity, one would have expected the CBA_{-B6} T cells to interact with B cells from the $F_1 \rightarrow$ CBA but not the $F_1 \rightarrow$ B6 chimeras. Thus, Sprent and Bruce found no evidence for self-restriction of B cell responses.

We extended the study of B cell repertoire development by examining responses controlled by Ir genes in unprimed B cells. Recently, we described a T cell-dependent B cell proliferative assay (10) in which unprimed B cells can be stimulated by primed T cells to proliferate through MHC-restricted polyclonal activation. This system also demonstrated Ir gene control of B cell responses because primed (responder × nonresponder)F_1 T cells stimulate proliferation of responder but not nonresponder B cells. We used this system to examine the immune response genes (Ir) phenotype of unprimed B cells from $F_1 \rightarrow$ parent and parent $\rightarrow F_1$ radiation-induced bone marrow chimeras. The results demonstrate that responder B cells developing in a nonresponder environment retain the capacity to respond in vitro, and nonresponder B cells developing in a responder environment are unable to make Ir gene-controlled responses, suggesting that the host environment has no effect on the cooperative interaction of B cells.

Materials and Methods

Animals. B10.Q and (B10.A × B10.Q)F_1 mice that originated from stocks generously supplied by Dr. J. H. Stimpfling (McLaughlin Research Institute, Great Falls, MT) and B10.T(6R) ($H\text{-}2^{v2}$, $K^qI^qD^d$) that originated from stocks generously provided by Dr. David Sachs (Transplantation Biology Section, Immunology Branch, National Cancer Institute, Bethesda, MD) were all bred in our own animal colony. B10.A mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Antigens. Poly(Glu^{60} Ala^{30} Tyr^{10})n (GAT) (lot 7) was purchased from Miles Laboratories, Inc., Miles Research Division, Elkart, IN. Poly(Glu^{56} Lys^{35} Phe^{9})n (GLϕ), originally purchased from Miles-Yeda, was the generous gift of Dr. Alan Rosenthal (Merck Institute, Rahway, NJ). Purified protein derivative of *Mycobacterium tuberculosis* (PPD) was purchased from Connaught Laboratory, Toronto, Ontario.

Immunization. GLϕ and/or GAT were emulsified in complete Freund's adjuvant containing 1 mg/ml of killed *M. tuberculosis* strain H37Ra (CFA) (Difco Laboratories, Detroit, MI). For priming of T cells, mice were injected subcutaneously at the base of the tail and in the hind footpads with 30–50 μg of the antigens.

Chimeras. Radiation-induced bone marrow chimeras were made as previously described (7). Mice were given 925–975 rad from a heavily filtered x-ray source and reconstituted with 10^7 bone marrow cells that had been depleted of T cells by in vivo treatment of donors with anti-thymocyte serum and cortisone and in vitro treatment with rabbit anti-mouse brain antiserum plus guinea pig complement. The chimeras were used no sooner than 3 mo after reconstitution and were individually H-2 typed before use. The spleen cells of each chimera were entirely of the donor origin. Parent $\rightarrow F_1$ chimeras were given 10^8 T cell-depleted bone marrow and spleen cells from the other parent intravenously as a source of antigen-presenting cells at the time of immunization. These cells were lysed with the appropriate antisera plus complement before assay.

T Cell and B Cell Proliferative Assays. 8 d after immunization, lymph node cells were harvested and passed over nylon wool columns (10). For the T cell proliferative response, 4×10^5 lymph node T cells eluted from the column were plated in 96-well flat-bottomed microtiter plates in EHAA medium supplemented with 10% fetal calf serum (FCS) along with soluble antigen and 10^5 irradiated (2,000 rad) F_1 spleen cells. For the B cell proliferative response, 10^5 primed T cells and 4×10^5 anti-Thy-1.2 (NEI-100; New England Nuclear, Boston, MA) plus complement-treated unprimed spleen cells were cultured in a modified Mishell-Dutton culture medium along with soluble antigen and 10^5 irradiated (2,000 rad) F_1 spleen cells. GAT and GLϕ were added at a final concentration of 100 μg/ml. PPD was used at 20 μg/ml. Cultures were maintained in 5% CO_2 at 37°C for 4 d. 16–20 h before harvesting, 1 μCi of tritiated thymidine (sp act 6.5 Ci/mmol; New England Nuclear) was added to each culture. Cultures were

TABLE I

Ir Gene Phenotype of Primed T Cells from Radiation-induced Bone Marrow Chimeras

Source of primed lymph node T cells	Source of APC in vivo	Source of APC in vitro	Proliferative response		
			Medium	GAT	GLϕ
				cpm	
B10.A → (B10.A × B10.Q)F_1*	B10.T(6R)	F_1	827	19,732	11,723
B10.T(6R) → (B10.A × B10.Q)F_1‡	B10.A	F_1	1,196	14,467	22,961
(B10.A × B10.Q)F_1 → B10.A	—	F_1	2,291	41,373	2,481
B10.A × B10.Q)F_1 → B10.Q	—	F_1	2,736	2,512	57,669

* Chimeras were given 10^8 T cell-depleted B10.T(6R) spleen and bone marrow cells intravenously at the time of immunization. All residual B10.T(6R) cells were killed before assay with (A/J × B10.A)F_1 anti-B10.Q antisera plus complement.

‡ Chimeras were given 10^8 T cell-depleted B10.A spleen and bone marrow cells intravenously at the time of immunization. All residual B10.A cells were killed before assay with (A.TH × B10.Q)F_1 anti-B10.A antisera plus complement.

harvested with a MASH II automatic harvester, and incorporation of tritiated thymidine was determined in a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, CA). All cultures were established in triplicate, and arithmetic means were determined.

Results

Ir Gene Phenotype of T Cells from Radiation-induced Bone Marrow Chimeras. To examine the effects of the developmental milieu on T cell antigen responses, we made radiation-induced bone marrow chimeras using strains with reciprocal Ir gene defects. The B10.A strain is a responder to GLϕ. The B10.Q and B10.T(6R) strains are responders to GLϕ and nonresponders to GAT. Four types of cimeras were prepared: H-2^{y2} → *H-*2^{axq}, *H-*2^{a} → *H-*2^{axq}, *H-*2^{axq} → *H-*2^{a}, and *H-*2^{axq} → *H-*2^{q}. The results of assaying the responsiveness of the T cells from these chimeras are shown in Table I. Chimeric animals of the type P_1 → (P_1 × P_2)F_1 were primed to antigen after the intravenous administration of 10^8 T cell-depleted spleen and bone marrow cells from P_2 as a source of APC. When B10.A cells (genotypic GLϕ nonresponder) develop in an F_1 animal, the mature T cells make a good proliferative response to GLϕ. Similarly, when B10.T(6R) (*I-*A^q) T cells (genotypic GAT nonresponder) develop in an F_1 animal, they became GAT responders. Thus, nonresponder stem cells can develop T cell responsiveness when they mature in a responder environment and are primed to antigen on responder APC. On the other hand, when responder F_1 stem cells develop in a B10.A (GLϕ nonresponder) environment, they fail to respond to GLϕ. In addition, responder F_1 T cells developing in a B10.Q (GAT nonresponder) environment become GAT nonresponders. Thus, the Ir gene phenotype of adult T cells is not determined by the T cell genotype, but by the environment in which the T cell matures.

Ir Gene Phenotype of Unprimed B Cells from Radiation-induced Bone Marrow Chimeras. To examine the effect of the host environment on B cell expression of Ir gene function, unprimed B cells from nonresponder parent → F_1 and F_1 → nonresponder parent chimeras were tested for their ability to be stimulated to proliferate by antigen-primed F_1 T cells. In this assay, monoclonal anti-Thy-1.2 plus complement-treated spleen cells from conventional or chimeric mice are used as the source of B cells, and primed F_1 lymph node cells passed over nylon wool columns are the source of T cells. All cultures are supplemented with F_1-irradiated spleen cells as a source of APC. As shown in Table II, normal B10.Q (GAT nonresponder) B cells cannot be recruited by F_1 T cells to prolifeate in response to the antigen GAT, whereas GAT responder B10.A and (B10.A × B10.Q)F_1 B cells both make good responses. B cells from all three

TABLE II

Ir Gene Phenotype of Unprimed B Cells from Radiation-induced Bone Marrow Chimeras

Source of primed T cells	Source of unprimed B cells	Irradiation of B cells (2,000 rad)	Proliferative response		
			Medium	GAT	PPD
			cpm		
—	B10.A	—	1,583	3,017	2,207
(B10.A × B10.Q)F_1	B10.A	—	1,658	28,762	19,993
—	B10.Q	—	2,749	2,796	2,545
(B10.A × B10.Q)F_1	B10.Q	—	3,012	4,408	18,506
—	(B10.A × B10.Q)F_1	—	2,341	3,067	2,651
(B10.A × B10.Q)F_1	(B10.A × B10.Q)F_1	—	2,639	23,781	11,615
(B10.A × B10.Q)F_1	(B10.A × B10.Q)F_1	+	199	1,383	1,285
—	(B10.A × B10.Q)F_1 → B10.A	—	1,364	1,135	924
(B10.A × B10.Q)F_1	(B10.A × B10.Q)F_1 → B10.A	—	1,739	16,897	15,163
—	(B10.A × B10.Q)F_1 → B10.Q	—	445	443	1,235
(B10.A × B10.Q)F_1	(B10.A × B10.Q)F_1 → B10.Q	—	1,395	17,041	12,903

(B10.A × B10.Q)F_1 mice were primed with GAT in CFA. 7 or 8 d later, T lymphocytes were prepared as described in Materials and Methods. B lymphocytes were obtained from anti-Thy-1.2 plus complement-treated spleen cells of strains, as indicated. T lymphocytes were used at 1×10^6 cells per well, whereas B lymphocytes were present at 4×10^6 cells per well. In certain cases, the B lymphocytes were irradiated at 2,000 rad. Cultures were assayed by tritiated thymidine incorporation on day 4.

TABLE III

Ir Gene Phenotype of Unprimed B Cells from Radiation-induced Bone Marrow Chimeras

Source of primed T cells	Source of unprimed B cells	Irradiation of B cells (2,000 rad)	Proliferative response		
			Medium	GAT	PPD
—	B10.A	—	2,895	2,821	3,141
(B10.A × B10.Q)F_1	B10.A	—	3,797	22,637	25,048
(B10.A × B10.Q)F_1	B10.A	+	187	9,978	4,048
—	B10.A → (B10.A × B10.Q)F_1	—	3,767	5,872	5,467
(B10.Q × B10.Q)F_1	B10.A → (B10.A × B10.Q)F_1	—	7,274	36,040	33,419
—	B10.T(6R) → (B10.A × B10.Q)F_1	—	4,194	5,594	4,582
(B10.A × B10.Q)F_1	B10.T(6R) → (B10.A × B10.Q)F_1	—	6,695	9,483	30,842

Experimental details were the same as in Table II.

strains respond to PPD antigen, the response to which is not under Ir gene control. Furthermore, (B10.A × B10.Q)F_1 B cells that developed in a GAT nonresponder B10.Q host still retain their phenotypic expression of responsiveness to GAT (Table II). This suggests that the host environment does not alter the Ir gene phenotype of donor B cells. In our assay, the behavior of chimeric B cells is indistinguishable from conventional B cells. This result is also seen in nonresponder parent → F_1 chimeras, as shown in Table III. Nonresponder B cells that develop in an F_1 responder host [i.e., B10.T(6R) → (B10.A × B10.Q)F_1] remain unresponsive to the antigen GAT. In all cases, the B cells were checked for their responses to PPD, and all were normally responsive. Unlike nonresponder T cells developing in a responder animal that became phenotypic responders, nonresponder B cells were unaffected by their development in a responder milieu.

Discussion

One of the difficulties in interpreting cell interaction experiments using primed B cells from chimeric mice (6, 9) is that such B cells might have been selected by host-restricted T cells during the priming processes. As a result, such B cells might also appear restricted to host MHC specificities. To avoid complications that might be introduced by in vivo priming, we used a B cell proliferative assay that is T cell

dependent but does not require primed B cells. Although this system does not assay the antigen specificity of the B cells (i.e., activation is polyclonal in nature), it does require histocompatibility at the I region between the interacting T and B cells (10, and unpublished observations). Our results indicate that phenotypic expression of Ir genes in chimeric B cells reflects the genotype of the donor B cells and is not affected by the genotype of the host. Responder F_1 B cells remain responders whether they develop in responder or nonresponder parental host (Table II). Similarly, nonresponder parental B cells developing in responder F_1 hosts do not acquire the ability to respond (Table III). These results are in contrast to those of Katz and co-workers (6) but agree with those of Sprent and Bruce (9). Our experiments suggest that the host environment has no effect on the cooperative interaction of B cells and argue against operation of adaptive differentiation at the level of B cells.

Previous experiments (1–7) have demonstrated that the T cell repertoire includes recognition of self-MHC gene products. Whether or not B cells need to recognize self-MHC products has not been determined unequivocally. Certainly, for MHC-restricted T cell-B cell interactions to occur, one does not require both the interacting cells to possess self-recognition capabilities. Schematic diagrams of MHC-restricted cell interactions have conventionally depicted the T cell-recognizing self-Ia antigens on the surface of both APC and B cells as the basis of the interaction. Because the Ir gene product is probably the Ia antigen (11–13) and because Ia antigens are not clonally expressed and seem not to change during development in a genetically different host, one would not expect the Ir gene phenotype of B cells to be altered if the conventional image is correct. The data presented by Sprent and Bruce (9) and our present study on the interaction of primed T cells and unprimed B cells are consistent with this notion. On the other hand, the implication that B cells might have a requirement to recognize self-MHC products was first suggested in the experiments of Katz et al. (6). Recently, Singer and his colleagues (14) have examined the B cell responsiveness of totally H-2 allogeneic chimeras of the sort A → B to the "T-independent" antigen trinitrophenyl-Ficoll. B cells from such animals seem to be restricted to cooperating only with accessory cells bearing host-type MHC. This apparent restriction is not overridden by providing F_1 T cells, indicating that the restriction does not simply reflect the failure to generate a T cell signal that requires an MHC-restricted APC-T cell interaction. In this primary T cell-independent response, the authors proposed that the responding B cells might recognize self-MHC products on the macrophage. Similar conclusions have been drawn by Nisbet-Brown et al. (15) in their recently published work on the antibody responses to the T-dependent antigens trinitrophenyl-keyhole limpet hemocyanin. It is interesting to note that the latter study suggests that B cells do not adaptively differentiate in parent → F_1 chimeras. Because APC and B cells always have the same genotype in our experiments, we cannot evaluate whether an H-2-restricted B cell-APC interaction mechanism can account for our observations. This possibility is currently under investigation.

Summary

We examined the effects of the developmental milieu on the capacity of B cells to undergo immune response gene-controlled, T cell-dependent polyclonal proliferation. Although *I-A*^{q} poly(Glu^{60} Ala^{30} Tyr^{10})n (GAT)-nonresponder T cells developing in a responder environment become phenotypic GAT-responders, *I-A*^{q} B cells remain unresponsive to GAT, even after maturation in a GAT-responder animal. Conversely,

(B10.A × B10.Q)F_1 ([GAT responder × GAT nonresponder]F_1) T cells developing in a B10.Q GAT nonresponder host fail to respond to GAT, but F_1 B cells from the same $F_1 \rightarrow$ parent chimeras make excellent proliferative responses in the presence of GAT and responder T cells. Thus, by this assay, B cell immune response gene function is genetically determined and is not affected by the developmental milieu.

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