

# Tingible Body Macrophages in Regulation of Germinal Center Reactions

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Tingible body macrophages (TBM), long thought simply as scavengers of apoptotic lymphocytes, are located in the unique microenvironment of germinal centers in close proximity to antigen-retaining follicular dendritic cells (FDC). Observations that TBM endocytose FDC-iccosomal (immune-complex coated bodies) antigen suggested that TBM might present this antigen and help regulate the germinal center reaction. To test for antigen presentation, the ovalbumin (OVA)-specific T<sub>H</sub> hybridoma, 3DO-54.8, which produces IL-2 on receiving effective presentation of OVA, were used as responders to OVA-bearing TBM. Results showed that OVA-bearing TBM failed to induce IL-2 production. Furthermore, addition of TBM to IL-2-inducing positive controls (B cells) not only failed to augment IL-2 production, but rather TBM significantly (55-90%) reduced B-cell induction of IL-2. We found that TBM were rich in prostaglandin by comparison with other nongerminal center lymph node macrophages and that addition of indomethacin to the cultures reversed the inhibitory effect of TBM. Depletion of TBM from enriched preparations, prior to addition to positive control cultures, also abrogated the inhibitory effect on IL-2 production. These data support the concept that TBM, within the unique microenvironment of germinal centers, may be specialized to downregulate the germinal center reaction.

*Keywords:* Antigen presentation, apoptosis, iccosomes, macrophage, microenvironment, prostaglandin

## INTRODUCTION

Macrophages represent a heterogenous population, and macrophage functions are influenced by the microenvironment in which they reside. By definition, tingibile body macrophages (TBM), a subset of the mononuclear phagocytes, are unique, large phagocytic cells that reside in germinal centers of secondary

lymphoid tissues (Flemming, 1885). TBM contain many phagocytized, apoptotic cells (referred to as tingibile bodies) (Flemming, 1885) in various states of degradation (Swartzendruber and Congdon, 1963). Unique to this subset of macrophages is the germinal center microenvironment, characterized functionally by long-term antigen retention on follicular dendritic cells, antigen presentation by B cells to T-helper cells,

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TABLE I Antigen Presentation of *In Vivo* Obtained and Exogenous Antigen by TBM-Enriched Cells

Experiment no.	TBM <sup>a</sup>	TBM + OVA <sup>b</sup>	IL-2 control <sup>c</sup>
1	1,240 ± 165 <sup>d</sup>	25,000 ± 1,800	322,000 ± 5,900
2	190 ± 50	250 ± 10	181,000 ± 1,400
3	300 ± 90	950 ± 120	364,000 ± 10,240
4	770 ± 100	4,340 ± 40	60,000 ± 3,600

<sup>a</sup>1.0 × 10<sup>5</sup> TBM-enriched cells were cultured for 24 hr with 3 × 10<sup>4</sup> 3DO-54.8 T<sub>H1</sub> cells. Results shown were always greater than control cultures of 3DO cells or TBM alone.

<sup>b</sup>1.0 × 10<sup>5</sup> TBM-enriched cells and 50 μg OVA were cultured for 24 hr with 3 × 10<sup>4</sup> 3DO-54.8 T<sub>H1</sub> cells.

<sup>c</sup>3 × 10<sup>4</sup> CTL cells were cultured with either 50 μl IL-2 containing Con A supernatant or 50 μM human rIL-2.

<sup>d</sup>Data represent <sup>3</sup>H-thymidine incorporation by 3 × 10<sup>4</sup> IL-2-dependent CTLL cells and are expressed as mean cpm ± standard errors of triplicate cultures.

a high rate of somatic mutations, affinity maturation, induction of antibody-forming cells and memory B-cell development (Coico *et al.*, 1983; Szakal *et al.*, 1989; Tew *et al.*, 1990). Although the scavenging function of TBM in germinal centers is obvious, it has also been suggested that TBM may be important in initiating the germinal center reaction (Kamperdijk *et al.*, 1978, 1982).

Regarding the germinal center reaction, it has been shown that germinal center B cells are extremely effective antigen-presenting cells. Three to eight days after a booster immunization, germinal center B cells obtain antigen from FDCs in the form of immune complex coated bodies (iccosomes) and are capable of processing and presenting the iccosomal antigen to T cells in an MHC-restricted manner (Kosco *et al.*, 1988; Szakal *et al.*, 1988). Typically, macrophages phagocytose antigens, process them, express them on class II molecules, and present the processed antigen to T-helper cells. Observations that TBM, like germinal center B cells, also endocytosed iccosomes (Szakal *et al.*, 1988) and that many TBM are class II positive (Smith *et al.*, 1988), suggest that TBM might also be able to present the FDC-derived antigen to T cells (Smith *et al.*, 1988).

The objectives of the present study was to determine if TBM can indeed present antigen and if antigen presentation by TBM may be implicated in the regulation of the germinal center reaction. The results showed that TBM-enriched preparations were not good antigen presenters but were highly effective inhibitors of T-cell lymphokine secretion induced by B cells. Selective depletion of TBM from the enriched

preparations and/or addition of the prostaglandin synthesis inhibitor indomethacin to the cultures restored lymphokine activity. Histochemical observations confirmed that TBM are a rich source of prostaglandins. Thus, these data are consistent with the concept that the microenvironment of germinal centers favors an inhibitory role for TBM in the regulation of the germinal center reaction via a prostaglandin-mediated mechanism.

## RESULTS

### Antigen Presentation

Germinal center B cells obtain antigen from FDC for processing and presentation to T cells during a period between 3 and 8 days after booster immunization. Typically, numerous germinal center B cells (20%) were present in the TBM preparation and both TBM and germinal center B cells take up iccosomes (Kosco *et al.*, 1988; Szakal *et al.*, 1988). Therefore, we reasoned that the addition of TBM-enriched cells from an OVA-immune animal to the OVA-specific T-cell hybridoma 3DO-54.8 should result in antigen presentation and IL-2 production. As shown in Table I, there was no evidence of presentation of *in vivo* obtained antigen by the TBM preparation. When antigen was added *in vitro* (TBM + OVA), to be certain that antigen was not limiting, a significant ( $p < 0.01$ ) but very modest level of IL-2 production was detected in two experiments (Table I, Experiments 1 and 4). In both cases, the production of IL-2 was less

TABLE II Effect of TBM-Enriched Cell Preparations on Antigen Presentation by B Cells

Experiment no.	B cells + OVA <sup>a</sup>	B cells + OVA + TBM <sup>b</sup>	% Suppression	IL-2 control <sup>c</sup>
1	≈165,000 <sup>d</sup> ± 9,300	16,000 ± 600	90.0	322,000
2	≈8,800 ± 330	2,700 ± 300	70.0	22,000
3	✓132,000 ± 8,300	13,550 ± 1,600	90.0	102,000
4	✓96,400 ± 5,700	42,700 ± 6,350	55.0	60,000

3DO-54.8 T<sub>H</sub> cells ( $3 \times 10^4$ ) were cultured for 24 hr with:

<sup>a</sup>Germinal center B cells ≈ ( $10^6$ ) or TA3 B-cell hybridoma ✓ ( $10^4$ ) and 50 μg OVA (positive control cultures).

<sup>b</sup>Footnote a and  $1 \times 10^5$  TBM-enriched cells.

<sup>c</sup> $3 \times 10^4$  CTLL cells were cultured with either 50 μl IL-2 containing Con A supernatant or 50 μM human rIL-2.

<sup>d</sup>Data represent <sup>3</sup>H-thymidine incorporation by  $3 \times 10^4$  IL-2-dependent CTLL cells and are expressed as mean cpm ± standard errors of triplicate cultures. In Experiments 1 to 3, the values for 3DO cells or TBM cultured alone were always less than 500. In Experiment 4, the values for cultures of 3DO alone and TBM alone were  $1532 \pm 67$  and  $1087 \pm 53$ , respectively.

TABLE III Indomethacin Relieves Suppression Associated with TBM-Enriched Cells

Exp. no.	B cells + OVA <sup>a</sup> (positive control)	B cells + OVA + TBM <sup>b</sup>	% of positive control cultures	B cells + OVA TBM + INDO <sup>c</sup>	% of positive control cultures
1	≈7,100 ± 700	2,600 ± 750	37	9,076 ± 650	129
2	≈8,800 ± 300	2,700 ± 320	30	7,260 ± 200	82
3	✓132,000 ± 8,000	13,500 ± 1,600	10	81,000 ± 1,000	61
4	✓96,400 ± 5,700	42,700 ± 6,350	40	66,300 ± 10,300	69

3DO-54.8 T<sub>H1</sub> cells ( $3 \times 10^4$ ) were cultured for 24 hr with:

<sup>a</sup>Germinal center B cells ≈ ( $10^6$ ) or TA3 B-cell hybridoma ✓ ( $10^4$ ) and 50 μg OVA.

<sup>b</sup>Footnote a and  $1 \times 10^5$  TBM-enriched cells. Values for 3DO cells and TBM alone were always less than 500 cpm.

<sup>c</sup>Footnote b and 100 μM indomethacin.

<sup>d</sup>Data represent <sup>3</sup>H-thymidine incorporation by  $3 \times 10^4$  IL-2-dependent CTLL cells and are expressed as mean cpm ± standard errors of triplicate cultures.

than 10% of the response attained in the control when a near optimal level of IL-2 was added.

To determine if TBM might suppress antigen presentation, TBM-enriched cells were cocultured with 3DO cells and B cells in the presence of OVA. The results from four experiments are shown in Table II. A 55-90% suppression of the IL-2 response was observed when TBM-enriched populations were added.

### Effect of Indomethacin on TBM Suppression of Antigen Presentation

Macrophages are a major source of prostaglandins and generally the larger most phagocytically active macrophages are the most prolific prostaglandin producers (Lee and Berry, 1977; Lee and Wong, 1982; Guidos et al., 1987). The striking phagocytic

activity of TBM and their large size prompted us to reason that TBM might suppress through a prostaglandin-dependent mechanism. To begin testing this, the prostaglandin synthesis inhibitor indomethacin was added to cultures containing TBM-enriched cells, B cells, and 3DO cells in the presence of OVA. As shown in Table III, the addition of 100 μM indomethacin relieved most of the suppression of IL-2 production in TBM-containing cultures. Controls showed that indomethacin did not inherently augment the IL-2 response in positive control cultures. Furthermore, indomethacin did not inherently increase the proliferation of CTLL over background levels (data not shown).

### TBM and Prostaglandins

PGE<sub>2</sub> is made by a membrane-bound prostaglandin synthetase. We reasoned that antibody specific for

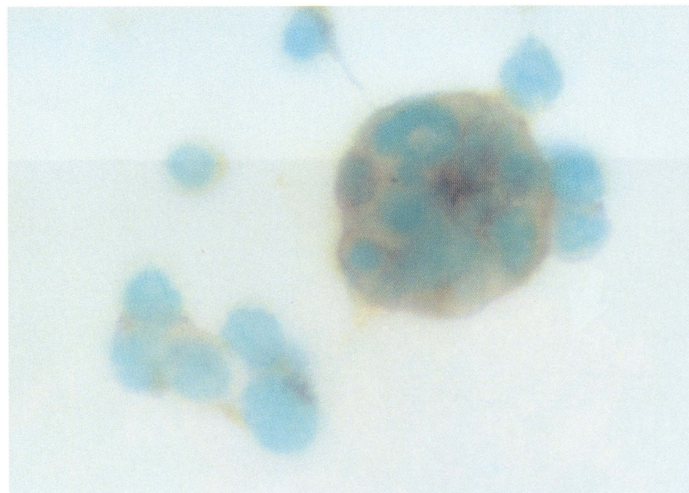


FIGURE 1 Immunocytochemical reactivity of TBM for prostaglandins. Prostaglandin reactivity was detected on enriched preparations of TBM using indirect peroxidase immunocytochemistry. Goat anti-human PGE<sub>2</sub> was used as the primary antibody and peroxidase-conjugated rabbit anti-Goat IgG was used as the secondary antibody. The reactivity of this TBM is demonstrated by the characteristic brown precipitate of DAB. Note that the surrounding lymphocytes are unlabeled. Methyl green background stain. Magnification:  $\times 1994$ . (See Color Plate XI)

PGE<sub>2</sub> might be able to react with membrane associated PGE<sub>2</sub> in the process of being made and might be detected by immunohistochemistry. The results are shown in Figure 1. TBM were clearly labeled, whereas no other cell type had levels above background. Controls included the addition of normal rabbit immunoglobulin, which did not label TBM, and soluble PGE<sub>2</sub>, which blocked labeling of TBM with the PGE<sub>2</sub>-specific antibody (not shown).

## DISCUSSION

In this study, we sought to determine whether TBM could be involved in regulating the germinal center reaction. Germinal center B cells obtain antigen from FDC and then process this antigen, which may be presented to T cells (Kosco *et al.*, 1988; Szakal *et al.*, 1988). It is believed that antigen presentation by germinal center B cells is a major event in initiating the germinal center reaction. In this study, only minimal antigen presentation was detectable when antigen was added to the TBM preparation even

though it contained germinal center B cells, which should have obtained antigen from follicular dendritic cells (Szakal *et al.*, 1988). More striking, the TBM-enriched cells markedly suppressed IL-2 production when added to cultures where germinal center B cells or other B cells were serving as the antigen-presenting cells. Indomethacin was able to alleviate this suppression, thus implicating a prostaglandin-mediated mechanism of suppression. Furthermore, depletion of TBM from the enriched population removed most of the suppressive activity, implying that TBM represent the major source of prostaglandin-mediated suppression of IL-2 production. The importance of this result is emphasized by the fact that TBM are typically the only macrophage type present in germinal centers and were the only cells that showed reactivity for prostaglandins in immunocytochemical tests. In addition, TBM may also limit the germinal center response by removing antigen released by FDC in the form of iccosomes (Szakal *et al.*, 1988). In short, these findings support the hypothesis that TBM are not just scavengers of apoptotic lymphocytes, but may serve as regulatory cells and may downregulate the germinal center reaction.

Depending on the stage of maturation, state of activation, and source, macrophages have been reported to either enhance or suppress immune responses (Stenson and Parker, 1980). Classically, macrophages are believed to be important accessory cells for antigen presentation (Rosenthal and Shevach, 1973a, 1973b; Unanue, 1981). A requirement for macrophages to function as accessory cells is the expression of MHC products. In contrast, in many cases, it has been shown that macrophages do not function as accessory cells but instead suppress immune reactions (Parkhouse and Dutton, 1966). In a series of publications by Lee and colleagues, it was suggested that the functional heterogeneity expressed by macrophages in the modulation of immune responses can be differentiated according to macrophage size as measured by velocity sedimentation rates (Lee and Berry, 1977; Lee and Wong, 1982; Guidos et al., 1987). Antigen-presenting activity was found to be characteristic of small macrophages, whereas large macrophages tended to be immunosuppressive. Furthermore, large macrophages that were induced to express class II molecules also exhibited suppressive effects on immune responses. Thus, the large size of TBM, even though they express class II (Smith et al., 1988) is compatible with the hypothesis that TBM may have a suppressive function to downregulate or limit the germinal center reaction.

Prostaglandins appear to be responsible for much of the immunosuppression mediated by macrophages. The suppressive effects of prostaglandins on immune responses are diverse and have been reviewed (Stenson and Parker, 1980; Goodwin and Ceuppens, 1983). The binding of immune complexes by macrophages stimulates phagocytosis, which in turn stimulates prostaglandin synthesis with the major product being PGE<sub>2</sub> with smaller amounts of PGF<sub>1</sub> (Bonney et al., 1979). This immune-complex stimulation may help explain the high level of prostaglandin in TBM since they may encounter immune complexes directly on the processes of FDC and in the form of iccosomes (Szakal et al., 1988). The data in this study show that indomethacin, an inhibitor of prostaglandin synthesis,

abrogates the suppressive effect of the TBM preparation on B-cell antigen presentation. Controls indicated that indomethacin did not inherently augment the IL-2 response in the assay system (data not shown). A known suppressive effect of prostaglandins on immune responses is the inhibition of lymphokine synthesis by T<sub>H</sub> cells. Whereas most of the lymphocytes in germinal centers are B cells, a small number of T cells are present and most of these T cells are of the helper phenotype (Rouse et al., 1982a, 1982b). Interestingly, a close association between TBM and germinal center T cells has been observed (Nieuwenhuis and Opstelten, 1984, and unpublished).

Germinal centers are sites of memory B-cell production (Coico et al., 1983) and production of memory B cells involves somatic mutation and maintenance of B cells that express high-affinity receptors for the stimulating antigen (Wysocki et al., 1986). It has been suggested that B cells with low-affinity antigen receptors cannot compete for antigen persisting on FDC and are clonally deleted (Wysocki et al., 1986). The tingible bodies in TBM may represent phagocytized low-affinity B cells that have been condemned to undergo apoptosis (Manser et al., 1987; Schad and Phipps, 1988). Consistent with this hypothesis, Odartchenko et al. (1966) and Flidner (1967) have reported that the majority of the tingible bodies located in TBM represent cells that have died either during late G<sub>2</sub> phase or just prior to a mitotic event.

It has also been suggested that TBM play an active role in initiating the germinal center reaction (Kampordijk et al., 1978, 1982). This suggestion was based on the observations that TBM initially appeared at the onset of germinal center development and that peak numbers of TBM were seen at peak germinal center development. However, in recent studies, it has been shown that TBM are not absolutely essential for germinal center development. Germinal centers can develop in old mice in the absence of TBM (Smith et al., 1990). The data shown in this report are consistent with a regulatory role for TBM and it appears that TBM are more likely to down regulate than to stimulate the germinal center reaction.

## MATERIALS AND METHODS

### Animals

Ten- to sixteen-week-old female Balb/C (H-2<sup>d</sup>) mice were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed under pathogen-free conditions in standard cages equipped with filter tops and allowed food and water *ad libitum*.

### Cell Lines and mAb

The T<sub>H1</sub> cell hybridoma 3DO-54.8 was a gift from J. Kappler and P. Marrack (National Jewish Hospital, Denver). Cultures of 3DO-54.8 cells (3DO) produce IL-2 when stimulated by the antigen ovalbumin (OVA) recognized in the context of the MHC molecule I-A<sup>d</sup>. The IL-2-dependent cytotoxic lymphocyte line CTLL used to assay IL-2 was provided by P. Allen and E. Unanue (Washington University, St. Louis). The B-cell hybridoma TA3 (I-A<sup>k,d</sup>) was a generous gift from Laurie Glimscher. Supernatants from the hybridoma M3/38 (obtained from Hybritech, San Diego) was used as the source of anti-Mac-2 antibody. Anti-Thy-1 antibody was obtained from Pharmingen (San Diego). Recombinant human IL-2 was obtained from Southern Biotech.

### Immunizations

#### Active immunization.

Germinal center B cells were obtained from ovalbumin (OVA; #A5503, Sigma Chemical, St. Louis)-immune mice. Mice were injected with 0.5 mg of OVA emulsified with complete Freund's adjuvant (CFA, Difco Laboratories, Detroit) behind the neck followed by a second 0.5-mg injection of OVA in CFA 14 days later. Seven days prior to germinal center B-cell isolation, mice received challenge injections of 0.5  $\mu$ g OVA in all footpads. Anti-OVA sera was prepared in rabbits immunized subcutaneously with 1.0 mg OVA emulsified in CFA followed by a booster immunization without CFA. Rabbits were bled via ear veins 7 days after booster immunization.

#### Passive immunization.

Phagocytosis of Freund's adjuvant by TBM and other macrophages can affect their densities and can cause erroneous isolation and the identification of non-TBM macrophages as TBM. Therefore, to obtain a population of TBM not contaminated by Freund's-adjuvant-containing macrophages, mice were passively immunized by intraperitoneal injections of 0.5 ml of rabbit anti-OVA. Eighteen to twenty-four hours later, challenge injections of 8-10  $\mu$ g of OVA diluted in 0.9% phosphate-buffered saline (PBS), pH 7.4, were injected into all four footpads. By using this immunization protocol, germinal center development and TBM were first evident 3 days after the antigenic challenge. Optimal numbers of TBM were present 8-10 days after challenge (Smith *et al.*, 1990; Tew *et al.*, 1990).

#### Isolation and Enrichment of TBM and B Cells

Techniques for obtaining enriched populations of TBM (Smith *et al.*, 1988) and germinal center B cells (Kosco *et al.*, 1988) were previously described in detail. Briefly, 8 to 10 days after antigen challenge of OVA immune mice, lymph nodes were collected, enzymatically dissociated, and enriched on a discontinuous Percoll (Pharmacia, Piscataway, NJ) gradient (densities 1.042 and 1.064). Cells at the 1.064 interface were collected, washed, and incubated in tissue-culture dishes (Costar #3100, Cambridge, MA) at 37°C for 45 min to remove strongly adherent cells, which were primarily typical macrophages. Weakly adherent cells, including TBM, were then flushed off the culture dishes using a Pasteur pipette and counted. This population consisted of 7-10% TBM, 50-60% B cells (including about 20% PNA<sup>hi</sup> GC B cells) and 30-35% T cells.

Enriched populations of germinal center B cells were obtained as previously described (Kosco *et al.*, 1988). Briefly, lymph nodes from actively immunized mice were collected at 3 and 5 days after challenge with OVA, enzymatically dissociated, and enriched on a continuous Percoll gradient. Further enrichment was achieved by panning for germinal center B cells on peanut agglutinin (PNA; Sigma)-coated tissue-

culture dishes (Costar #3100). Unbound cells were removed by gentle swirling and PNA-positive germinal center B cells were then eluted from the dishes by adding 0.2 M d-galactose (Sigma) for 1 hr before vigorous flushing with a pipette. By using this technique, 70-80% enriched populations of highly PNA<sup>+</sup> germinal center B cells were commonly attained.

### Depletion of TBM Using Dynabeads

It is known that TBM are the only Mac-2-bearing cells in lymph nodes (Flotte et al., 1983; Smith et al., 1990), and, in addition, TBM also express Thy-1 (Smith et al., 1988). Consequently, TBM can be selectively removed from TBM-enriched cell populations using anti-Mac-2 and anti-Thy-1 on immunomagnetic beads (Dynal, Great Neck, NY). The beads were purchased precoated with Fc-specific sheep anti-rat IgG. The beads were washed five times, incubated with a cocktail of anti-Mac-2 (1:4 dilution) and mouse anti-rat Thy-1 (1:20) for 1 hr at 4°C with intermittent agitation, and gently washed three times with HBSS. The beads were then incubated with the Mac-2/Thy-1-treated TBM-enriched preparation at a bead-to-cell ratio of 40:1 for 30 min on ice with gentle intermittent agitation. Following incubation, the Dynabead rosetted TBM were removed using the Dynal magnetic chamber. The remaining cells were collected with a Pasteur pipette, washed, and adjusted to  $2 \times 10^5$  cells/50  $\mu$ l in complete media. This preparation was used as the TBM-depleted population.

### IL-2 Assay for Antigen Presentation

Antigen presentation was assessed using an assay system similar to the one originally described by Shimonkevitz et al. (1983). In this system, the OVA-specific T<sub>H</sub>-cell hybridoma 3DO-54.8 (3DO) will produce the lymphokine IL-2 when cocultured with OVA and a source of antigen-presenting cells. The production of IL-2 can then be quantitated by transferring aliquots of media from the cocultured

cells to the IL-2-dependent T-cell line CTLL. The presence of IL-2 in the media stimulates the proliferation of CTLL cells, which can be measured by <sup>3</sup>H-thymidine incorporation. Ultimately, then, the proliferation of CTLL serves as an index for antigen presentation. From  $5 \times 10^4$  to  $1 \times 10^6$  antigen-presenting cells (germinal center B cells or TA3 cells) were cocultured with  $3.0 \times 10^4$  3DO cells in 96-well flat-bottom plates (Falcon #3596) at 37°C, in 5% CO<sub>2</sub> atmosphere for 24 hr in the presence of 50  $\mu$ g of OVA. RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% pyrogen-free fetal calf serum, 100  $\mu$ g/ml gentamicin (GIBCO), 0.2 mM glutamine (GIBCO), and 10 mM Hepes buffer (GIBCO) was used as the culture media. Positive controls for antigen presentation were either (1) cocultures of  $5 \times 10^5$  to  $1.0 \times 10^6$  germinal center B cells with  $5.0 \times 10^4$  3DO cells in the presence of 50  $\mu$ g of OVA or (2)  $5.0 \times 10^4$  TA3 cells with  $3.0 \times 10^4$  3DO cells in the presence of 50  $\mu$ g OVA.

To assess the production of IL-2 by 3DO cells, 150- $\mu$ l aliquots of coculture media were transferred to  $3 \times 10^4$  CTLL cells in 96-well U-bottom plates (Costar #3799) and cultured for 24 hr at 37°C, at 5% CO<sub>2</sub> atmosphere. At 16-20 hr, each well was pulsed with 1.0  $\mu$ Ci <sup>3</sup>H-thymidine (New England Nuclear, Boston) and harvested (PHD Harvester, Cambridge Technical) at 24 hr onto glass filter strips (Costar, #240-1). Proliferation of CTLL cells induced by IL-2 in the coculture media was then measured as thymidine incorporation using a Packard 2200CA scintillation counter. All culturing were performed in triplicate and the data were expressed as the mean <sup>3</sup>H-thymidine uptake. Cultures of CTLL cells alone and supernatant from 3DO cells alone in the presence or absence of OVA were used as controls and were consistently negative (mean cpm  $\pm$  standard error =  $461 \pm 76$ ). As a control for T-cell contamination in the germinal center B-cell preparation, germinal center B cells were cultured alone in the presence of antigen. The germinal center B-cell preparation appeared to contain some T cells, as indicated by slight but inconsequential IL-2 production when cultured in the presence of antigen.

### Influence of TBM on Antigen Presentation by Germinal Center B Cells

To determine if TBM have an affect on the capacity of B cells to present antigen,  $1-2 \times 10^5$  TBM-enriched cells (containing 14,000-16,000 TBM) were cocultured with B cells (either  $5.0 \times 10^5$  to  $1.0 \times 10^6$  germinal center B cells or  $5.0 \times 10^4$  TA3 cells) and  $3.0 \times 10^4$  3DO cells in the presence of 50  $\mu$ g OVA. As in the antigen-presenting assay, in some cultures, TBM were removed from the enriched preparations using Mac-2-coated Dynabeads prior to culturing. In some experiments, 50  $\mu$ l of 100  $\mu$ M indomethacin (Sigma, St. Louis), a prostaglandin inhibitor, was added. Prostaglandins are known to inhibit IL-2 synthesis. As a control, to ensure that indomethacin did not inherently affect the assay, indomethacin was also added to cocultures of germinal center B cells,

antigen, and 3DO cells in the absence of TBM-enriched cells.

### Immunocytochemistry

Reactivity for prostaglandins was detected on enriched preparations of TBM using indirect peroxidase immunocytochemistry. Paraformaldehyde/glutaraldehyde-fixed cytobucket (IEC) slide preparations of TBM-enriched populations were incubated for 30 min at 4°C with goat anti-human PGE<sub>2</sub> (1:30) (ICN, Irvine, CA) as the primary antibody. The slides were washed 3  $\times$  5 min with 0.1 M cacodylate buffer followed by incubation with a peroxidase-conjugated rabbit anti-goat IgG (1:100) as the secondary antibody. Specific peroxidase activity was localized, using DAB as chromogen, and endogenous perox-

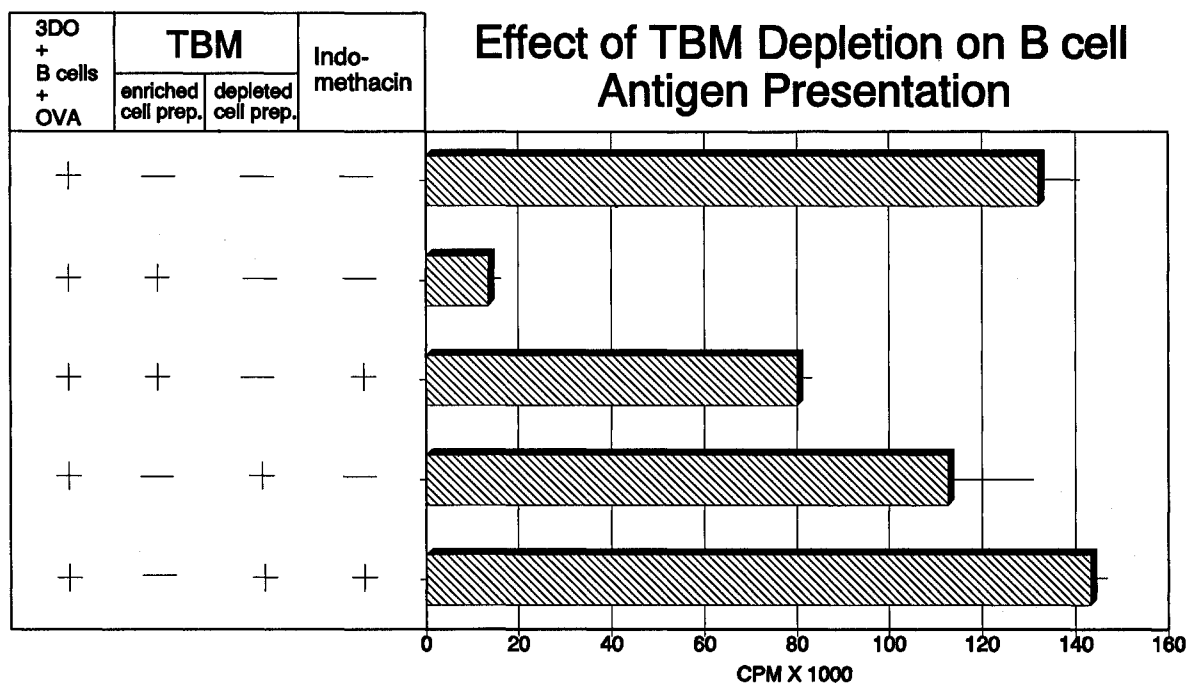


FIGURE 2 Effect of depletion of TBM from TBM-enriched populations on lymphokine secretion. TBM-enriched cells ( $1 \times 10^5$ ), depleted of TBM by MAC-2/THY-1-coated Dynabeads (-TBM), or nondepleted (+TBM) were cocultured with positive antigen-presenting cultures (3DOs + B cells + OVA) in the presence or absence of 100  $\mu$ M of indomethacin. Data represent proliferation of CTLL cells in response to IL-2 produced in coculture supernatants and are expressed as mean cpm  $\pm$  standard error of triplicate cultures.



idase activity was inhibited according to the method of Herzog and Miller (1972).

### Depletion of TBM Relieves Suppression of IL-2 Activity

If the TBM were responsible for depressing the IL-2 production, depletion of the TBM from the enriched preparation prior to addition to the control cultures should alleviate the suppression. Results from a representative experiment using TBM-depleted cells are shown in Fig. 2. Addition of the TBM-enriched population to positive controls resulted in dramatic depression of IL-2 production—about 10% of positive control cultures. Addition of indomethacin to cultures containing TBM-enriched cells resulted in a restoration of most of the IL-2 activity. Furthermore, depletion of TBM from TBM-enriched populations restored IL-2 production to a level insignificantly less than that of the positive controls.

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