

ANTIGEN-SPECIFIC HELPER T CELLS REQUIRED FOR  
DOMINANT PRODUCTION OF AN IDIOTYPE (ThId) ARE  
NOT UNDER IMMUNE RESPONSE (Ir) GENE CONTROL\*

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Anti-hapten antibody responses to hapten-protein conjugates have been shown to involve the cooperative interaction of hapten-specific B lymphocytes and helper T (Th)<sup>1</sup> lymphocytes specific for the protein carrier molecule (1). In addition to specificity for the carrier, helper T cells display specificity for self determinants encoded in the I-region of the major histocompatibility complex (MHC), such that they collaborate efficiently only with B lymphocytes also bearing these determinants (2-4). Also mapping in the I-region of the MHC are a family of genes known collectively as immune response (Ir) genes that control the immune response to many protein and synthetic polypeptide antigens (5, 6). When antigens under the control of Ir genes are used as carriers, only B cells from responder animals can be activated to produce antibody by T cells from mice which themselves are responders (7, 8).

Recently, it has been shown that certain antibody responses require two distinct sets of antigen-specific helper T cells (9-12). For instance, dominant production of the idiotypic associated with the BALB/c phosphorylcholine (PC) binding myeloma protein TEPC 15 (T15) involves two sets of helper T cells (11, 12). One set is specific for carrier, requires a physical linkage of hapten and carrier molecules, and activates hapten-specific B cells independent of idiotypic. The other set, also specific for antigen, does not require physical linkage of the hapten and the carrier. These latter helper T cells are deficient in mice having low levels of circulating antibody bearing the T15 idiotypic (11-13). Optimal activation of PC-specific B cells bearing the T15 idiotypic by such Th cells occurs only in the presence of the other Th cell set (12). This finding that B cell activation can involve helper signals from two distinct sets of antigen-specific T cells raises the question of whether both types of helper T cells recognize antigen in the context of I-region-encoded determinants, or more specifically are both under the control of MHC-linked Ir genes. This question seems particularly relevant in the case of the idiotypic-recognizing helper T cell set as these cells appear to have

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† *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; DTH, delayed type hypersensitivity; GLPhe, poly-(L-glutamic acid, L-lysine, L-phenylalanine); HRPO, horse radish peroxidase; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; NP, 4-hydroxy-3-nitrophenyl acetyl; OVA, ovalbumin; PC, phosphorylcholine; PFC, plaque-forming cell; (T,G)-A--L, poly(Tyr,Glu)-poly(DL-Ala)--poly-L-Lys; T15, TEPC 15; Th, helper T cell(s).

specificity both for antigen and for self idiotypic determinants, and it would seem unlikely that they bear a receptor for yet another specificity.

In order to approach this question, the synthetic polypeptide antigen poly-(L-glutamic acid,L-lysine,L-phenylalanine) (GLPhe) was used. Responsiveness to GLPhe in inbred mice has been shown to be under the control of Ir genes mapping in the I region of the MHC (14, 15). Effective T-B collaboration in response to hapten conjugates of GLPhe has been shown to require that both the B cell and the T cell be derived from responder animals (8). In the present experiments, responder and nonresponder mice were primed with GLPhe. T cells from these animals were analyzed by adoptive transfer for both types of helper activity needed for a T15 dominated anti-PC response. Although conventional GLPhe-primed T cells helping via a hapten-carrier linkage were found only in responder animals as expected (8), GLPhe-primed helper Th acting selectively on T15-bearing B cells in the presence of GLPhe were found equally in both responder and nonresponder animals. This demonstrates that responses of these antigen-specific idiotype-recognizing helper T cells are not regulated by the same Ir genes as are the conventional helper T cells.

### Materials and Methods

*Mice.* BALB/cByJ mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, and were used between 8 and 12 wk of age. BALB.B and BALB.K mice were provided by the core mouse breeding facility in the Comprehensive Cancer Center at Yale, New Haven, Conn. by Dr. D. B. Murphy.

*Antigens.* The preparation of antigens used in these experiments has been described previously (11, 16). The preparation of GLPhe utilized in these studies was prepared and analyzed as previously described (14).

*Antisera.* The preparation, purification, and testing of anti-T15 idiotype antibodies have been described previously (11, 16). Hybridoma anti-Thy-1.2 was kindly provided by Dr. J. Sprent, Wistar Institute, Philadelphia, Pa.

*Anti- $\mu$ -treated Mice.* Anti-mouse  $\mu$ -chain-antibody-treated mice were generously supplied by Dr. C. A. Janeway, Jr., Yale University School of Medicine, New Haven, Conn. These mice were injected three times weekly starting within 24 h of birth with ammonium-sulfate-precipitated anti- $\mu$ -chain antibody prepared in a goat. This antibody reacted only with IgM in normal mouse serum by immunoelectrophoresis. The anti- $\mu$ -treated mice had no surface-immunoglobulin-positive cells in their spleens and no circulating T15 idiotype at the time of killing (12).

*Immunizations.* BALB/c and BALB.B mice to be used as B cell donors were immunized with 100  $\mu$ g of PC-horse radish peroxidase (HRPO) in complete Freund's adjuvant (CFA). Mice to be used as T cell donors were immunized with either 50  $\mu$ g ovalbumin (OVA), keyhole limpet hemocyanin (KLH), or GLPhe in CFA.

*Isolation of Spleen Cell Populations and Adoptive Transfer.* This was carried out as previously described (11, 16). Briefly, graded numbers of primed T and B cells, 25  $\mu$ g of PC-KLH, and, as appropriate, 25  $\mu$ g OVA or 50  $\mu$ g GLPhe were injected intravenously into syngeneic irradiated recipients. Their spleens were assayed for plaque-forming cells (PFC) 8 d after cell transfer.

*Hemolytic PFC Assay.* Spleen cells were assayed for direct anti-PC PFC by the modified Jerne hemolytic plaque technique (17). The proportion of anti-PC PFC of the T15 idiotype was determined by inhibition of plaque formation using rabbit anti-T15 antibodies in the agarose suspension medium. Only PFC that could be inhibited by  $10^{-3}$  M PC-Ficoll were considered to be PC specific.

### Results

It has been previously established that the dominant production of T15 idiotype in the antibody response to PC-proteins requires two distinct Th (11, 12). One Th cell

set is present in both carrier-primed normal mice and mice treated from birth with anti- $\mu$  antibody. This T cell set induces an anti-PC response which is mainly non-T15 in nature and requires the hapten PC to be physically linked to the carrier. The second Th set is present in normal BALB/c mice but lacking in carrier-primed anti- $\mu$  suppressed mice, and it is necessary for predominant production of the T15 idiotypic. The missing T cell set necessary for T15 production in anti- $\mu$  suppressed BALB/c mice can be restored by adding carrier-primed T cells from normal BALB/c donors along with the priming antigen (12).

To approach the question of whether or not Ir genes influence carrier recognition and subsequently T-B interactions, the ability of both helper cell sets to be activated by GLPhe and to induce PC-specific B cells to produce antibody was determined both in responder BALB/c mice and nonresponder BALB.B mice.

*Helper Activity for the T15 Idiotypic from BALB/c Responder Mice Primed with GLPhe.* The experiments represented in Table I (lines 1-4) demonstrate again that T cells from KLH-primed, anti- $\mu$ -treated mice were unable to induce PC-primed syngeneic B cells to produce a T15-dominated anti-PC response (line 1). The missing Th cell set needed for predominant T15 production could only be restored by transferring T cells from KLH-primed, anti- $\mu$ -treated mice along with T cells from OVA-primed normal BALB/c mice and boosting with PC-KLH plus OVA (lines 2-4). It can be seen that whereas the Th cell set needed for predominant T15 production existed among the OVA-primed T cell pool, it required activation by the addition of the appropriate priming antigen, in this case OVA. Neither KLH nor GLPhe could activate this cell (lines 2 and 4). It should be noted that the activity of the idiotypic-specific Th cells did not require that the hapten (PC) be linked to the priming carrier (OVA) (line 3).

TABLE I  
Activity of Th Cell Subsets from BALB/c Responder Mice Primed with GLPhe

	T cells from donors*			Antigen boost	Geometric mean PC-PFC/spleen‡			
	Anti- $\mu$ KLH	Normal OVA	Normal GLPhe		Total	T15 <sup>+</sup>	T15 <sup>-</sup>	T15
	$\times 10^6$				mean $\times$ / $\pm$ relative SE			%
1.	2	—	—	PC-KLH	1,774 (1.28)	682 (1.58)	1,002 (1.16)	38
2.	2	2	—	PC-KLH	1,772 (1.15)	772 (1.57)	913 (1.18)	44
3.	2	2	—	PC-KLH + OVA	4,772 (1.25)	3,531 (1.01)	1,214 (1.11)	74
4.	2	2	—	PC-KLH + GLPhe	2,361 (1.21)	1,062 (1.32)	1,403 (1.29)	45
5.	—	—	5	PC-GLPhe	1,465 (1.12)	1,204 (1.17)	251 (1.31)	82
6.	2	—	5	PC-KLH + GLPhe	4,169 (1.12)	3,251 (1.37)	811 (1.23)	78
7.	2	—	5	PC-KLH + OVA	1,480 (1.09)	715 (1.17)	726 (1.21)	48

\*  $5 \times 10^6$  B cells from PC-primed BALB/c donors were transferred along with T cells from anti- $\mu$  treated, KLH-primed, and normal OVA and GLPhe-primed BALB/c donors into 500-rad irradiated BALB/c recipients.

‡ The number of PC-specific PFC was determined on day 8 after cell transfer. The proportion of anti-PC PFC shown to be T15<sup>-</sup> was determined by plaque inhibition with rabbit anti-T15 antibodies. The number of T15<sup>+</sup> PFC was determined by subtracting the T15<sup>-</sup> PFC response from the total anti-PC response. The background PFC response of T and B cells transferred alone was subtracted.

§ The total number of PC-PFC represents only those PFC inhibited by  $10^{-3}$  M PC and therefore considered to be PC specific.

To determine whether or not both helper cell sets needed for T15<sup>+</sup> anti-PC antibody production could be primed to the synthetic antigen GLPhe, the helper activity of T cells from GLPhe-primed normal BALB/c mice was evaluated. It can be seen in Table I, line 5 that T cells from GLPhe-primed BALB/c responder donors could effectively collaborate with PC-primed B cells to generate a T15-dominated anti-PC response to PC-GLPhe. It is clear that even though it takes a greater number of GLPhe-primed Th to generate a substantial anti-PC response, GLPhe-like large protein carriers can induce an anti-PC antibody response dominated by the T15 idiotype in responder BALB/c mice. This confirms that at least the helper T cell requiring hapten (PC) and carrier (GLPhe) linkage is present in BALB/c mice primed with GLPhe. Likewise, T cells from GLPhe-primed donors were tested directly for their ability to replace the helper cell set required for predominant T15 production and missing in anti- $\mu$  treated, KLH-primed BALB/c mice. The results shown in Table I, lines 6 and 7 indicate that T cells from GLPhe-primed donors do include a T cell set, which in the presence of T cells from anti- $\mu$  treated, KLH-primed donors can preferentially induce B cells bearing the T15 idiotype. The helper cells for T15 idiotype production are activated only when the system is boosted with PC-KLH and GLPhe (compare lines 6 and 7).

These results indicate that priming of responder BALB/c mice with GLPhe activates both helper T cell subpopulations involved in an optimal T15 dominated anti-PC antibody response. Furthermore, this provides further evidence as to the antigen-specificity of the helper T cell required for dominant T15 idiotype production, in that OVA, KLH, and GLPhe are all discriminated by such T cells.

*Helper Activity for the T15 Idiotype from BALB.B Nonresponder Mice Primed with GLPhe.* Previous studies have shown that the immune response to GLPhe is under the control of MHC-linked Ir genes at the T helper cell level (8). Because both T cell subsets involved in an optimal anti-PC antibody response were found to be present in GLPhe-primed BALB/c responder mice, it was of interest to determine whether or not the same Ir genes controlled the ability of the two distinct helper T cells to collaborate effectively with PC-primed B cells. To do this, helper activity was evaluated in BALB.B mice primed with GLPhe, an antigen to which they are nonresponders. The results obtained using BALB.B mice are similar to those found using BALB/c mice in that anti- $\mu$  treated, KLH-primed BALB.B mice are missing a Th cell set needed for dominant T15 production (Table II, line 1) and this cell set can be replaced by addition of OVA-primed T cells from normal BALB.B mice provided the recipients are boosted with both PC-KLH and OVA (line 2). By contrast, T cells from GLPhe-primed BALB.B donors were unable to induce PC-specific B cells to produce antibody to PC-GLPhe (line 3), thus confirming previous studies demonstrating ineffective T-B collaboration when the carrier molecule used to prime nonresponder T cell donors is under Ir gene control (7, 8). More important, however, is the finding that T cells from GLPhe-primed BALB.B donors were able to provide effective antigen-specific helper activity to T15-idiotype-producing B cells when transferred along with T cells from anti- $\mu$ -treated, KLH-primed donors in the presence of PC-KLH and GLPhe (line 4). The findings in lines 5 and 6 demonstrate the specificity of this added helper T cell for the immunizing and boosting antigen GLPhe. In some experiments the total PC-PFC response increases when a mixture of T cells from anti- $\mu$ , KLH-primed, and normal GLPhe-primed donors is transferred along with PC-

TABLE II  
*Th Needed for Dominant T15 Idiotype Production is Present in BALB.B Nonresponder Mice Primed with GLPhe*

	T cells from donors*			Antigen‡ boost	Geometric mean PC-PFC/spleen§			
	Anti- $\mu$ KLH	Normal OVA	Normal GLPhe		Total	T15 <sup>+</sup>	T15 <sup>-</sup>	T15
	$\times 10^6$				mean $\times / +$ relative SE			%
1.	2	—	—	PC-KLH	2,261 (1.28)	855 (1.34)	1,401 (1.23)	38
2.	2	2	—	PC-KLH + OVA	9,465 (1.22)	7,968 (1.21)	1,372 (1.47)	84
3.	—	—	6	PC-GLPhe	0	0	0	—
4.	2	—	4	PC-KLH + GLPhe	7,095 (1.24)	5,741 (1.46)	1,226 (1.30)	81
5.	2	—	4	PC-KLH + OVA	4,591 (1.03)	1,965 (1.04)	2,498 (1.02)	43
6.	2	—	4	PC-KLH	2,430 (1.07)	842 (1.07)	1,564 (1.15)	35

\*  $5 \times 10^6$  B cells from PC-primed BALB.B donors were transferred along with T cells from anti- $\mu$  treated, KLH-primed, and normal OVA- and GLPhe-primed BALB.B donors into 500-rad irradiated BALB.B recipients.

‡ Each recipient received 25  $\mu$ g of PC-KLH or 50  $\mu$ g PC-GLPhe intravenously plus 25  $\mu$ g OVA or 50  $\mu$ g GLPhe, as appropriate.

§ See footnotes to Table I.

KLH plus the inappropriate carrier, OVA (line 5). Although the reason for this finding is unclear, there is no selective activation of those B cells bearing the T15 idiotype. The finding shown in lines 3 and 4 demonstrates that T cells requiring a hapten-carrier-linkage are under the control of MHC-linked Ir genes, whereas antigen-specific activation of those helper T cells that selectively activate idiotype-bearing B cells and that do not require a hapten-carrier linkage is not under the control of the same Ir genes. Similar results have been obtained in the H-2<sup>k</sup> nonresponder strain BALB.K (data not shown).

### Discussion

It has been clearly demonstrated that determinants encoded by the I-region of the major histocompatibility complex have an important restrictive role in interactions between T lymphocytes and macrophages and between T lymphocytes and B lymphocytes (2-4, 18-20). Many studies have indicated that the ability of helper T cells to recognize antigen and subsequently activate B cells depends on I-region, particularly I-A, similarity between the cell types (2-4). Furthermore, studies evaluating the role of MHC-linked Ir genes in regulating specific immune responses to a variety of antigens have shown that these genes can control T-dependent immune responses, and also play a role in T-B interactions in such responses (7, 8, 14, 15, 20). In this context, the studies reported here have shown that the activity of one of the two distinct sets of helper T cells involved in B cell responses to PC is indeed under the control of specific Ir genes that have been shown to be in the I-A and I-E/C subregions of the MHC (8, 15). By contrast, the second T helper cell set required for predominant T15 production is either independent of Ir gene control or under the control of genes distinct from those regulating conventional helper T cell activation.

These observations were obtained in a system that has allowed an examination of two Th required for a T15 dominated anti-PC response. As shown previously (12), helper T cells from mice suppressed from birth with anti- $\mu$  antibody provide effective

help for anti-PC PFC responses, but lack a helper T cell required for dominant production of anti-PC antibody bearing the T15 idiotype. The helper T cell set present in anti- $\mu$ -treated mice is identical to the carrier specific Th cells described by Mitchison (1) in that the optimal activity of these cells depends on the hapten being physically linked to the carrier. These conventional helper cells have been shown to be I-region restricted (2-4) and under Ir gene control (7, 8). In the present in vivo studies, T-B collaboration for an anti-PC response requires the presence of this cell set. The failure of the conventional Th cells from anti- $\mu$ -treated mice to induce an idiotype-dominated anti-PC response can be reconstituted by adding Ly-1<sup>+</sup> T cells from normal antigen-primed donors, provided the appropriate priming antigen is also given to the recipients (11, 12). From these and previous studies, it has been concluded that the helper T cells that selectively activate T15-bearing B cells are also antigen-specific, but their activation does not require that PC be coupled to the carrier. The present experiments extend these studies and clearly demonstrate that the antigen-specific responses of this unique set of helper T cells are not under the control of known Ir genes, in that both responder and nonresponder mice can provide such helper T cell activity to syngeneic responder or nonresponder B cells after priming with GLPhe, an antigen that is under MHC-linked Ir gene control. The same GLPhe-primed T cells were shown to behave as nonresponders when tested for their ability to help syngeneic nonresponder B cells make an anti-PC antibody response to PC-GLPhe. In addition, the use of both protein and synthetic polypeptides as antigen in these studies renders unlikely the possibility that idiotype-recognizing helper T cells are activated by each of these antigens through a resemblance of the antigen either for the hapten PC or for the T15 idiotype. These results lend additional weight to the concept that idiotype-recognizing helper T cells bear a distinct recognition unit for the antigen.

It can be envisioned that the antigen-specific T cell population needed for predominant T15 production selectively activates T15-bearing B cells through the use of an anti-idiotypic receptor. Previous studies have demonstrated that T cells activating B cells bearing a particular idiotype can be depleted on plastic plates coated with idiotypic molecules, suggesting that idiotype-specific Th cells can bind idiotype directly (21, 22). Therefore, it seems reasonable to conclude that such helper T cells recognize at least two distinct specificities: antigen and the T15 idiotype.

One can propose several possible explanations for the inability of known Ir genes to regulate the antigen-specific response of idiotype-recognizing Th to GLPhe. The interpretation we favor is that idiotype-recognizing helper cells do not bear recognition sites for self I-region determinants (in this case Ir gene products), but rather bear recognition sites for self idiotypic determinants. This would lead one to predict that the Th idiotype (ThId) cell set would not recognize antigen in the context of MHC-linked Ir gene products, and would therefore not be under the control of known Ir genes, as was indeed found in these experiments. The present results would favor the notion that Id-recognizing Th cells bear receptors only for antigen and for self idiotype. Alternatively, the activity of the Id-specific Th cell set may be regulated either by an as yet undescribed set of Ir genes or by other genes encoded in the MHC. This would suggest that this cell set either has three specificities (Id-, antigen-, and MHC-encoded determinants), or that there are two interacting T cells responsible for the specificities determined for this cell population. We believe the latter possibility

TABLE III  
*Characteristics of Th Involved in the T15-dominated Anti-PC Response*

Characteristic	ThMHC	ThId	Reference
Antigen specificity	+	+	(this paper; 11, 12)
Antigen recognition under Ir gene control	+	-	(this paper)
Selective activation of T15 <sup>+</sup> B cells	-	+	(this paper, 11, 12)
Requirement for hapten-carrier linkage	+	-	(this paper, 11, 12)
Presence in low T15 idiotype strains	+	-	(11-13)
Cell surface antigens			
Lyt-1	+	+	(12)
Lyt-2	-	-	(12)
Activates B cells by itself	?	-	(this paper)

to be unlikely because additional studies using F<sub>1</sub> into parent chimeras indicate that the ThId cell set is apparently not MHC restricted because ThId cells can collaborate effectively with B cells from both parental haplotypes. In these same chimeras, the activity of conventional Th cells requiring a hapten-carrier bridge is MHC restricted as expected (K. Bottomly and D. Mosier. Manuscript in preparation.). A third possibility that can not be ruled out by either of these sets of experiments is that ThId cells recognize a highly conserved, MHC-encoded structure found in all the haplotypes tested.

If one accepts the argument that ThId cells are indeed not MHC-recognizing,<sup>2</sup> then one can propose a general framework within which to consider such helper T cells. Because both sets of Th cells recognize antigen, it is convenient and informative to describe these cells in terms of the known self specificity each recognizes during responses to antigen. Therefore, one can refer to those helper T cells that recognize antigen in the context of self MHC gene products (in this case the products of Ir genes) as ThMHC, and those helper T cells that selectively activate B cells bearing the T15 idiotype as ThId. Table III lists the salient characteristics of each of these types of helper T cells as revealed in studies of the anti-PC antibody response. The ThMHC and ThId cell sets have some striking similarities. For instance, both cell sets are Lyt-1<sup>+</sup>, 2<sup>-</sup>, both have a specificity for self and a specificity for antigen, and both have the ability to activate B cells. These similarities have suggested (23) that the development of these two Th sets may be analogous. It has been shown that ThMHC cells are selected for recognition of self MHC specificities by radioresistant elements of the thymus, and the selected cells are subsequently expanded by contact with the same MHC specificities in the periphery (24). Although nothing is known about the role of the thymus in the ontogeny of ThId cells, it is clear that peripheral contact with idiotype is required for their development. This has been shown by noting the absence of ThId cells in mice with low levels of circulating T15 idiotype (11, 12).

Studies from other laboratories have also demonstrated anti-idiotypic T cells involved in helper (21, 22, 25-27) and suppressor (28-33) cell activity. It might be envisioned that the anti-idiotypic T cell population could be divided into two distinct groups based on differences in the immunization procedures used to induce these cells and ultimately on differences in the specificity of the T cells. Several studies have

<sup>2</sup> Bottomly, K. and C. A. Janeway, Jr. Selected populations of alloreactive T cells contain helper T cells but lack ThId, an antigen-specific helper T cell required for dominant production of the T15 idiotype. Manuscript submitted for publication.

demonstrated that immunization with idiotype-bearing antibody generates Th cells specific for the idiotypic determinants (25-27). In vivo, such cells have been shown to function as carrier-specific Th cells activating hapten-specific B cells only when the hapten is linked physically to idiotype-bearing antibody (26, 27). Moreover, these idiotype specific Th cells are capable of activating B cells to secrete antibody in vivo (26, 27) and in vitro (25). It might be speculated that Th cells immunized with idiotype recognize idiotypic determinants in association with MHC-encoded antigens, as has been shown for other antigen-specific Th cells (2-4). Although this question has not been resolved for such anti-idiotypic Th cells, the function of effector-phase suppressor T cells involved in 4-hydroxy-3-nitrophenylacetyl (NP)-specific delayed type hypersensitivity (DTH) responses depends on cells which are both anti-idiotypic and MHC-restricted (33). In the case of helper T cells, this class of idiotype-immunized anti-idiotypic Th cells may most likely be MHC restricted and may recognize Id by the use of an anti-idiotypic antigen receptor.

By contrast, anti-idiotypic Th cells involved in selective activation of B cells bearing germ-line idiotypes are normally present in mice (11, 12, 21, 22) and can be shown to function only in the presence of conventional Th cells (ThMHC) (11, 12, 21). These cells can be activated by immunization with antigens unrelated to the idiotype in question (11, 12, 21) and have been shown to be antigen (11, 12) and idiotype (21, 22) specific. The ability of these anti-idiotypic cells to recognize idiotypic determinants appears not to involve the receptor for antigen, and, furthermore, it might be proposed that this recognition involves a receptor for self determinants. Further experiments are planned to test this hypothesis. Clearly, T cells bearing anti-idiotypic receptors can be generated in a variety of ways and can be demonstrated to have differing functions. Further studies should resolve the question of whether specificity and function are related in such cells.

Although the influences of Ir genes on carrier recognition by the conventional helper T cell has been well documented (7, 8, 15, 20), certain T cell responses to Ir-controlled antigens, in addition to those described in this paper, have been generated in nonresponder strains. Both proliferating T cell (34) and DTH responses (35) could be elicited by haptened Ir-controlled antigens in nonresponder strains if the nonresponders were first primed with hapten conjugated to an immunogenic carrier. There seems to be little similarity between these cells and the ThId cell set, because responses of proliferating and DTH cells could be elicited but could not be primed in nonresponder strains, whereas ThId could be primed and shown to function in a nonresponder environment. Furthermore, T cells from low- and high-responder strains have been shown to produce antigen-specific T cell factors in response to poly(Tyr,Glu)-poly(DL-Ala)--poly-L-Lys [(T,G)-A--L] (36). By contrast with the Th activity of GLPhe-specific ThId cells, these factors will only trigger high-responder and not low-responder B cells to produce antibody. The relationship between the ThId cell set and such T cell factors, both of which can be generated in nonresponder strains, is not known.

These experiments do not specifically address the possible physiological role of the ThId cell set. It seems most likely, however, that ThId cells function specifically early in an immune response, to activate those B cells most frequently represented; namely, those bearing germ-line encoded idiotypes. Several studies have shown that the expression of germ-line idiotypes may be only dominant early in an antibody response



(37, 38). Furthermore, experiments using helper T cells from normal and anti- $\mu$ -treated mice demonstrate that the Ig-dependent T cells were only active early during an anti-DNP response (9). Taken together, these data lend support to the concept that preferential activation of frequently represented B cells may be a result of the ThId cell set. Furthermore, the shift from idiotypic dominance to a more heterogeneous response seen with some antigens could be explained by suppression of ThId function as the response proceeds. Thus, ThId would play a critical role in protective immunity, causing the early activation of B cells with receptors bearing frequently represented idiotypes. Current experiments are aimed at confirming this role of ThId cells in the anti-PC antibody response.

### Summary

Responder and nonresponder mice primed with poly-(L-glutamic acid,L-lysine,L-phenylalanine) (GLPhe), the response to which is under the control of immune response (Ir) genes, were used as a source of both types of helper T cells required for a T15 idiotypic dominated T-dependent anti-phosphorylcholine (PC) response. It was found that the activity of one of the helper T cells needed for an anti-PC response was under major histocompatibility complex (MHC)-linked Ir gene control, and only GLPhe-primed responder mice could be used as a source of these cells. These T cells (ThMHC) whose presence is required for in vivo T-B collaboration are found in normal and anti- $\mu$ -treated mice, and their activity depends on the hapten being physically linked to the carrier molecule. By contrast, the activity of the second helper T cell (ThId) required for a T15-dominated anti-PC response was present in both GLPhe-primed responder and nonresponder mice. The ThId cell set that is missing or deficient in anti- $\mu$  treated mice can be restored by the addition of T cells from normal, carrier-primed donors and restimulating with the priming carrier. When T cells from GLPhe-primed donors are used as a source of ThId cells, both responder and nonresponder donors provide helper cells capable of inducing syngeneic B cells to produce a T15 dominated anti-PC response. These results are interpreted to suggest that idiotypic recognizing helper T cells (ThId) recognize antigen independent of known Ir gene products.

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### References

1. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* 1:18.
2. Katz, D. H., M. Graves, M. E. Dorf, H. DiMuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VII. Cooperative responses between lymphocytes are controlled by genes in the I region of the H-2 complex. *J. Exp. Med.* 141: 263.
3. Sprent, J. 1978. Restricted helper function of F<sub>1</sub> T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper

- cell induction and T-B collaboration, both mapping to K-end of the H-2 complex. *J. Exp. Med.* **147**:1159.
4. Sprent, J. 1978. Restricted helper function of  $F_1 \rightarrow$  parent bone marrow chimeras controlled by K-end of H-2 complex. *J. Exp. Med.* **147**:1838.
  5. McDevitt, H. O., and A. Chinitz. 1969. Genetic control of antibody response: relationship between immune response and histocompatibility (H-2) type. *Science (Wash. D. C.)*. **163**:1207.
  6. McDevitt, H. O., B. D. Deak, D. C. Shreffler, J. Klein, J. H. Stimpfling, and G. D. Snell. 1972. Genetic control of the immune response. Mapping of the Ir.1 locus. *J. Exp. Med.* **135**:1259.
  7. Katz, D. H., T. Hamaoka, M. E. Dorf, P. H. Maurer, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (Ir) gene in the control of lymphocyte interactions in responses controlled by the gene. *J. Exp. Med.* **138**:734.
  8. Katz, D. H., M. E. Dorf, and B. Benacerraf. 1976. Control of T lymphocyte and B lymphocyte activation by two complementing Ir-GL $\phi$  immune response genes. *J. Exp. Med.* **143**:906.
  9. Janeway, C. A., Jr., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4582.
  10. Tada, T., T. Takemori, K. Okumura, M. Nonaka, and T. Tokuhisu. 1978. Two distinct types of helper T cells involved in the secondary antibody response. Independent and synergistic effects of Ia<sup>-</sup> and Ia<sup>+</sup> helper T cells. *J. Exp. Med.* **147**:446.
  11. Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T15 idiotype also lack an antigen-specific helper T cell required for T15 expression. *J. Exp. Med.* **150**:1399.
  12. Bottomly, K., C. A. Janeway, Jr., B. J. Mathieson, and D. E. Mosier. 1980. Absence of an antigen-specific helper T cell required for the expression of the T15 idiotype in mice treated with anti- $\mu$  antibody. *Eur. J. Immunol.* **10**:159.
  13. Bottomly, K., B. J. Mathieson, H. Cosenza, and D. E. Mosier. 1979. Idiotype specific regulation of the response to phosphorylcholine by T cells from mice with high and low levels of circulating idiotype. In *B Lymphocytes in the Immune Response*. M. Cooper, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier North Holland, Inc. New York. 323.
  14. Merryman, C., P. H. Maurer, and D. W. Bailey. 1972. Genetic control of immune response in mice to a glutamic acid, lysine, phenylalanine copolymer. III. Use of recombinant inbred strains to establish association of immune response genes with H-2 genotype. *J. Immunol.* **108**:937.
  15. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1975. Requirement for two H-2 complex Ir genes for the immune response to the L-Glu,L-Lys,L-Phe terpolymer. *J. Exp. Med.* **141**:1459.
  16. Bottomly, K., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotype induced regulation of helper cell function for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* **148**:1216.
  17. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Wash. D. C.)*. **140**:405.
  18. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* **138**:1194.
  19. Schwartz, R. H., A. Yano, and W. E. Paul. 1978. Interaction between antigen-presenting cells and primed T lymphocytes. *Immunol. Rev.* **40**:153.

20. Benacerraf, B., and R. N. Germain. 1978. The immune response genes of the major histocompatibility complex. *Immunol. Rev.* **38**:70.
21. Woodland, R., and H. Cantor. 1978. Idiotype-specific T-helper cells are required to induce idiotype B memory cells to secrete antibody. *Eur. J. Immunol.* **8**:600.
22. Adorini, L., M. Harvey, and E. E. Sercarz. 1979. The fine specificity of regulatory T cells. IV. Idiotypic complementarity and antigen-bridging interactions in the anti-lysozyme response. *Eur. J. Immunol.* **9**:906.
23. Bottomly, K., and D. E. Mosier. Analogous dual specificity of helper T cells cooperating in the generation of clonally-restricted antibody responses. In *Strategies for Immune Regulation*. E. Sercarz and A. Cunningham, editors. Academic Press, Inc., New York. 487.
24. Zinkernagel, R. M. 1978. Thymus and lymphohemopoietic cells: their role in T cell maturation in selection of T cells H-2-restrictions-specificity and in H-2 linked Ir gene control. *Immunol. Rev.* **42**:224.
25. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte interactions. II. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8**:853.
26. Janeway, C. A., Jr., N. Sakato, and H. N. Eisen. 1975. Recognition of immunoglobulin idiotypes by thymus-derived lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* **72**:2357.
27. Cosenza, H., M. H. Julius, and A. A. Augustin. 1977. Idiotypes as variable region markers: analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* **34**:3.
28. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Binding to idiotypic determinants of large proportions of thymus-derived lymphocytes in idiotypically suppressed mice. *Proc. Natl. Acad. Sci. U. S. A.* **74**:204.
29. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **149**:1559.
30. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149**:592.
31. Sy, M.-S., B. A. Bach, A. Brown, A. Nisonoff, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. II. Induction of suppressor T cells with idiotype-coupled syngeneic spleen cells. *J. Exp. Med.* **150**:1229.
32. Sy, M.-S., M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. IV. Idiotype-bearing I-J<sup>+</sup> suppressor T cell factors induce second-order suppressor T cells which express anti-idiotypic receptors. *J. Exp. Med.* **151**:1183.
33. Weinberger, J. Z., B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. III. Interaction of effector suppressor T cells is restricted by H-2 and Igh-V genes. *J. Exp. Med.* **151**:1413.
34. Janeway, C. A., Jr., P. H. Maurer, M. O. Dailey, and J. K. Inman. 1976. The specificity of cellular immune responses in guinea pigs. II. The structure of antigenic determinants leading to T-lymphocyte stimulation. *J. Exp. Med.* **144**:1621.
35. Weinberger, J. Z., B. Benacerraf, and M. E. Dorf. 1979. Ir gene controlled carrier effects in the induction and elicitation of hapten-specific delayed-type hypersensitivity responses. *J. Exp. Med.* **150**:1255.
36. Mozes, E., and J. Haimovich. 1979. Antigen-specific T-cell helper factor cross reacts idiotypically with antibodies of same specificity. *Nature (Lond.)*. **278**:54.
37. Mäkelä, O., and K. Karjalainen. 1976. Inheritance of antibody specificity. IV. Control of related molecular species by one V<sub>H</sub> gene. *Cold Spring Harbor Symp. Quant. Biol.* **41**:735.
38. Dzierzak, E. A., C. A. Janeway, Jr., R. W. Rosenstein, and P. D. Gottlieb. 1980. The expression of an idiotype (Id-460) during in vivo anti-dinitrophenyl antibody responses. I.

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Mapping of the genes for Id-460 expression to the variable region of the immunoglobulin heavy-chain locus and to the variable region of the immunoglobulin  $\kappa$ -light-chain locus. *J. Exp. Med.* **152**:720.