

PRODUCTION OF TUMOR NECROSIS  
FACTOR/CACHECTIN BY HUMAN B CELL LINES  
AND TONSILLAR B CELLS

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TNF/cachectin has diverse physiological functions and is produced by a wide variety of cell types (reviewed in reference 1). Recently, evidence has accumulated that TNF may play regulatory roles in T and B lymphocyte activation, proliferation, and differentiation (2, 3). Activated T cells have been shown to produce a considerable amount of TNF (4-6). However, the capacity of B cells to produce TNF has not been documented fully. Bersani, et al. (7) could detect only lymphotoxin (LT)<sup>1</sup> but not TNF in the supernatants of tonsillar B cells activated by *Staphylococcus aureus* Cowan I strain (SAC). In the present study, we found that a number of B leukemia and EBV-transformed B cells expressed TNF at both the mRNA and protein levels. Tonsillar B cells could also be induced by mitogens to synthesize TNF mRNA and to secrete TNF. TNF production by these B cells correlated with their proliferative responsiveness to the mitogenic stimuli, and it may play important roles in B cell activation, growth, and development.

Materials and Methods

*Reagents.* Goat anti- $\mu$  IgG was affinity purified after absorption with mouse, rabbit, and fetal calf sera, and human IgG and IgA. Anti-TNF mAbs C16, F12, and E43 have been described (5). The concentrations of stimulants used were as follows: anti- $\mu$  IgG, 25  $\mu$ g/ml; SAC (Calbiochem-Behring Corp., La Jolla, CA), 0.01%; B cell growth factor (BCGF; Cellular Products, Buffalo, NY), 5%; PMA (Sigma Chemical Co., St. Louis, MO), 10 ng/ml; LPS (from *S. typhosa*; Sigma Chemical Co.), 5  $\mu$ g/ml; IFN- $\gamma$  (Cellular Products), 100 U/ml; cyclosporin A (CsA; Sandoz, East Hanover, NJ), 5  $\mu$ g/ml; anti-CD3, 1  $\mu$ g/ml; and A23187 (Calbiochem-Behring Corp.), 0.1  $\mu$ M.

*Cells.* Cells were maintained in RPMI 1640 with 10% FCS as described (5). The B cell lines used were from Dr. Jun Minowada (Fujisaki Cell Center, Hayashibara Biochem. Labs, Inc., Fujisaki, Okayama, Japan), the American Type Culture Collection (Bethesda, MD), or derived in our own laboratory (8). The Ig expressions of cell lines derived from Burkitt's lymphoma patients are as follows: Daudi,  $\mu^+ \delta^+ \kappa^+$ ; Raji,  $\mu^+$ ; and Ramos,  $\mu^+ \lambda^+$ . The leukemia cell line Ig expressions are SeD,  $\mu^+ \delta^+ \kappa^+$ ; Nalm-6, cytoplasmic  $\mu^+$ ; and Nalm-12, cytoplasmic  $\mu^+$ . The EBV-transformed cell line Ig expressions are Josh 7, Ig<sup>-</sup> (9); 32a1,  $\alpha^+ \lambda^+$ ; 8866P,  $\gamma^+ \kappa^+$ ; RPMI 1788,  $\mu^+ \delta^+ \lambda^+$ . Other EBV-transformed B cell lines were derived from T

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A preliminary account of this work has been reported as an abstract (26).

<sup>1</sup> *Abbreviations used in this paper:* BCGF, B cell growth factor; CsA, cyclosporin A; LT, lymphotoxin; SAC, *Staphylococcus aureus* Cowan I strain.

cell-depleted human bone marrow cells as described (9). These cells express: WIH8,  $\gamma^+ \lambda^+$ ; WIH10,  $\alpha^+ \kappa^+$ ; WIH14,  $\alpha^+ \kappa^+$ ; WIH20,  $\mu^+ \kappa^+$ ; WIH67,  $\gamma^+ \lambda^+$ ; and KAW15,  $\gamma^+ \lambda^+$ .

Tonsillar B cells were isolated by Ficoll-Hypaque gradient centrifugation, followed by T cell depletion by E-rosetting twice (5). Monocytes/macrophages were depleted from B cells by counterflow centrifugal elutriation as described (5). The final B cell preparations contained <0.1% macrophages and >95%  $Ig^+$  and >98%  $CD20^+$  cells, as determined by fluorescence measurements on a flow cytometer (Cytofluorograf IIs, model 50H; Ortho Diagnostic Systems, Inc., Westwood, MA). Tonsillar T cells were isolated by E-rosetting twice. These cells were >95%  $CD2^+$  and  $CD3^+$ .

**Northern Blot Analyses.** Northern blot analyses using RNA prepared from guanidinium thiocyanate ultracentrifugation have been described (5). A 3.4-Kb human genomic TNF Pst-I fragment (5) and a human TNF cDNA clone from Drs. B. Beutler (Howard Hughes Medical Institute, Dallas, TX) and D. Caput (Chiron Research Laboratories, Emeryville, CA) were used in Northern blots. The  $C\kappa$  (Sac I fragment; reference 10) and  $C\lambda$  (Bam HI-Hind III fragment; reference 11) probes were subcloned from genomic clones generously provided by Dr. Phillip Leder (Harvard Medical School, Boston, MA). Other cDNA probes have been described (5). In some experiments, equal amounts of exogenous globin mRNA (Bethesda Research Laboratories, Bethesda, MD) were added to each of the samples before guanidinium thiocyanate ultracentrifugation as an internal standard. The recoveries of RNA in the individual samples were compared by probing the blots with an  $\alpha$ -globin probe (pJW101, ATCC) and were found to be essentially identical.

**TNFR1A.** TNF in culture supernatants was quantitated by a sandwich assay using E-43 anti-TNF mAb as the immobilized primary antibody and  $^{125}I$ -labeled F12 anti-TNF mAb (sp act  $\sim 5 \times 10^7$  cpm/ $\mu$ g protein) as the secondary tracer antibody. rTNF (Amgen, Thousand Oaks, CA) was used as standards. The assay was linear up to 500 pg rTNF and the detection limit was  $\sim 2.5$  pg. Nonspecific binding in samples containing goat anti- $\mu$  antibody was blocked by 5% mouse serum during incubation of the plates with the secondary iodinated antibody.

## Results

**B Cell Lines Express TNF mRNA.** A group of well established B cell lines were analyzed for TNF mRNA expression (Fig. 1 A). Three patterns were apparent. The Burkitt lines Daudi, Raji, and Ramos, the pre-B leukemia line Nalm 12, and the EBV-transformed precursor B lymphoblastoid line, Josh 7, did not constitutively express TNF mRNA. PMA induced TNF mRNA in these lines readily. Low levels of TNF mRNA were detected in the unstimulated B lymphoblastoid lines SeD, 8866P, 32a1, and RPMI 1788. Higher levels of TNF mRNA were induced with PMA. The pre-B leukemia line Nalm 6 did not produce TNF mRNA even when stimulated with PMA. An actin probe was included as a control.  $\kappa$  and  $\lambda$  L chain probes confirmed that L chain mRNA was readily detectable. Low levels of L chain mRNA were shown in four pre-B cell lines. They may represent aberrant mRNA because no L chain proteins were detected. Newly established EBV-transformed B cell lines from bone marrow expressed TNF mRNA constitutively, and PMA induced slight to moderate increases in TNF mRNA accumulation (Fig. 1 B).

**Activated Tonsillar B Cells Express TNF mRNA.** Highly purified tonsillar B cell populations with >98%  $CD20^+$  cells were used for analysis. Unstimulated tonsillar B cells in control medium for 24 h did not make TNF mRNA, as shown by Northern blot analysis. B cell mitogens such as PMA, anti- $\mu$  antibody, BCGF, and SAC were used in various combinations. Our goat anti- $\mu$  antibody was weakly mitogenic. It was synergistic with either BCGF or PMA to induce B cell proliferation. The anti- $\mu$  antibody did not induce TNF mRNA (Fig. 2 A, lane 2), nor did BCGF at 5% (data

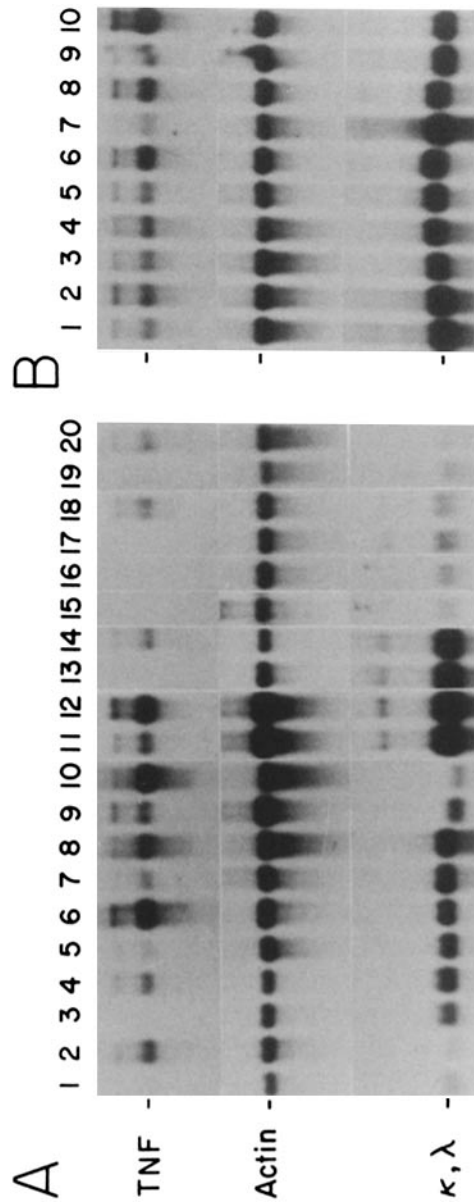


FIGURE 1. Expression of TNF mRNA by human B cell lines. (A) Poly(A)<sup>+</sup> RNA (5 μg/lane) from Raji (lanes 1 and 2), Daudi (lanes 3 and 4), SeD (lanes 5 and 6), 8866P (lanes 7 and 8), 32a1 (lanes 9 and 10), RPMI 1788 (lanes 11 and 12), Ramos (lanes 13 and 14), Nalm-6 (lanes 15 and 16), Nalm-12 (lanes 17 and 18), and Josh-7 (lanes 19 and 20) were analyzed by Northern blot hybridization. Nick-translated TNF, actin, and a mixture of κ and λ inserts were sequentially hybridized to the blot and washed as described (5). Odd number lanes were mRNA from unstimulated cells and even number lanes were mRNA from cells (10<sup>6</sup>/ml) stimulated for 4 h with PMA. (B) Northern blots of poly(A)<sup>+</sup> RNA from bone marrow-derived EBV-transformed cell lines WIH8 (lanes 1 and 2), WIH10 (lanes 3 and 4), WIH14 (lanes 5 and 6), WIH20 (lanes 7 and 8), and WIH67 (lanes 9 and 10). The stimulation of cells and the analyses of mRNA were as in A.

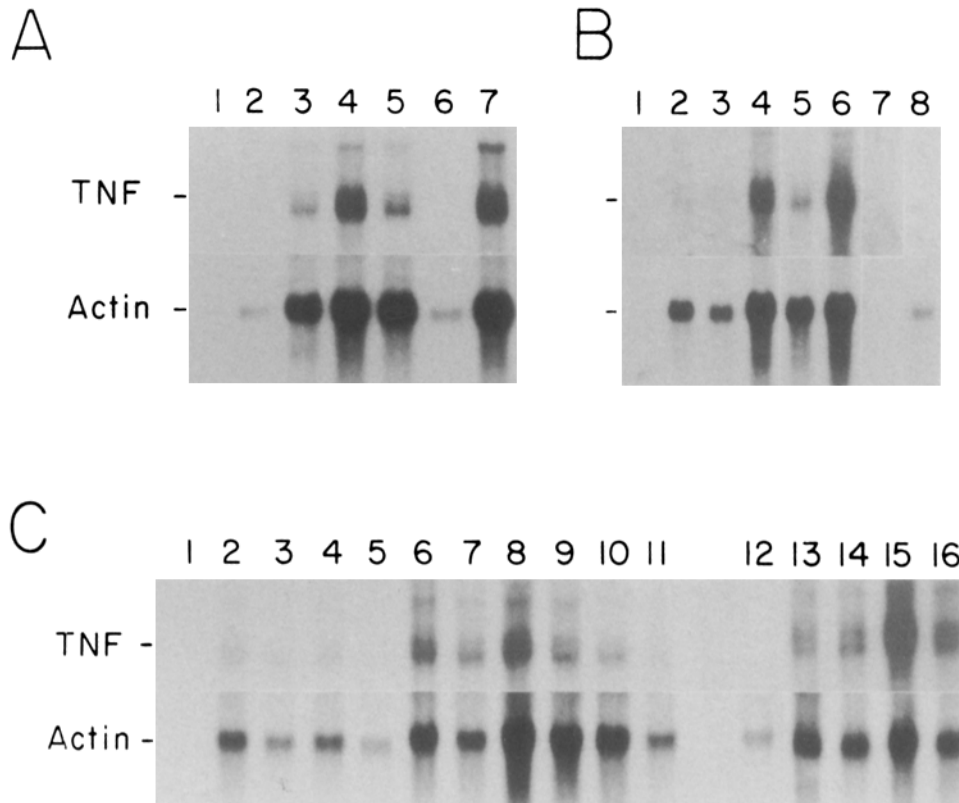


FIGURE 2. TNF mRNA expression in tonsillar B cells stimulated by anti- $\mu$  (A) and SAC (B), and inhibition of these stimulations by CsA (C). Purified tonsillar B cells were resuspended at  $10^6$ /ml and treated for 24 h with the drugs indicated. The total RNA was isolated by ultracentrifugation and analyzed as in Fig. 1. One representative experiment of four similar experiments for A and B, and one of two experiments for C, are shown. (A) Tonsillar B cells ( $1.7 \times 10^7$  cells/sample) were treated with control medium (lane 1), anti- $\mu$  (lane 2), PMA (lane 3), PMA plus anti- $\mu$  (lane 4), PMA plus BCGF (lane 5), anti- $\mu$  plus BCGF (lane 6), and PMA plus anti- $\mu$  plus BCGF (lane 7). (B) Tonsillar B cells ( $2.8 \times 10^7$  cells/sample) were treated with control medium (lane 1), SAC (lane 2), PMA (lane 3), PMA plus SAC (lane 4), SAC plus BCGF (lane 5), PMA plus SAC plus BCGF (lane 6), SAC plus anti- $\mu$  (lane 7), and SAC plus anti- $\mu$  plus BCGF (lane 8). (C) Tonsillar B cells (lanes 1-11) and T cells (lanes 12-16) at  $2.1 \times 10^7$  cells/sample were treated with the following reagents: lanes 1 and 12, control medium; lanes 2 and 13, PMA; lane 3, SAC; lanes 4 and 14, PMA plus CsA; lane 5, SAC plus CsA; lane 6, PMA plus SAC; lane 7, PMA plus SAC plus CsA; lane 8, PMA plus anti- $\mu$ ; lane 9, PMA plus anti- $\mu$  plus CsA; lane 10, SAC plus BCGF; lane 11, SAC plus BCGF plus CsA; lane 15, PMA plus anti-CD3; and lane 16, PMA plus anti-CD3 plus CsA. In the lower panel showing actin hybridizations, lanes 1-11 were exposed twice as long as lanes 12-16.

not shown). PMA (Fig. 2 A, lane 3; B, lane 3; C, lane 2) and SAC (Fig. 2 B, lane 2; C, lane 3) stimulated B cells to accumulate low levels of TNF mRNA. Anti- $\mu$  (Fig. 2 A, lane 4; C, lane 8) and SAC (Fig. 2 B, lane 4; C, lane 6) were strongly synergistic with PMA and induced 5-10-fold higher accumulation of TNF mRNA than PMA alone. BCGF increased PMA stimulation about two-fold (Fig. 2 A, lane 5), and SAC stimulation two- to threefold (Fig. 2 B, lane 5). BCGF also slightly

increased (more than twofold) stimulations by PMA plus SAC (Fig. 2 *B*, lane 6) and PMA plus anti- $\mu$  (Fig. 2 *A*, lane 7), but did not increase anti- $\mu$  stimulation (Fig. 2 *A*, lane 6). The effects of anti- $\mu$  and SAC on TNF mRNA accumulation in tonsillar B cells were not additive (Fig. 2 *B*). In fact, anti- $\mu$  abolished the stimulation by SAC (lane 7), and BCGF plus SAC induced no detectable TNF mRNA in the presence of anti- $\mu$  (lane 8). Total mRNA in these B cells, shown as actin mRNA accumulation, increased with B cell proliferation but did not strictly correlate with TNF accumulation. For example, BCGF plus SAC (Fig. 2 *B*, lane 5) caused a relatively higher increase in actin than TNF mRNA accumulation.

*Time Course of TNF mRNA Accumulation in B Cells.* The kinetics of TNF mRNA accumulation in PMA plus SAC-treated tonsillar B cells was examined (Fig. 3 *A*). Control B cells had no detectable TNF mRNA. mRNA was detectable at 4 h (not shown). At 24 h, substantial amounts of TNF message were accumulating. Maximal accumulation was observed at 32 h. This peak accumulation was maintained up to 48 h, and the message level decreased slowly thereafter until 120 h when a level comparable with that at 8 h was detected (not shown). No IL-2 mRNA could be detected in these stimulated B cells.

To rule out the possibility that the TNF mRNA detected in these B cell preparations was due to contaminating T lymphocytes, the kinetics of TNF mRNA accumulation in T cells stimulated with PMA plus SAC, and PMA plus anti-CD3 were examined. In contrast to B cells, T cells barely responded to PMA plus SAC (Fig. 3 *A*). The small increase in TNF mRNA detected was probably due to the effects of PMA on T cells. However, when stimulated with PMA plus anti-CD3, tonsillar T cells exhibited an increase in TNF mRNA with similar kinetics as B cells, showing peak accumulation at 32–48 h, and a gradual decrease in TNF mRNA over the next 72 h. The peak TNF mRNA accumulation was about twofold over that of B cells on a per cell basis. IL-2 mRNA accumulation showed similar kinetics to TNF mRNA accumulation. The kinetics of TNF mRNA accumulation in B cells stimulated with PMA plus anti- $\mu$  was also examined. Maximal accumulation also occurred at  $\sim$ 48 h (not shown).

The B cell line RPMI 1788, which produced high levels of TNF mRNA when stimulated with PMA, was used to study the time course of TNF mRNA accumulation in B cell lines. PMA-stimulated TNF mRNA accumulation was maximal at 1 h and decreased rapidly with time (Fig. 3 *B*). By 6–8 h, a very low message accumulation above control level was observed. Cells treated with A23187 alone showed no increase in TNF mRNA (not shown). A23187 plus PMA induced two- to threefold increase in TNF mRNA accumulation over that attained with PMA stimulation alone. The maximal accumulation with the combination treatment occurred at between 1 and 2 h. The elevated TNF mRNA level was also more sustained than that in PMA-treated cells.

*Production of TNF by B lymphocytes.* The production of TNF by B lymphocytes was examined by Western blots and a quantitative RIA. The production of TNF by the three cell lines, SeD, RPMI 1788, and WIH8, stimulated with PMA, was examined by Western blots (Fig. 4). The 17-kD TNF protein band absent in blots probed with a control mAb could be detected in the supernatants of all three cell lines. The band at 25 kD in SeD supernatants (Fig. 4, lanes 1 and 2) was due to the crossreactivity of Ig L chain with the secondary goat anti-mouse antibody, since

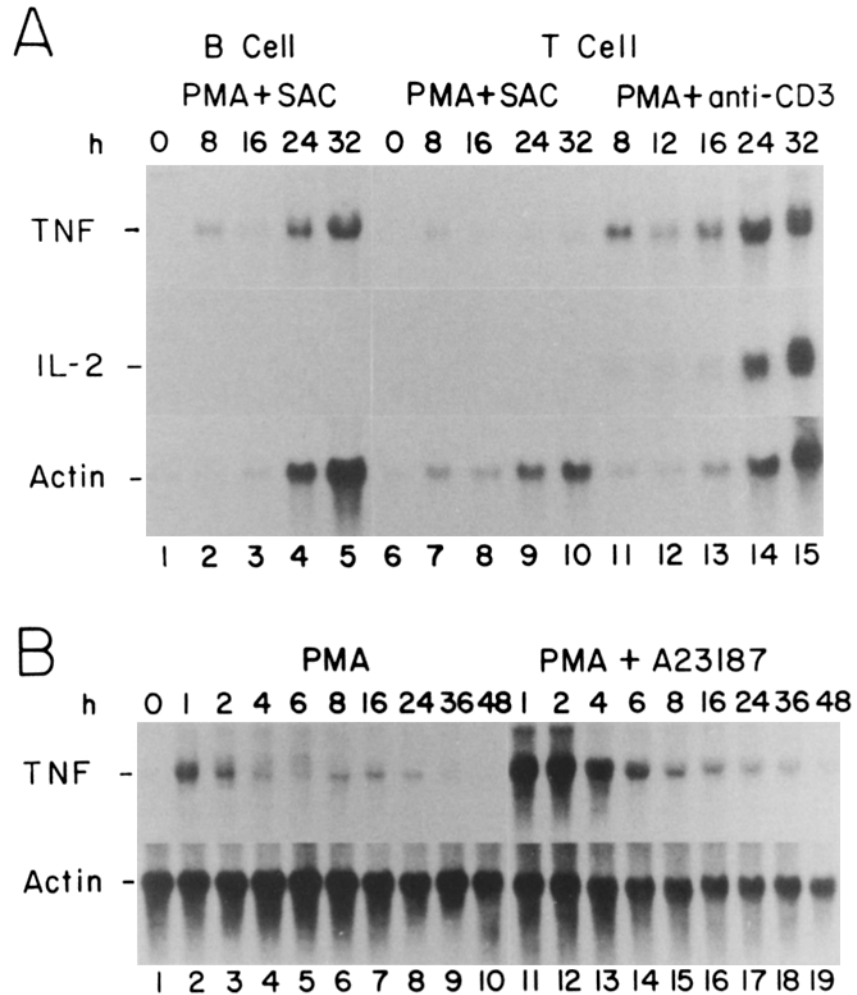


FIGURE 3. Time course of TNF mRNA expression in tonsillar B and T cells (A) and the B cell lines RPMI 1788 (B). (A) Tonsillar B cells (lanes 1-5) and T cells (lanes 6-15) at  $10^6$  cells/ml (20 ml per sample) were treated for the indicated time period with PMA plus SAC (lanes 1-10) or PMA plus anti-CD3 (lanes 11-15), and their total RNA was analyzed by Northern blots. The same blot was probed successively with TNF, IL-2, and actin probes. One of three similar experiments is shown. (B) RPMI 1788 cells were suspended at  $2.5 \times 10^5$ /ml and treated with PMA (lanes 2-10) or PMA plus A23187 (lanes 11-19) for the indicated time period. Total RNA (20  $\mu$ g/lane) was analyzed by Northern blots. The figure shows one of two similar experiments.

the presence of human Ig fraction during incubation with the secondary antibody blocked this staining (Fig. 4, lane 3). Tonsillar B cells stimulated with PMA plus SAC also produced the 17-kD TNF detected by Western blotting (Fig. 4 B). The quantity was less than that produced by the three B cell lines examined, as discussed later.

A quantitative RIA was used to measure the amount of TNF produced by B cells. The B cell lines RPMI 1788, and the EBV-transformed bone marrow-derived B

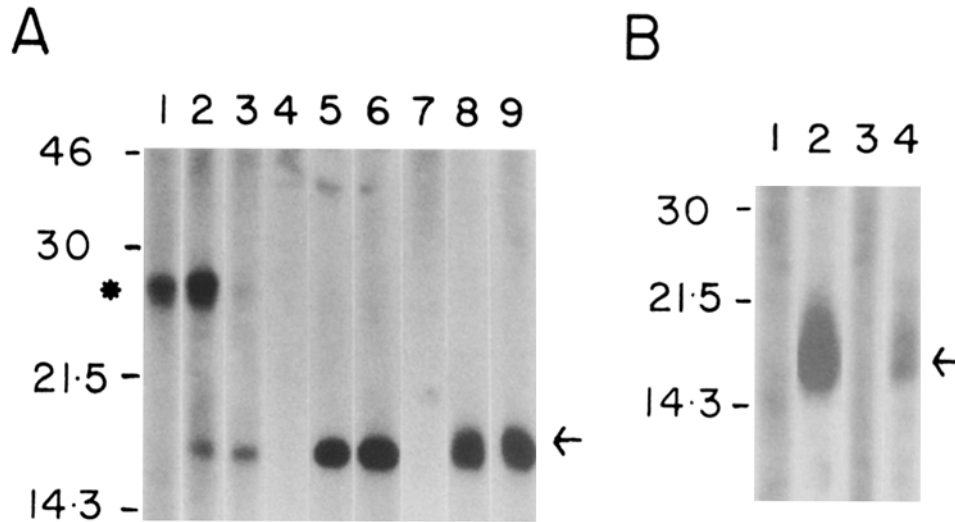


FIGURE 4. Western blot of supernatant proteins isolated from B cell lines and tonsillar B cells. (A) Supernatants from SeD (lanes 1-3), RPMI 1788 (lanes 4-6), and WIH8 (lanes 7-9) stimulated with PMA in serum-free RPMI 1640 medium for 24 h were concentrated and analyzed by Western blots (5). Lanes 1, 4, and 7 were treated with a control antibody (HDP-1) and lanes 2, 3, 5, 6, 8, and 9 were treated with C16 anti-TNF mAb. In lanes 3, 6, and 9, crossreactivities with the secondary antibody were blocked with human gamma globulin fraction (1%). (B) Tonsillar B cells ( $10^8$  total) were resuspended at  $2 \times 10^6$ /ml and prestimulated with PMA plus SAC in RPMI 1640 with 10% FCS for 24 h. The cells were washed and resuspended in serum-free medium containing stimulants. Serum-free supernatants collected over a 3-d period with daily medium changes were concentrated and analyzed by Western blots. Lanes 1 and 2 contained protein from WIH8 supernatants and lanes 3 and 4 were tonsillar B cell supernatant proteins. Lanes 1 and 3 were probed with control mAb HDP-1 while lanes 2 and 4 were probed with C16 anti-TNF mAb.

cell lines WIH8 and KAW15 at  $10^6$  cells/ml, produced 5-7 ng/ml TNF in 24 h when cultured in medium containing 10 ng/ml PMA (Table I). The combination of PMA and A23187 did not further increase the amount of TNF produced within this 24-h period, although the TNF mRNA level in these cells was higher (Fig. 3 B). The amount of TNF produced by these cell lines is comparable with that produced by a myeloid cell line, ML-1. Molt-4, a T cell line that can be stimulated to synthesize LT, but not TNF mRNA, did not produce any detectable TNF. Interestingly, little TNF could be detected in the supernatants of the unstimulated B cell lines, though they accumulated moderate levels of TNF mRNA (Fig. 1).

The production of TNF by tonsillar B cells stimulated with various mitogens was examined (Table II). Control B cells and B cells stimulated with anti- $\mu$ , or anti- $\mu$  plus SAC, did not produce any detectable amount of TNF. PMA or SAC alone stimulated B cells to produce 100-300 pg/ml of TNF. PMA plus anti- $\mu$  and PMA plus SAC, which stimulated the accumulation of high levels of TNF mRNA in B cells, induced the synthesis of TNF, and 400-1,500 pg/ml TNF was found in the medium. The combination of the three stimulants, PMA, anti- $\mu$ , and SAC, caused a further twofold increase in medium TNF to a maximal concentration of 2,700 pg/ml. The maximal TNF concentration in medium occurred on the second day of culture.

TABLE I  
*TNF Concentration in Supernatants of B Cell Lines*

Supernatants	TNF concentration		
	No stimulus	PMA	PMA + A23187
		<i>pg/ml</i>	
RPMI 1788	38 ± 3	4,730 ± 110	3,920 ± 300
WIH8	470 ± 10	7,150 ± 70	7,540 ± 30
KAW15	120 ± 3	5,160 ± 90	5,380 ± 30
ML-1	<25	6,630 ± 450	5,530 ± 180
Molt-4	<25	<25	<25

Cells were resuspended in medium containing 10% FCS at  $10^6$ /ml and treated as indicated for 24 h. TNF concentrations in supernatants were measured by RIA and expressed as mean ± SD.

The production of TNF by B cells was compared with that by T cells and macrophages. T cells stimulated with PMA plus anti-CD3 or PMA plus 9.3 (an mAb against CD28), accumulated 5–10-fold more TNF in the medium. These values are comparable with those published by Cuturi et al. (4). Peripheral blood monocytes stimu-

TABLE II  
*Concentrations of TNF in the Supernatants of Tonsillar B Cells and T Cells, and Peripheral Blood Monocytes*

Supernatants	TNF concentration*		
	1 d	2 d	3 d
		<i>pg/ml</i>	
<b>Tonsillar B cells</b>			
C	<25	<25	<25
Anti- $\mu$	<25	<25	<25
Anti- $\mu$ + SAC	<25	<25	<25
PMA	140 ± 6	260 ± 8	160 ± 1
SAC	270 ± 8	280 ± 10	170 ± 18
PMA + anti- $\mu$	370 ± 1	1,550 ± 17	650 ± 9
PMA + SAC	1,050 ± 11	1,450 ± 53	400 ± 14
PMA + anti- $\mu$ + SAC	840 ± 10	2,700 ± 118	1,440 ± 45
<b>Tonsillar T cells</b>			
C	<25	<25	<25
Anti-CD3	<25	<25	<25
PMA	ND	ND	221 ± 50
PMA + anti-CD3	7,660 ± 260	14,090 ± 192	22,840 ± 526
<b>Peripheral blood monocytes</b>			
C	130 ± 3	ND	ND
LPS	480 ± 51	ND	ND
IFN- $\gamma$	250 ± 5	ND	ND
LPS + IFN- $\gamma$	3,140 ± 100	ND	ND
PMA†	11,770 ± 310	ND	ND

B and T cells were purified as described in Materials and Methods. Monocytes were purified by percoll gradients (5). Cells were suspended at  $10^6$ /ml and treated with the indicated reagents. The results represent one of three similar experiments.

\* Cell supernatants (100  $\mu$ l) in duplicates were assayed for TNF by an RIA as described in Materials and Methods. Values represent mean ± SD.

† PMA was used at 1 ng/ml.



lated with IFN- $\gamma$  produced little TNF. LPS, or IFN- $\gamma$  plus LPS, stimulated monocytes to produce 0.5–3 ng/ml TNF. PMA, though toxic to monocytes, caused the release of  $\sim$ 12 ng/ml TNF. Monocytes without added stimuli also secreted some TNF.

*Inhibition of B Cell TNF Expression by CsA.* Since CsA inhibited the expression of IL-2 and IFN- $\gamma$  mRNA in T lymphocytes and B cell proliferation (12–14), the effects of CsA on TNF mRNA expression were examined. CsA did not affect the TNF mRNA level in cells stimulated with PMA alone in both B and T cells but abolished the expression of TNF mRNA in cells stimulated with SAC or BCGF plus SAC (Fig. 2 C, compare lane 3 with 5 and lane 10 with 11). The potentiation effects of PMA stimulation of TNF mRNA accumulation by anti- $\mu$  or SAC in tonsillar B cells and anti-CD3 in tonsillar T cells were markedly depressed by 5  $\mu$ g/ml CsA (Fig. 2 C, compare lanes 6, 8, and 15 with lanes 7, 9, and 16, respectively).

### Discussion

In this study, TNF production by human B cells at both the mRNA and protein levels were documented. A large panel of B cell lines at differing stages of differentiation was shown to make TNF either constitutively or with the addition of PMA. The report of Steffen et al. (6) similarly showed that RPMI 1788 made TNF mRNA. Nalm-6, a pre-B leukemia cell line, was the only line found not to make TNF even when stimulated with PMA. Tonsillar B cells extensively depleted of monocytes and T cells were shown to synthesize TNF in response to B cell mitogens. The purity of B cell populations and their responsiveness to B cell mitogens in this system made it unlikely that TNF production was due to very minor contaminating cells. Thus, TNF production by activated B cells is demonstrated.

Recently, TNF has been shown to enhance the proliferation of SAC-activated tonsillar B cells (2). Our finding that activated B cells produce TNF further implicates the importance of this factor in B cell responses. The decrease in TNF concentrations in supernatants of stimulated tonsillar B cells on day 3 may indicate that TNF produced by these B cells was used. Limited attempts to block the proliferative responses of B cells to PMA plus anti- $\mu$  antibody or PMA plus SAC with anti-TNF mAb were not successful. TNF has been shown to enhance the proliferation induced by allogeneic cells, and polyclonal anti-TNF antibody block the MLR (3). Thus, it remains possible with different anti-TNF antibodies and more physiological stimuli to demonstrate that TNF plays some role in B cell proliferation.

LT is closely related to TNF in structure and function. It has been shown to be important in B cell proliferation (15). We have also shown that LT is produced by activated B cells (Sung, S., L. Jung, J. Walters, E. Jeffes, III, G. Granger, and S. Fu, unpublished results). In addition to TNF and LT, activated B cells are capable of secreting IL-1 (16, 17), IFN- $\alpha$  (18), and BSF-2/IL-6 (19). Thus, it appears that B cells are an important cell type for cytokine production.

The TNF concentrations measured in the supernatants of B cells reflect the difference between the rates of synthesis and utilization of TNF by the B cells. Though the levels of TNF mRNA in B cells and T cells and their rates of TNF secretion are not directly comparable because of the requirement of different stimuli for different cell types, these values do provide an estimate of the potential of each cell type to synthesize TNF under immunologically responsive conditions *in vivo*. It seems that T lymphocytes have a much higher potential to synthesize TNF, and along with

macrophages, may indeed be the major cell types responsible for TNF production in general. However, under situations in which B cell-specific stimuli are present, or in areas of high B cell concentration, TNF production by B cells may be important. Of relevance to the latter situation is the finding that TNF occurs in a membrane bound form on macrophages (20, 21). Whether this form is on B cell membranes and acts on adjacent cells via cell-cell contact is currently under investigation.

The kinetics of TNF mRNA accumulation in activated tonsillar B and T cells are similar, peaking at 32-48 h (Fig. 3 A). The time course for IL-2 mRNA accumulation in tonsillar T cells and for LT mRNA accumulation in both tonsillar B and T cells is similar to that of TNF. With a different mitogen, namely PHA, peak IL-2 mRNA accumulation has been reported to be at 24 h for tonsillar T cells (22). In human peripheral blood T cells, PMA plus anti-CD3 caused maximal TNF mRNA accumulation at ~4 h (5), and PMA plus PHA-stimulated T cells accumulated IFN- $\gamma$  and IL-2 mRNA at ~12 h (13). PHA-primed T lymphoblasts expressed IFN- $\gamma$  and IL-2 mRNA maximally 3 h after restimulation (13). These kinetics of cytokine accumulation in peripheral blood T cells are quite different from that in tonsillar B and T cells. The kinetics of TNF mRNA accumulation is also different in B and T cell lines. The B cell line RPMI 1788 (Fig. 3 B) and the T cell lines CEM and SKW3 (5) responded to stimuli much more quickly and showed peak TNF accumulation at 1-2 h. The response of these cell lines to PMA is more pronounced than either tonsillar or peripheral T and B cells. Similarly, the T cell line Jurkat showed peak IL-2 and IFN- $\gamma$  accumulation 4-6 and 4-8 h after PHA plus PMA stimulation, respectively (23). The different kinetics in cell lines may be due to the fact that transformation makes cells respond faster to stimuli in expressing their lymphokine genes. Thus, the kinetics of cytokine mRNA expression is related to cellular origin, stimuli used, and the state of activation of the cells.

The accumulation of TNF mRNA in a number of B cell lines with different Ig phenotypes was examined in an attempt to correlate TNF expression with different stages of B cell differentiation. Three interesting observations were made in this set of experiments. First, the pre-B cell lines, Josh-7, Nalm-6, and Nalm-12, made little or no detectable TNF mRNA even when stimulated with PMA. These results would lend support to the hypothesis that TNF expression in B cells is developmentally regulated. Second, a number of recently established EBV-transformed bone marrow-derived B cell lines accumulated higher levels of TNF mRNA even when they were not stimulated. This is in agreement with reports showing that EBV activates B cell to express their cytokine genes (17, 18, 24). Third, the TNF levels in certain EBV-transformed cell line supernatants did not correlate with the cellular TNF mRNA level (Fig. 1 B and Table I). This may be due to the same translational control of TNF production that is operational in mouse macrophages (25).

CsA selectively inhibits the responses of cells to agents that raise the level of intracellular-free  $Ca^{2+}$  (12). B cell proliferation in response to anti- $\mu$ , SAC, or BCGF was inhibited by CsA (14). Our results showed that the responses of B lymphocytes to CsA in cytokine production were similar to that of T cells. CsA inhibited the direct effect of SAC and the synergistic effects of anti- $\mu$  or SAC with PMA to increase TNF mRNA expression in much the same way as the inhibition of anti-CD3 responses in T cells, but did not affect the stimulation of tonsillar B and T cells by PMA alone (Fig. 2 C). These results support the notions that stimulation by anti- $\mu$

or SAC in B cells is analogous to stimulation by anti-CD3 in T cells and that CsA has less effects on cellular events related to PKC activation.

### Summary

The production of TNF/cachectin by human B cell lines and tonsillar B cells was examined. Of the 15 B cell lines examined, 9 cell lines synthesize TNF mRNA constitutively. PMA stimulated most cell lines to accumulate increased amounts of TNF. SeD, 8866P, 32a1, RPMI 1788, and four bone marrow-derived EBV-transformed cell lines accumulated high levels of TNF mRNA when stimulated by PMA. TNF production by these cell lines was examined. RPMI 1788 and WIH8 produced little TNF constitutively, but synthesized 5–7 ng/ml TNF when stimulated by PMA. A pre-B cell line, Nalm-6, did not synthesize any detectable amount of TNF mRNA, even with PMA stimulation. Tonsillar B cells could also be stimulated to produce TNF. PMA or *Staphylococcus aureus* Cowan I strain (SAC) alone stimulated some TNF mRNA accumulation, whereas B cell growth factor (BCGF) or anti- $\mu$  did not. This accumulation was synergistically elevated by the combinations of PMA and SAC, or PMA and anti- $\mu$ . BCGF increased PMA-, SAC-, PMA plus SAC-, or PMA plus anti- $\mu$ -induced TNF mRNA accumulations about twofold. The accumulation of TNF mRNA in tonsillar B cells stimulated by PMA plus SAC was between 32 and 48 h, the same peak interval as the accumulation of TNF and IL-2 mRNA in tonsillar T cells. This is in contrast to PMA or PMA plus A23187-stimulated RPMI 1788 cells in which TNF mRNA accumulation was maximal at 1–2 h. TNF activities found in tonsillar B cell supernatants correlated with the TNF mRNA levels in the cells. However, more TNF activity was found on the second-day than the third-day supernatants, indicating active TNF uptake by the B cells. Cyclosporin A (CsA) inhibited SAC and anti- $\mu$  responses in B cells in much the same way as the anti-CD3 responses in T cells. SAC-, PMA plus SAC-, and PMA plus anti- $\mu$ -stimulated, but not PMA-stimulated, increases in TNF mRNA accumulations in tonsillar B cells were inhibited by CsA. TNF production seems to increase in parallel with B cell proliferation, but the relationship of these two functions needs to be further examined.

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