

HELPER T CELL-DEPENDENT HUMAN B CELL  
DIFFERENTIATION MEDIATED BY A MYCOPLASMAL  
SUPERANTIGEN BRIDGE

By JOSEPH R. TUMANG,\* DAVID N. POSNETT,\*† BARRY C. COLE,§  
MARY K. CROW,\*† AND STEVEN M. FRIEDMAN\*†

*From the Departments of Medicine, \*The Hospital for Special Surgery, and  
†The New York Hospital-Cornell University Medical College, New York, New York 10021;  
and the §Division of Rheumatology, Department of Internal Medicine,  
University of Utah College of Medicine, Salt Lake City, Utah 84132*

mAbs recognizing TCR V gene products have helped to define a unique group of potent T cell activators termed superantigens (1). Recognized superantigens include murine self antigens (2, 3) and a group of microbial toxins, the staphylococcal enterotoxins (SE) (1), and a soluble product of *Mycoplasma arthritidis* mitogen (MAM) (4). Superantigens share a number of intriguing characteristics. For example, superantigen recognition is almost entirely a function of TCR V  $\beta$  gene usage (1, 2). In addition, superantigens bind selectively and with high affinity to MHC class II molecules (6). In the absence of antigen processing and in an MHC-nonrestricted manner, superantigen-MHC class II complexes on the APC surface trigger the proliferation of T cells expressing the relevant TCR V gene product (5). Finally, the microbial toxins that function as superantigens are among the most potent mitogens known.

The dual affinity of superantigens for MHC class II and TCR V gene products suggests that this class of antigen could promote a form of abnormal Th-B cell collaboration analogous to that observed during graft-vs.-host disease (GVHD). In GVHD, adoptive transfer of donor Th cells, specific for recipient MHC class II antigens, triggers polyclonal B cell activation. In selected strains of nonautoimmune prone mice, GVHD is characterized by an autoimmune diathesis remarkably similar to SLE (6). It has been suggested that in GVHD, the presence of both self antigens and donor-derived Th cells, reactive against recipient MHC class II antigens, provides a combination of stimuli that selectively drives autoreactive B cell differentiation (6).

The experiments described below were designed to determine if a superantigen bridge between Th cells and B cells would result in an analogous form of polyclonal human B cell activation. MAM was selected for study in view of the chronic inflammatory arthritis that *M. arthritidis* induces in rodents (7), and scattered reports of *M. arthritidis* cultured from the bone marrow of patients with SLE (8).

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Address correspondence to Steven M. Friedman, Department of Medicine, The Hospital for Special Surgery, The New York Hospital-Cornell University Medical College, 535 East 70th Street, New York, NY 10021.

## Materials and Methods

**Reagents.** PHA (Gibco Laboratories, Grand Island, NY) and PWM (Gibco Laboratories) were used at a final concentration of 1 and 10  $\mu\text{g/ml}$ , respectively. Staphylococcal enterotoxin B (SEB) (Toxin Technology, Madison, WI) was used at 0.1  $\mu\text{g/ml}$ . Partially purified MAM (isolated from *M. arthritis* culture supernatants as described [4]) was used at a final concentration of 1:4,000, predetermined to be optimal for T cell proliferation.

**Generation of T cell lines (TCL).** To optimize conditions for detecting B cell activation, MAM-reactive CD4<sup>+</sup> TCL were analyzed rather than unselected resting T cell populations. PBL, obtained from normal donors, were cultured at  $2 \times 10^6/\text{cc}$  in final medium supplemented with a 1:4,000 dilution of partially purified MAM, or 10  $\mu\text{g/ml}$  of the anti-TCR V $\beta$ 5 mAb, C37 (9). After 3 d, cultures were supplemented with partially purified human IL-2 (Electro-Nucleonics Inc., Fairfield, NJ) and, 16–24 h later, blast and resting cells separated by centrifugation on discontinuous Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradients. TCL were maintained in IL-2 and expanded by weekly retriggering with MAM or mAb C37 plus X-irradiated autologous non-T cells (APC<sub>xr</sub>). Purified CD4<sup>+</sup> TCL were derived by immune rosette depletion of CD8<sup>+</sup> T cells.

**B Cell Helper Assay.** B cell-enriched populations were isolated from fresh PBL or tonsils. Briefly, T cells were removed by rosette formation with neuraminidase-treated sheep erythrocytes, followed by complement-mediated lysis of contaminating T cells by the mAb OKT3. Resting B cells were isolated from monocytes and activated cells by density centrifugation on discontinuous Percoll gradients. Responder B cells ( $5 \times 10^5$ ) were cultured in round-bottomed culture tubes in 0.5 cc of final medium alone or with fresh autologous T cells ( $5 \times 10^5$ ), autologous CD4<sup>+</sup> MAM reactive, or mAb C37<sup>+</sup> TCL cells ( $10^5$ ). Where indicated, cultures were supplemented with PWM or MAM. TCL populations were X-irradiated (xr) with 500 rad to prevent overgrowth, a procedure that does not affect helper activity. After 10 d, cultures were terminated and supernatants assayed for total IgM by ELISA.

## Results and Discussion

After 4 d of in vitro sensitization with the partially purified superantigen, MAM-responsive T cell blasts were isolated from the nonresponsive, resting T cell population by discontinuous Percoll gradient centrifugation. Table I demonstrates that the T cell blast population was enriched, and the resting T cell population depleted, of MAM-reactive T cells, as determined by proliferation. The T cell blasts respond to MAM with a vigorous and accelerated (day 2) proliferative response compared with fresh PBL. In contrast, the resting T cell population proliferates to control stimuli, either PHA or a distinct bacterial superantigen, SEB, but not to MAM.

Phenotypic analysis of the MAM blast population revealed approximately equal distribution of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. To maximize the likelihood of detecting MAM-dependent Th cell activity, the CD4<sup>+</sup> helper/inducer subset of MAM-reactive T cell blasts was isolated by immune rosette depletion of CD8<sup>+</sup> cells. This procedure yielded a T cell population that was >98% CD4<sup>+</sup>, and maintained an accelerated proliferative response to MAM in the presence of autologous, allogeneic, or xenogeneic APC (Table I, Exp. 3).

MAM-reactive CD4<sup>+</sup> T cell blasts were assayed for helper activity. As shown (Fig. 1 A), little IgM is produced in B cell cultures supplemented with MAM alone or MAM plus unselected autologous T cells. In contrast, a striking IgM response is induced in cultures containing both MAM-reactive CD4<sup>+</sup> T cell blasts and MAM. This B cell response is MAM dependent, but not MHC restricted. Thus, B cells derived from all donors tested, regardless of HLA haplotype, are activated by CD4<sup>+</sup> MAM-reactive T cells in the presence of MAM (data not shown).

TABLE I  
Isolation of MAM-reactive T Cells by Percoll Density Gradient Centrifugation

Exp.	Responder cell population	<sup>3</sup> H[TdR] incorporation								
		Day 2			Day 4			Day 6		
		APC <sub>xr</sub>	MAM + APC <sub>xr</sub>	PHA + APC <sub>xr</sub>	APC <sub>xr</sub>	MAM + APC <sub>xr</sub>	PHA + APC <sub>xr</sub>	APC <sub>xr</sub>	MAM + APC <sub>xr</sub>	PHA + APC <sub>xr</sub>
<i>cpm</i>										
1	T blasts	445	13,366	7,717	187	5,210	1,944	274	731	1,142
	T resting		ND		220	151	11,340		ND	
	PBL	140	378	8,115	397	1,186	38,410	743	4,844	1,572
2	T blasts	128	2,207	ND	141	3,314	ND	650	220	ND
	T resting	189	299	1,359	124	274	2,946	483	328	1,066
3	CD4 <sup>+</sup> MAM blasts	180	727	931	19,504	386	14,370	434	5,542	

PBL were cultured with MAM for 96 h, then separated into "blasts" and "resting" T cell populations by centrifugation on discontinuous Percoll gradients. The separated populations were cultured for several days in final medium supplemented with IL-2, washed extensively, and  $2 \times 10^4$  cells assayed in triplicate for proliferation (<sup>3</sup>H]TdR incorporation) triggered by  $10^5$  X-irradiated autologous non-T cells (APC<sub>xr</sub>) alone or in combination with a 1:4,000 dilution of MAM, 1 μg/ml of PHA (Exp. 1), or 0.1 μg/ml of SEB (Exp. 2). In Exp. 1, freshly isolated autologous PBL was also assayed for proliferation. In Exps. 1 and 2, [<sup>3</sup>H]TdR incorporation was assayed on days 2, 4, and 6 of culture. In Exp. 3, an aliquot of the MAM-reactive TCL, described in Exp. 1, was depleted of CD8<sup>+</sup> cells and assayed for proliferation against X-irradiated APC: either autologous (AUTO<sub>xr</sub>), allogeneic (ALLO<sub>xr</sub>), or xenogeneic rat splenocytes (XENO<sub>xr</sub>) alone or in the presence of MAM. [<sup>3</sup>H]TdR incorporation was assessed 36 h later.

In preliminary studies, we noted that MAM-reactive TCL, comprised of both CD4<sup>+</sup> and CD8<sup>+</sup> cells, often trigger little Ig production. This suggested restriction of helper activity to the CD4<sup>+</sup> MAM-reactive T cell subset. The experiment presented in Fig. 1 B confirms this impression. While both CD4<sup>+</sup> and CD8<sup>+</sup> MAM-reactive T cell blasts proliferate in response to MAM, only CD4<sup>+</sup> cells provide MAM-dependent help for B cell activation, as assessed by Ig production.

In related studies, we examined TCR V-β gene family usage by MAM-reactive human T cells. We noted a strong negative correlation between MAM-reactive T cells and T cells expressing TCR V-β 5 gene products recognized by mAb C37 (9), and V β 6.7 recognized by mAb OT145 (10). To prove that MAM-dependent B cell activation requires superantigen-specific Th cells, CD4<sup>+</sup> TCL were obtained from cultures of PBL stimulated with either MAM or the mAb C37. As expected, MAM-reactive TCL were depleted of C37-expressing cells, while the C37-stimulated culture contained >95% C37<sup>+</sup> T cells. In functional assays (Fig. 2 A), the CD4<sup>+</sup>, C37<sup>+</sup> TCL provides help for B cell differentiation in the presence of PWM. However, only the MAM-reactive CD4<sup>+</sup> TCL drives B cell differentiation in the presence of MAM.

Antibody production by resting B cells requires direct cognate cell-cell interaction with antigen-specific Th cells, as well as the action of lymphokines (11). Recent studies of GVHD in chimeric mice have demonstrated that autoantibody produc-

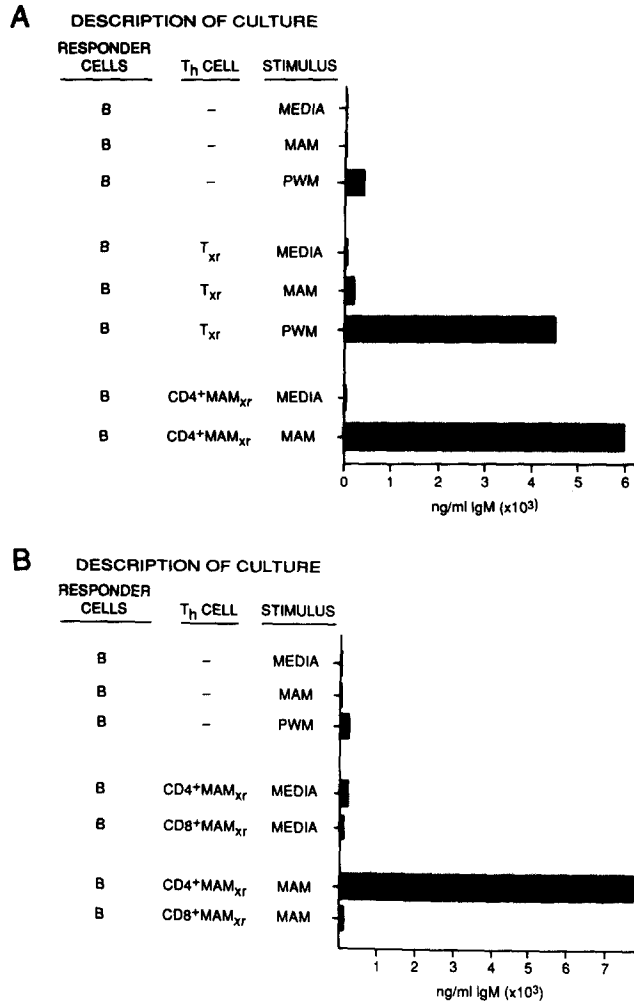


FIGURE 1. MAM-dependent polyclonal B cell activation mediated by CD4<sup>+</sup> MAM-reactive T cells. (A)  $5 \times 10^5$  peripheral blood B cells, isolated as described above, were cultured in round-bottomed culture tubes in 0.5 cc of final medium alone, with  $5 \times 10^5$  autologous T cells, or with  $10^5$  autologous CD4<sup>+</sup> MAM-reactive TCL cells. Where indicated, cultures were supplemented with PWM (10  $\mu$ g/ml), or MAM (1:4,000). T cell populations were X-irradiated (xr) with 500 rad to prevent overgrowth. After 10 d, cultures were terminated and supernatants assayed for total IgM by ELISA, as described previously. (B) MAM-reactive TCL cells were depleted of CD4<sup>+</sup> or CD8<sup>+</sup> cells by the immune rosetting technique described above. Cytofluorographic analysis of the unselected MAM-reactive TCL blasts was 46% CD4<sup>+</sup>, 52% CD8<sup>+</sup>, the CD8-depleted fraction was >98% CD4<sup>+</sup>, <1% CD8<sup>+</sup>, and the CD4-depleted fraction was >95% CD8<sup>+</sup>, <1% CD4<sup>+</sup>. The CD4<sup>+</sup> and CD8<sup>+</sup> fractions were assessed for B cell help as described above. [<sup>3</sup>H]TdR incorporation by the CD4<sup>+</sup> and CD8<sup>+</sup> populations to MAM in the presence of autologous APC<sub>xr</sub> was 9,253 cpm (background control, 229 cpm) and 7,940 cpm (background control, 324 cpm), respectively.

tion in this model is also dependent on cognate Th-B cell interaction (12). To postulate a role for superantigens in the *in vivo* activation of normally quiescent, autoreactive B cells, it is crucial to demonstrate that superantigens can promote a productive Th-resting B cell interaction. To address this question, high density resting tonsillar B cells were incubated with MAM or medium alone for 1 h at 37°C, then washed extensively. These B cell populations were subsequently cultured with MAM-specific Th cells and polyclonal IgM secretion assayed by ELISA. As shown in Fig. 2 B, MAM-pulsed resting B cells are excellent activators of and targets for MAM-reactive CD4<sup>+</sup> T cell help in the absence of exogenous MAM. These data demonstrate that a mycoplasmal superantigen, bound to the surface of resting human B cells, is an effective means of activating superantigen-reactive Th cells that, in turn, trigger polyclonal Ig production by the superantigen-bearing B cell population.

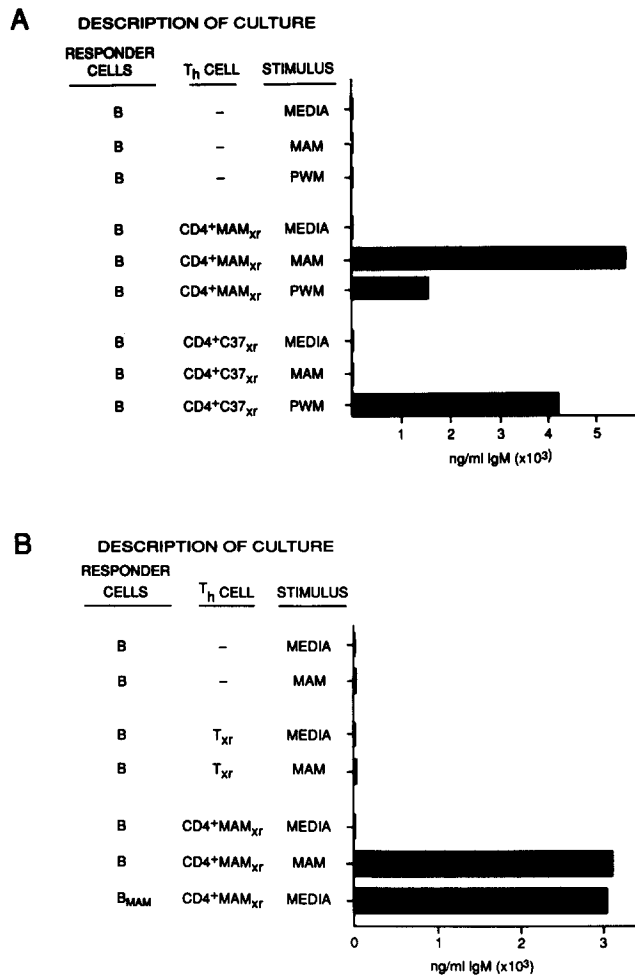


FIGURE 2. Resting B cells bind and present MAM to specific Th cells. (A) CD4<sup>+</sup> MAM-reactive or C37<sup>+</sup> TCL were generated as described above. Aliquots of these TCL were assayed for polyclonal B cell help as described in Fig. 1. At the time of assay, the MAM-reactive line was >98% CD4<sup>+</sup> and <0.1 C37<sup>+</sup>; and the C37<sup>+</sup> line was >98% CD4<sup>+</sup> and >96% C37<sup>+</sup>. The C37<sup>+</sup> T cell line did not proliferate or secrete lymphokines in response to autologous APC<sub>xr</sub> and MAM, but proliferated vigorously and secreted >100 μg/ml of IL-4 in response to autologous APC<sub>xr</sub> and mAb C37 (data not shown). (B) Resting tonsillar B cells were preincubated at 37°C with final medium alone (B) or with a 1:4,000 dilution of MAM (B<sub>MAM</sub>). After 1 h, the B cells were washed extensively before use as responder cells in the polyclonal IgM production assay.

The observation that superantigens activate subsets of T cells based on TCR V gene usage has led to speculation that these substances may induce organ-specific autoimmunity by activating normally quiescent autoreactive T cells (1). In addition, however, the affinity of superantigens for MHC class II molecules and their recognition by T cells in the absence of processing suggested to us that bacterial superantigens may also perturb the immune system in a manner analogous to that seen in GVHD. For example, any B cell, regardless of its specificity for antigen, may bind a superantigen and be rendered a target of cognate T cell help by superantigen-specific Th cells. The studies reported here support this hypothesis. While this manuscript was in preparation, Mourad et al. reported similar results using the Staphylococcal toxic shock syndrome toxin (13). Together, these two reports suggest that productive Th-B cell bridging may be a common feature of microbial superantigens.

### Summary

Experimentally induced murine graft-vs.-host disease may be characterized by hypergammaglobulinemia, autoantibody formation, and immune complex-mediated organ system damage that mimics SLE. These autoimmune phenomena are mediated by abnormal Th-B cell cooperation, across MHC disparities, in which donor-derived allospecific Th cells recognize and interact with MHC class II antigens on the surface of recipient B cells. Microbial toxins, termed superantigens, which bind to MHC class II molecules and activate selected T cells based on TCR variable gene usage, may induce a similar form of Th-B cell interaction. In the present study, we generated and characterized human Th cell lines reactive with the *Mycoplasma arthritidis* superantigen (MAM). The essential observation is that resting human B cells bind MAM and present it to superantigen-reactive autologous or allogeneic Th cells, resulting in both Th cell activation and a consequent polyclonal Ig response by the superantigen-bearing B cells.

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