

EFFECT OF TUMOR NECROSIS FACTOR α ON
MITOGEN-ACTIVATED HUMAN B CELLS

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Several monocyte/macrophage-derived factors have been reported to influence human and murine B cell function. Interleukin 1 (IL-1), a product of activated monocytes, has been shown (1, 2) to augment both the proliferation and differentiation of human B cells. Additionally, stimulated monocytes have been shown (3) to release a factor that induces the differentiation of antigen-activated murine B cells to Ig secretion. Such a monocyte-derived B cell differentiation factor (BCDF) has been reported to be functionally identical to a factor that also triggered the selective differentiation of murine B cells and was present in the serum of LPS-treated *Bacillus Calmette-Guerin* (BCG)-infected mice (3, 4).

Besides this BCDF activity, the serum of such treated mice has been found (5) to cause hemorrhagic necrosis and regression of certain tumors in mice. The factor responsible for this activity has been termed tumor necrosis factor α (TNF- α). Subsequently, TNF- α has been found to be a product of activated monocytes and it has been purified, sequenced, and cloned by recombinant DNA techniques (6–8). Purified and recombinant TNF- α is cytotoxic or cytostatic against a variety of tumor cell lines.

The availability of recombinant TNF- α has allowed us to directly test whether this cytokine modulates human B cell function.

Materials and Methods

Cell Preparation. B cell-enriched populations were obtained from tonsillar mononuclear cells as previously described (1). These preparations of B cells were routinely >95% surface immunoglobulin (sIg)-positive, <1% esterase positive, and <1% OKT3⁺ (Ortho Pharmaceutical Corp., Raritan, NJ) as determined by immunofluorescence and esterase staining.

Factors. Recombinant IL-1- β was purchased from Genzyme Corporation (Boston, MA). Recombinant IL-2 (lot LP2) was kindly provided by the Cetus Corporation (Emeryville, CA). TNF- α (lot TD4) was also kindly provided by Cetus Corp. and contained a bioactivity of 1.3×10^7 U/mg, an endotoxin level of 0.089 ng/mg, and was >95% pure by SDS-PAGE. Recombinant IFN- γ was kindly provided by Genentech Corporation (South San Francisco, CA). B cell growth factor (BCGF), lot number 15117, was purchased from Cellular Products, Inc. (Buffalo, NY).

Culture Condition and Assays. Purified B cells were cultured in RPMI 1640 supplemented with 10% FCS (Dutchland Laboratories, Inc., Denver, PA) and gentamicin (50 μ g/ml) in 75 cm² culture flasks (Costar Data Packaging, Cambridge, MA) at a concentration of 10^6 cells/ml with a 1:20,000 vol/vol dilution of *Staphylococcus aureus* Cowan (SAC)

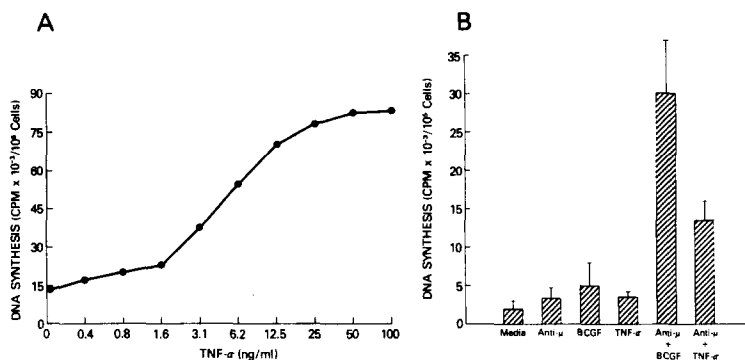


FIGURE 1. (A) Dose response to TNF- α . Purified tonsillar B lymphocytes were cultured for 2 d in the presence of SAC in medium containing 10% FCS. The cells were harvested, washed, and recultured in serum-free media with varying concentrations of TNF- α . [3 H]Thymidine incorporation was measured 3 d later. (B) TNF- α costimulates with anti- μ . Purified tonsillar B lymphocytes were cultured in the presence of media alone, 15 μ g/ml of rabbit anti-human IgM coupled to beads, 10% BCGF, 40 ng/ml of TNF- α , anti- μ plus BCGF, or anti- μ plus TNF- α . [3 H]Thymidine incorporation was measured during the last 18 h of a 72-h culture. Data represents the mean \pm SEM of two experiments.

strain I (Bethesda Research Laboratories, Bethesda, MD). Because we found that serum-containing media often suppressed the subsequent response of in vitro-activated B cells to IL-2, TNF, and BCGF (our unpublished observation), on the second day of culture the cells were harvested, washed twice with RPMI 1640, and recultured in Ventrex HL-1 serum-free media (Ventrex Laboratories, Inc., Portland, ME) at a concentration of 5×10^5 cells/ml in 96-well Costar plates in the presence or absence of various cytokines. The culture was continued for the indicated time periods, and DNA synthesis was measured during the last 18 h of the culture period by the incorporation of [3 H]thymidine using standard liquid scintillation counting techniques. The anti- μ costimulation assay was performed as previously described (1). IgG and IgM levels in culture supernatants were measured by an isotype-specific ELISA.

Receptor Studies. Purified recombinant TNF- α (10 μ g) was radiolabelled using iodo-beads (Pierce Chemical Co., Rockford, IL) and separated from free iodide by filtration over a Sephadex G-25 column. The radiolabelled TNF had a specific activity of 5.7 mCi/mg and was biologically active when assayed on L929 cells. 125 I-TNF radioreceptor binding assays were performed by suspended 10^6 B cells in 100 μ l of binding buffer (Ventrex media plus 25 mM Hepes) and incubating them with increasing amounts of 125 I-TNF in the presence or absence of a 500 molar excess of unlabelled TNF. After 2 h of binding at 23°C with agitation, cell associated and free 125 I-TNF were separated by overlaying the reaction mixture on a cushion of 84% silicon oil (Dexter Hysol, Olean, NY) and 16% paraffin oil (Fischer Scientific, Fair Lawn, NJ) and centrifuging at 12,000 $\times g$ for 1 min. The resultant binding data were analyzed by the LIGAND computer program developed by Munson and Rodbard (9).

Results

TNF- α Maintains the Proliferation of SAC-activated B Cells and Costimulates with Anti- μ in the Induction of B Cell DNA Synthesis. Stimulation of human B lymphocytes with the polyclonal B cell activator, SAC, is only sufficient to trigger an initial round of B cell proliferation. However, in the presence of an appropriate growth factor such as IL-2 or BCGF, the proliferation is maintained (10, 11). The effects of various concentrations of TNF- α on the incorporation of [3 H]-thymidine by B cells preactivated with SAC is shown in Fig. 1A. The addition of

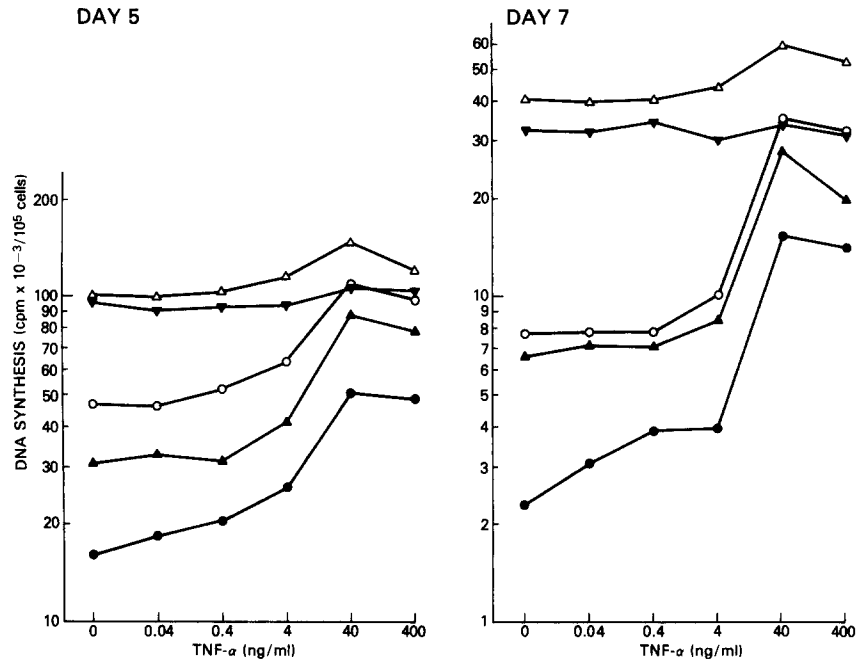


FIGURE 2. Effects of TNF- α plus IL-1, IFN- γ , BCGF, or IL-2 on B cell proliferation. Preactivated tonsillar B cells were cultured with varying concentrations of TNF- α in the presence or absence of the above factors, medium (●), 10 U/ml of IL-1 (▲), 100 U/ml of IFN- γ (○), 10% vol/vol of BCGF (▼), or 100 U/ml of IL-2 (Δ). [³H]Thymidine incorporation was measured 3 d (day 5 of culture) or 5 d later (day 7 of culture).

25 ng/ml of TNF- α to these cultures resulted in a fivefold increase in thymidine incorporation compared to B cells cultured with media alone. Half-maximal stimulation occurred at a concentration of 5 ng/ml (285 pM) of TNF- α .

Using an anti- μ costimulation assay, we next examined whether TNF- α could enhance anti- μ -triggered B cell proliferation. TNF- α could costimulate with anti- μ in this assay, but not as efficiently as a BCGF preparation (Fig. 1B).

TNF- α Enhances the Effects of IL-1, IL-2, and IFN- γ on SAC-activated B Cell Proliferation. To test whether TNF- α modulated the effects of factors known to enhance B cell function (1, 10–12), optimal concentrations of BCGF, IL-1, IL-2, and IFN- γ were added to 2-d SAC-activated B cells in conjunction with increasing concentrations of TNF- α (Fig. 2). DNA synthesis was measured 3 and 5 d later. TNF- α augmented the B cell proliferation compared to a media control at both days 5 and 7. The addition of TNF- α to either IL-1 or IFN- γ resulted in a greater increase in DNA synthesis than did either factor alone at both day 5 and day 7. Additionally, TNF- α increased IL-2-induced B cell proliferation at both days 5 and 7 by 40%, but it did not augment BCGF induced proliferation at either time. Subsequently, the BCGF preparation was found to contain a moderate concentration of TNF (data not shown).

TNF- α Itself Does Not Induce Ig Secretion by SAC-activated Human B Cells, But It Can Augment IL-2-induced IgM Secretion of These Cells. Because of the original report that serum from BCG-infected and LPS-challenged mice, in conjunction

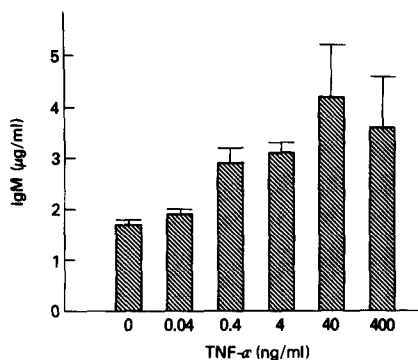


FIGURE 3. TNF- α augments IL-2 induced B cell differentiation. B cells were activated with SAC for 2 d, washed, and recultured in serum-free medium in the presence or absence of 100 U/ml IL-2 with varying concentrations of TNF- α . Supernatant IgM levels were measured 7 d later using an ELISA (mean \pm SEM of two experiments).

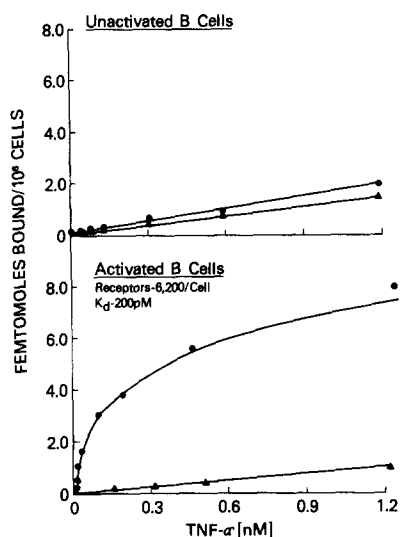


FIGURE 4. 125 I-TNF- α binding to unstimulated or SAC stimulated B cells. Purified tonsillar B cells were cultured for 48 h in the presence or absence of SAC. Binding assays were performed as described in Materials and Methods. (\blacktriangle), binding in the presence of cold TNF- α ; (\bullet), binding in the absence of cold TNF- α .

with antigen, would induce murine B cell Ig secretion (3, 4), we were interested in examining the effects of TNF- α on human B cell differentiation to Ig secretion. Using B cells preactivated in vitro with SAC, we added various concentrations of TNF- α to cultures and measured supernatant Ig concentration 5 d later. TNF- α by itself did not promote preactivated B cells to secrete Ig but it did enhance (twofold) the IgM production induced by IL-2 (Fig. 3). Of note, the effects of TNF- α on IgM production were more pronounced than its effects on IgG secretion (data not shown).

Expression of TNF- α Receptors on Human B Cells. To further characterize the role of TNF- α in B cell function, receptor binding studies with 125 I-TNF- α were performed. Unstimulated B cells had low levels of receptors not quantifiable using the LIGAND program; however, B cells that were cultured with SAC for 2 d had an average of 6,000 receptors per cell, with a K_d of 2.0×10^{-10} M (200 pM) (Fig. 4). A linear Scatchard was found, which suggests a single affinity class of receptors. The binding of TNF- α to SAC-activated B cells was not secondary to the presence of SAC in the culture, since the addition of SAC to the unactivated B cells before the binding assay did not alter the results (data not shown).

Discussion

TNF- α has been generally defined by its cytotoxic or cytostatic activity against certain transformed cell lines. However, recently, Sugarman et al. (13) found that TNF- α actually enhanced the growth of certain normal cell lines. Additionally, Vilcek et al. (9) have noted that the growth of human FS-4 diploid fibroblasts was stimulated by TNF- α . In the present study we demonstrate that TNF- α can also significantly enhance the proliferation of activated B cells. These bifunctional effects of TNF- α on cell growth, although unusual, are not unique, as several other growth factors that can provide positive or negative growth signals, such as transforming growth factor β , have been identified (15). The growth-promoting effects of TNF- α for normal human B cells gives further credence to the hypothesis (14) that TNF- α is a growth factor for certain types of normal cells and that its antiproliferative effects on transformed cells is the result of an aberrant response to growth stimulation in cells that lack normal growth regulatory mechanisms.

Binding studies with radiolabelled TNF- α have been previously performed using cell lines that are sensitive targets for the cytotoxic effects of TNF. Tsujimoto et al. (16) examined the binding of ^{125}I -labelled TNF- α to the TNF-sensitive cell line, L929, and the TNF-resistant cell line, FS-4. Scatchard analysis of the binding data indicated the presence of 2,200 receptors per L929 cell, with a K_d of 6.1×10^{-10} M, and 7,500 receptors per FS-4 cell, with a K_d of 3.2×10^{-10} M. In this study activated B cells had approximately three times as many receptors per cell, with a similar affinity as those reported for L929 cells.

Summary

In this study we demonstrate that the monocyte/macrophage product, tumor necrosis factor α (TNF- α), has significant in vitro effects of B cell function. It costimulated with anti- μ in the induction of B cell DNA synthesis, and it prolonged the DNA synthesis initiated in B cell cultures stimulated with the human B cell mitogen, *Staphylococcus aureus* Cowan strain I (SAC). The addition of either IL-1 or IFN- γ to TNF- α resulted in a substantial further increase in DNA synthesis. The addition of TNF- α to IL-2, a known inducer of SAC-activated B cell Ig secretion, resulted in a twofold enhancement in the amount of IL-2 stimulated B cell Ig secretion. Receptor binding studies with ^{125}I -TNF- α demonstrate a marked increase in TNF- α binding sites after B cell activation ($\sim 6,000$ sites per cell, with an apparent K_d of 2.0×10^{-10} M). Thus, TNF- α may be an important factor in human B cell function and is likely to interact with other T cell and monocyte derived cytokines in the regulation of human B cell proliferation and Ig production.

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References

1. Falkoff, R. J. M., A. Muraguchi, J.-X. Hong, J. L. Butler, C. A. Dinarello, and A. S. Fauci. 1983. The effects of interleukin 1 on B cell proliferation. *J. Immunol.* 131:801.

2. Falkoff, R. J. M., J. L. Bulter, C. A. Dinarello, and A. S. Fauci. 1984. Direct effects of a monoclonal B cell differentiation factor and of purified interleukin 1 on B cell differentiation. *J. Immunol.* 133:692.
3. Hoffmann, M. K., S. Koenig, R. S. Mittler, H. F. Oettgen, P. Ralph, C. Galanos, and U. Hammerling. 1979. Macrophage factor controlling differentiation of B cells. *J. Immunol.* 122:497.
4. Hoffmann, M. K., S. Green, L. J. Old, and H. F. Oettgen. 1976. Serum containing endotoxin-induced tumor necrosis factor substitutes for helper T cells. *Nature (Lond.)*. 263:416.
5. Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA.* 72:3666.
6. Pennica, D., G. E. Nedwin, J. S. Hayflick, P. H. Seeburg, R. Derynck, M. A. Palladino, W. J. Kohr, B. B. Aggarwal, and D. V. Goeddel. 1984. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature (Lond.)*. 312:724.
7. Shirai, T., H. Yamaguchi, H. Ito, C. W. Todd, and R. B. Wallace. 1985. Cloning and expression in *Escherichia coli* of the gene for human tumor necrosis factor. *Nature (Lond.)*. 313:803.
8. Wang, A. M., A. A. Creasey, M. B. Ladner, L. S. Lin, J. Strickler, J. N. Van Arsdell, R. Yamamoto, and D. F. Mark. 1985. Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science (Wash. DC)*. 228:149.
9. Munson, P. J., and D. Rodbard. 1980. Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107:220.
10. Muraguchi, A., and A. S. Fauci. 1982. Proliferative responses of normal human B lymphocytes. Development of an assay system for human B cell growth factor (BCGF). *J. Immunol.* 129:1104.
11. Muraguchi, A., J. H. Kehrl, D. L. Longo, D. J. Volkman, and A. S. Fauci. 1985. Tac expression on activated B cells and on a human T-cell leukemia-infected B-cell line. *J. Exp. Med.* 161:181.
12. Romagnani, S., M. G. Guidizi, R. Biagiotti, F. Almerigogna, C. Mingari, E. Maggi, C. Liang, and L. Moretta. 1986. B cell growth factor activity of interferon- γ . Recombinant human interferon- γ promotes proliferation of anti- μ activated human B lymphocytes. *J. Immunol.* 136:3513.
13. Sugarman, B. J., B. B. Aggarwal, P. E. Hass, I. S. Figari, M. A. Palladino, Jr., and H. M. Shepard. 1985. Recombinant human tumor necrosis factor- α : Effects on proliferation of normal and transformed cells in vitro. *Science* 230:943.
14. Vilcek, J., V. J. Palombella, D. Henriksen-Destefano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto. 1986. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *J. Exp. Med.* 163:632.
15. Roberts, A. B., M. A. Anzano, L. M. Wakefield, N. S. Roche, D. F. Stern, and M. B. Sporn. 1985. Type B transforming growth factor: a bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci. USA.* 82:119.
16. Tsujimoto, M., Y. K. Yip, and J. Vilcek. 1985. Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. *Proc. Natl. Acad. Sci. USA.* 82:7626.