A variety of helper factors necessary for in vitro antibody response by B cells have been identified in the supernates of antigen-stimulated T cells of experimental animals (1–6). These factors fall into two broad categories: nonspecific helper factors (1–4), and antigen specific helper factors (5, 6). Nonspecific factors of T-cell origin behave like polyclonal B-cell activators and stimulate syngeneic as well as allogeneic B cells to synthesize antibodies against a variety of antigens (1–3). On the other hand antigen-specific factors induce only autologous B cells (or B cells that share a haplotype or Ia determinants with autologous B cells) to synthesize antibodies with specificity restricted to the antigen used to elicit the T-cell helper activity. It has also been shown that antigen-specific factors express Ia determinants and contain or can bind to antigen or antigen fragments (5, 6).

Supernates of human T cells stimulated with tetanus toxoid antigen (TT) have been shown to contain a helper factor(s) termed lymphocyte mitogenic factor (LMF) which can induce blastogenesis and immunoglobulin synthesis in as many as 50–60% of autologous as well as allogeneic B cells (7). A fraction (10–20%) of the IgG secreted was shown to have specificity against TT. The specificity of human T-cell derived LMF is examined in this paper.

Materials and Methods

Antigens and Antibodies. TT and diphteria toxoid (DT) were a gift from Dr. Leo Levine of the Massachusetts Biological Laboratories. These antigens were purified by Sephadex G 200 chromatography (8). (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) In both instances more than 90% of the purified material was precipitable by the corresponding specific rabbit antiserum. Streptokinase-streptodornase antigen was obtained from The Lilly Research Laboratories Eli Lilly and Co., Indianapolis, Ind.; Monilia antigen was obtained from Hollister-Stier Laboratories Inc., Yeadon, Pa.; and keyhole limpet hemocyanin (KLH) was purchased from Sigma Chemical Co., St. Louis, Mo. Antigens were dialyzed against tissue culture medium before use. Iodination of TT, DT, and KLH was carried with carrier-free iodine 125I by the chloramine-T method (9). Anti-TT and anti-DT antisera were raised in rabbits immunized with these antigens in complete Freund's adjuvant. Antibody titers were determined by hemagglutination of sheep erythrocytes coated...
with antigen by using chromic chloride (10). They were 1/16,000 and 1/8,000 for the anti-TT and the anti-DT antisera, respectively. Rabbit anti-human IgG and anti-human IgM antisera specific to heavy chains were obtained from Behring Diagnostics, American Hoechst Corp., Somerville, N. J.

**Immunoadsorbents.** TT and DT antigens were cross-linked to Sepharose 4B and to Sepharose 6MB beads (Pharmacia Fine Chemicals, Inc.) by the cyanogen bromide activation method of Axen and Ernback (11). The IgG fraction from rabbit anti-human IgG, from rabbit anti-human IgM, and from rabbit anti-TT antisera was purified by diethylaminoethylcellulose (DEAE 32, Whatmann Chemicals, Div. W & R Balston, Maidstone, Kent, England) chromatography by using a 0.01-M, pH 6.8 phosphate buffer as eluting buffer. The purified IgG was then dialyzed against 0.1 M NaHCO₃ and cross-linked to Sepharose beads. The efficiency of protein coupling to Sepharose beads exceeded 80% in all instances.

**Preparation of Lymphocytes.** Mononuclear cells were isolated from heparinized blood on Ficoll-Hyapque gradients or by the means of an IBM cell separator (Haemonetics, model 10, Haemometrics Corp., Natick, Mass.). 90% of the cells were mononuclear and viability exceeded 90% as determined by trypan blue exclusion. Mononuclear cells were washed three times before use with medium 199 (Microbiological Associates, Rockville, Md.). Tonsil lymphocytes were obtained by mincing the tonsils with forceps and scissors, sedimenting the debris, and passing the cell-rich suspension over a column of glass beads prewarmed to 37°C (12).

**Preparation of B Lymphocytes.** Lymphocyte suspensions rich in B cells were obtained by fractionating tonsil or blood lymphocytes on gradients of 17-35% bovine serum albumin (BSA), as described previously (7), and taking the cells that sediment in fraction 7 of the gradient (interface of 29-31% BSA). About 80-90% of the cells in this fraction carry membrane-bound immunoglobulins and form rosettes with EAC₁₄₂₃.

**Preparation of B Cells Enriched in Specific Antigen Binding Cells.** 5 ml Sepharose 6MB beads cross-linked to antigen (DT or TT) were packed into a column in a 10-ml syringe over a support of glass wool. The column was placed at 4°C and washed extensively with medium 199 containing 1% human serum albumin (M199-HSA). 5 ml of a B-cell suspension at a concentration of 4 × 10⁶ cells/ml in M199-HSA were applied to the column and the cell suspension was allowed to stay in contact with the beads for 2 h, after which the column was washed with a large volume (100 ml) of cold M199-HSA. 4-ml fractions were collected from the column eluate. The cells that eluted in the first five tubes were pooled and washed. The column was then washed with 50 ml of medium 199 containing antigen (DT or TT) at a concentration of 200 μg per ml and the cells eluted (1-2% of the starting cell number) with the antigen containing medium were collected and washed. Total cell recovery from the column averaged 70-80% and viability exceeded 90% as determined by trypan blue exclusion.

**Tissue Culture.** Lymphocytes were cultured in medium 199 or medium RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 15% heat inactivated human serum obtained from donors with type AB, Rh⁺ erythrocytes. Cultures were supplemented with penicillin G (50 U/ml), streptomycin (50 μg/ml), kanamycin (50 μg/ml), and Mycostatin (50 μg/ml). Cultures were placed in a humidified incubator at 37°C in an atmosphere of 5% CO₂ in air.

**Production of T-Cell Supernates.** The basic design for production of T-cell supernates is described in reference 6. Peripheral blood lymphocytes were cultured at a concentration of 1 × 10⁷ cells/ml for 3 days. One set of cultures received antigen (TT 5-10 μg/ml; or DT 5-10 μg/ml) at the onset of the incubation period and were designated as preincubated (P) cultures. A duplicate set of cultures received an equivalent amount of antigen at the end of the incubation period and were designated as reconstituted (R) cultures. A duplicate set of cultures received an equivalent amount of antigen at the end of the incubation period and were designated as reconstituted (R) cultures. The incubation was terminated by pelleting the cells (200 g for 10 min). Supernates were collected, filtered through a 0.22-μm filter (Millipore Corp., Bedford, Mass.), and stored at -20°C until tested.

**Fractionation of T-Cell Supernates on Sephadex G 200.** 50 ml of T-cell supernates were concentrated fivefold by ultrafiltration by using an Amicon XM50 membrane (Amicon Corp., Lexington, Mass.) then applied to a 1.5 × 100-cm G 200 column equilibrated in 0.15 M NaCl. The material eluting after albumin but before free salts (fraction IV of Sephadex G 200 eluate) was collected, dialyzed against water, and concentrated by embedding the dialysis tubing in dry Sephadex G 200. The concentrated material was diluted 1:2 in medium RPMI-1640 (Grand Island Biological Co.) containing 5% human serum albumin and designated LMF-TT (fraction IV).

**Depletion of T-Cell Supernates from Antigen.** Crude T-cell supernates as well as Sephadex G 200 fractionated supernates were depleted of TT antigen by passage over anti-TT-immunosorbent
columns. 5 ml of Sepharose 4B beads cross-linked to anti-TT were packed into a column in a 10-ml syringe plugged with glass wool. The column was washed extensively with culture medium, then 5 ml of supernates were applied to the column. After an incubation period of 2 h at 4°C the supernates were eluted from the column and millipore filtered. Parallel experiments were carried where 125I-labeled TT was added to the supernates to ascertain the extent of antigen depletion. Less than 0.1% of the radioactivity was eluted from the column. In some experiments Sephadex G 200 fractionated supernates were passed sequentially through TT and anti-TT-immunosorbent columns.

**Assay of T-Cell Supernates on B Lymphocytes**

**Mitogenesis.** B lymphocytes were cultured in 0.2-ml cultures in the wells of microtiter plates (Falcon Plastics, Oxnard, Calif.) at a concentration of 1 x 10⁶ cells/ml and in various dilutions of T-cell supernates. After an incubation period of 6 days the degree of [3H]thymidine incorporated into DNA was determined by using a tissue culture automatic harvester (Skatron, Flow Laboratories, Inc., Rockville, Md.). Results were expressed as counts per minute of [3H]thymidine incorporated per culture and as mitogenic index (MI) where

\[ MI = \frac{cpm \text{ of } [3H] \text{thymidine incorporated in P-stimulated culture}}{cpm \text{ of } [3H] \text{thymidine incorporated in R-stimulated culture}} \]

**Immunoglobulin Synthesis.** B lymphocytes were cultured at 2 x 10⁶ cells/ml in 16 x 125-mm sterile Falcon plastic culture tubes (Falcon Plastics) in the presence of T-cell supernates. After a 5-day incubation period, cell pellets were thoroughly washed in Hank's medium and then resuspended at 4 x 10⁶/ml in fresh RPMI-1640 medium (Grand Island Biological Co.) deficient in l-valine, l-leucine, and l-isoleucine, and supplemented with C¹⁴-radioactive amino acids (2 μCi/ml) and with dialyzed fetal calf serum (Microbiological Associates) 48 h later the supernates of these cultures were harvested, filtered through a 0.22-μm Millipore filter, (Millipore Corp.) and frozen at -20°C until tested.

**Determination of De Novo Synthesis of Immunoglobulins.** Newly made IgG, present in supernates of B-cell cultures, was determined by a sandwich radioimmunoassay by using 20 μg human IgG and rabbit anti-human IgG at equivalence. The precipitate was washed three times in phosphate-buffered saline and redissolved twice in 0.1 N acetic acid and reprecipitated each time with an equal volume of 0.1 N sodium hydroxide. This procedure minimizes the amount of radiolabeled material trapped nonspecifically in the immune precipitate. Precipitates were finally dissolved in 0.1 N NaOH and their radioactivity was counted in a liquid scintillation counter (Tri Carb, Packard Instrument Co., Inc., Downers Grove, Ill.) by using Aquasol (New England Nuclear, Boston, Mass.) for scintillation fluid. Controls consisted of supernates of fibroblast cultures grown in medium containing radioactive amino acids.

**Determination of De Novo Synthesis of Specific IgG Antibodies.** Antibodies of the IgG class, specific for TT and DT, were determined by two different methods. The first method consisted of a direct coprecipitation: to 0.1 ml of supernate an equivalent amount of antigen (200 μg of TT or DT) and of antigen-specific rabbit antibody (rabbit anti-TT or rabbit anti-DT) were added. The amount of radioactivity present in the precipitate was determined. Specifically precipitated radioactivity was obtained by subtracting the radioactivity precipitated in an unrelated antigen-antibody system (ovalbumin-antiovalbumin).

The second method consisted of adding to 0.1 ml of supernates, 0.1 ml of 125I radiolabeled antigen (30,000 cpm), carrier human IgG, and an equivalent amount of rabbit anti-human IgG preabsorbed with antigen cross-linked to Sepharose 4B.

**Electron Microscopy.** Cell pellets of lymphocytes were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 0.1 M sucrose, and 4.5 x 10⁻³ M CaCl₂, for 1 h at 4°C. They were then rinsed three times in 0.1 M cacodylate buffer, pH 7.4 containing 0.1 M sucrose and postfixed in 2% aqueous OsO₄, for 1 h at 4°C. The cells were stained en bloc with 1.5% uranyl acetate in 0.05 M maleate buffer, pH 6.2, and dehydrated in graded ethanol and embedded in Epon. Thin sections were cut with a diamond knife (Dupont Instruments, Wilmington, Del.) on an LKB Ultratome III (LKB Instruments, Inc., Rockville, Md.) and picked up on carbon coated grids. They were stained with lead citrate and examined in a Philips 300 electronmicroscope (Philips Electronic Instruments, Inc., Mount Vernon, N. Y.). Between 50 and 200 cells were counted from each cell pellet which had been incubated with varying concentrations of LMF. The size of the lymphocytes was
Table I
Response of B Cells from TT-Immune and TT-Nonimmune Donors to LMF-TT

| Source of B cells | MI | Total IgG synthesis* | IgG anti-TT synthesis* |
|------------------|----|---------------------|------------------------|
| TT-immune donors |    |                     |                        |
| 1                | 62.3 | +8,548              | +1,171                 |
| 2                | 31.5 | +7,206              | +1,015                 |
| 3                | 40.7 | +5,374              | +884                   |
| TT-nonimmune donors |    |                     |                        |
| 1                | 39.5 | +4,316              | +54                    |
| 2                | 23.3 | +5,660              | −115                   |
| 3                | 55.8 | +6,323              | +70                    |

* IgG synthesis was determined on 0.1-ml portions from supernates of B-cell cultures containing 4 x 10⁶ cells/ml and therefore correspond to the IgG secreted by 4 x 10⁵ B cells.

Results

Effect of T-Cell Supernates on B Cells From TT-Immune and TT-Nonimmune Donors. Lack of immunity to TT was ascertained in three individuals by negative history for TT immunization, absence of lymphocytes transformation in response to TT, and absence of anti-TT antibodies in the serum as determined by hemagglutination (10). When B-cell cultures obtained from TT immune and TT nonimmune donors were incubated in an equal volume of fresh culture medium and of T-cell supernates, containing LMF-TT, an equal degree of blastogenesis and of IgG synthesis was observed. However, only B cells from TT-immune donors made IgG with specificity to TT (Table 1).

Effect of Antigen Depletion on the Activity of T-Cell Supernates. When LMF-TT was depleted of TT by passage through an anti-TT immunosorbent column the same degree of mitogenesis and IgG synthesis was observed in B-cell cultures as with LMF-TT not depleted of TT. No TT-specific antibodies were detected in the supernates of B-cells cultures when the antigen was absent. Anti-TT antibodies were only detected if TT were readded to the B-cell cultures (Table II).

Similar findings were obtained when LMF-TT was fractionated on Sephadex G 200. The activity of the material eluted after albumin, LMF-TT (fraction IV), remained undiminished after sequential passage over TT and anti-TT immunosorbent columns (Table II).

Effect of anti-μ and anti-γ Chain Immunosorbents on LMF Activity. LMF-TT (fraction IV) was passed over anti-μ and anti-γ chain immunosorbent...
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TABLE II
Effect of Antigen Depletion on the Activity of LMF-TT on B-Cell Cultures from Immune Donors

| Source of supernate | Passage over immunosorbent | Antigen present in B-cell cultures | MI | IgG synthesis* | IgG anti-TT synthesis |
|---------------------|----------------------------|----------------------------------|----|---------------|----------------------|
| LMF-TT              |                            | TT                               | 78.7 | + 7,150       | + 1,105              |
| LMF-TT              | anti-TT                    | --                               | 66.5 | + 6,732       | + 47                 |
| LMF-TT              | anti-TT                    | TT                               | 65.3 | + 6,880       | + 810                |
| LMF/T-T fraction IV |                            | --                               | 38.3 | + 4,067       | + 110                |
| LMF/T-T fraction IV | anti-TT                    | --                               | 32.8 | + 3,712       | - 30                 |
| LMF-T-T fraction IV | anti-TT                    | TT                               | 30.5 | + 4,312       | + 677                |

* IgG synthesis was determined on 0.1-ml portions from supernates of B-cell cultures containing $4 \times 10^5$ cells/ml and therefore correspond to the IgG secreted by $4 \times 10^5$ B cells.

TABLE III
Effect of Anti-μ and Anti-γ Immunosorbent Columns on the Activity of LMF-TT on B-Cell Cultures

| Supernatant source | Passage over immunosorbent | MI | IgG synthesis* | Δ(P-R) 14C cpm |
|--------------------|----------------------------|----|---------------|---------------|
| LMF-TT fraction IV |                            | 40.3 | + 3,990       |
| LMF-TT fraction IV | anti-μ chain               | 33.6 | + 3,572       |
| LMF-TT fraction IV | anti-γ chain               | 37.5 | + 3,445       |
| LMF-TT fraction IV | anti-HSA                   | 34.8 | + 3,724       |

* IgG synthesis was determined on 0.1-ml portions from supernates of B-cell cultures containing $4 \times 10^5$ cells/ml and therefore correspond to the IgG secreted by $4 \times 10^5$ B cells.

columns. These passages did not affect the mitogenic activity of LMF-TT nor its capacity to induce IgG synthesis in B-cell cultures (Table III).

Antibody Synthesis Against an Unrelated Antigen Added to the B-Cell Culture. B cells incubated with LMF-TT in the presence of a second antigen DT, made IgG antibodies to DT provided the B-cell donor was immune to that antigen (Table IV). When KLH was used as a second antigen, antibodies to KLH were not detected. In all cases the B-cell donors were immune to DT but not to KLH. Similar findings were true for both unfractionated and fractionated T-cell supernates.

Dose-Response Curve of B Cells to Various Dilutions of T-Cell Supernates. Fig. 1 shows the dose-response curve of B cells to various dilutions of LMF-TT T-cell supernates in fresh culture medium. Optimal mitogenesis of B cells was induced by a 50% concentration of LMF-TT. Maximal IgG synthesis occurred in cultures incubated with a 20% concentration of LMF-TT. These results correlated with electron microscopic examination of cell pellets. The percent of large blast cells was highest (35%) in pellets of cultures made with 50% LMF (Table V). These pellets contained many mitotic figures but very few
TABLE IV

Effect of the Addition of a Second Antigen on Specific Antibody Synthesis by B-Cell Cultures Stimulated with LMF-TT

| Source of T-cell supernate | Antigen present in B-cell cultures* | IgG synthesis by B-cell culture† |
|---------------------------|-----------------------------------|---------------------------------|
|                           |                                   | Total IgG $\Delta$(P-R) cpm $^{14}$C | anti-TT $\Delta$(P-R) cpm $^{14}$C |
| LMF-TT                    | TT                                | 8,077                           | 3,073 | 128 | 212 |
| LMF-TT                    | TT, DT                            | 8,345                           | 2,688 | 1,077 | 259 |
| LMF-TT                    | TT, KLH                           | 8,212                           | 2,839 | 307 | 184 |
| LMF-DT                    | DT                                | 6,033                           | 261   | 1,854 | 3 |
| LMF-DT                    | DT, TT                            | 6,533                           | 1,926 | 1,665 | 6 |

* B cells were derived from donors immune to TT and DT but not to KLH.
† IgG synthesis was determined on 0.1-ml portions from supernates of B-cell cultures containing $4 \times 10^6$ cells/ml and therefore correspond to the IgG secreted by $4 \times 10^6$ B cells.

Fig. 1. DNA synthesis and IgG synthesis induced in B cells by various concentrations of LMF-TT supernates in fresh culture medium. IgG and anti-TT were determined on 0.1-ml aliquots from the supernates of $4 \times 10^6$ B cells/ml stimulated with paired P and R supernates and the results are expressed as $\Delta$(P-R) $^{14}$C cpnm. MI, mitogenic index as defined in the text.

plasma cells (<1%). Pellets from cultures incubated with 10% LMF-TT contained a high proportion (11%) of plasma cells and fewer large blast cells (21%). High concentrations (>50%) of T-cell supernates became inhibitory to B-cell responses. At low concentrations of LMF-TT (10%) the proportion of antigen-specific antibodies (anti-TT) over total IgG increased (Fig. 2). The absolute amount of anti-TT antibodies remained rather constant until extremely low dilution of T-cell supernates were used (1%).

At low concentration of LMF-TT (10%) addition of one or more antigen to the B-cell cultures caused an increase in the amount of DNA synthesis provided the B-cell donor was immune to those antigens (Table VI).

Sensitivity of B-Cell Population Specifically Enriched for Antigen-Binding Cells. B-cell populations enriched for TT binding cells exhibited a vigorous
TABLE V
Morphology of Lymphoid Cells in B-Cell Cultures Incubated with Different Concentrations of T-Cell Supernates as Determined by Electron Microscopy

| Concentration of LMF-TT (%) | Large lymphocytes | Plasma cells |
|-----------------------------|-------------------|--------------|
| 50                          | 35                | <0.4         |
| 10                          | 21                | 11.0         |
| 5                           | 23                | 3.1          |
| 1                           | 13                | 2.8          |
| 0                           | 4                 | <0.5         |

Fig. 2. Fraction of newly made IgG that has specificity against TT as a function of the concentration of LMF-TT used to stimulate B-cell cultures.

mitogenic and IgG synthetic response to low concentrations of LMF-TT which are normally nonmitogenic for unfractionated B cells (Table VII). This vigorous response was not due to nonspecific activation of the TT antigen binding cells as these cells failed to respond to equally low concentration of LMF-DT. At all concentrations of LMF-TT more anti-TT antibodies were secreted by TT binding B cells than by unfractionated B cells. B cells that eluted from the TT-immunosorbent column failed to secrete anti-TT antibodies but secreted normal amounts of anti-DT antibodies when incubated with LMF-DT.

Discussion
The experiments described in this paper demonstrate that supernates of TT-activated human T lymphocytes contain a helper factor for B cells that is not antigen specific. This factor, termed LMF, induces DNA and immunoglobulin synthesis in autologous as well as allogeneic B cells and in B cells of TT immune as well as of TT nonimmune donors. LMF is active in the absence of antigen (TT) and does not contain immunoglobulin determinants.

The nonspecific nature of LMF was suspected very early in our studies (7) because it induced DNA synthesis in a large proportion of B cells (50–60%) and was equally active on autologous as well as on allogeneic B cells, while all
TABLE VI

| Source and concentration of supernate | Antigens present in B-cell cultures* | Mitogenic response of B cells to | MI |
|--------------------------------------|-------------------------------------|---------------------------------|----|
|                                      |                                     | P supernate | R supernate |    |
| LMF-TT; 1:2                         | TT, DT                              | 63,130      | 872         | 72.4 |
|                                      | TT, Monilia                         | 56,340      | 795         | 70.9 |
|                                      | TT, SKSD                            | 64,470      | 858         | 75.1 |
|                                      | TT, DT, SKSD, Monilia               | 58,550      | 922         | 63.5 |
|                                      | TT, KLH                             | 55,610      | 816         | 67.6 |
|                                      | TT, 4                                | 54,290      | 781         | 69.5 |
|                                      | TT, DT                              | 4,895       | 762         | 6.42 |
|                                      | TT, Monilia                         | 7,312       | 745         | 9.81 |
|                                      | TT, SKSD                            | 7,833       | 703         | 11.14 |
|                                      | TT, DT, SKSD, Monilia               | 7,105       | 691         | 10.28 |
|                                      | TT, KLH                             | 8,246       | 728         | 11.3 |
|                                      | TT, 4                                | 4,756       | 732         | 6.49 |

* B-cell donors were immune to TT, DT, Monilia, and SKSD but not to KLH.

antigen-specific helper factors described to date are restricted in their activity to syngeneic B cells or to B cells that share Ia determinants with the T cells that generated the factor (5, 6, 13). Initial studies of the IgG secreted by B cells activated with LMF-TT demonstrated that only a small fraction of that IgG (10–20%) had specificity against TT. The rest of the IgG could have been the result of polyclonal B-cell activation or could have consisted of low affinity anti-TT antibody not detectable by our methods. The availability of three donors who had never been immunized with TT provided the opportunity to resolve this issue. The sera of these donors lacked hemagglutinating activity against sheep erythrocytes coated with TT and their lymphocytes failed to transform in vitro when incubated with TT antigen. LMF-TT caused the B cells of these TT nonimmune donors to synthetize DNA and to secrete IgG in normal amounts as compared to B cells from TT-immune donors. However, anti-TT activity was not detected in the culture supernates of B cells from TT-nonimmune donors.

Nonantigen-specific helper factors behave as polyclonal B-cell activators. They trigger B cells in the presence as well as in the absence of antigen (3, 14, 15). LMF-TT containing supernates were depleted of TT by passage through anti-TT immunosorbent columns. TT-depleted supernates caused the same degree of DNA and IgG synthesis in B cells as antigen containing supernates. However, B cells activated with TT-depleted supernates made no detectable anti-TT antibodies (Table II). Addition of TT antigen to these supernates after their passage through anti-TT immunosorbent columns restored their ability to induce anti-TT synthesis by B cells of immune donors.

Similar results were obtained by using LMF-TT purified on Sephadex G 200. LMF activity elutes on Sephadex G 200 after albumin (LMF-TT fraction IV) free of both TT and of TT complexed to the anti-TT which is present in the normal AB+ serum used in the culture medium. LMF-TT (fraction IV) induced DNA and IgG synthesis in B cells but no anti-TT synthesis. Synthesis of anti-TT
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TABLE VII
Response of B-Cell Cultures Enriched for Antigen-Binding Cells

| B Cells          | Source of supernate | Concentration of supernate | MI | Response of B-cell cultures IgG synthesis* |
|------------------|---------------------|----------------------------|----|-------------------------------------------|
|                  |                     |                            |    | Total IgG ant-TT anti-DT                  |
|                  |                     |                            |    | Δ(P-R) 14C cpm  | Δ(P-R) 125I cpm |
| Untreated        | LMF-TT              | 50                         | 2.1| 106.3 10,040 | 4,082 148       |
| Cells binding to | LMF-TT              | 5                          | 2.1| 2,336   4,635 | 39               |
| TT-Sepharose     |                     |                            |    |          |                  |
|                   | LMF-DT              | 50                         | 2.0| 58.4    7,065 | 121 2,316       |
|                   |                     |                            |    | 1,822   4,635 | 39               |
|                   | LMF-DT              | 5                          | 2.0| 58.4    7,065 | 121 2,316       |
|                   |                     |                            |    | 1,822   4,635 | 39               |
|                   |                     |                            |    |          |                  |
| Cells eluting from | LMF-TT              | 50                         | 1.8| 88.7    9,370 | 11,230 153      |
| TT-Sepharose     |                     |                            |    | 6,884   13,780 | 166               |
|                   | LMF-DT              | 50                         | 1.8| 22.3    6,551 | 1,406 248       |
|                   |                     |                            |    | 906     189 | 203               |
|                   |                     |                            |    |          |                  |
|                   | LMF-DT              | 50                         | 2.8| 93.6    8,860 | 393 71           |
|                   |                     |                            |    | 1,163   351 | 80               |

* IgG synthesis was determined on 0.1-ml portions from supernates of B-cell cultures containing 4 × 10⁶ cells/ml and therefore correspond to the IgG secreted by 4 × 10⁶ B cells.

occurred only if TT were added to LMF-TT (fraction IV). Passage of LMF-TT (fraction IV) over either TT or anti-TT immunosorbent columns did not result in loss of its activity. Furthermore, LMF was shown not to contain μ- or γ-heavy chain determinants because passage of LMF (fraction IV) over anti-μ or anti-γ chain immunosorbents did not affect its activity. The failure of TT and anti-TT immunosorbents to remove LMF activity provides further evidence that LMF is nonspecific because such immunosorbents are known to remove the activity of antigen specific factors (16).

The synthesis of anti-TT antibody only in the presence of TT antigen can be explained in one of two ways. The supernates under study contained in addition to the nonspecific helper factor an antigen-specific helper factor which expresses its activity only in the presence of antigen. Alternatively, the nonspecific helper factor preferentially activates B cells that bind antigen. That the second possibility exists was demonstrated by the ability of LMF-TT to induce specific antibody synthesis to a second antigen added to the B-cell cultures provided the B cells were immune to that second antigen. Addition of DT to B cells incubated with LMF-TT resulted in the synthesis of specific anti-DT antibodies while addition of KLH did not result in the synthesis of anti-KLH antibodies (Table IV). In this instance B-cell donors were immune to DT but not KLH. This finding was not peculiar to LMF-TT, but occurred with LMF-DT as well. The ability of nonspecific helper factors to induce antibody synthesis to antigen that do not cross-react with the antigen used to generate these factors is well
documented in the mouse (1, 3, 17). Recently, supernates of human T cells activated with pokeweed mitogen were shown to induce human B cells to form plaque-forming cells against sheep erythrocytes (18, 19).

Preferential triggering of antigen binding B cells was seen when low concentrations of LMF-TT containing supernates were used (Fig. 1). At these low concentrations (<10% LMF-TT) total IgG synthesis dropped much more than anti-TT synthesis and the proportion of anti-TT over total IgG synthesized by B cells increased up to 70% (Fig. 2). These findings are similar to those obtained by Coutinho and Müller (19–21) with submitogenic concentrations of lipopolysaccharide a mouse polyclonal B-cell activator. Our observations can, however, be explained by the simultaneous presence in our T-cell supernates of an antigen-specific factor whose activity dilutes less readily than that of the nonspecific helper factor. The latter alternative is unlikely to have occurred in the present set of experiments as allogeneic B cells were used throughout. Under such conditions, antigen-specific factors are unlikely to be active because their activity would be restricted to B cells that are histocompatible or that share Ia-like antigens with the T cells that made the factors (5, 6, 13). It is, however, known that T cells stimulated with antigen can secrete simultaneously both antigen-specific as well as antigen nonspecific helper factors into their supernates (21, 22). The present experiments do not, therefore, rule out the presence of antigen-specific helper factor(s) in the supernates under study. Use of autologous B cells would be necessary to investigate the presence of antigen-specific factors in these supernates.

The preferential activation of antigen-binding B cells by LMF is further illustrated in the experiments described in Table VI and VII. At low concentrations of LMF-TT (10%) addition to the B-cell cultures of antigen(s) to which the B-cell donor is immune resulted in an increment in the extent of [3H]thymidine incorporated into DNA (Table VI). Furthermore, concentrations of LMF-TT that were subinitogenic for unfractionated B cells (5%) induced DNA, IgG, and anti-TT synthesis in B-cell populations that were enriched in cells capable of binding to TT (Table VII). This phenomenon was not due to nonspecific activation of the B cells during their enrichment over TT immunosorbent columns as these B cells failed to respond to low concentrations of LMF-DT.

B cells exposed to graded concentrations of LMF-TT exhibited dose-response curves that differed for DNA synthesis and for IgG synthesis (Fig. 1). Optimal mitogenic concentrations of LMF was 50% LMF in fresh culture medium while the optimum concentration of LMF for IgG synthesis was 20%. These results correlated with morphologic evidence obtained by electron micrographic examination of the cell pellets. Maximal number of plasma cells were found in B-cell pellets incubated in 10% LMF while lymphoblasts as well as mitotic figures were most abundant in pellets incubated with 50% LMF (Table V). These observations raise the possibility that there are two nonspecific helper factors, one causing mitogenesis and the other causing IgG synthesis in B cells. Alternatively one could be dealing with only one factor which causes mitogenesis in a subpopulation of B cells and IgG synthesis and secretion in a second subpopulation of B cells with the latter subpopulation having a lower threshold of response. That certain B-cell activators selectively trigger B-cell subpopulations into blastogenesis and/or Ig synthesis is well documented in the mouse model.
Resolution of this question in man will have to await chemical purification of LMF.

Summary

Supernates of human T cells stimulated with TT antigen contain a factor that induces mitogenesis and immunoglobulin synthesis in autologous as well as allogeneic B cells. A fraction of the IgG produced has specificity against TT. The T-cell-derived LMF-TT eluted after albumin on Sephadex G 200 and did not contain immunoglobulin heavy chain determinants. LMF-TT was active on B cells from TT immune as well as TT-nonimmune individuals but in the latter instance the IgG secreted had no specificity against TT. B cells incubated with LMF-TT in the presence of a second antigen (DT) made IgG with specificity to that antigen provided the B-cell donor was immune to that second antigen.

LMF-TT-containing supernates were depleted of TT antigen by Sephadex G 200 chromatography followed by passage over anti-TT immunosorbent columns. The antigen-free supernates were able to induce mitogenesis and IgG synthesis in B cells but the IgG produced failed to exhibit specificity against TT unless the TT antigen was readded to the B-cell cultures.

The optimal concentration of LMF-TT (50%) inducing B-cell mitogenesis was different from the optimal concentration (20%) causing IgG synthesis by B cells. At low LMF concentrations (≤10%) addition of a second antigen to which the cell donor was immune caused an increase in the degree of B-cell mitogenesis. Submitogenic concentrations of LMF-TT (≤5%) were still capable of inducing immunoglobulin synthesis in B cells. At these low concentrations of LMF-TT the proportion of anti-TT IgG over total IgG increased sharply.

B cells from TT immune donors were separated on TT immunosorbent columns. Cells that bound to the column were more sensitive to the mitogenic and IgG synthetic effects of LMF-TT than unfractionated B cells.

Thus, LMF is a nonspecific human T-cell helper factor which behaves like a polyclonal B-cell activator. However, in the presence of specific antigen (TT) the antigen-specific B cell is preferentially triggered by LMF. The experimental design of the present study does not rule out the additional presence of an antigen-specific helper factor in the supernates of TT-stimulated human T cells.

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