ANTIGEN-SPECIFIC HELPER T CELLS REQUIRED FOR DOMINANT IDIOTYPE EXPRESSION ARE NOT H-2 RESTRICTED*

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Cooperation of two different antigen-specific helper T (Th)[1] lymphocytes in the induction of antibody synthesis by B lymphocytes has been inferred from many studies (1–4). We have recently reported (5, 6) that two antigen-specific Th populations are required for an antibody response to phosphorylcholine (PC) that is dominated by the TEPC15-like (T15) idiotype. One Th population is required for any response to PC coupled to a protein carrier and is activated only when carrier-primed T cells, PC-primed B cells, and PC coupled to the homologous carrier are present simultaneously. This Th cell thus resembles the classic carrier-specific population first defined by Mitchison (7) and later studied by many workers. The second Th population is required for optimal expression of the T15 idiotype and can be activated when primed T cells are exposed to unconjugated carrier protein; i.e., there is no requirement for conjoint recognition of PC and carrier determinants for the expression of helper activity. This Th cell thus seems similar to idiotype-specific Th populations described by others (8–11).

One way of directly distinguishing between these two T lymphocyte subpopulations may be by their self-specificities (12). The cells that are carrier specific have been shown by several studies (13–15) to be restricted in their activity by self-recognition of major histocompatibility complex (MHC)-encoded products (ThMHC). Recognition of autoidiotypic determinants as well as carrier protein can be viewed as an analogous example of self-restriction. However, if Th cells for idiotype dominance (ThId) also recognize MHC products, then two restricting elements as well as three potential receptors must be postulated. The experiments reported here were designed to test the hypothesis that ThId are not MHC restricted.

To approach this question, both ThMHC and ThId cells from $F_1 \rightarrow$ parent radiation bone marrow chimeras were tested for their ability to collaborate with B cells bearing MHC-encoded determinants of either parental haplotype. Previous studies using Th cells from $(A \times B)F_1 \rightarrow$ parent chimeras have shown that the ability of Th cells to interact with B cells expressing a given parental set of MHC-encoded determinants depends on exposure of the Th cells to these same determinants during

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[1] *Abbreviations used in this paper:* KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; OVA, ovalbumin; PC, phosphorylcholine; PFC, plaque-forming cell(s); T15, TEP15; Th, helper T; ThId, Th cells for idiotype dominance; ThMHC, major histocompatability complex-encoded products.

T cell differentiation in the thymus (16), and, conversely, that (A × B)F_1 → parent A chimeras lack T cells capable of cooperating with B cells from parent B.

We find that ThMHC cells from (A × B)F_1 → parent A chimeras can collaborate only with B cells of the parent A recipient haplotype and not with B cells of the parent B haplotype. In contrast, the ThId cell set from the same F_1 → parent chimeras does provide helper activity for idiotype expression to B cells of both parental haplotypes. These studies therefore demonstrate that, in contrast to the ThMHC cell set, ThId cells are not required to recognize MHC-encoded determinants for successful T-B collaboration.

Materials and Methods

Previously Described Methods and Materials. The use and preparation of PC-coupled carrier antigens (keyhole limpet hemocyanin [KLH] and ovalbumin [OVA]) as well as the procedure for immunization, adoptive transfer experiments, plaque-forming cell (PFC) assays, and the determination of Id-positive PFC using rabbit anti-T15 Id antibody are all as previously described (5, 17).

Preparation of Radiation Bone Marrow Chimeras. All mice used in these studies were bred at the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. Recipient mice were given 950 rad x-irradiation 1 d before receiving donor cells. Bone marrow donors were pretreated with 50 μl rabbit anti-mouse thymocyte serum (18). Bone marrow cells from these donors were treated with anti-Thy-1.2 antibodies and absorbed guinea pig complement, as previously described (17). 5–15 million bone marrow cells were injected intravenously into the irradiated recipients. The recipients were used as a source of Th cells 2–5 mo after reconstitution. The extent of chimerism was determined by indirect immunofluorescence on purified T cells using anti-H-$2K^k$ and anti-H-$2D^d$ antisera kindly provided by Dr. D. B. Murphy, Yale University, New Haven, Conn.; positive cells were detected using fluorescein-conjugated rabbit anti-mouse immunoglobulin (Ig) antibody. By this criterion, >95% of the T cells from the chimeras used in these experiments were of donor origin in that they bore H-2^d determinants derived from the donor bone marrow. Staining of these cells with fluoresceinated rabbit anti-mouse Ig revealed <5% contaminating Ig^+ cells.

Results

Helper Activity of F_1 T Cells for Parental Strain B Cells. Previous studies have shown (5, 6) that the dominant production of the T15 Id by PC-primed B cells requires the presence of two distinct Th cells sets. One of the Th cell sets, which we refer to as ThMHC, is present in both (CBA/N × BALB/c)F_1 male and female mice. This Th cell set induces an anti-PC antibody response that is characterized by the expression of a low percentage of anti-PC PFC producing the T15 Id (20). The presence of a second cell set (ThId) is necessary for dominant production of the T15 Id. The ThId cell set is present in (CBA/N × BALB/c)F_1 female mice but is deficient or lacking in unimmunized F_1 male mice which because of their X-linked immune defect do not normally have circulating $T15^+$ anti-PC antibody (19). In adoptive transfer experiments, the lack of ThId cells within the Th cell populations obtained from F_1 male donors could be restored by the addition of F_1 female T cells provided they are primed and restimulated with a second protein antigen (5). Provision of ThId function by use of a second antigen-specific T cell population is a standard feature of the experiments below.

Although it has been established that both ThMHC and ThId cell sets from F_1 donors can provide helper activity for F_1 B cells, it was necessary to evaluate the activity of both Th cell sets from F_1 mice for B cells of the two parental haplotypes.

The results of these studies are shown in Fig. 1. As shown previously (5, 20), Th cells from the defective F_1 male donors were able to induce a substantial anti-PC PFC response but were unable to induce the pool of F_1 B cells to produce a response dominated by the T15 Id. The lack of ThId cell function was evident when (CBA/N × BALB/c)F_1 (k × d) female, BALB/c (d), or BALB.K (k) B cells were used as a source of responding B cells (group 2). In contrast, Th cells from normal F_1 female donors provided both ThMHC and ThId cell function, inducing F_1 or parental B cells to produce a T15-dominated anti-PC PFC response (group 1).

If T cells from F_1 female donors were used as a source of ThId cells, then the Th defect of F_1 male donors could be corrected by the addition of carrier-primed F_1 female T cells in the presence of the priming antigen (group 4). The mixture of Th cells permitted the induction of a T15-dominated anti-PC response by F_1 or by either parental strain B cells. Furthermore, the activation of the KLH-primed ThId cell set required reexposure of the cells to the priming antigen, in this case KLH (compare groups 3 and 4).

These results indicate that primed T cells from F_1 donors can provide not only ThMHC, but also can provide ThId function for F_1 as well as for both parental strain B cells.

ThMHC and ThId Activity of T Cells from (k × d)F_1 → Parent k Bone Marrow Chimeras. It has been established that Th cells recognize antigen in association with self-MHC-encoded determinants (13–15). Because an optimal $T15^+$ anti-PC response requires the presence of two distinct Th cell sets, it was important to determine whether cells of each Th cell set required recognition of self-MHC encoded determi-

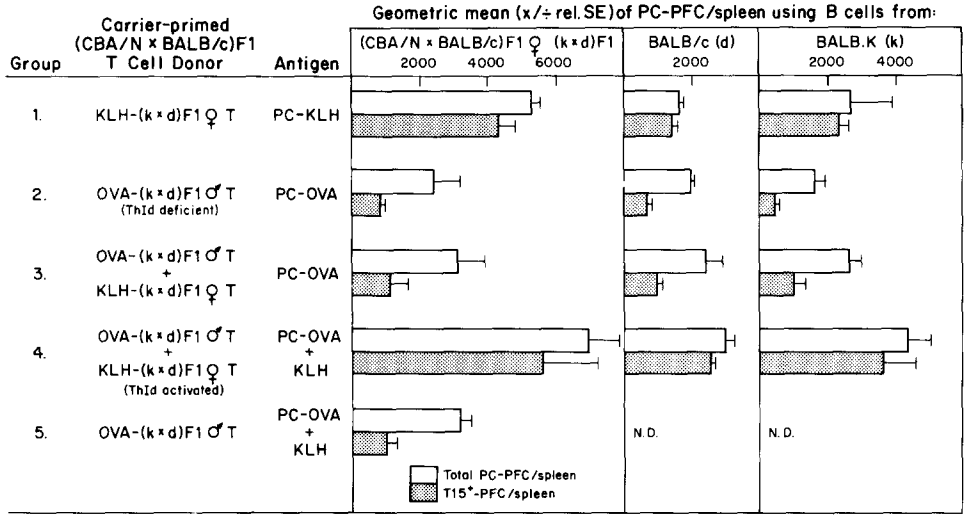

FIG. 1. Antigen-specific Th cells are required for dominant T15 production by F_1 or parental B cells. 5 million B cells from PC-primed donors were transferred along with 1 million T cells from KLH and/or OVA-primed donors and 25 μg antigen into 650 rad irradiated (CBA/N × BALB/c)F_1♀ recipients. The number of PC-specific PFC per spleen was determined 8 d after cell transfer. The proportion of anti-PC PFC shown to be $T15^-$ was determined by plaque inhibition with rabbit anti-T15 antibodies. The number of $T15^+$-PFC was determined by subtracting the $T15^-$-PFC response from the total anti-PC response. The background PFC response of T and B cells transferred alone was subtracted. The geometric mean (relative standard error) for each group represents the responses of three to five mice in at least four experiments, with similar results.

nants during a response to antigen. To evaluate this question, T cells from (CBA/N × BALB/c)F_1 female (k × d) → CBA/N (k) chimeras were tested for their ability to cooperate with F_1 B cells or with B cells from either parental strain. The B cell donors in these experiments were either (CBA/N × BALB/c)F_1 (k × d), BALB/c (d), or BALB.K (k). Whereas CBA/N mice normally express low levels of T15 Id, the CBA/N recipients reconstituted with (CBA/N × BALB/c)F_1 ♀ bone marrow cells used in the experiments expressed levels of circulating T15 Id equivalent to the F_1 female donor. The results shown in Fig. 2, group 2 demonstrate that whereas T cells from KLH-primed (k × d)F_1 → parent k chimeras collaborate effectively with PC-primed B cells from (k × d)F_1 or parent k donors, the chimeric T cells are unable to induce B cells from parent d to make anti-PC PFC when stimulated with PC-KLH. The inability of chimeric T cells to collaborate with B cells from H-2^d donors demonstrates that one of the Th cell sets must recognize antigen in association with self-MHC-encoded determinants, and we have designated such cells ThMHC. In addition, these results indicate that whereas increased numbers of chimeric T cells are required to generate anti-PC responses equivalent to the response generated by F_1 T cells (data not shown), the response generated by chimeric T cells is dominated by the T15 Id at all cell doses. These results confirm previous findings demonstrating that ThId cells are measurable in mice with high levels of circulating T15 Id (5, 6, 20). This is also true in (CBA/N × BALB/c)F_1 female → CBA/N chimeras expressing substantial levels of circulating T15 Id, even though the CBA/N recipient expresses little or no naturally occurring T15-bearing anti-PC antibody. The expression of ThId activity therefore seems not to depend on the T15 phenotype of the recipients.

Having shown that one Th subset (ThMHC) must recognize self-MHC-encoded determinants to function, the same T cells were used to determine whether ThId also had to recognize self-MHC-encoded molecules to express their function. To accomplish this, T cells from the same (k × d)F_1 → k chimeras were tested for their ability to reconstitute ThId activity missing from T cells of F_1 male mice (group 1) and necessary for a T15-dominated anti-PC response. As seen in group 3, T cells from KLH-primed (k × d)F_1 → k chimeras include a T cell set that, in the presence of T

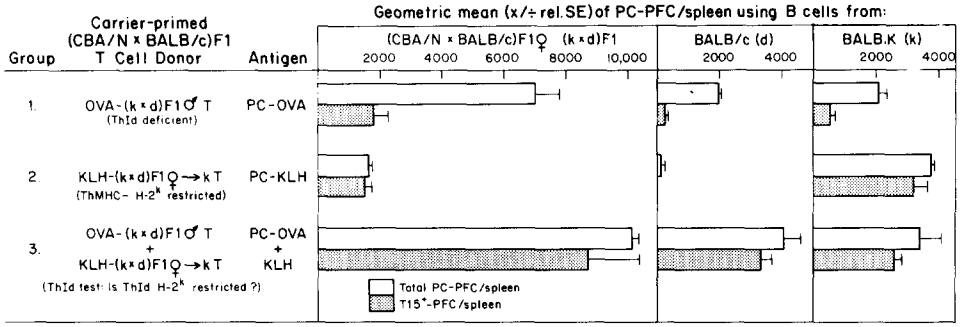

FIG. 2. Demonstration of two Th cell sets in (CBA/N × BALB/c)F_1 → CBA/N (k × d → k) chimeras: H-2 restriction of ThMHC and ThId cell sets. 5 million B cells from PC-primed B cell donors were transferred along with 1 million T cells from OVA and KLH-primed donors and 25 μg of PC-KLH, PC-OVA, and, as appropriate, 25 μg KLH into 650 rad irradiated (CBA/N × BALB/c)F_1♀ recipients. 2 million T cells from the KLH-primed (k × d)F_1 → k chimera were transferred (see key on Fig. 1). All PFC represented are inhibitable by 10^{-3} M PC and are considered to be PC specific.

cells from OVA-primed F_1 male mice and PC-OVA plus KLH, induces a T15-dominated anti-PC antibody response. Moreover, T cells from $F_1 \rightarrow$ parent k chimeras induce T15-bearing B cells of F_1 and of either parental strain (H-2^k and H-2^d) to secrete antibody and thus fail to show a haplotype preference. The ability of ThId cells from (k × d) → k chimeras to induce dominant T15 Id production by BALB/c (H-2^d) B cells strongly suggests that the function of the ThId cell set does not depend on the ability of this cell set to recognize self-MHC-encoded determinants.

Lack of ThId Activity in (k × d)F_1 → Parent k Chimeras Generated from Bone Marrow Donors Expressing Low Levels of Circulating T15. It has been previously demonstrated that the generation and/or expansion of the ThId cell population depends on the presence of B cells producing circulating Id-bearing Ig (5, 6, 20). Therefore, $F_1 \rightarrow$ parent chimeras generated using the T15 Id-deficient strain (CBA/N × C.B20)F_1 female bone marrow cells as donors and using CBA/N as recipients were tested for their ability to provide both ThMHC and ThId cell function. Because (CBA/N × C.B20)F_1 → CBA/N chimeric mice expressed low levels of circulating T15 Id-bearing Ig, it was expected that T cells from these (k × d)F_1 → k chimeras might contain only the ThMHC cell set. As can be seen in Fig. 3, T cells from the (k × d)F_1 (low T15) → k chimeras induced substantial anti-PC PFC responses from B cells of F_1 but not from parent d donors (group 3). The anti-PC responses of B cells from F_1 donors were predominantly non-T15. Similarly, T cells from (k × d)F_1 (low T15) → k chimeras were unable to restore a T15-dominated anti-PC response when mixed with T cells from OVA-primed F_1 male donors (groups 1 and 2). These data confirm the finding that ThId cells are deficient in mice having low levels of circulating Ig bearing the T15 Id and indicate that chimeric T cells cannot provide nonspecific or allogeneic signals to B cells from F_1 and parent d haplotypes as a possible explanation for the lack of MHC restriction observed in the function of the ThId cells demonstrated in Fig. 2.

ThMHC and ThId Activity of T Cells from (b × d)F_1 → Parent b Bone Marrow Chi-

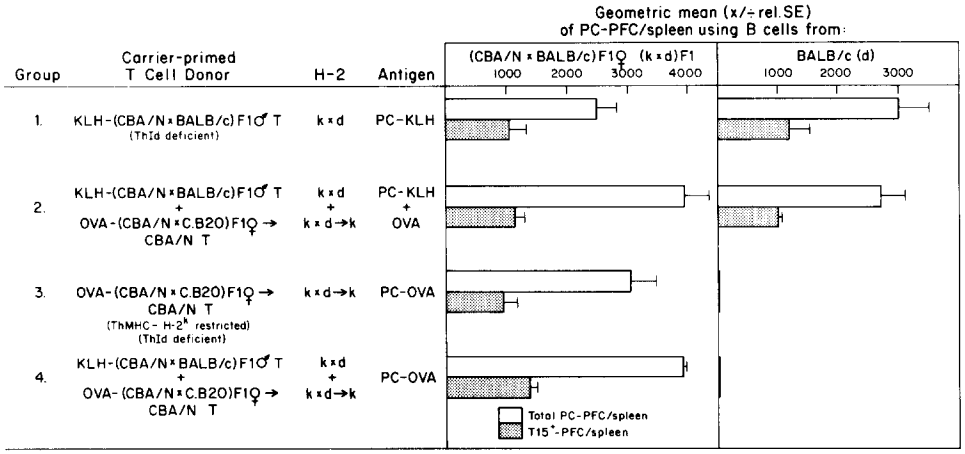

FIG. 3. ThId cells are deficient in chimeras generated from donors expressing low levels of circulating T15 (see key on Figs. 1 and 2). Primed cells were transferred along with antigen into 650 rad irradiated (CBA/N × BALB/c)F_1♀ donors. In groups 2–4, 4 million T cells from OVA-primed (CBA/N × C.B20)F_1♀ → CBA/N were transferred.

meras. To confirm the finding that the ThId cell set does not recognize self-MHC-encoded determinants expressed by B cells, these studies were repeated using: (*a*) chimeras generated from mice with different MHC haplotypes, and (*b*) a system for evaluating ThId cell function other than by reconstitution of the ThId activity deficient in defective (CBA/N × BALB/c)F_1 male mice.

Because mice with low T15 levels are deficient in ThId cells, mice of the low T15 Igh^b haplotype were used as T cell donors. To avoid total H-2 mismatches, the appropriate F_1 mice were used as T and B cell donors. As seen in Fig. 4 (group 1), T cells from (C.B20 × B6)F_1 ($H\text{-}2^{b \times d}$, Igh^b) mice appeared deficient in the ThId cell set because the induction of both (BALB/c × BALB.B)F_1 B cells and BALB/c B cells resulted in an anti-PC response characterized by low T15 expression. Similarly, the deficiency of ThId cells seen in (C.B20 × B6)F_1 T cell donors could be restored by the addition of T cells from carrier-primed (BALB/c × BALB.B)F_1 ($H\text{-}2^{b \times d}$, $Igh^{a \times b}$) donors (group 2). ThId cell function could be provided by either (BALB/c × BALB.B)F_1 donors (group 2) or by (BALB/c × B6)F_1 donors (data not shown), both of which produce substantial levels of circulating T15-bearing Ig.

This second experimental approach allowed the generation of $F_1 \rightarrow$ parent chimeras differing in H-2 haplotype. T cells from (BALB/c × BALB.B)$F_1 \rightarrow$ (BALB.B × B6)F_1 chimeras (d × b)$F_1 \rightarrow$ b were tested for their ability to provide both the Th cell functions to (BALB/c × BALB.B)F_1 ($H\text{-}2^{d \times b}$) and BALB/c ($H\text{-}2^d$) PC-primed B cells. As seen in group 4, T cells from OVA-primed (d × b)$F_1 \rightarrow$ b chimeras generated a T15-dominated anti-PC response with (b × d)F_1 B cells in response to PC-OVA. By contrast, they failed to provide helper function for the PC-OVA response of BALB/c ($H\text{-}2^d$) B cells, even at higher T cell numbers. However, T cells from these same OVA-primed (d × b)$F_1 \rightarrow$ b chimeras could restore ThId cell function missing in the T cell population from KLH-primed (C.B20 × B6)F_1 donors (group 3). This helper activity from chimeras was evident with both (b × d)F_1 and BALB/c ($H\text{-}2^d$) B cell populations.

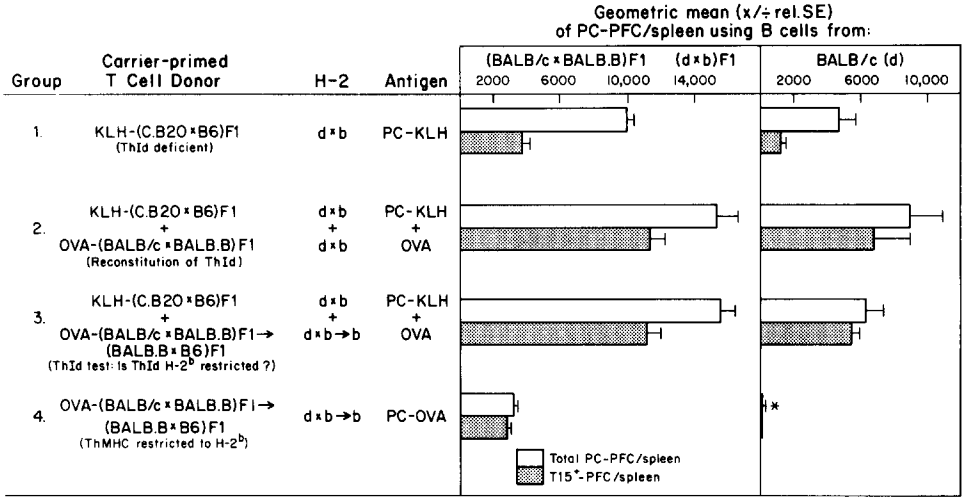

FIG. 4. Demonstration of two Th cell sets in (d × b)$F_1 \rightarrow$ b chimeras: H-2 restriction of ThMHC and ThId cell sets. 5 million B cells from PC-primed donors plus 2 million T cells from primed donors were transferred along with 25 μg antigen into 700 rad irradiated (BALB/c × B6)F_1 recipients (see key on Figs. 1 and 2). *4 million T cells were transferred.

These findings confirm those above and also confirm that the function of the ThId cell set, in contrast to that of the ThMHC cell set, does not depend on self-MHC recognition.

Discussion

This study has demonstrated that two distinct helper T cells involved in an anti-PC response differ in their requirements for recognition of self-MHC-encoded determinants. The activity of one of the helper T cells was shown to depend on MHC matching for T-B collaboration in that Th cells from (A × B)F_1 → A chimeras provided help for B cells from F_1 and from parent A but not for B cells from parent B. By contrast, the interactions between the second helper T cell needed for predominant T15 production and PC-specific B cells clearly did not require self-MHC recognition.

These findings extend our previous studies in which the optimal activation of T15-bearing B cells has been shown to depend on the presence of two distinct helper T cells. Both of these Th cells need to be primed by antigen (carrier) and both must be activated by the priming carrier to express helper activity. Whereas both Th cell subpopulations influence B cells, one of the Th cell sets activates PC-specific B cells only if the hapten, PC is physically linked to the carrier. The other Th cell preferentially activates T15-bearing B cells and does not require hapten-carrier association; moreover, its function is only apparent in the presence of the first Th cell set. The presence of both of these Th cell sets results in an anti-PC response that is dominated by the T15 Id. Previous studies have also shown that mice that naturally express low levels of T15-positive antibody are deficient in one of the two Th cell sets that are necessary for T15-dominated anti-PC response. This missing Th cell set can be restored by the addition of antigen-primed T cells from donors expressing high levels of circulating T15-bearing Ig. We have taken advantage of two such systems to analyze the requirement for self-MHC recognition for both Th cell sets during the various cell interactions leading to B cell activation. In one set of experiments, T cells from (CBA/N × BALB/c)F_1 male and female donors were used to induce an anti-PC response. Whereas Th cells from (CBA/N × BALB/c)F_1 male mice lack those Th cells needed for predominant T15 production, this deficiency could be restored by the addition of T cells from antigen-primed F_1 female donors. Th cells from F_1 → parent chimeras generated from (CBA/N × BALB/c)F_1 female donors into CBA/N irradiated recipients behaved similarly to (CBA/N × BALB/c)F_1 females in that they induce an optimal T15-dominated anti-PC response. Moreover, T cells from (k × d) → k chimeras could induce (CBA/N × BALB/c)F_1 female (k × d) or BALB.K (k) B cells but not BALB/c (d) B cells to make an anti-PC PFC response. These results confirm previous studies (13–15) and demonstrate in these studies that at least the Th cell set requiring a hapten-carrier association needs to recognize self-MHC-encoded determinants for effective T-B collaboration. Similarly, it was important to determine whether the Id-recognizing T cell set also depended on MHC recognition to provide helper signals. The present results demonstrate that T cells from the same chimeras could provide the Th cells needed for a dominant T15 response to recipients of T cells from F_1 male donors. Most important, however, is the finding that (k × d)F_1 → k chimeras could provide Th activity for selective activation of T15-bearing B cells of F_1 and both parental H-2 types (H-2^d, H-2^k). From these experiments we conclude

that whereas the activity of conventional Th cells depends on the recognition of self-MHC-encoded determinants, the distinct population of Id-recognizing cells from the same chimeric donors appears to recognize antigen independent of MHC-encoded determinants.

Whereas the present data demonstrate that MHC-encoded determinants do not regulate the antigen-specific response of Id-recognizing Th, there are several possible explanations for the apparent lack of "restriction" in these cell interactions. We envision that Th cell sets involved in an anti-PC response are analogous in their specificity requirements. Both Th cell sets have specificity for antigen and for self-determinants. According to this concept, one of the two Th cell sets recognizes self-MHC determinants, and the other recognizes self-Id determinants. We therefore discriminate between these cells based on their self-specificities as ThMHC and ThId. The data presented in this paper are consistent with this concept because the activity of ThId cell set does not depend on the recognition of MHC-encoded determinants.

An alternative explanation of our results might be based on other studies that have suggested that the failure of ThMHC cells from (A × B)F_1 into parent A chimeras to cooperate with B cells from parent B is due to a lack of macrophages bearing MHC-encoded parent A determinants in these cell mixtures (21). Based on such results, it could be argued in the present experiments that (*a*) ThMHC cells from (A × B)F_1 → parent A chimeras were unable to collaborate with B cells from parent B due to absence of a source of parent A macrophages and that (*b*) once the activation of ThMHC occurs in an H-2-restricted manner, ThMHC-B collaboration is unrestricted. Similarly, one could argue that (*a*) the ThId cell set from (A × B)F_1 → parent A chimeras do recognize antigen in association with parent A macrophages because such macrophages are absent in the assay for ThMHC function, but could be present in the F_1 T cells used as a source of ThMHC cells missing the ThId cell set (compare Fig. 2, groups 2 and 3; Fig. 4, groups 3 and 4); and that (*b*) as described for ThMHC activation, once the ThId is activated by recognition of antigen in association with MHC-encoded determinants on macrophages, Th-B collaboration is not MHC restricted. We think that this explanation is unlikely because the addition of a similar source of T cells from KLH-primed F_1 donors (Fig. 3, group 4), which by this argument should serve as a source of parent k macrophages, to T cells from (k × d)F_1 → parent k chimeras did not result in effective T-B collaboration between chimeric ThMHC cells and B cells from H-2^d parental donors. This result was also found in several other studies (13–15). In the context of these experiments, MHC homology between the ThMHC cells and the B cell seems to be required. Consequently, it seems equally unlikely that the ThId cell set would recognize antigen in the context of self-MHC determinants present on F_1 cells and subsequently would activate B cells in a nonrestricted fashion.

Alternatively, it is possible to envision a mechanism whereby the ThId cell set does not directly activate T15-bearing B cells but activates instead an as yet uncharacterized Th cell in the ThMHC cell source. One might thus propose that the mixing of ThId cells from (k × d)F_1 → parent k chimeric donors and Th cells deficient in ThId from defective F_1 male donors would result in the activation of T cells of F_1 male origin. This interaction between chimeric Th and F_1 T cells could require recognition of MHC-encoded determinants on these latter T cells themselves. Once activated, the F_1 Th cells would activate B cells of either parental haplotype. Although this

explanation for our results cannot be ruled out at this time, it is difficult to envision the cellular interactions that would then lead to selective activation of those B cells bearing the T15 Id by such a mechanism, or why this would not also function when the chimeric T cells are derived from low T15 mice. Furthermore, our interpretation of these results, namely, that the ThId cell set is indeed not MHC restricted, is strongly supported by the finding that the ThId cell set is not under the control of known MHC-linked Ir genes (22). That ThId cell function can be primed in nonresponder mice to the Ir-gene-controlled antigen, GLPhe, and can help syngeneic nonresponder B cells make a T15-dominated anti-PC response adds support to the concept that ThId cells do not recognize MHC gene products and mitigates against alternative explanations of the present results that would require the ThId cell set to be MHC restricted.

An important remaining question is the setting in which ThId recognize antigen and preferentially activate T15-bearing B cells. One could argue that ThId cells recognize antigen on B cells or macrophages also bearing the appropriate self-Id determinant. This interpretation seems unlikely because the probability of a T cell finding a cell bearing both specificities simultaneously would be small, given the heterogeneity of Id. Alternatively, antigen and Id could be recognized independently by two distinct receptors. Thus, a ThId cell could become activated by antigen and could deliver its helper signal to Id-bearing B cells at a later time. Although this question cannot be answered definitively, our studies favor the latter interpretation because the antigens used to activate ThId cells do not need to bear the ligand PC, which would be necessary to promote an association of the antigen with cells bearing the T15 Id. However, if this sequential activation concept were true, Th cells that could recognize B cells bearing virtually any self-Id would be represented among the antigen-activated ThId cells. It might be expected that the observed activation of idiotypic B cells that bind PC is due to the presence of ThMHC cells. As shown here, ThId cannot activate PC-specific B cells in the absence of ThMHC cells. Thus, one might propose a sequence of events in which ThMHC cells activate hapten-binding B cells and ThId cells selectively expand those activated B cells that bear the appropriate Id.

Summary

Two synergizing antigen-specific helper T (Th) cell populations are required for an optimal TEPC15 (T15)-dominated antiphosphorylcholine (PC) plaque-forming cell response. In these studies, the two Th cell sets are shown to differ in their requirements for recognition of self-major histocompatibility complex (MHC)-encoded determinants by testing the ability of Th cells from $F_1 \rightarrow$ parent bone marrow chimeras to collaborate with PC-specific B cells bearing MHC-encoded determinants of either parental haplotypes. Previous studies have shown that one antigen-specific Th cell population is required for T-dependent anti-PC responses and activates PC-specific B cells only if the hapten, PC, is physically linked to the priming antigen. This Th cell, referred to as ThMHC, induces anti-PC responses that are mainly non-T15 in character, and it appears to be identical to the conventional antigen-specific Th cell. In these experiments, using T cells from $(A \times B)F_1 \rightarrow$ parent A chimeras, ThMHC cells requiring hapten-carrier association provide help for F_1 and parent A B cells but not for B cells from parent B, thus confirming that the activity of the conventional Th

cell is H-2 restricted. The second antigen-specific Th cell population, whose function is measured in the presence of the ThMHC cell set, preferentially activates T15-bearing B cells. This Th cell set (ThId) is missing in mice expressing low levels of T15-bearing antibody and can be restored by the addition of antigen-specific T cells from donors expressing high levels of circulating T15 Id. These studies demonstrate that T cells from $F_1 \rightarrow$ parent chimeras that express substantial levels of T15-bearing anti-PC antibody could provide ThId cell activity for the selective activation of T15-bearing B cells of F_1 and both parental H-2 types. These results imply that whereas the activity of conventional, ThMHC, cells is clearly H-2 restricted, ThId cells from the same chimeric donors are not required to recognize antigen in association with self-MHC-encoded determinants for successful T-B collaboration.

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