HUMAN T CELL HYBRIDOMAS SECRETING FACTORS FOR IgA-SPECIFIC HELP, POLYCLONAL B CELL ACTIVATION, AND B CELL PROLIFERATION*

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Substantial evidence has been accumulated for a major role of T cells or their factors in B cell proliferation and maturation. Recent reports have suggested that certain T cells may be responsible for specific isotype expression on B cells (1-3). This has been most clearly demonstrated for IgE (1), but recent evidence has also implicated such cells in IgG and IgA systems (2, 3). There is also evidence that these factors may act by inducing an Ig class switch (2, 4).

Immtortalization of functional T cells through T cell hybridomas has proved of considerable use in the mouse system, and some results have also been obtained with human T cell hybridomas (5-7). This methodology was used in our laboratory in an attempt to obtain different T cell factors involved in B cell activation and compare their effect on isolated normal B cells and monoclonal B cell leukemias.

Materials and Methods

Cell Separation and Culture Conditions. Leukocyte concentrate packs obtained from the New York Blood Center, New York, were used as a source of peripheral blood mononuclear cells (PBMC). Tonsillar tissue was obtained from routine tonsillectomy specimens. Mononuclear cells and T/B separations were prepared as previously described (8). Non-T cells were rosetted twice before use, resulting in <1% OKT 3+ (pan T cell marker; Ortho Pharmaceutical, Raritan, NJ) cells in this population. Isolated T cells were cultured in complete medium (8) or subjected to a further rosetting procedure to enrich for OKT-4+ (helper/inducer T cell marker) cells used for fusion. An indirect rosetting technique was used; 40 × 10^6 T cells were incubated in OKT-8 antibody (suppressor/cytotoxic T cell marker) and rosetted with goat anti-mouse Ig-coated ox erythrocytes (RBC), coated by a chromium chloride technique (8). The resulting interface, after Ficoll-Hypaque gradient centrifugation, was 90-95% OKT-4+ and <1% OKT-8+ by indirect immunofluorescence. The OKT-4+ cells were incubated with concanavalin A (Con A) (Sigma Chemical Co., St. Louis, MO), 10 μg/ml, for 72 h at a cell concentration of 1-2 × 10^6/ml. These stimulated cells were washed three times in serum-free medium before use in cell fusion.

Establishment of Hypoxanthine Guanine Phosphoribosyl Transferase (HGPR T)-deficient Human T Cell Lines for Fusion. Jurkat, a human T cell lymphoma line, and KE 37, a human T cell ALL line, were mutagenized with ethylmethane sulfonate at a concentration of 200 μg/ml according to Epstein et al. (9). 80% of treated cells died from this treatment, but after 2 wk in culture, the remaining 20% were ≥95% viable. HGPR T-deficient mutants were selected in 6-thioguanine and cloned on soft agar with human 6-thioguanine-resistant fibroblasts (Gm 1362; The Human Genetic Mutant Cell Repository, Camden, NJ). Resultant clones were tested for aminopterin...
sensitivity with dose ranges of 10^{-7} - 10^{-9} M. Clones were selected on the basis of their cloning efficiency and the presence of a clear-cut level of aminopterin sensitivity.

**Fusion Procedure.** Fusion of peripheral blood (PB) -activated T cells was performed with mutagenized Jurkat 3 or KE 37.3.2 clones, or a nonmutagenized Jurkat 2 T cell line. In the former case, 2 × 10^{7} Con A-stimulated PB T cells or OKT-4+ T cells were pelleted with 1 × 10^{7} KE 37.3.2 or Jurkat 3, respectively. Pellets were resuspended in polyethylene glycol (PEG) 1000 (Merck Chemical Div., Merck & Co., Inc., Rahway, NJ) and centrifuged for 8 min at room temperature at 150 g. The cells were washed free of PEG with serum-free medium and cultured in 96-well, flat-bottomed microwell plates (Linbro Chemical Co., Hamden, CT) at a concentration of 1 × 10^{5} mutant cells/well. Hybrid culture medium consisted of RPMI 1640, 20% fetal calf serum, penicillin/streptomycin, and 2 mM glutamine. In addition, hypoxanthine, 10^{-4} M, and thymidine, 1.6 × 10^{-5} M, were added. After 24 h at 37°C, 5% CO_{2}, and 95% room air, aminopterin, 3 × 10^{-8} M, was added. After 14-17 d in culture, with feeding every 3rd d, all nonfused control cultures were nonviable. Growth-positive wells were transferred out of aminopterin and into 24-well, flat-bottomed macrowell plates (Linbro Chemical Co.). With continued growth, cultures were maintained in 25-cm^{2} culture flasks (3013; Falcon Labware, Oxnard, CA), and cell-free supernatants were obtained and tested for functional capabilities.

Fusion of activated T cells with a nonmutagenized T cell line, Jurkat 2, was performed in a similar manner except that no hypoxanthine, aminopterin, thymidine (HAT) was added to the cultures. Donor cells in this case were known to HLA-B27+ as measured by staining with a monoclonal antibody (N. L. Cappel Laboratories, Cochranville, PA). Because all wells showed positive growth by this method, single wells were cloned on soft agar, 0.6% (SeaKem, Rockland, ME) using human fibroblasts as a feeder layer (Gm 1604). Resultant clones were stained with the HLA B27 antibody, with the positive clones being selected for further study.

**HLA Typing.** HLA typing of parental lines and fusion products was kindly performed by Dr. M. Fotino of the New York Blood Center.

**Immunofluorescence and Fc Receptors.** Direct and indirect staining methods for surface and intracytoplasmic staining was performed as previously described (8). Fc receptors were determined by the ability of T cells to form rosettes with human IgG, IgA, or IgM chromium chloride-coupled ox RBC.

**Plaque Assay.** Purified B or non-T cell populations were placed in culture medium with 10–30% hybrid supernatant at a concentration of 1 × 10^{6} cells/ml, in an effort to determine the supernatant's ability to induce B cell maturation. Cultures were maintained at 37°C, 5% CO_{2}, and 95% room air. A reverse hemolytic plaque was performed to evaluate Ig isotype production on day 6 (10).

**B Cell Proliferation.** Resting B cells were incubated as above in 10–30% hybrid supernatant in triplicate 0.1-ml cultures at a cell concentration of 1 × 10^{6} cells/ml. On days 2–5, 2 μCi of aqueous methyl [3H]thymidine (sp act 2.5 mCi; Schwartz/Mann Div., Becton, Dickinson & Co., Orangeberg, NY) were added, and after 12 h the cells were harvested and processed for scintillation counting. Average counts per minute of the triplicate cultures divided by counts per minute of the media control was the stimulation index.

**Results**

**Characterization of T-T Hybrids.** 63 out of 180 wells showed positive growth after 4–6 wk in HAT medium (31/90 from the Jurkat 3 fusion and 32/90 from the KE 37.3.2 fusion). Four of these hybrids showed potent activity of interest when tested functionally, as described below, including one B27+ clone from the fusion with the nonmutagenized Jurkat line. The parental lines, Jurkat 2 and 3, were typed as HLA A2, A4, B7, and CW4. The normal donor T cells expressed HLA A1, A30, B35, B17, and CW4. Fusion product J1 and its clone J1.3, expressed A1 and B17 from the donor cells as well as A2 and B7 from the Jurkat line. Surface marker studies revealed that J1.3, as well as Jurkat 3, was OKT-3+, T4+, T8+, Ia−, Fcγ−, and Fcα−. However, the hybrid was Fcγ+, whereas the parental line was not. Hybrids K1 and K8 expressed HLA A1 and B17 from the donor T cells, and were OKT-4+, whereas the KE37.3.2 line was not. J2S1 expressed HLA B27 from its donor parent.
Functional Characteristics of the Supernatants

B cell differentiation. Supernatants from hybrid cultures were added to a purified population of tonsil B cells. In the control cultures, with media alone, no IgA-specific PFC were seen (Table I). Addition of supernatant from hybrid clone J1.3 resulted in 700 IgA-specific plaque-forming cells (PFC)/well. The addition of T cells without supernatant yielded only 100 IgA-specific PFC and a different isotypic pattern. Similar data were obtained using a different tonsil B cell preparation, with an eightfold increase in IgA PFC over control. This effect could still be demonstrated with the supernatant diluted to 1:100 (Table I). PB non-T cells, cultured in the presence of supernatant, also resulted in an increase in IgA-specific PFC, approaching that seen with autologous T cells and pokeweed mitogen (PWM).

The J1.3 supernatant also caused differentiation of monoclonal leukemic B lymphocytes that had membrane IgA. These cells were derived from a case of B cell leukemia with a leukocyte count of 100,000/mm³, due to membrane IgA-bearing lymphocytes. A few plasma cells with cytoplasmic IgA were also seen in the peripheral blood. Table II shows the marked increase in IgA PFC as well as plasma cells, determined by fluorescent analysis with anti-IgA antiserum, after the addition of J1.3 supernatant. Similar differentiation was observed after addition of allogeneic T cells and PWM, or addition of J2S1 supernatant (see below).

Hybrid J2S1 had a different effect. Addition of this supernatant induced polyclonal B cell differentiation with large increases in PFC of all isotypes (Table I). These supernatants appeared to be especially potent, with activity present, although diminished, at a dilution of 1:200 (Table I). Supernatants from the parental line Jurkat 3, as well as hybrids K1 and K8, which promote B cell proliferation (see below), failed to induce B cell differentiation (Table I).

### Table I

| B Cells        | Media control | Supernatant* | Plus autologous T cells |
|----------------|---------------|--------------|-------------------------|
|                | G/A/M         | G/A/M        | G/A/M                   |
| T cell hybridoma J1.3 |               |              |                         |
| Tonsil B₁      | 0/0/100       | 200/700      | 0/0/100                 |
| Tonsil B₂      | 0/300/0       | 2,400/0      | 0/0/0                   |
| Tonsil B₃      | 20/0/0        | 20/0/0       | 0/0/0                   |
| PB non-T₁      | 0/0/0         | 100/700      | 0/0/0                   |
| PBMC           | 0/0/0         | 500/0        | 0/0/0                   |
| T cell hybridoma J2S1 |               |              |                         |
| Tonsil B₁      | 40/0/90       | 520/450      | 190/30                  |
|                | GAM           | GAM          | GAM                     |
| T cell hybridoma J2S1 |               |              |                         |
| Tonsil B₁      | 30/0/1170     |              |                         |
| Tonsil B₂      | 50/170**      |              |                         |
| PB non-T₁      | 100/700       |              |                         |
| Hybrids K1     |               |              |                         |
| Tonsil B₁      | 0/0/0         |              |                         |
| K8             | 0/0/0         |              |                         |
| Parent line Jurkat 3 |       |              |                         |
| Tonsil B₁      | 0/0/0         |              |                         |

* Supernatant at 1:3 dilution.
‡ PFC/10⁶ cells at initiation of culture. Ig isotypes measured by reverse plaque assay at day 6.
§ Supernatant at 1:100 dilution.
¶ Reverse plaque assay recognizing all isotypes.
** Supernatant at 1:200 dilution.
TABLE II

Differentiation of Monoclonal IgA-bearing Leukemic Lymphocytes in the Presence of Hybrid Supernatants

| Added cells or supernatant | PFC/well* | Fluorescence‡ |
|---------------------------|-----------|---------------|
|                           | G         | A  | M  | G | A | M |
| Media control             | 0         | 600| 0  | 0 | 7 | 0 |
| J1.3                     | 200       | 18,000| 0 | 0 | 86 | 1 |
| J2S1                     | 0         | 16,000| 600| 0 | 70 | 2 |
| Allogeneic T cells        | 200       | 1,800| 0  | 0 | 7  | 2 |
| Allogeneic T cells and PWM| 400       | 22,800| 600| 1 | 50 | 2 |
| PWM (1:100)               | ND        | ND | ND | 1 | 7 | 2 |

* PFC/well on day 6 of culture, as per Table I.
‡ Intracytoplasmic staining in fixed preparations on day 6 after addition of supernatant at a 10% concentration.
§ Very bright fluorescence.
∥ Not done.

Fig. 1. Stimulation index (experiment cpm/control cpm) of resting B cells at days 2-5, incubated in hybrid supernatants K1 (■), K8 (●), J1.3 (○), J2S1 (▲), or parental line KE37.3.2 (△).

B CELL PROLIFERATION. [³H]thymidine uptake of purified tonsil B cells incubated with or without supernatant were measured at days 2-5 (Fig. 1). Supernatants from hybrids K1 and K8 induced B cell proliferation on day 3, with stimulation indices of 20.6 and 31.4, respectively. As noted above, this proliferation was not associated with B cell maturation. Hybrids J1.3 and J2S1 induced only modest increases in [³H]thymidine uptake, and the parental line KE 37.3.2 did not stimulate at all (Fig. 1).

Discussion

In the present study we have generated a series of human T cell hybridomas by fusion of normal activated T cells with T cell lines, either rendered HGPRT deficient
by treatment with ethylmethane sulfonate or used intact. HLA typing and other surface characteristics revealed that the hybrids expressed HLA A, B, or C determinants or surface markers of both parental cells. Two different methods used for selecting T-T hybrids proved satisfactory in the production of functional hybrids. Our experience with the hybrids showed that they were not stable in continuous culture and repeated subcloning was necessary to maintain function. In this manner, the hybrids were maintained for 6-8 mo.

One particular hybrid, J1, and its clone, J1.3 were shown to constitutively produce a factor that enhanced IgA secretion by normal human tonsil or PB B cells, as well as by monoclonal leukemic B cells with membrane IgA. It had little effect on the production of IgG and IgM and was therefore clearly class specific. The effect on monoclonal leukemic B cells with membrane IgA was of special interest. Virtually complete differentiation was observed. This provided strong evidence for a direct effect of the J1.3 factor on B cells. The cells of this patient were also inducible with allogeneic T cells and PWM as well as the polyclonal activator J2S1. Also of interest is the fact that the J1.3 hybrid had Fc receptors for $\mu$ and not $\alpha$, in contrast to the IgA helper T cells described by others (4, 11).

T cell influence on Ig isotype expression has been described for several classes of Ig. Helper T cells for IgG and IgE (1) as well as IgG subclasses have been demonstrated (12). Isotype switching is influenced by T cells (13), and as shown more recently (2), factors from murine T cell lines appear to induce a switch from IgG$^-$ to IgG$^+$ cells. IgA class-specific T cell help has been described in murine Peyer's patches (3) and in the peripheral blood of humans using isolated Fc$\alpha^+$ T cells (11). Clones of Peyer's patch T cells have been shown to cause a switch from surface IgM$^+$ to surface IgA$^+$-bearing B cells, without increase in IgA production (4). This suggests that several factors may be necessary for the final secretion of IgA.

Exactly where our hybrid J1.3 acts in the stages of B cell differentiation is unclear at present. Data have been obtained showing that depletion of surface IgA-bearing B cells eliminates the effect of the supernatant (L. Mayer, unpublished observation). This suggests that the factor acts on a post-switch, IgA-committed B cell.

The other hybrids described above differ from J1.3 in several important respects. J2S1 induces polyclonal differentiation with increases in IgG and IgM as well as IgA, in striking contrast to J1.3. This hybrid may be similar to that described by Irigoyen et al. (5), except that prestimulation of the B cells with PWM was not necessary with J2S1. In addition, two other hybrids were studied that did not induce B cell differentiation, but rather caused active proliferation of resting B cells. The factors from these clones may be analogous to B cell growth factors described in Con A- and phytohemagglutinin-stimulated T cell supernatants (14, 15).

Current efforts are being directed toward defining the molecular nature of these factors and their specific B cell target. The monoclonal leukemic B cells were stimulated to differentiate by both the IgA-specific and polyclonal factors. It would be of interest to determine whether the same receptor site is involved. The use of monoclonal B cell targets appears to be of special value in these studies.

Summary

Human T-T hybridomas were established by fusion of concanavalin A-activated OKT-4$^+$ T cells with hypoxanthine guanine phosphoribosyl transferase-deficient as well as nondeficient T cell lines. Four hybrids were selected for further study.
Supernatant from hybrid clone J1.3 specifically enhanced IgA production and secretion by isolated human B cells, with increases in IgA plaque-forming cells approaching those seen with addition of autologous T cells and pokeweed mitogen. A monoclonal lymphocytic leukemia with membrane IgA also differentiated to IgA plasma cells by this supernatant. Evidence suggests that this hybrid supernatant acts on post-switch IgA-committed B cells. The other hybrids were not isotype specific; hybrid J2S1 enhanced polyclonal Ig secretion and hybrids K1 and K8 induced B cell proliferation without induction of Ig secretion.

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References
1. Ishizaka, K., and A. Adachi. 1976. Generation of specific helper cells and suppressor cells in vitro for the IgE and IgG antibody responses. J. Immunol. 117:40.
2. Isakson, P. C., E. Pure, E. S. Vitetta, and P. H. Krammer. 1982. T cell derived B cell differentiation factor(s). Effect on isotype switch of murine B cells. J. Exp. Med. 155:734.
3. Elson, C. O., J. A. Fleck, and W. Strober. 1979. T cell regulation of murine IgA synthesis. J. Exp. Med. 149:632.
4. Kawanishi, H., L. E. Saltzman, and W. Strober. 1982. Characteristics and regulatory function of murine Con A induced cloned T cells obtained from Peyer's patches and spleen: mechanisms regulating isotype specific immunoglobulin production by Peyer's patch B cells. J. Immunol. 129:475.
5. Irigoyen, O., P. V. Rizzolo, Y. Thomas, L. Rogozinski, and L. Chess. 1981. Generation of functional human T cell hybrids. J. Exp. Med. 154:1827.
6. Grillot-Courvalin, C., J. C. Brouet, R. Berger, and A. Berheim. 1981. Establishment of a human T cell hybrid line with suppressive activity. Nature (Lond.). 292:844.
7. Okada, M., N. Yoshimura, T. Kaieda, Y. Yamamura, and T. Kishimoto. 1981. Establishment and characterization of human T hybrid cells secreting immunoglobulin and regulatory molecules. Proc. Natl. Acad. Sci. U. S. A. 78:7717.
8. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1980. Stimulation of human B lymphocytes by antibodies to IgM and IgG: Functional evidence for the expression of IgG on B lymphocyte surface membranes. Clin. Immunol. Immunopath. 15:301.
9. Epstein, J. A., Leyva, W. N. Kelley, and J. W. Littlefield. 1977. Mutagen-induced diploid human lymphoblast variants containing altered hypoxanthine guanine phosphoribosyl transferase. Somatic Cell Genet. 3:135.
10. Ginsburg, W. W., F. D. Finkelman, P. E. Lipsky, 1978. Circulating and mitogen induced immunoglobulin secreting cells in human peripheral blood: evaluation by a modified reverse hemolytic plaque assay. J. Immunol. 120:33.
11. Endoh, M., H. Sakai, Y. Nomoto, Y. Tomino, and H. Kaneshige, 1981. IgA-specific helper activity of T cells in human peripheral blood. J. Immunol. 127:2612.
12. Mongini, P. K. A., K. E. Stein, and W. E. Paul. 1981. T cell regulation of IgG subclass antibody production in response to T independent antigens. J. Exp. Med. 153:1.
13. Braley-Mullen, H. 1974. Regulatory role of T cells in IgG antibody formation and immune memory to type III pneumococcal polysaccharide. J. Immunol. 113:1909.
14. Sredni, B., D. G. Sieckman, S. Kumagai, S. House, I. Green, and W. E. Paul. 1981. Long-term culture and cloning of nontransformed human B lymphocytes. J. Exp. Med. 154:1500.
15. Andersson, J., and F. Melchers. 1981. T cell dependent activation of resting B cells: requirement for both nonspecific unrestricted and antigen-specific Ia restricted soluble factors. Proc. Natl. Acad. Sci. U. S. A. 78:2497.