TWO DISTINCT TYPES OF HELPER T CELLS INVOLVED IN THE SECONDARY ANTIBODY RESPONSE: INDEPENDENT AND SYNERGISTIC EFFECTS OF Ia^- AND Ia^+ HELPER T CELLS*

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Since the original discovery of T- and B-cell collaboration in the antibody response, it has been generally accepted that the helper T cell represents a single subset in the multimember family of T cells. There are, however, some controversial findings on the nature of the help with respect to its specificity, class preference, and genetic restrictions. In the antibody response to a hapten-carrier conjugate, the helper T cell is believed to recognize the carrier determinant and then to help antibody synthesis by B cells which recognize another determinant (hapten) linked to the same molecule. This type of interaction is supported by a phenomenon known as the carrier effect, in which the hapten-specific secondary antibody response is successfully elicited by the hapten coupled to the same carrier by which animals have been primarily immunized (1–4). A similar cooperative interaction between hapten-specific B cells and carrier-specific T cells is readily demonstrable in the adoptive secondary antibody response (5–8). Since in these cases hapten and carrier determinants must be present on a same single molecule, the T-B collaboration should occur upon recognition of the hapten by B cells, and recognition of the adjacent carrier determinants by T cells in a cognate form.

There are certainly no denials of the presence of this type of interaction. Nevertheless, there are several examples in which the cognate interaction is not likely to occur in certain T-cell-dependent antibody responses. In fact, various antigen-nonspecific factors derived from T cells can trigger the B-cell response, in which the factors themselves do not recognize carrier determinants (9–13). Several investigators are also aware that the hapten-specific B cells can be triggered under certain circumstances in which B and T cells are independently stimulated by corresponding determinants present on two distinctly separate molecules (8, 12, 14, 15). Hence, cognate interaction is not the only pathway for the effective T-B cell collaboration. One could ask whether the same or different helper T cells are involved in these diverse pathways of T-B cell collaboration.

Such problems are now even more complicated by the growing evidence suggesting that the helper T cell may recognize not only the carrier determinants, but also the products of major histocompatibility gene complex (MHC).[1] One of the major controver-

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[1] *Abbreviations used in this paper:* Ad, nylon adherent; BAT, mouse brain-associated T-cell antigen; C, complement; DNP, 2,4-dinitrophenyl; EA, egg albumin; FCS, fetal calf serum; KLH, keyhol limpet hemocyanin; MEM, Eagle's minimal essential medium; MHC, major histocompatibility gene complex; MIg, mouse immunoglobulin; P, nylon nonadherent; PFC, plaque-forming cells; Th_1, nylon-nonadherent helper T cells without Ia antigen; Th_2, nylon-adherent helper T cells with Ia antigen.

sies is that in some experimental systems, an effective stimulation occurs between allogeneic T and B cells (16–20), but in other systems there exists a strict histocompatibility requirement (21–23). One further question to be resolved is whether or not the helper T cell in fact recognizes immunoglobulin V or C regions on the responding B cells, thus leading to the class- or idiotype-specific stimulation of B-cell clones (24–26). There has been much controversy over the presence of Ia antigen on helper T cells (27–29). Our primary concern is to learn whether or not such divergent helper effects can be ascribed to a single category of the helper T cell.

In the course of our experiments on the antigen-specific T-cell factors, we have repeatedly found that the purified T-cell fraction obtained by passage through a nylon wool column has far less helper activity than expected from the recovery of T cells from original, primed spleen cells. The addition of a small number of nylon-adherent cells often increased the helper activity, and such an effect of adherent cells was abrogated by treating the cells with anti-Thy-1 and complement. These observations led us to study the nature of both nylon-purified and nylon-adherent T cells which are involved in the triggering of B cells. Here we describe the presence of two distinct types of helper T cells which act either independently or synergistically to help the B-cell response. The first type of helper T cell (Th_1) has on it no Ia antigen and acts to augment the antibody response via the cognate interaction with B cells; and the other (Th_2) possesses Ia antigen and helps the B-cell response to an unrelated antigen. Both of these belong to the Lyt-1^+ subclass and perhaps act synergistically to induce the secondary IgG antibody response under the physiologic condition.

Materials and Methods

Animals. Mice of C3H/He and BALB/cNCr were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu City, Japan. Breeding pairs of B10.A(3R) and B10.A(5R) were kindly provided by Dr. C. S. David of the Department of Genetics, Washington University, St. Louis, Mo. They were maintained in our animal facility by the brother-sister mating.

Antigens. Keyhole limpet hemocyanin (KLH; Calbiochem, San Diego, Calif.) and egg albumin (EA) recrystallized 5 times (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) were used as carrier proteins. 2,4-Dinitrophenyl (DNP) groups were introduced to these proteins by the method of Eisen et al. (30). DNP_{770}-KLH and DNP_{18}-EA were used as hapten-carrier conjugates.

Immunization. Mice were immunized with 100 μg of DNP-KLH, KLH, or EA intraperitoneally with 1×10^9 *Bordetella pertussis* vaccine. Spleen cells were taken 6–10 wk after the immunization.

Antisera. A polyvalent anti-mouse immunoglobulin (anti-MIg) was raised in rabbits by repeated injections of normal mouse gamma globulin in complete Freund's adjuvant. The pooled, heat-inactivated antiserum was repeatedly absorbed with normal mouse thymocytes to remove nonspecific cytotoxicity. A rabbit anti-mouse brain-associated T-cell antigen (anti-BAT) was prepared according to the method of Sato et al. (31). After appropriate absorption with mouse erythrocytes and bone marrow cells, this antiserum specifically killed ≅50% of normal spleen cells at 1:100 dilution.

The following alloantisera directed at restricted subregions of *H-2* complex were the gifts of Dr. C. S. David; A.TH anti-A.TL, (A.TH × B10.A(5R))F_1 anti-A.TL, (A.TH × B10.S(9R))F_1 anti-A.TL, (B10.HTT × A.BY)F_1 anti-A.TL, B10.A(4R) anti-B10.A(2R), B10.S(7R) anti-B10.HTT, and (C3H.Q × B10.D2)F_1 anti-B10.AQR. An antiserum, B10.A(3R) anti-B10.A(5R), was prepared by repeated injections of B10.A(5R) spleen and thymus cells into B10.A(3R).

Anti-Lyt-1.2 and anti-Lyt-2.2,3.2 alloantisera were supplied by Dr. D. B. Murphy of the Department of Medicine, Stanford University, Stanford, Calif. A congenic anti-Thy-1.2 antiserum

was the gift of Dr. H. Sato of the Asahikawa Medical School, Hokkaido, Japan.

Preparation of Splenic B Cells. Splenic B cells from either DNP-KLH-primed or normal mice were prepared by the following procedure. The spleen cell suspension containing 3×10^7 cells/ml was treated with 1:100 dilution of anti-BAT antiserum and guinea pig complement (C) at 37°C for 30 min. The live cells were collected by centrifugation on a layer of fetal calf serum (FCS; Grand Island Biological Co., Grand Island, N. Y.).

Depletion of B Cells from Primed Spleen Cells. This was performed by the method used by Yuan et al. (32). 3×10^7 KLH- or EA-primed spleen cells were first incubated with 1:10 dilution of anti-MIg antiserum in the presence of 0.1% of sodium azide at room temperature for 30 min. They were washed once with Eagle's minimal essential medium (MEM), and then further incubated with a 1:10 dilution of C and azide at 37°C for 30 min. After centrifugation on an FCS gradient, ≅50% of the original cells were recovered, which consisted of >85% Thy-1 antigen-positive cells and <10% surface Ig-positive cells.

Separation of Nylon-Adherent (Ad) and Nonadherent (P) T Cells. The B-cell depleted spleen cells obtained by the above procedure were fractionated with the nylon wool column by the method of Julius et al. (33). The column was made with 4 g of nylon wool (Leuko-pak; Fenwal Laboratories, Morton Grove, Ill.) in a 100-ml glass syringe, and it was equilibrated with MEM fortified with 5% FCS at 37°C. 5×10^8 cells were applied to the column and incubated at 37°C for 45 min. P cells (denoting the nylon column-passed cells) were eluted with 37°C MEM. The Ad cells were obtained by vigorously pressing the nylon wool with chilled MEM. The yields of both P and Ad cells were ≅20% of original spleen cells. Usually, ≅95% of P cells and 70–80% of Ad cells were killed by anti-Thy-1 and C.

Cytotoxic Treatment of T Cells with Alloantisera. Both P and Ad cells were treated with various alloantisera and C. In general, 2×10^7 cells in 0.5 ml of MEM were incubated with an equal volume of a 1:5 dilution of alloantisera for 30 min at room temperature. After washing with MEM, the cells were further incubated with 1:10 dilution of well-selected rabbit C for 30 min at 37°C. The cells were centrifuged on an FCS gradient and washed three times with MEM. All procedures were carried out under strict sterile conditions.

In Vitro Secondary Antibody Response with B and Nylon Column-Separated T Cells. The in vitro secondary antibody response was induced in the modified Marbrook culture system as described previously (34). 5×10^6 DNP-primed B cells were mixed with graded numbers of T cells in 1 ml of enriched MEM. The total number of cells per culture was adjusted to 1×10^7/ml by adding appropriate numbers of B cells from unprimed animals. This was found to be necessary for obtaining uniform results in preliminary experiments, since lowering the density of cells often diminished the expected antibody response. We also confirmed that the addition of normal B cells did not influence the number of DNP-specific plaque-forming cells (PFC). The cells were stimulated with 0.1 μg/ml of DNP-KLH or DNP-EA. The culture was maintained at 37°C in 10% CO_2 for 5 days, and the number of DNP-specific IgG PFC was determined by the method of Cunningham and Szenberg (35).

Results

Independent and Synergistic Helper Effects of Ad and P Splenic T Cells. KLH-primed anti-MIg-treated spleen cells were separated into Ad and P populations. Graded numbers of both cells were admixed with 5×10^6 DNP-primed B cells, and the mixture was stimulated in vitro with 0.1 μg/ml of DNP-KLH. The dose-response curves for the column-separated helper T cells are shown in Fig. 1 in comparison with that for unseparated T cells. 2×10^6 P cells already gave a maximal response which showed a plateau level with larger numbers of P cells. In contrast, when the Ad-cell population was used as the source of the helper, increasing the number of Ad cells resulted in a proportional increase of helper activity as determined by the number of PFC per culture. However, in neither case was the helper activity found to reach the level obtained with T cells before separation, suggesting that only some of the B cells were triggered by either one of the column-separated T cells. In

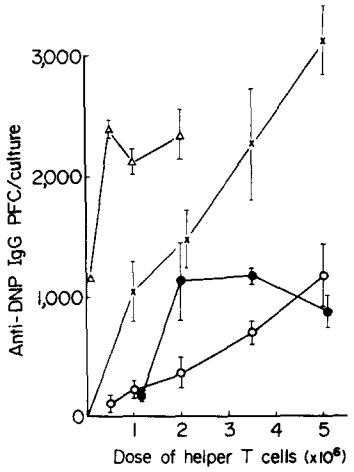

FIG. 1. Independent and synergistic effects of P and Ad helper T cells. Graded numbers of P (●) and Ad (○) cells were mixed with DNP-primed B cells, and were stimulated in vitro with 0.1 μg of DNP-KLH. A synergism between P and Ad cells is seen when the graded numbers of Ad cells are added to a constant number (3.5×10^6) of P cells (△). (×) shows a dose-response curve of unseparated T cells. Note that the unseparated T cells and the mixture of P and Ad cells produce far higher helper effect than does either P or Ad cells alone.

addition, the slopes of the dose-response curves indicate that the quality of the helper effect produced by P and Ad cells is different.

Based upon these findings, we added small graded numbers of Ad cells to a fixed optimal number of P cells (3.5×10^6), and the helper activity of the mixture of P and Ad cells was examined by coculturing with DNP-primed B cells. As is evident from Fig. 1, the addition of even 0.5×10^6 Ad cells to the optimal number of P cells produced a far higher helper effect than that of either single cell type alone. The magnitude of the response with the mixture of P and Ad cells was comparable to that obtained with the unseparated T cells of approximately the same number. The helper effect by the mixture of P and Ad cells was higher than the sum of the effects of each single type at any point, suggesting that a synergistic rather than additive effect between these two cell types gives maximal help. By this and other evidence described below, we tentatively refer to the nylon-nonadherent helper T cell as Th_1, and the adherent helper T cell as Th_2.

Both Th_1 and Th_2 Belong to the Lyt-1$^+$,2$^-$,3$^-$ Subclass. To determine whether these two populations of helper T cells have the same or different Lyt phenotype, we treated the optimal number (3.5×10^6) of P and Ad cells from KLH-primed BALB/c mice with anti-Lyt-1.2 or anti-Lyt-2.2,3.2 alloantiserum and C, and the treated T cells were mixed with DNP-primed B cells. The total viable cell number per culture was adjusted to 1×10^7 by adding normal B cells as described in Materials and Methods. The cells were stimulated with DNP-KLH for 5 days and the number of DNP-specific PFC was measured.

It is clear from Table I that the helper effects of both P (Th_1) and Ad (Th_2) cells were abrogated by treatment with anti-Lyt-1.2 but not with anti-Lyt-2.2,3.2, indicating that both helper T cells belong to the Lyt-1$^+$,2$^-$,3$^-$ subclass.

TABLE I

Lyt Phenotype of Th_1 and Th_2 as Determined by Treatment of P and Ad T Cells with Anti-Lyt Antisera

DNP-primed B cells*	T cells treated with‡	Anti-DNP IgG PFC/culture§
Th_1		
5×10^6	NMS‖	1,357 ± 247
5×10^6	Anti-Lyt-1	307 ± 53
5×10^6	Anti-Lyt-2	1,397 ± 267
Th_2		
5×10^6	NMS	1,762 ± 248
5×10^6	Anti-Lyt-1	35 ± 12
5×10^6	Anti-Lyt-2	2,044 ± 354
5×10^6	Not added	0

* Anti-BAT-treated splenic B cells from DNP-KLH-primed BALB/c mice.

‡ 3.5×10^6 each of nylon column-separated fractions were treated with anti-Lyt antisera and C, and were cocultured with DNP-primed B cells with 0.1 μg/ml of DNP-KLH. Total cell number per culture was adjusted to 1×10^7 by adding B cells from normal unimmunized mice.

§ Arythmetic means and standard deviations from five cultures.

‖ NMS, normal mouse serum.

In addition, the treatment of Ad cells with anti-Lyt-2.2,3.2 resulted in a slight increase of the PFC response in some repeated experiments, suggesting that the treatment had eliminated the suppressor T cell present in the Ad-cell population.

Presence of Ia Antigen on Th_2 but not on Th_1. P- and Ad-cell populations from KLH-primed C3H mice were treated with anti-Ia antiserum to learn whether or not Th_1 or Th_2 possess *I*-region determinants. Cells were treated with 1:10 final dilution of A.TH anti-A.TL and C, and the remaining live cells were cocultured with DNP-primed B cells. In general, <10% of either cells was killed by the anti-Ia antiserum.

The results presented in Table II clearly show that the helper activity of Th_1 was not affected by anti-Ia treatment, whereas that of Th_2 was almost completely abrogated. This killing of Th_2 by A.TH anti-A.TL was specific, since the same antiserum after absorption with C3H spleen cells showed no effect on Th_2 helper activity (Table II).

To assign the *I* subregion which determines the Ia antigen on Th_2, the Ad cells were treated with various anti-Ia antisera having different subregion specificities. The combination of animal strains and putative subregion specificities are listed in Table III. Some, but not all anti-Ia antisera were effective in eliminating the Th_2 activity. The close analysis of *I*-subregion specificity of these antisera indicates that the sera having *I-J*-subregion specificity were in all cases capable of removing the Th_2 helper activity, although the ability varied from one serum to another. This may reflect the difference in titers of antibody contained in these antisera which is directed at the specific determi-

TABLE II
Presence of Ia Antigen on Th_2 but not on Th_1

DNP-primed B cells*	T cells treated with‡	Anti-DNP IgG PFC/culture§
Th_1		
5×10^6	NMS‖	931 ± 180
5×10^6	A.TH anti-A.TL	960 ± 163
Th_2		
5×10^6	NMS	713 ± 86
5×10^6	A.TH anti-A.TL	36 ± 14
5×10^6	A.TH anti-A.TL absorbed with C3H	1,126 ± 102
5×10^6	Not added	25 ± 15

* Anti-BAT-treated splenic B cells from DNP-KLH-primed C3H mice.
‡ 3.5×10^6 each of KLH-primed T cells of separated fractions were treated with anti-Ia and C, and were cocultured with DNP-primed B cells with 0.1 μg/ml of DNP-KLH.
§ Arythmetic means and standard deviations from five cultures.
‖ NMS, normal mouse serum.

TABLE III
Subregion Assignment of Ia Antigen Expressed on Th_2

Th_2 treated with*	Subregion specificity	Anti-DNP IgG PFC/culture‡
NMS§	–	2,156 ± 213
A.TH anti-A.TL	A,B,J,E,C	253 ± 86
(C3H.Q × B10.D2)F_1 anti-B10.AQR	A,B,J,E	304 ± 24
(B10.HTT × A.BY)F_1 anti-A.TL	A,B,J	58 ± 30
B10.A(4R) anti-B10.A(2R)	B,J,E	953 ± 312
B10.A(3R) anti-B10.A(5R)	J	419 ± 129
B10.S(7R) anti-B10.HTT	E,C	2,490 ± 348
(A.TH × B10.A(5R))F_1 anti-A.TL	A,B, C	2,506 ± 227
(A.TH × B10.S(9R))F_1 anti-A.TL	A,B, C	2,187 ± 609
Not added	–	29 ± 20

* 3.5×10^6 KLH-primed adherent T cells after treatment with one of the alloantisera were cocultured with 5×10^6 DNP-primed B cells.
‡ Arythmetic means and standard deviations from five cultures.
§ NMS, normal mouse serum.

nants on Th_2. In sharp contrast, none of the antisera lacking *I-J* specificity could remove the Th_2 helper activity. In addition, the presence or absence of *I-A*, *I-E*, and *I-C* subregion specificities did not correlate with the activity to kill Th_2. The results strongly suggest that the Ia antigen expressed on Th_2 is controlled by genes in the *I-J* subregion.

Th_2 but not Th_1 Can Trigger B-Cell Response to a Hapten Coupled to a Heterologous Carrier. To learn if there is any difference in the mode of the help given by Th_1 and Th_2, P and Ad cells were prepared from KLH- or EA-primed mice. They were admixed with DNP-primed B cells, and were stimu-

TABLE IV
Ad Helper T Cell Can Stimulate B Cells with DNP Coupled to a Heterologous Carrier

DNP-primed B cells*	T cells primed with‡	Antigen in vitro	Anti-DNP IgG PFC/culture§
Th_1			
5×10^6	KLH	DNP-KLH	1,284 ± 121
5×10^6	EA	DNP-KLH	163 ± 17
5×10^6	EA	DNP-KLH + EA	192 ± 51
5×10^6	EA	DNP-EA	794 ± 164
Th_2			
5×10^6	KLH	DNP-KLH	849 ± 155
5×10^6	EA	DNP-KLH	48 ± 27
5×10^6	EA	DNP-KLH + EA	496 ± 128
5×10^6	EA	DNP-EA	426 ± 89
5×10^6	Not added	DNP-KLH + EA	30 ± 25

* Anti-BAT treated splenic B cells from DNP-KLH primed BALB/c mice.
‡ 3.5×10^6 each of KLH-primed T cells of separated fraction.
§ Arythmetic means and standard deviations from five cultures.

lated in vitro with the mixture of DNP-KLH and uncoupled EA. The rationale of this experiment was to examine whether or not the hapten and carrier determinants must be present on a single molecule to induce the collaborative interaction between B and both helper T cells.

The combinations of T cells and stimulating antigens are listed in Table IV. With nonadherent Th_1 population, optimal help was obtained only if B and T cells were stimulated by corresponding hapten and carrier determinants linked on a single molecule (cognate interaction). For example, KLH-primed Th_1 can help DNP-primed B cells only when stimulated with DNP-KLH, and EA-primed Th_1 can do so with DNP-EA. EA-primed Th_1 cells cannot, however, exert a helper effect when stimulated with unlinked hapten and carrier, i.e., DNP-KLH and uncoupled EA.

By contrast, Th_2 was found not to require a cognate interaction: as shown in the lower part of Table IV, EA-primed Th_2 can exert a significant helper effect to produce the DNP-specific antibody response, even if the DNP-primed B cells and EA-primed Th_2 cells were stimulated with two separate molecules, i.e., DNP-KLH and uncoupled EA. This helper effect observed with DNP-KLH and EA was comparable to that obtained with DNP-EA, a molecule having both hapten and carrier determinants. It is also clear that this second helper effect is produced by a specific antigenic stimulation of Th_2, since in the absence of EA no B-cell stimulation has occurred with DNP-KLH alone. The results indicate that the helper activity of Th_1 and Th_2 are mediated through different pathways.

Discussion

The data presented here clearly indicate that there exist two distinct types of carrier-specific helper T cells which act either independently or synergistically to help the B-cell response to a hapten. A simple procedure of passing the

carrier-primed splenic T cells through a nylon wool column could separate these two distinguishable helper T cells, and enabled us to analyze the genetic distinctions and modes of their interaction with B cells. Th_1, we found, had no detectable Ia antigen and could help the B-cell response to a hapten only if the hapten was present on the carrier molecule by which Th_1 was generated (cognate interaction). By contrast, Th_2 was killed by anti-Ia antiserum and C, and could help the B-cell response to a hapten coupled to an unrelated carrier (polyclonal interaction). The addition of a very small number of Th_2 augmented the antibody response mounted by the B cell and Th_1, and thus Th_1 and Th_2 were found to act synergistically to give optimal help to B-cell clones. It was also found that both Th_1 and Th_2 belong to the Lyt-1^+,2^-,3^- subclass.

These results, though not complete, would explain several phenomenological findings which have not been coherently examined. The classical concept of T-B cell collaboration, in which carrier-specific T cells and hapten-specific B cells interact in the presence of the relevant hapten-carrier conjugate (1–8), is indeed supported by the cognate interaction between Th_1 and B cells. One may ask, however, why the cognate interaction is needed for the help by Th_1. The first explanation is that Th_1 and B cells are linked by the antigen, which results in the close contact between these cell types. This could also be mediated by an antigen-specific cooperative T-cell factor, which is perhaps released from Th_1 after antigenic stimulation. Since such a factor has been shown to be the product of *I-A* subregion gene(s) (36, 37), Th_1 may express Ia antigen only at a certain point of time after antigenic stimulation. Alternatively, Th_1 may have Ia antigen in a quantity undetectable by the usual cytotoxic procedure. In any event, such an *I*-region product, which is probably associated with the Th_1 helper effect, is different from that detected on Th_2, as the loci coding for these molecules are mapped in different *I* subregions.

The second hypothetical explanation is that Th_1 recognizes not only carrier determinants, but also the idiotype of surface immunoglobulins possessed by B cells specific for the given hapten. This latter type of interaction would mimic the T-B cell link by antigen, and is thus an apparent cognate interaction. This may in turn explain the strict antigen-specificity of the Th_1 effect which may be directed at only certain restricted B-cell clones. Ward et al. (26) have recently shown evidence that the helper T cell which shares identical or complementary idiotype to that of a certain B-cell clone does indeed exist. As increasing the dose of Th_1 did not result in the maximal stimulation of the B-cell response, it is probable that only a part of B-cell clones can be triggered by Th_1.

On the other hand, there are several examples in which the cognate interaction is not the essential event for the B-cell response. Hamaoka et al. (8), as well as Kishimoto and Ishizaka (12), have demonstrated that under certain circumstances, B cells can be triggered by unlinked hapten and carrier determinants. Interestingly, in both cases there were some class preferences in the antibody produced which belonged mainly to the IgE class. One mere suggestion from these studies is that the stimulation of Th_2 by carrier determinants results in the triggering of a broader spectrum of B cells than in the strict cognate interaction, and thus we would refer to this type of interaction as polyclonal interaction. One can predict that many of the nonspecific T-cell

factors mediate this type of polyclonal interaction, and the quality of the response would depend upon the nature of such mediators. These postulates raise the important question of whether the B-cell clones triggered by Th_1 and Th_2 are the same or different. Our current efforts are directed towards answering this question.

There have been some observations which suggest the presence of two synergizing helper T cells. Marrack (Hunter) and Kappler (38, 39) have shown that there are two factors, clone-activating and clone-expanding T-cell factors, perhaps derived from different T cells. Janeway (40) described that increasing the dose of helper T cell leads to an unexpectedly steep rise in B-cell response (premium effect), which could be ascribed to the cooperative interaction between two helper T cells. More recently, Janeway et al. (41) reported that one helper T cell recognizes antigen in association with immunoglobulins, and the other probably with Ia antigen. Our present experiments do not directly verify their postulates, but would support them in certain aspects. As discussed before, Th_1 seems to selectively stimulate B-cell clones of a restricted nature which may be characterized by a surface moiety on B cells. We have not yet obtained any data on the class-, idiotype-, or allotype-specific help, either by Th_1 or Th_2. On the other hand, Th_2 acts as a polyclonal activator, and is perhaps involved in the clonal expansion. It is quite obvious that the synergism between Th_1 and Th_2 determines both the magnitude and quality of the antibody response.

There have been some controversial findings regarding the presence of Ia antigen on helper T cells. Okumura et al. (27) showed strong evidence that carrier-specific helper T cells can be killed by certain anti-Ia antisera and C in an adoptive secondary antibody response, whereas Hämmerling et al. (29) could not abrogate the helper activity of nylon wool-purified T cells with anti-Ia antisera. McDevitt et al. (28) also found that in some experiments using carrier-primed total spleen cells, anti-Ia could deplete the helper T cell, whereas in other experiments using nylon wool-purified helper T cells, no reduction of helper activity was observed. This apparent discrepancy is perhaps completely resolved by our present experiments. It is probable that the failure to detect Ia antigen on helper T cells is the result of the purification of T cells over a nylon wool column which eliminated the Ia^+ Th_2. In contrast, the total spleen cells as the source of helper T cells should contain both Th_1 and Th_2, and the treatment with anti-Ia may have resulted in the reduction of helper activity mounted by Th_2. In fact, Okumura et al. (27) were aware that the removal of helper activity with anti-Ia antisera is not always complete, and leaves some activity behind. These results will also warn that the semipurification of T cells by passage through a nylon wool column would loose an important compartment of T cells.

Although it is firmly established in the present experiment that the Ia locus controlling the determinants on Th_2 maps in *I-J* subregion, it is not known whether the locus is identical to the Ia-4 locus which controls determinants on the suppressor T cell and suppressive T-cell factor. However, since the activity of alloantisera to remove Th_2 activity greatly differed from one antiserum to the next, which was not observed in the absorption of suppressive T-cell factor (42), it is probable that the *I-J* determinants on the suppressor and helper T cells are different. Okumura et al. (27) also arrived at the same conclusion,

since potent antisera which could invariably kill the suppressor T cell had no effect on helper T cell, and some other antisera having no cytotoxic effect on the suppressor T cell could effectively remove the helper activity. These results suggest that *I-J* subregion contains at least two or more loci which determine distinct surface molecules on different subsets of T cells.

It was surprising to us that the Ia antigen expressed on Th_2 was controlled by the *I-J* subregion, which was originally defined by surface determinants on the suppressor T cells. This would, in turn, give a clue for analyzing the complex process of suppressive cell interactions in which T-cell Ia antigens would act as the communication devices in antigen-driven chain reactions. We have previously shown that the suppressive T-cell factor with *I*-region determinants is successfully absorbed with nylon-adherent T cells, but not with B and nylon-passed T cells (43). It is tempting to assume that the Th_2 is one of the targets of the suppressive T-cell factor, and that the interaction between the factor and Th_2 is determined by complementary structures encoded by closely linked genes mapped in the same *I-J* subregion.

The presence of two helper T cells as well as two helper pathways opens a new vista for analyzing a complex network of immunocompetent cell interactions. One could now safely ask which of the Th_1 and Th_2 is involved in idiotype-, allotype- and class-specific helper effects, and in MHC-restricted and unrestricted cell collaborations. The question is also raised as to which cell type is the target of antigen-specific and nonspecific suppressor T cells. Answering these questions would lead to a better understanding of the immunoregulatory system which involves two polymorphic structures, i.e., immunoglobulins and MHC products.

Summary

We have described here two distinct types of carrier-specific helper T cells which act independently and synergistically to augment the B-cell response to a hapten. They are separable by passage through a nylon wool column. The first type of helper T cell, which we designate as Th_1, is nylon nonadherent, and can help the response of hapten-primed B cells only if the haptenic and carrier determinants are present on a single molecule (cognate interaction). The second type of helper T cell, Th_2, adheres to the nylon wool column, and can help the B-cell response to a hapten coupled to a heterologous carrier upon stimulation with unconjugated relevant carrier (polyclonal interaction). The addition of a small number of Th_2 to the mixture of Th_1 and B cells significantly augmented the net response to the hapten carrier conjugate. Both Th_1 and Th_2 cells belong to the Lyt-1^+,2^-,3^- subclass. Th_1 has no detectable Ia antigen, whereas Th_2 is killed by certain anti-Ia antisera and complement. The Ia antigen detected on Th_2 was found to be controlled by a locus in the *I-J* subregion. The results clearly established the fact that there are two distinct pathways in the T- and B-cell collaboration, which involves two different subsets of carrier-specific helper T cells.

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