# A CLONED MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED TRINITROPHENYL-REACTIVE HUMAN HELPER T CELL LINE THAT ACTIVATES B CELL SUBSETS VIA TWO DISTINCT PATHWAYS*

BY MARY ANN PRINCIPATO, GARY S. THOMPSON, AND STEVEN M. FRIEDMAN

*From the Division of Rheumatology, Department of Medicine, Columbia University College of Physicians and Surgeons, New York 10032*

Recent studies in the murine system have demonstrated that the activation signals required to trigger antibody production by B cells vary considerably depending on the nature of the B cell subpopulation under study. For example, Asano et al. (1) used major histocompatibility complex (MHC)[1]-restricted keyhole limpet hemocyanin-specific cloned T helper (Th) cells to study B cell subsets distinguished by the presence or absence of the Lyb-5 differentiation antigen. They determined that the activation of hapten-specific Lyb-5$^-$ B cells requires both hapten-carrier linkage and MHC-restricted Th cell-B cell interaction, while the triggering of hapten-specific Lyb-5$^+$ B cells is unrestricted and does not require carrier-hapten linkage. Other investigators have isolated murine B cell subsets by virtue of differences in cell size or density, and analyzed their interactions with Th cells specific for B cell surface determinants, including male-associated (HY) antigen (2–4), allogeneic Ia antigens (5), minor histocompatibility determinants (6), or covalently bound hapten (7). These experimental systems, in which most if not all B cells bear the target antigen recognized by the Th cell, offer the unique advantage of measuring antigen-specific T-B collaboration as a polyclonal plaque-forming cell (PFC) response. The results of these studies establish that large (partially activated) murine B cells respond readily to nonspecific, non–MHC-restricted, T cell-derived helper factor(s). In contrast, Th cells must interact directly with antigen on the surface of small (resting) B cells before these B cells become competent to respond to nonspecific helper factor(s).

Heterogeneity within the human peripheral B cell pool has also been described, both with respect to cell surface markers and functional responses to mitogens and T cell-derived helper factors. Thus, the pokeweed mitogen (PWM)-responsive human B cell is large, of low density, and lacks both surface membrane IgD

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[1] *Abbreviations used in this paper*: APC, antigen-presenting cell; BCGF, B cell growth factor; ELISA, enzyme-linked immunoabsorbent assay; FITC, fluorescein isothiocyanate; GVHD, graft-vs.-host disease; IL-2, interleukin 2; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PFC, plaque-forming cell; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus; TCL, T cell line; Th, helper T cell; TNP, trinitrophenyl.

(8, 9) and receptors for mouse erythrocytes (10). In contrast, small peripheral B cells bear receptors for mouse erythrocytes, are surface IgD positive, and respond poorly to PWM but relatively well to lipopolysaccharide (LPS) (8, 9). Moreover, Muraguchi et al. (11) recently determined that while large human tonsillar B cells respond directly to B cell growth factor (BCGF), small B cells require activation by anti-$\mu$ antibody before they become competent to proliferate in response to BCGF.

Taken together, these observations serve to define distinct stages in the ontogeny of human B cells and suggest strong analogies to the murine B cell differentiative scheme. To date, however, the precise mechanisms by which antigen-specific human Th cells interact with these B cell subpopulations have not been clearly delineated. To address this important question, we have established and functionally characterized a cloned, trinitrophenyl (TNP)-specific, MHC-restricted human helper T cell line (TCL). Our results demonstrate that human B cell subpopulations, isolated by discontinuous density gradient centrifugation, differ with respect to their requirements for T cell help. Thus, large B cells are highly responsive to nonspecific helper signals generated by the antigen-specific, MHC-restricted interaction of Th cells and TNP-modified antigen-presenting cells (APC). In contrast, polyclonal activation of small peripheral B cells requires direct interaction of Th cells with antigen (TNP) on both the B cell and the APC surface. These results are discussed in the context of normal human B cell physiology and possible abnormal Th-B cell interactions that may lead to autoimmunity.

## Materials and Methods

*Lymphocyte Preparation and Fractionation.* Fresh peripheral blood mononuclear cells were isolated from healthy donors by Ficoll-Hypaque gradient centrifugation. Adherent cells were partially depleted by incubation on plastic petri dishes for 1 h at 37°C. Nonadherent cells were fractionated into T cell-enriched ($E^+$) and B cell-enriched ($E^-$) populations by the formation of rosettes with neuraminidase-treated sheep erythrocytes. In some studies, the $E^-$ population was depleted of any contaminating T cells by complement-mediated lysis in the presence of OKT3, a monoclonal anti-human T cell antibody (12), or subjected to further fractionation on discontinuous Percoll gradients.

*Separation of B Cell Subpopulations on Density Gradients.* $E^-$ cells were fractionated by density centrifugation on a discontinuous gradient of Percoll (Pharmacia, Uppsala, Sweden) as previously described (9). Briefly, 20–60 × $10^6$ $E^-$ cells in 0.5 cc of 30% Percoll in phosphate-buffered saline (PBS) were layered on a discontinuous gradient of 45–60% Percoll in PBS consisting of 5% increments (2.5 ml vol). Gradients were centrifuged at 5,000 *g* for 5 min at 4°C, and layers routinely observed at the 30–45%, 45–50%, and 50–55% interfaces. The 45–50% and 50–55% layers were designated large and small B cell populations, respectively (8, 9). The 30–45% layer was comprised largely of macrophages, and produced very few PFC under the culture conditions used (data not shown).

*Induction, Isolation, and Propagation of Cloned TNP Altered Self-reactive TCL.* As previously described (13), in vitro sensitizations were performed in sterile Linbro MR-2 microplates (Linbro Chemical Co., Hamden, CT). Each microwell contained 2 × $10^5$ responder T cells and an equal number of TNP-modified, x-irradiated $E^-$ autologous stimulators in a volume of 0.2 cc of final medium, consisting of Iscove's modified Dulbecco's medium (Gibco Laboratories, Grand Island, NY) supplemented with 100 mM penicillin-streptomycin, 200 mM L-glutamine, and 10% fetal bovine serum (Microbiological Associates, Bethesda, MD). As in previous studies, cells were modified with trinitrobenzene sulfonic acid (10 mM, pH 7.4, in PBS) for 10 min at 37°C (13). After 10 d of

culture at 37°C in a humid 95% air, 5% $CO_2$ atmosphere, 100 μl of spent medium was aspirated and replaced with an equal volume of fresh final medium containing $2 \times 10^5$ TNP-modified, x-irradiated autologous stimulators. After three additional days of culture, responder T cells were resuspended, washed, and propagated in the presence of interleukin 2 (IL-2). After 2 wk of growth as a bulk TCL, the culture was cloned by limiting dilution. Briefly, TCL cells were seeded at 1 or 10 cells/well in flat bottomed microtiter wells (3596; Costar, Cambridge, MA) in a volume of 0.2 cc of final medium supplemented with IL-2 and $1 \times 10^5$ x-irradiated, TNP-modified autologous cells. Microwells were fed fresh final medium containing IL-2 every 3–4 d and, after 2–3 wk, growing cultures were transferred to macrowells and expanded in the presence of IL-2 and x-irradiated, TNP-modified autologous cells. Of 96 wells seeded at 10 TCL/well, 14 cultures were grown, whereas only 4 of 96 seeded at 1 TCL/well were successful. These cultures have been designated cloned TCL.

*Preparation of IL-2.* As recently reported (14), the IL-2 used to propagate TCL cells was obtained by phytohemagglutinin activation of tumor cells derived from a patient with T cell chronic lymphocytic leukemia. This tumor cell preparation has been termed OT-CLL. The active moiety(ies) produced by OT-CLL cells have proven to be both functionally and biochemically similar to conventional human IL-2 but is produced in titers >20-fold higher than those obtained from conventional sources. Although each batch of OT-CLL-derived IL-2 varied slightly in potency, the crude supernatant was usually added to cell cultures at a final concentration of 2.5–5%.

*Phenotypic Analysis of TCL Cells.* The cell surface phenotype of the TCL cells was assessed by indirect immunofluorescence using monoclonal antibodies generously supplied by Dr. Gideon Goldstein of Ortho Pharmaceutical Corp., Raritan, NJ and a fluorescein-conjugated goat anti-mouse IgG (Meloy Laboratories Inc., Springfield, VA). Samples were assayed using a model 30-H cytofluorograf (Ortho Instruments, Westwood, MA). The antibodies used in this study included: OKT3 and OKT11, two pan-T cell antibodies (12, 15), the latter of which recognizes the E rosette receptor; OKT4, which recognizes an antigen expressed by the "helper" or "inducer" T cell subset (16); OKT8, which identifies suppressor/cytotoxic T cells (17); OKI1, which binds to the human Ia antigen analog (18); and OKM1, which identifies peripheral blood monocytes (19).

*Proliferative Assay (13).* $1 \times 10^4$ cloned TCL cells, suspended in 0.1 ml of final medium, were co-cultured with an equal volume of final medium alone or final medium containing $1 \times 10^5$ x-irradiated stimulator cells. Cultures were performed in Linbro flat-bottomed microtiter plates and all experimental points assayed in triplicate. After 48 h, cultures were pulsed for 16 h with 0.2 μCi of [$^3$H]thymidine (5 μCi/ml) (New England Nuclear, Boston, MA). Incorporation of radioactivity was measured by liquid scintillation counting and data expressed as cpm ± SEM.

*B Cell Helper Assay.* $1 \times 10^5$ responder B cells were cultured in flat-bottomed microwells in a volume of 0.2 ml of final medium alone or final medium supplemented with varying numbers of T cells, TCL cells, x-irradiated APC, or PWM (10 μgm/ml), according to experimental design. Each culture consisted of three replicate microwells which, after 6 d of culture, were pooled and assayed in triplicate for polyclonal immunoglobulin production using the reverse hemolytic plaque assay (13). Data is presented as PFC/$10^6$ initially cultured B cells. SEM was <15% for all data points. In some studies, total IgM and IgG immunoglobulin production were quantitated using class-specific, enzyme-linked immunoabsorbent assays (ELISA) as previously described (20).

## Results

*Establishment of Cloned TNP-specific TCL.* Hapten-specific T cells were isolated by limiting dilution culture of an uncloned TNP-altered self-reactive TCL. Briefly, TCL cells were seeded at either 1 or 10 cells/well in microwells containing x-irradiated, TNP-modified autologous stimulators and IL-2. Growing cultures were transferred to macrowells, expanded by repetitive stimulations with IL-2 in conjunction with TNP-modified feeder cells, and screened for TNP-

specific proliferation. Because of excellent growth characteristics, one clone, designated E-11, was selected for more detailed study. This cloned TCL has been in continuous culture for >1 yr with maintenance of TNP-specific functional activity (Table I). E-11 cells have been cryopreserved by standard rate-controlled freezing procedures, and thawed with retention of functional, phenotypic, and growth characteristics. Currently, $>300 \times 10^6$ cryopreserved E-11 TCL cells are available in our laboratory.

*E-11 Is a TNP-specific MHC-restricted Helper TCL.* Phenotypic characterization

TABLE I
*E-11 Is a TNP-specific Cloned TCL*

| Experiment | Description of culture | | [$^3$H]thymidine incorporation |
|---|---|---|---|
| | Responder | x-irradiated stimulator | |
| | | | *cpm ± SEM* |
| 1 | E-11 | — | 326 ± 17 |
| | | A | 444 ± 82 |
| | | $A_{TNP}$ | 24,492 ± 1,826 |
| 2 | E-11 | — | 339 ± 86 |
| | | A | 896 ± 144 |
| | | $A_{TNP}$ | 9960 ± 289 |
| | | $A_{FITC}$ | 824 ± 152 |
| 3 | E-11 | — | 166 ± 38 |
| | | A | 140 ± 47 |
| | | $A_{TNP}$ | 2,824 ± 371 |
| | | — + IL-2 | 1,624 ± 188 |
| | | A + IL-2 | 3,155 ± 124 |
| | | $A_{TNP}$ + IL-2 | 8,499 ± 522 |

$1 \times 10^4$ E-11 cells were cultured in triplicate with final medium alone (—) or $1 \times 10^5$ unmodified (A), TNP-modified ($A_{TNP}$), or fluorescein isothiocyanate-modified ($A_{FITC}$) x-irradiated autologous peripheral blood mononuclear cells as stimulators. Some cultures were supplemented with IL-2 (Exp. 3). After 48 h of culture, [$^3$H]TdR incorporation was assayed as described above. These three experiments represent the proliferative response of E-11 after 1, 3, and 7 mo of culture, respectively.

TABLE II
*Phenotypic Analysis of E-11*

| Monoclonal antibody | Percent reactivity by immunofluorescence |
|---|---|
| Ascites control | 4 |
| T 3 | 92 |
| T 11A | 93 |
| T 4 | 95 |
| T 8 | 6 |
| I 1 | 70 |
| M 1 | 5 |

E-11 cells were characterized with respect to cell surface markers by indirect immunofluorescence using the panel of monoclonal antibodies indicated.

of E-11 with a panel of monoclonal antibodies (Table II) demonstrates that the line is homogeneous, comprised of activated ($Ia^+$) T cells belonging exclusively to the $T3^+$, $T4^+$, $T8^-$ subset. We recently reported that cloned HLA-DR-specific human TCL, which express this phenotypic profile, can induce a polyclonal PFC response by B cells that bear the relevant DR antigen (21). We were therefore interested to determine if the E-11 TCL also mediates antigen-specific help for B cells. To this end, E-11 cells were co-cultured with unmodified, TNP-modified, or, in some studies, fluorescein isothiocyanate (FITC)-modified autologous B cells. After 6 d, polyclonal B cell activation was determined in the reverse hemolytic PFC assay. The results of two representative experiments, depicted in Fig. 1, demonstrate clearly that E-11 provides potent antigen-specific help for TNP-modified autologous B cells. To rule out the possibility that this apparent antigen-specific B cell activation merely reflects differences in the kinetics of E-11-induced PFC responses, day 10 culture supernatants were assayed for total IgM and IgG production using class-specific ELISA systems. As shown in Table III, these studies serve to confirm that E-11 is a TNP-specific helper TCL. Finally, E-11 helper activity is radioresistant and independent of contaminating T cells (data not shown).

To evaluate whether E-11 help is MHC restricted, as well as antigen specific, E-11 cells were co-cultured with unmodified or TNP-modified $E^-$ cells obtained from a panel of allogeneic donors of known HLA-DR haplotype. The results (Table IV) demonstrate that help is observed only when E-11 is co-cultured with

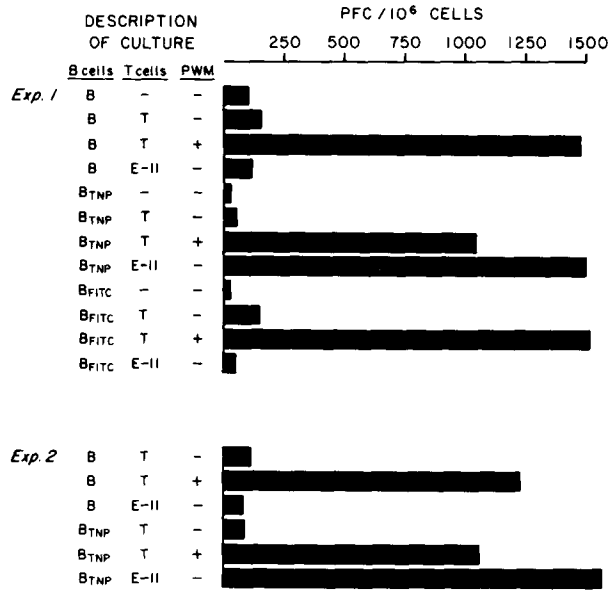


FIGURE 1. E-11 is a TNP-specific helper TCL. Unmodified (B), TNP modified ($B_{TNP}$), or FITC modified ($B_{FITC}$) $E^-$ cells were cultured alone, with 20% autologous T cells, or an equal number of E-11 TCL cells. As a positive control, some cultures were supplemented with PWM. After 6 d, aliquots of each culture were assayed in triplicate for PFC activity. Data is expressed as PFC/$10^6$ initially cultured $E^-$ cells. In both experiments, the $E^-$ responder cells are autologous to E-11.

TABLE III
*E-11 Is a TNP-specific Helper TCL*

| Description of culture | IgM | IgG |
|---|---|---|
| | *ngm/ml* | |
| B + T + media | <125 | <10 |
| B + T + PWM | 65,000 | 290 |
| B + E-11 | <125 | <10 |
| $B_{TNP}$ + T + media | <125 | <10 |
| $B_{TNP}$ + T + PWM | 131,660 | 375 |
| $B_{TNP}$ + E-11 | 94,830 | 615 |

Unmodified (B) or TNP-modified ($B_{TNP}$) $E^-$ cells, obtained from a DR $5^+$ donor, were cultured with final medium alone, 20% autologous T cells, or an equal number of E-11 cells. As a positive control, some cultures were supplemented with PWM. After 10 d of culture, supernatants were harvested and assayed for total IgM and IgG using class-specific ELISA systems.

TNP-modified allogeneic cells that express the DR5 allele. This pattern of restriction was not anticipated, since the donor of E-11 types is DR 2,8. Although the explanation for this apparently anomalous result remains to be found, two points should be emphasized. First, E-11 does not provide help for TNP-modified DR 2-positive B cells (Table III, donor G). Second, the DR 8 antigen remains poorly characterized and is known to cross-react with some DR 5-specific alloantisera. Therefore it is reasonable to suggest that E-11 is restricted by a "self" determinant, present perhaps on the DR 8 antigen, which is either coexpressed on the DR 5 allele, or expressed on a polymorphic structure distinct from but closely linked to the DR 5 allele. Although more detailed immunogenetic studies are required to clarify this point, our results strongly suggest that E-11 recognizes the nominal antigen, TNP, in the context of a class II MHC determinant.

*E-11 Help Is Restricted at the Inductive Level.* Antigen specificity and MHC restriction of E-11 helper activity may operate at the inductive level (Th-APC interaction), the effector level (Th-B cell interaction), or both. To distinguish among these possibilities, we first asked if E-11 could provide help for irrelevant (bystander) B cells and, if so, under what conditions. To this end, E-11 was cocultured with unmodified allogeneic responder B cells, either $DR5^+$ or $DR5^-$, and the cultures further supplemented with unmodified or TNP-modified x-irradiated stimulator cells. The results of two representative studies (Fig. 2) demonstrate that E-11 can trigger a polyclonal PFC response by unmodified responder B cells, regardless of their MHC haplotype. It should be emphasized, however, that this result requires the presence of x-irradiated stimulator cells that bear both TNP and the appropriate MHC-restriction antigen (DR5). These results demonstrate that E-11 helper activity is antigen specific and MHC restricted at the inductive (Th-APC) level, but appears to be at least partially unrestricted and nonspecific at the effector level.

*E-11 Help Is Partially Restricted at the Effector Level.* Inductive level restriction of E-11 helper activity is consistent with our data concerning allospecific helper TCL (21). In those studies, we noted that the level of help that allospecific Th cells can provide for appropriate allogeneic B cells is often significantly greater

TABLE IV

*E-11 Is an Antigen-specific MHC-restricted Helper TCL*

| Description of culture | PFC/$10^6$ cells | Description of culture | PFC/$10^6$ cells |
|---|---|---|---|
| Donor A (DR 5,7) | | Donor C (DR 1,3) | |
| B + T | 135 | B + T | 75 |
| B + T + PWM | 4,585 | B + T + PWM | 1,725 |
| B + E-11 | 150 | B + E-11 | 150 |
| $B_{TNP}$ + T | 192 | $B_{TNP}$ + T | 45 |
| $B_{TNP}$ + T + PWM | 3,010 | $B_{TNP}$ + T + PWM | 425 |
| $B_{TNP}$ + E-11 | 2,270 | $B_{TNP}$ + E-11 | 75 |
| Donor B (DR 1,7) | | Donor D (DR 1,3) | |
| B + T | 15 | B + T | 70 |
| B + T + PWM | 970 | B + T + PWM | 1,135 |
| B + E-11 | 84 | B + E-11 | 170 |
| $B_{TNP}$ + T | 25 | $B_{TNP}$ + T | 220 |
| $B_{TNP}$ + T + PWM | 570 | $B_{TNP}$ + T + PWM | 2,260 |
| $B_{TNP}$ + E-11 | 68 | $B_{TNP}$ + E-11 | 165 |
| Donor E (DR -,5) | | Donor G (DR 2,3) | |
| B + T | 165 | B + T | 52 |
| B + T + PWM | 1,420 | B + T + PWM | 1,028 |
| B + E-11 | 140 | B + E-11 | 68 |
| $B_{TNP}$ + T | 250 | $B_{TNP}$ + T | 24 |
| $B_{TNP}$ + T + PWM | 7,840 | $B_{TNP}$ + T + PWM | 780 |
| $B_{TNP}$ + E-11 | 6,620 | $B_{TNP}$ + E-11 | 59 |
| Donor F (DR 3,5) | | Donor H (DR 1,4) | |
| B + T | 180 | B + T | 140 |
| B + T + PWM | 1,240 | B + T + PWM | 980 |
| B + E-11 | 170 | B + E-11 | 120 |
| $B_{TNP}$ + T | 24 | $B_{TNP}$ + T | 110 |
| $B_{TNP}$ + T + PWM | NT | $B_{TNP}$ + T + PWM | 720 |
| $B_{TNP}$ + E-11 | 940 | $B_{TNP}$ + E-11 | 120 |

Unmodified (B) or TNP-modified ($B_{TNP}$) $E^-$ cells, obtained from a panel of allogeneic donors of known HLA-DR haplotype, were co-cultured with 20% autologous T cells or an equal number of E-11 cells. As a positive control, some cultures were supplemented with PWM. After 6 d, PFC activity was assayed as described for Fig. 1. NT, not tested.

than that obtained with PWM. This observation suggested that B cells which did not respond to PWM-induced T cell help may be activated by direct interaction with the allospecific Th cell. In this regard, E-11 may also trigger PFC responses by TNP-modified autologous or DR $5^+$ allogeneic B cells that are greater than those induced by PWM (Fig. 1). To determine if this result reflects preferential interaction of E-11 with TNP-modified B cells, the following studies were performed. Varying numbers of E-11 cells were cultured with DR $5^+$ allogeneic $E^-$ populations comprised of equal numbers of either unmodified responders and TNP-modified x-irradiated stimulators, or TNP-modified responders and unmodified x-irradiated stimulators. After 6 d, polyclonal PFC responses were assessed. We observed (Fig. 3) that although E-11 can recruit a PFC response by unmodified bystander cells, TNP-modified B cells are preferentially activated. Similar evidence for preferential interaction of E-11 with TNP-modified B cells is demonstrated in the experiment presented in Table V. Taken together, these

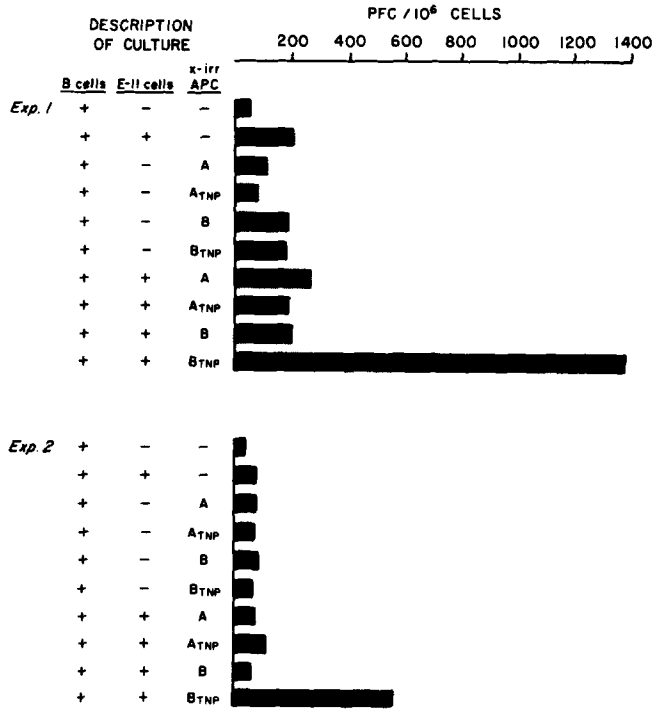


FIGURE 2. E-11 is TNP specific and MHC restricted at the inductive level. Unmodified $E^-$ responder cells, obtained from a DR $5^+$ (experiment 1) or a DR $5^-$ (experiment 2) donor, were cultured alone or with 20% E-11 TCL cells. Some cultures were supplemented with 20% x-irradiated $E^-$ cells as APC. The APC were DR $5^-$ (*A*) or DR $5^+$ (*B*) and either unmodified or TNP modified, as indicated. After 6 d, PFC activity was assayed as described for Fig. 1.

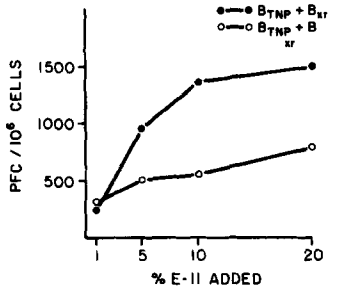


FIGURE 3. E-11 preferentially activates TNP-modified B cells. Varying numbers of E-11 TCL cells were cultured with DR $5^+$, $E^-$ allogeneic responder cells. The responder populations consisted of equal numbers of TNP-modified cells and unmodified x-irradiated $E^-$ cells ($B_{TNP}$ + $B_{xr}$), or TNP-modified x-irradiated cells and unmodified $E^-$ cells ($B_{TNPxr}$ + B). After 6 d, PFC activity was assayed as described for Fig. 1. To determine maximal PFC activity, both $E^-$ responder populations were cultured with 20% autologous T cells and PWM. These PFC responses were 1,260 ($B_{TNP}$ + $B_{xr}$) and 1,057 ($B_{TNPxr}$ + B).

results strongly suggest antigen-specific interaction at the Th-B cell level, and served as the impetus for the studies which follow.

*E-11 Helps B Cell Subsets through Distinct Pathways.* As described above, studies in the murine system have shown that partially activated (large) B cells respond

TABLE V
*E-11 Preferentially Activates TNP-modified B Cells*

| Description of culture | | | PFC/$10^6$ cells |
|---|---|---|---|
| Responder B cells | x-irradiated APC | Helper TCL | |
| B | — | — | 190 |
| | B | — | 270 |
| | $B_{TNP}$ | — | 170 |
| | B | E-11 | 160 |
| | $B_{TNP}$ | E-11 | 515 |
| $B_{TNP}$ | — | — | 215 |
| | B | — | 295 |
| | $B_{TNP}$ | — | 200 |
| | B | E-11 | 1,900 |
| | $B_{TNP}$ | E-11 | 3,520 |

Unmodified (B) or TNP-modified ($B_{TNP}$) $E^-$ cells, obtained from a DR $5^+$ donor, were cultured alone or in the presence of 20% unmodified or TNP-modified x-irradiated autologous $E^-$ cells as APC. Some cultures were further supplemented with 20% E-11 TCL cells. After 6 d, PFC activity was assayed as described for Fig. 1.

directly to nonspecific helper signals (factors) released by antigen-activated Th cells, while resting (small) B cells require direct interaction with Th cells before they can be triggered to produce antibody. We were therefore interested to determine if the differences that we observe between "recruited" and "direct" help by E-11 reflect distinct pathways of activation by which the Th cell interacts with different human B cell subpopulations. To this end, B cell-enriched populations were fractionated by discontinuous density gradient centrifugation and the subsets obtained assayed for their response to E-11 helper activity. The results of two separate experiments are presented in Table VI. As shown in experiment 1, antigen-activated E-11 cells readily trigger a PFC response by both unmodified and TNP-modified large B cells. In marked contrast, TNP-modified, but not unmodified, small B cells are selectively driven to antibody production by E-11. In experiment 2, E-11 TCL cells were "preactivated" at the time of assay, presumably by interaction with TNP-modified APC during IL-2-dependent expansion. Interestingly, although both unmodified and TNP-modified large B cells are polyclonally triggered by co-culture with these activated Th cells, only TNP-modified small B cells are responsive. It should be noted that the interaction of E-11 with TNP-modified small B cells is required, but not sufficient, to trigger a PFC response; TNP-modified APC are also necessary. Moreover, the requirement for TNP-modified APC is also observed in the activation of large B cells (Table VI, experiment 1) and cannot be overcome by merely increasing the percentage of E-11 cells in culture (Fig. 4). Taken together, these results demonstrate that large human peripheral blood B cells are readily induced to antibody secretion by E-11 in an unrestricted and nonspecific manner, provided that E-11 has been activated by TNP on the appropriate APC. In contrast, small B cells require an additional signal, which appears to be delivered by direct antigen-specific interaction of E-11 and TNP-modified B cells.

TABLE VI
*Antigen-specific Th-Small B Cell Interaction*

| Exp. | Description of culture | | | PFC/$10^6$ cells |
|---|---|---|---|---|
| | Responder B cells | x-irradiated APC | Helper TCL | |
| 1 | lg B | $E^-$ | — | 330 |
| | | $E^-_{TNP}$ | — | 315 |
| | | $E^-$ | E-11 | 320 |
| | | $E^-_{TNP}$ | E-11 | 1,837 |
| | lg $B_{TNP}$ | $E^-$ | — | 40 |
| | | $E^-_{TNP}$ | — | 50 |
| | | $E^-$ | E-11 | 50 |
| | | $E^-_{TNP}$ | E-11 | 1,075 |
| | sm B | $E^-$ | E-11 | 155 |
| | | $E^-_{TNP}$ | E-11 | 330 |
| | sm $B_{TNP}$ | $E^-_{TNP}$ | — | 100 |
| | | $E^-$ | E-11 | 260 |
| | | $E^-_{TNP}$ | E-11 | 1,425 |
| 2 | lg B | $E^-$ | — | 660 |
| | | $E^-_{TNP}$ | — | 475 |
| | | — | E-11 | 4,415 |
| | lg $B_{TNP}$ | $E^-$ | — | 450 |
| | | $E^-_{TNP}$ | — | 305 |
| | | — | E-11 | 4,550 |
| | sm B | $E^-$ | — | 55 |
| | | $E^-_{TNP}$ | — | 120 |
| | | — | E-11 | 65 |
| | sm $B_{TNP}$ | $E^-$ | — | 50 |
| | | $E^-_{TNP}$ | — | 65 |
| | | — | E-11 | 750 |

Responder populations, in both experiments, consisted of unmodified or TNP-modified large (lg B) and small (sm B) DR $5^+$ B cells, isolated by discontinuous density gradient centrifugation. As indicated, some cultures were supplemented with 30% unmodified ($E^-$) or TNP-modified ($E^-_{TNP}$) x-irradiated autologous $E^-$ APC. 30% E-11 TCL cells were added as indicated. After 6 d, PFC activity was assayed as described for Fig. 1.

## Discussion

In this report we have investigated human Th-B cell interactions through the isolation and functional characterization of a cloned TNP-altered, self-reactive helper TCL, termed E-11. Our results show that co-culture of E-11 cells with TNP-modified, but not unmodified or FITC-modified, autologous B cells results in a vigorous polyclonal PFC response. Moreover, in panel studies involving allogeneic responder B cells, E-11 selectively triggers a PFC response by TNP-modified allogeneic B cells that express the DR 5 allele, suggesting that E-11 recognizes the nominal antigen TNP in the context of a class II MHC determi-

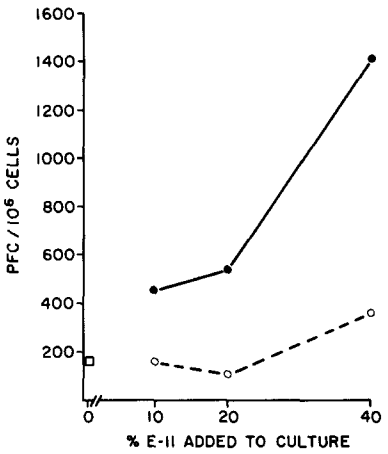


FIGURE 4. E-11 help requires TNP-modified APC. Large TNP-modified DR $5^+$ B cells isolated by discontinuous density gradient centrifugation were cultured with 20% unmodified (○) or TNP modified (●) autologous x-irradiated $E^-$ cells. Cultures were supplemented with varying percentages of E-11 TCL cells. After 6 d, PFC activity was assayed as described for Fig. 1. In the absence of E-11 cells, both populations gave essentially identical PFC responses (□).

nant. Analysis of the mechanisms by which E-11 mediates antigen-specific, MHC-restricted help demonstrated that the interaction of E-11 with TNP-modified DR $5^+$ APC is a necessary and sufficient trigger for the induction of a polyclonal PFC response by bystander B cells (e.g., unmodified and DR $5^-$ B cells). This observation suggests that E-11 is not a constitutive helper cell, but requires antigen-specific, MHC-restricted activation. Further, it shows that E-11 help for B cells is at least partially nonspecific and unrestricted at the effector (Th-B cell) level.

Although the precise nature of the APC that activates E-11 help remains to be defined, it appears that the relevant cell is a macrophage. This conclusion is suggested by the observation that although E rosette-negative cells can function as APC, removal of macrophages by plastic adherence and density gradient centrifugation yields a highly purified B cell population that can respond to but not induce E-11 help. It should be emphasized that in this experimental system, hapten is covalently coupled to the cell surface; therefore, both TNP-modified macrophage and B cell populations express the target antigen recognized by E-11. The most likely explanation for the crucial role of macrophages in activating E-11 is that interaction of E-11 with hapten (TNP)- and MHC-restricting elements on the macrophage surface triggers the macrophage to release IL-1, which, in turn, signals antigen-activated E-11 cells to secrete nonspecific helper factor(s) capable of recruiting bystander B cells. In this regard, we have previously reported that after antigen-specific activation, TNP-altered, self-reactive human helper TCL cells elaborate nonspecific helper factor(s) that induce a polyclonal PFC response (13).

Perhaps the most interesting point to emerge from these studies is the demonstration that a cloned, antigen-specific Th cell activates human B cell subpopulations via two distinct mechanisms. Thus, large B cells, isolated by Percoll

density centrifugation, respond directly to the helper signals provided by the interaction of E-11 with TNP-modified DR $5^+$ APC. This response is nonspecific, nonrestricted, and does not involve direct Th-B cell contact. In contrast, polyclonal activation of small B cells by E-11 requires that the B cells bear TNP on their surface and that TNP-modified APC be also present. These data suggest that direct interaction between Th cells and antigen on the B cell surface provides an important activation signal for the small B cell which allows responsiveness to helper signals generated by Th-APC interaction. This scheme of Th-B cell collaboration is depicted in Fig. 5. We would emphasize that these findings are consistent with published data concerning human B cell subset activation. For example, polyclonal activation of large B cells by helper signals (factors) generated during the Th-APC interaction is in accord with the observations that large B cells are responsive to T cell-derived factor(s) (e.g., BCGF) (11), and the T cell-dependent lectin PWM (8, 9), which is known to trigger helper factor production by T cells (22). In contrast, small resting B cells are refractory to T cell helper factor(s) unless they receive an additional activation signal. This signal may be provided by: (*a*) cross-linking of the B cell receptors for antigen by anti-IgM (11); (*b*) LPS (9), a T-independent lectin that is known to substitute for the Th-B cell interaction signal in the activation of murine B cells (2); and (*c*) as shown in this report, direct Th cell interaction with antigen on the B cell surface. It has been suggested that, together, Th-APC and Th-B cell interactions are sufficient to

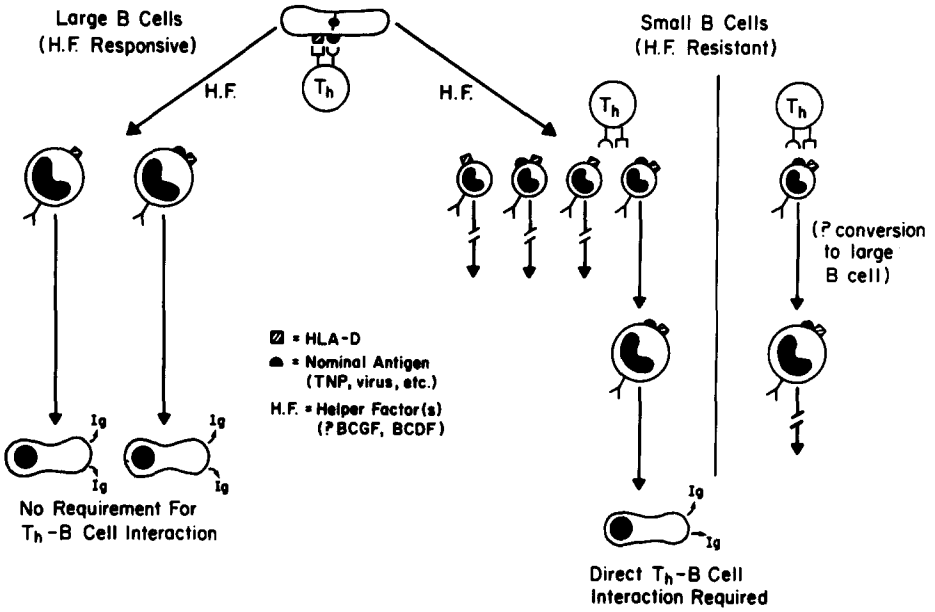


FIGURE 5. Pathways of Th-B cell subset interaction. Large human peripheral B cells are driven to Ig secretion by the helper signals (factors) produced during the interaction of Th cells with antigen- and MHC-restricting elements on the surface of antigen presenting macrophages ($\phi$). Small B cells are resistant to these helper signals unless an additional trigger is provided: the direct cell-cell interaction of Th cells with antigen on the B cell surface. Although Th-small B cell interaction alone is not sufficient to trigger Ig production, it is not known if this signal can drive the small B cell to a more activated stage of differentiation (e.g., a large B cell).

trigger the maturation of resting murine B cells to immunoglobulin-secreting cells, while antigen functions to selectively expand antigen-specific B cell clones (2). By extension, one might predict that the combined stimulus of anti-IgM antibody and antigen-specific Th cells would synergize in the induction of a polyclonal immunoglobulin response by antigen-bearing resting human B cells. Moreover, it is possible that the addition of a particular antigen to the Th-B cell system we have described may induce a specific antibody response against any antigen for which specific B cells are present.

The biologic significance of human Th cells that recognize, interact with, and polyclonally activate modified autologous B cells remains to be elucidated. It is intriguing, however, that similar interactions, by adoptively transferred allospecific murine Th cells, are postulated to be responsible for the polyclonal immunoglobulin response that characterizes chronic graft-vs.-host disease (GVHD) (23). Of particular interest is the observation that in certain strain combinations the abnormal T-B collaboration induced during chronic GVHD leads to the appearance of autoantibodies and immune complex disease characteristic of systemic lupus erythematosus (SLE) (24, 25). Moreover, autoimmune phenomenon characteristic of the "collagen vascular" diseases are known sequelae of chronic GVHD in man (26). Our results provide an experimental basis for explaining how abnormal T-B cell collaboration could operate in the pathogenesis of "spontaneously arising" human SLE. For example, one may envision that in some individuals the presence of exogenous antigen (e.g., viral determinants) on cell surfaces may result in a predominantly helper, rather than cytotoxic, T cell response. Collaborative interaction between these Th cells and virally modified small B cells would lead to polyclonal activation. Moreover, the continuous presence of self-antigens may serve to specifically expand autoreactive B cell clones, yielding a preponderance of autoantibody in the polyclonal response. To evaluate this hypothesis, we are currently investigating the capacity of E-11 to induce, in vitro, anti–double-stranded DNA antibody production by normal and SLE patients' B cells.

## Summary

A cloned, trinitrophenyl (TNP)-specific helper T cell line (TCL), termed E-11, has been established in long-term, interleukin 2-dependent culture and used to study human T helper (Th)-B cell collaboration. Co-culture of E-11 with TNP-modified, but not unmodified or FITC-modified, autologous B cells results in a vigorous, polyclonally plaque-forming cell (PFC) response. E-11 helper activity is not constitutive, but requires antigen-specific, major histocompatibility complex-restricted activation of the TCL cells by interaction with TNP-modified autologous or DR $5^+$ allogeneic macrophages. Using B cell subsets isolated by discontinuous density gradient cengrifugation as responder populations, we determined that E-11 activates B cell subsets via two distinct mechanisms: (*a*) E-11 polyclonally activates large B cells in an unrestricted and nonspecific manner; and (*b*) E-11 preferentially induces a PFC response by TNP-modified small B cells. These results suggest that the large B cell subset is activated by helper signals generated during the Th-antigen-presenting cell interaction, while small B cells require an additional stimulus that is provided by antigen-specific Th-B

cell contact.

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