Characterization of Human T Cells Reactive with the *Mycoplasma arthritidis*-derived Superantigen (MAM): Generation of a Monoclonal Antibody against Vβ17, the T Cell Receptor Gene Product Expressed by a Large Fraction of MAM-reactive Human T Cells

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Summary

While all known microbial superantigens are mitogenic for human peripheral blood lymphocytes (PBL), the functional response induced by *Mycoplasma arthritidis*-derived superantigen (MAM) is unique in that MAM stimulation of PBL consistently results in T cell-dependent B cell activation characterized by polyclonal IgM and IgG production. These immunostimulatory effects of MAM on the humoral arm of the human immune system warranted a more precise characterization of MAM-reactive human T cells. Using an uncloned MAM reactive human T cell line as immunogen, we have generated a monoclonal antibody (mAb) (termed C1) specific for the T cell receptor Vβ gene expressed by the major fraction of MAM-reactive human T cells, Vβ17. In addition, a Vβ17$^-$ MAM-reactive T cell population exists, assessed by MAM, induced T cell proliferation and cytotoxic T cell activity. mAb C1 will be useful in characterizing the functional properties of Vβ17$^+$ T cells and their potential role in autoimmune disease.

The term superantigen (SA)[1] has been applied to a class of T cell mitogens, including a group of endogenous murine retroviral gene products (1–4) and several microbial toxins (5–7), which share a unique set of characteristics. Specifically, SA bind with high affinity to regions of MHC class II molecules distinct from the conventional peptide binding groove (8–14), and are mitogenic for T cells in the absence of processing (15). T cell responsiveness to SA is dictated exclusively by TCR Vβ gene family usage, with little influence by other TCR variable elements (5, 16–18). In addition, SA are among the most powerful mitogens known, functioning at nanomolar to fentamolar concentrations (7, 19, 20). Together, these characteristics render the SA capable of profound effects on immune system function.

To date the clinical impact of microbial SA has been most clearly associated with acute systemic illnesses, often manifest by shock and immunosuppression which are thought to result from the massive release of cytokines following SA activation of a substantial fraction of the T cell pool (21). Examples include toxic shock syndrome, caused by the *Staphylococcus aureus*-derived toxin (TSST-1) (22–24), and food poisoning or shock induced by the *Staphylococcal* enterotoxins (SEs) (21, 25). The potential for SA to initiate more subtle perturbations of the immune system, leading to B cell activation, hypergammaglobulinemia and, perhaps, autoimmunity has been suggested (5, 26–28). In this context, the *Mycoplasma arthritidis*-derived SA, termed MAM, is of particular interest. The in vitro response of human PBL to MAM is distinct from that induced by the *Staphylococcus aureus*-derived SA in at least two respects: (*a*) the level of T cell proliferation induced by MAM is much more modest (29) and (*b*) the re-

[1] *Abbreviations used in this paper:* EAE, experimental allergic encephalomyelitis; MAM, *Mycoplasma arthritidis*-derived superantigen; MBP, myelin basic protein; MS, multiple sclerosis; RA, rheumatoid arthritis; SA, superantigen; SE, staphylococcal enterotoxin; TSST, toxic shock syndrome toxin.

sponse is characterized by striking T cell dependent polyclonal immunoglobulin production (Crow, M.K., Z. Chu, B. Ravina, G. Zagon, J.R. Tumang, B.C. Cole, and S.M. Friedman, manuscript submitted for publication). These in vitro observations in man, coupled with the unique capacity of *M. arthritidis* to induce a chronic, inflammatory polyarthritis in genetically-susceptible strains of rodents (30), suggest that the identification and characterization of MAM-reactive human T cells may prove useful for evaluating the potential role of MAM-like SA in autoimmune responses. Here, we describe the generation of a mAb to the TCR Vβ gene product used by the major fraction of MAM reactive human T cells, which was determined to be Vβ17.

Materials and Methods

Reagents. Staphylococcal enterotoxins SEA, SEB, SEC_1, SEC_2, SEC_3, and SEE, as well as toxic shock syndrome toxin (TSST-1), were obtained from Toxin Technology (Madison, WI). Partially purified MAM was isolated from *M. arthritidis* culture supernatants as previously described (7). All SA were used at a final concentration predetermined to be optimal for T cell proliferation, 1:4,000 for MAM and 10–25 ngm/ml for the Staphylococcal-derived SA.

Isolation and Fractionation of Lymphocytes. Fresh peripheral blood or tonsil lymphocytes were isolated by Ficoll-Hypaque centrifugation. T cells were isolated from non-T cells by E-rosette formation with neuraminidase-treated sheep red blood cells and a second Ficoll-Hypaque centrifugation. Residual T cells were removed from the non-T cell fraction by treatment with anti-CD3 followed by the addition of magnetic beads coated with goat anti-mouse antibody (Dynal, Inc., Great Neck, NY) and physical separation of the bead-bound T cells utilizing a magnet.

Generation of SA-reactive T Helper (T_h) Cell Lines. $CD4^+$ peripheral blood T cells were isolated from unselected T cell populations by incubating T cells with an excess of anti-CD8 mAb followed by washing and physical removal of T cells binding antibody to CD8 using magnetic beads coated with goat anti-mouse antibody (Dynal, Inc.) and a magnet. The $CD4^+$-enriched populations were cocultured with x-irradiated autologous APC and either MAM or SEE. After 5 d, semi-purified human IL-2 (Electro-Nucleonics, Inc., Fairfield, NJ) was added. Cultures were retriggered weekly with APC_x, and the relevant SA, and expanded in the presence of IL-2. Cell lines were maintained in culture media consisting of RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% fetal bovine serum (Whittaker, M.A. Bioproducts, Walkersville, MD), penicillin and streptomycin (50 U/ml, Gibco Laboratories), and 2 mM glutamine (Gibco Laboratories).

CD23 Induction Assay. The induction of B cell surface CD23 expression by T_h cells has been detailed previously (31). Briefly, 5×10^5 purified tonsillar B cells were cultured in final medium with 1.5×10^5 x-irradiated $CD4^+$ MAM- or SEE-reactive TCL cells. Cultures were supplemented with final medium alone or medium containing an optimal concentration of the various SA. After 16 h, B cells were assayed for CD23 expression by indirect immunofluorescence staining using mAb $EBVCS_2$ (generously donated by Dr. Bill Sugden and Stan Metzenberg, Madison, WI) and counterstained with fluorescein-conjugated $F(ab')_2$ fragments of goat anti-mouse IgG (Tago, Inc., Burlingame, CA). The percentage of positively staining cells was determined by analysis on an Ortho IIs cytofluorograph (Ortho Diagnostic Systems, Inc., Westwood, MA).

Generation of TCL Enriched for Specific TCR V Gene Usage. Tonsil T cell aliquots were incubated at room temperature with saturating concentrations of TCR V gene specific mAb: C37 (Vβ 5.2/5.3) (32) (unpublished data, DN Posnett), OT145 (Vβ6.7a) (33, 34), S511 (Vβ12) (17), Ti3a (Vβ8) (35), and C1. After 30 min, cells were washed three times, resuspended in final medium and cultured at a final concentration of 0.5×10^6/ml in the presence of goat anti-mouse antibody-coated magnetic beads (Dynal, Inc.). Beads were added at a ratio of 20 beads to 1 target T cell. After 5 d, magnetic beads were removed, the T cells washed and recultured with IL-2 alone for 48 h. Cultures were maintained with IL-2 and weekly feeding with periodate-treated allogeneic non-T feeder cells. These cultures become highly enriched in T cells expressing the relevant Vβ gene, depending on the initial mAb used for stimulation. Usually, this occurs over a 6-d period. Occasionally, a second cycle of stimulation was required to achieve greater than 95% specific Vβ expression. At the time these TCL were utilized as effectors in the cytolytic assay or for RNA isolation, each was virtually 100%$^+$ for T cells expressing the appropriate TCR V gene products.

Assay of SA-dependent Cytolysis. TCL cells were assayed for cytolytic activity in a 4-h ^{51}Cr release assay (36), using MHC class II antigen-bearing target cells, B cell lymphoblastoid cell line 8866. Briefly, 8866 cells were incubated for 2 h at 37°C with ^{51}Cr in the presence of final medium alone, or the indicated SA. Target cells were washed and mixed in triplicate with varying numbers of TCL effector cells. Data is presented as mean percent lysis of target cells at each effector to target cell ratio.

Immunoprecipitation of TCR Utilizing mAb C.1. A MAM-reactive TCL with 60% $C1^+$ T cells was radioiodinated with lactoperoxidase and peroxide, using 25×10^6 cells and 2.5 mCi ^{125}I. Cell lysis and immunoprecipitations with SPA-Sepharose and monoclonal antibodies were performed as described (37).

Analysis of TCR Vβ Gene Usage by Polymerase Chain Reaction (PCR). Three T cell lines were prepared by stimulating normal peripheral blood T cells with either OT145 (Vβ6.7), C37 (Vβ5.2/5.3) or C1 MAb (see above). Total cellular RNA was isolated from each cell line by the acid guanidinium thiocyanate-phenolchloroform method (38). cDNA was synthesized with reverse transcriptase, using an anti-sense Cβ primer (see below) (34). The PCR was performed with a panel of Vβ specific sense primers, in parallel reactions where each Vβ primer was matched with the Cβ anti-sense primer situated 55 bp from the 5′ end of the C region. Two paired Cα primers were used as a positive control. Each cDNA preparation was tested for the optimal dilution. PCR conditions included primers at 0.5 μM. Replinase (Dupont) 2 U, buffer containing 3.0 mM MgCl (20× buffer, Dupont), $^{32}dCTP$ 20 μCi, cold dNTPs at 0.2 mM, in a final volume of 0.02 ml. Amplification was done for 1 min at 94°C, 1 min at 51°C and 1 min at 72°C for 25 cycles.

PCR products were analyzed on a 5% polyacrylamide gel. The gel was dried and exposed to film.

PCR primers used in the experiment shown in Fig. 7 were: Cβ (anti-sense) 5′ CTTCTGATGGCTCAAACAC 3′; Cα 5′ (sense) 5′ GAACCCTGACCCTGCCGT 3′; Cα 3′ (anti-sense) 5′ TCATAAATTCGGGTAGGATC 3′; Vβ2 (sense) 5′ GTTTCTCATCAACCATGCAA 3′; Vβ6 (sense) 5′ TCAGGTGTGATCCAATTTC 3′; Vβ5.3/5.2 (sense) 5′ GTCAGGGGCCCCAGTTTAT 3′; Vβ17 (sense) 5′ ACAGCGTCTCTCGGGAGA 3′.

Immunofluorescence. Single color immunofluorescence was performed as previously described (31). Two-color immunofluorescence was prepared by first incubating 5×10^5 cells with various mAb for 30′ at room temperature. This was followed by three washes in PBS-BSA 1%, azide 0.02%, and goat anti-mouse Ig-FITC (GAM-FITC) for 30′ at room temperature. The cells were washed

3× and incubated with a negative control IgG1 mAb to quench free GAM-FITC binding sites for 30′ at room temperature. The cells were washed 3× and incubated with PE-labeled anti-CD4 or anti-CD8 mAb (UBI/Olympus, Lake Success, NY) for 30′ at room temperature. The cells were finally washed 3× and analyzed on an ORTHO cytofluorograph. The results shown in Table 1 represent the ratio of double positive (FITC + PE) cells over total CD4 or CD8 positive cells expressed as a percentage.

Results

Rationale and Strategy for the Development of mAb Specific for MAM-reactive Human TCRs. The *Mycoplasma arthritidis*-derived SA, termed MAM, became of special interest during studies which compared the effects of a panel of microbial SA on human PBL function (Crow, M. K., Z. Chu, B. Ravina, G. Zagon, J. R. Tumang, B. C. Cole, and S. M. Friedman, manuscript submitted for publication). These experiments demonstrated that MAM is unique among the SA tested, including TSST-1 and the SEs, in its capacity to reproducibly trigger polyclonal T cell-dependent Ig production by unselected human PBL. It should be emphasized that the *Staphylococcus aureus*-derived SA can support a polyclonal Ig response, but, in most instances, require culture conditions in which T cell proliferation is inhibited, e.g., cultures containing purified responder B cells and small numbers of x-irradiated autologous T cells (26).

To understand how MAM activation of PBL so effectively drives the humoral arm of the immune system, and to characterize MAM-reactive human T cells, a mAb against the MAM binding TCR was generated. Since no MAM-reactive T cell clones were available, it was reasoned that a polyclonal MAM-reactive TCL could serve as a successful immunogen if MAM-responsive T cells represent a fraction of the T cell repertoire, which is highly restricted with respect to TCR Vβ gene usage. To this end, we took advantage of the fact that the specific interaction of SA-reactive CD4$^+$ human T cells and SA-bearing B cells results in the rapid expression of the CD23 activation antigen on a fraction of the resting B cell pool (28), Using this assay, we compared the CD23 induction profiles of CD4$^+$ MAM-reactive and a number of *Staphylococcus aureus* SA-reactive TCL. CD4$^+$ MAM-reactive TCL induce maximal CD23 expression on B cells which have bound MAM, SEA, SEB, SEC1, SEC2, or SEC3, while a minimal CD23 response is induced on B cells bearing either SEE or TSST-1 (data not shown). Conversely, CD4$^+$ SEE-reactive TCL cells induce optimal CD23 expression on B cells bearing TSST-1 or any of the SEs, but trigger little CD23 expression by MAM bearing B cells (data not shown). This functional evidence of crossreactivity by SA-activated TCL cells is consistent with reports that activated human T cells are somewhat promiscuous in their proliferative responses to the staphylococcal-derived SA (39). Importantly, however, the pattern of CD23 expression observed suggest that MAM and SEE-specific human T cells show little cross-reactivity and may therefore utilize different TCR Vβ gene products. Thus, the SEE-reactive TCL should provide an excellent comparison for screening MAM-specific TCR mAbs.

Given these considerations, Balb/c mice were immunized on four occasions with MAM-reactive TCL cells which had been expanded in long-term culture (7 wk) by weekly restimulation with autologous APC and MAM. Three days after the final immunization, the mice were sacrificed and their splenocytes fused with SP2/0 myeloma cells. Hybridomas which demonstrated reactivity with a small fraction of freshly isolated (resting) peripheral T cells were screened against the MAM-reactive TCL used for immunization and an SEE-reactive TCL-derived from the same donor. In this manner, a mAb termed C1, was identified. As shown in Fig. 1, C1 stains between 3–6% of peripheral T cells, >60% of the MAM-reactive TCL used as immunogen, and virtually no SEE-reactive TCL cells. Immunoprecipitation studies (Fig. 2) showed that mAb C1 recognizes a disulfide-linked heterodimer consistent with the α/β TCR. Finally, as with other TCR mAb specific for V gene products, C1 recognizes a small subset of peripheral T cells from all donors tested, including samples of cord blood T cells. While C1$^+$ cells are found among both CD4$^+$ and CD8$^+$ T cells, some donors show selective enrichment of C1$^+$ T cells in one or the other T cell subset (Table 1).

mAb C1 was used to screen a number of SA-reactive TCL propagated in vitro. As shown (Table 2) short term activation of peripheral T cells with a panel of SA demonstrates a clear enrichment of C1$^+$ cells among the T cells activated by MAM and several of the SE with which MAM-specific TCL cells cross-react in the CD23 induction assay, in particular, SEB, SEC1, SEC2, SEC3. In contrast, C1$^+$ T cells are not well represented among non-crossreactive TSST-1 or SEE-activated T cells. T cells expanded by weekly retriggering with SEB or SEC 1 and autologous APC show a marked fall off in the percentage of C1$^+$ T cell (Table 2). We inter-

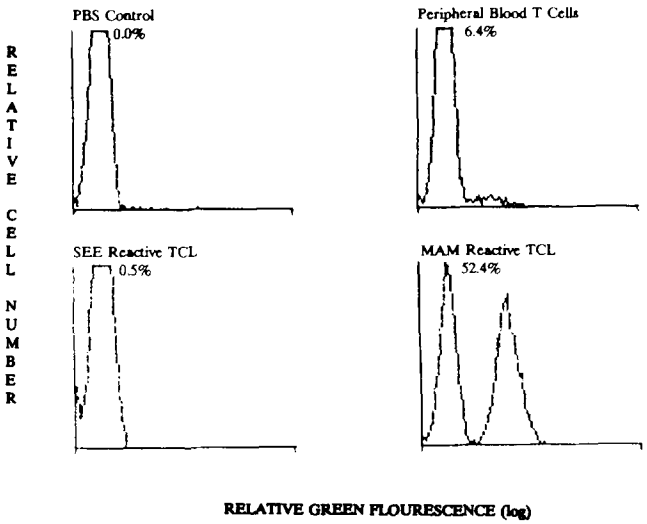

Figure 1. PBL (*upper right*); the CD4$^+$ MAM-reactive T cell line used for immunization (*lower right*); or a CD4$^+$ SEE-reactive T cell derived from the same donor (*lower left*) were analyzed by indirect immunofluorescence staining for reactivity with the C1 mAb. Background staining of PBL with PBS and fluoresceinated anti-mouse Ig is shown (*upper left*).

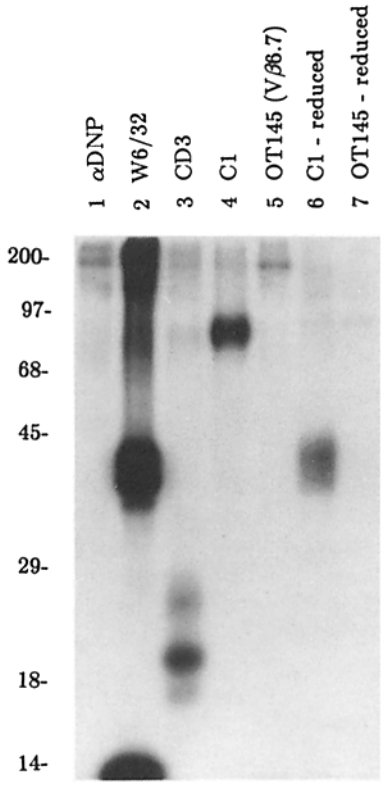

Figure 2. Immunoprecipitation of TCR by C1 mAb: Radioiodinated MAM-reactive TCL cells were lysed and precipitated with the indicated mAb. αDNP is a negative control mAb (IgG1); W6/32 (anti-class I MHC); CD3 was precipitated with mAb 454 (59); OT145 is specific for TCR expressing Vβ6.7a gene products.

Table 1. *Percentage of C1⁺ T Cells in CD4 and CD8 Subpopulations*

		C1⁺/CD4⁺	C1⁺/CD8⁺
Adult bloods	1	14.00	3.77
	2	6.40	2.30
	3	7.98	5.56
	4	5.65	7.87
	5	6.63	11.32
Cord bloods	1	7.69	3.76
	2	4.75	3.98
	3	3.99	3.20
	4	3.45	4.48
	5	5.65	7.87
Mean ± SD		6.62 ± 2.83	5.41 ± 2.64

Cord blood lymphocytes and PBL obtained from normal adult donors were analyzed by two-color immunofluorescence staining for distribution of C1⁺ T cells in the CD4⁺ and the CD8⁺ T cell subsets.

pret this to indicate that SA such as SEB are recognized by T cells expressing several TCR Vβ gene family products among which C1⁺ T cells are a minor component with a relatively low binding affinity for SEB. In contrast, C1⁺ T cells are greatly expanded in short-term cultures of MAM-activated T cells and remain well represented. In the experiment presented in Table 2, the percentage of C1⁺ T cells decreases somewhat over time in the culture stimulated weekly by MAM. However, in most experiments, C1⁺ T cells represent between 50 and 60% of TCL repetitively triggered with MAM (Fig. 1). Finally, it is of interest that C1⁺ T cells comprise a stable population of 15–20% of SEC_2 reactive TCL cells. These results suggest that C1⁺ T cells represent the major population of human T cells reactive with MAM, and a significant fraction of the SEC_2-responsive T cell pool.

Table 2. *Peripheral Blood T Cells Triggered with MAM Are Highly Enriched in C1*

		Percentage of T cells staining positively with anti-TCR mAbs				
	Superantigen stimulus	C1	C37	OT145	S511	Ti3a
Primary culture	MAM	55.6	0.0	2.5	2.5	5.6
	TSST	0.6	1.3	2.8	0.8	1.9
	SEA	5.1	0.6	2.9	2.9	5.9
	SEB	26.5	0.4	2.7	9.0	1.5
	SEE	3.0	3.4	1.2	0.2	12.5
	SEC1	17.8	4.8	3.0	7.3	5.2
	SEC2	15.1	1.8	0.0	7.1	1.7
	SEC3	9.2	4.1	1.9	4.5	6.2
Secondary culture	MAM	39.4	1.2	0.2	2.8	2.7
	TSST	0.2	0.0	1.1	0.0	0.0
	SEA	1.2	0.0	0.0	0.0	0.0
	SEB	10.1	0.6	2.2	6.4	0.7
	SEE	0.6	1.2	0.5	0.1	15.3
	SEC1	9.4	1.0	1.4	2.0	3.1
	SEC2	15.5	1.6	0.0	5.0	0.4
	SEC3	6.9	0.0	0.0	0.8	0.0
Tertiary culture	MAM	32.4	0.2	0.0	1.5	2.7
	TSST	0.0	0.1	0.3	2.6	1.6
	SEA	0.7	0.3	0.4	0.7	0.9
	SEB	7.3	0.5	0.0	3.3	3.3
	SEE	0.1	0.3	2.1	1.3	11.9
	SEC1	8.7	0.6	0.7	2.5	0.9
	SEC2	17.0	0.0	0.0	7.4	0.0
	SEC3	6.2	0.0	0.1	1.8	0.2

PBL were activated weekly with x-irradiated autologous APC and the indicated SA. The percentage of T cells staining with each of the anti-TCR MAbs was assessed each week 6 d after triggering.

Table 3. *C1 Depleted T Cells Proliferate in Response to MAM*

	Description of responder population		[^{3}H]Tdr incorporation induced by: Media	APC_{xr}	MAM APC_{xr}	SEE APC_{xr}	TSST-1 APC_{xr}
		% C1⁺			*cpm*		
Exp. 1	E^+	3.5	85	6,681	37,978	39,313	69,033
	E^+ $C1^-$	0.3	53	1,159	24,226	25,667	55,629
	E^+ $C37^-$	2.8	2,969	1,837	25,509	23,847	59,855
Exp. 2	E^+	4.6	82	1,170	26,748	122,655	54,603
	E^+ $C1^-$	0.9	267	1,348	27,721	99,509	46,956
	E^+ $C37^-$	4.1	239	1,184	29,513	77,742	58,827

Peripheral blood T lymphocytes were depleted of T cells reacting with anti-TCR MAbs C1 or C37 using magnetic beads coated with anti--mouse Ig. Untreated or MAb-depleted T cells were assayed, in triplicate, for proliferative responses against medium alone, autologous APC_{xr}, or the indicated SA in the presence of autologous APC_{xr}. The percentage of $C1^+$ T cells present in each responder T cell population was detected by immunofluorescence staining. Experiments 1 and 2 describe the results of separate studies involving two different normal donors.

$C1^+$ T Cells are MAM-reactive. To formally prove that $C1^+$ T cells are MAM-reactive, we took advantage of the fact that anti-TCR mAbs are mitogenic, allowing the selective activation and expansion of T cells expressing the relevant TCR epitope. Thus, aliquots of tonsillar T cells were treated with saturating concentrations of mAb C1 or either of two non cross-reactive TCR Vβ gene product specific mAb: C37 (Vβ 5.2/5.3), and OT145 (Vβ 6.7a), TCL were generated as described in the methods section. These lines are virtually pure with respect to reactivity with the relevant anti-TCR mAb (see legend to Fig. 3).

While each line contained a $CD4^+$ T cell fraction, $CD8^+$ T cells predominated, comprising 60–80% of the TCL population. We therefore assessed a $CD8^+$ T cell-dependent function, by asking if these TCL cells could lyse MHC class II positive target cells in a SA dependent manner. In the experiment depicted in Fig. 3, no significant lysis of untreated 8866 target cells by any of the TCL is observed. However, the $C1^+$ TCL selectively lyses MAM-bearing 8866 cells, while both the $OT145^+$ and $C37^+$ TCL cells effectively lyse TSST-1 bearing, but not MAM-bearing, targets. It should be noted that the proliferative response of human T cells to TSST-1 is reportedly dominated by the $V\beta2^+$ fraction (40). We interpret the lysis of TSST-1 bearing targets by $V\beta6.7a^+$ and $V\beta\ 5.2/5.3^+$ TCL cells to represent another example of the cross-reactivity of activated human T cells to *S. aureus*-derived SA (39). The experiment depicted in Fig. 3 has been performed on three separate occasions, using $C1^+$ TCL independently derived from different donors. In all studies, the results are similar to those shown. Thus, $C1^+$ T cells, activated and expanded with mAb C1, demonstrate functional specificity for MAM.

MAM-reactive Human T Cells Include a C1 Negative Population. The expansion of $C1^+$ cells in short-term cultures of SE-activated T cells (Table 2) indicates that $C1^+$ T cells can account for the pattern of SA responsiveness associated with MAM-reactive TCL. However, this data does not rule out the existence of $C1^-$ MAM-reactive T cells. Indeed, the observation that repetitive triggering of MAM-reactive T cells with MAM results in a TCL that is is maximally 50–60% $C1^+$ provides indirect evidence that a $C1^-$ MAM-reactive T cell population exists. To address this point, aliquots of

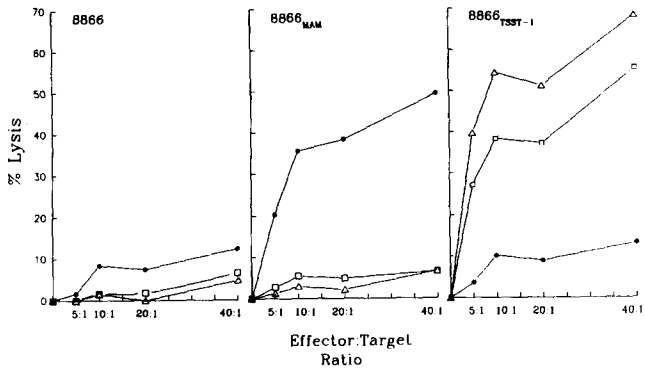

Figure 3. Tonsillar T cells, expanded in culture by weekly activation with an anti-TCR mAb, goat anti-Ig-coated magnetic beads, and IL-2, triplicate at the effector to target ratios indicated for cytotoxic activity against ^{51}Cr release assay. Target cells consisted of a lymphoblastoid B cell line 8866 either untreated (8866) or "pulsed" for 1 h at 37°C with MAM (8866_{MAM}) or TSST-1 (8866_{TSST-1}). Depicted are the cytotoxic activities of mAb C37 activated T cells (△); mAb OT145 activated T cells (□); and mAb C1 activated T cells (●). The phenotype of these three cell lines at the time of assay are as follows: mAb C37 activated = 99% $C37^+$, 22% $CD4^+$, 77% $CD8^+$; mAb OT145 activated = 100% $OT145^+$; 16% $CD4^+$; 85% $CD8^+$; mAb C1 activated = 99% $C1^+$, 43% $CD4^+$, 61% $CD8^+$.

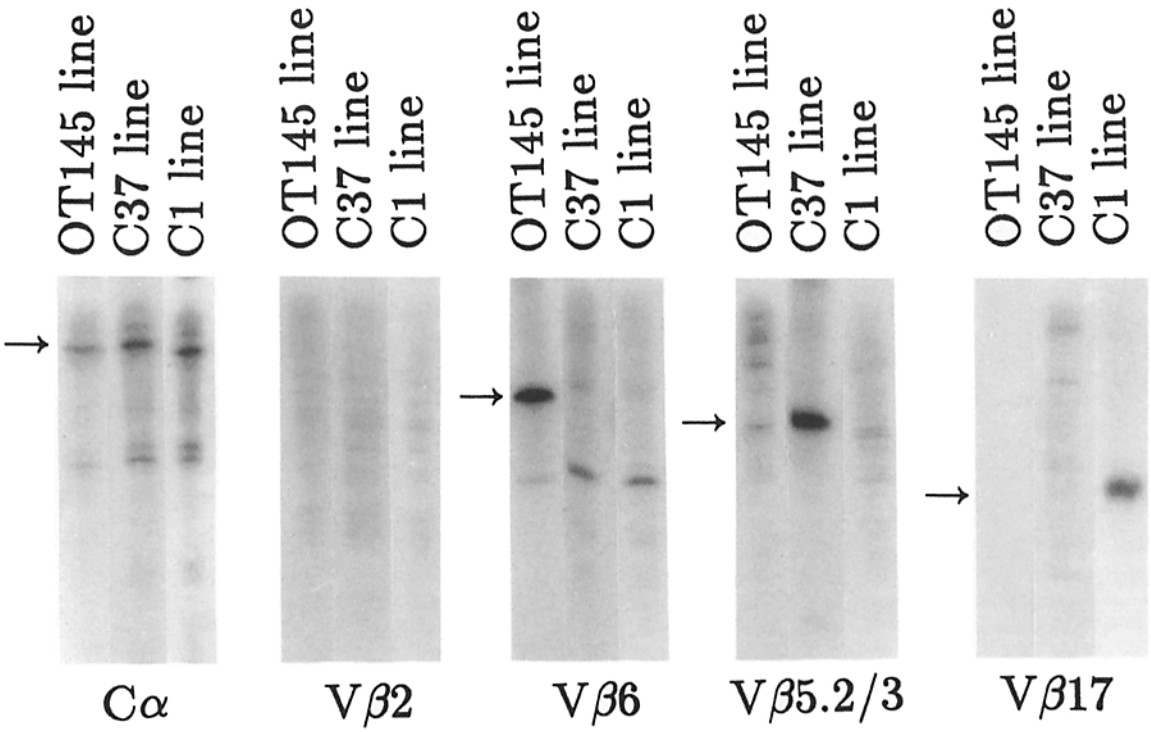

Figure 4. Specific PCR amplification of Vβ17 gene products from a C1⁺ TCL. The cDNA from three cell lines (OT145⁺, C37⁺, C1⁺) was amplified with Cα primers, Cβ-Vβ2 primers, Cβ-Vβ5.2/5.3 primers, Cβ-Vβ6 primers, or Cβ-Vβ17 primers. Specific bands are indicated with arrows. In each case, the band migrates as expected based on the estimated size of the amplified segment.

fresh peripheral T cells were depleted of C1⁺ or C37⁺ T cells by treatment with the relevant mAb followed by physical removal of the reactive T cells using magnetic beads bearing goat anti-mouse IgG. As shown in Table 3, while this procedure efficiently reduces or eliminates the C1⁺ T cell pool, as detected by immunofluorescence staining, the proliferative response to MAM was not affected. We should point out that T cell populations depleted of C1⁺ cells maintain strong proliferative responses over a wide range of MAM concentrations (6 log dilutions). In additional studies, we determined that both C1⁻ MAM-reactive TCL and the C1⁺ TCL specifically and efficiently lyse the MAM bearing 8866 target cells (data not shown). Taken together, these findings support the existence of a MAM-reactive human T cell population distinct from that which expresses the C1 epitope.

mAb C1 Is Specific for TCR Vβ 17. TCL were prepared with three mAb: OT145 (Vβ6.7a), C37 (Vβ5.2/5.3), and C1, as described above. Each of these polyclonal T cell lines contained >98% cells positive with the relevant mAb. RNA was isolated and cDNA synthesized with reverse transcriptase. Aliquots of cDNA were PCR amplified with different primer combinations. Fig. 4 shows that each cell line expressed a specific Vβ. As expected, the OT145⁺ cells expressed Vβ6, and the C37⁺ cells expressed Vβ5.2/5.3. The C1⁺ cells expressed Vβ17. None of these TCL expressed Vβ2 and all of them expressed Cα. In other experiments (not shown) the C1⁺ cell line was analyzed with primers specific for Vβ1 - Vβ20. No primer combinations other than Vβ17-Cβ amplified a β chain product. Thus, Vβ17 appears to represent the sole Vβ gene product recognized by C1. Vβ17 is thought to represent a Vβ family with a single gene copy based on counting bands on Southern blots (41–43).

Discussion

While all microbial SA are mitogenic for human PBL, the functional response induced by MAM is unique in that MAM stimulation of PBL consistently results in T cell-dependent B cell activation characterized by polyclonal IgM and IgG production (Crow, M. K., Z. Chu, B. Ravina, G. Zagon, J. R. Tumang, B. C. Cole, and S. M. Friedman, manuscript submitted for publication). This evidence of the immunostimulatory effects of MAM on the humoral arm of the human immune system suggested that a more precise characterization of MAM-reactive human T cells is warranted. In the mouse, MAM has been shown to behave as a classic microbial SA, selectively inducing the proliferation of Vβ 8⁺ and Vβ 6⁺ murine T cells (18). While MAM is mitogenic for human T cells, the level of proliferation induced is quite modest compared to that triggered by the *Staphylococcus aureus*-derived SA, and no data exists regarding TCR V gene dependence of MAM recognition. The mAb described in this report, C1, provides the first direct evidence that MAM-reactive human T cells utilize a restricted group of TCR Vβ gene products. mAb C1 recognizes a disulfide linked heterodimer, consistent with the α/β TCR, on approximately 3–6% of peripheral T cells. As with other mAb specific for TCR V gene products, C1 reacts with a small fraction of both the CD4⁺ and CD8⁺ subsets of all donors tested, including cord blood T cells. With respect to SA recognition, it was

determined that MAM-reactive TCL are greatly enriched in $C1^+$ cells, while TCL responsive to SA with which MAM-reactive TCL are less crossreactive, SEE and TSST-1, are depleted of $C1^+$ T cells. Studies employing the polymerase chain reaction to amplify TCR β chain cDNA from $C1^+$ TCL cells demonstrate that C1 identifies an epitope expressed on the Vβ 17 gene product. Taken together, these results show that MAM recognition by human T cells is restricted by TCR Vβ gene usage and that a major fraction of MAM-reactive human T cells are $V\beta17^+$.

From a technical standpoint, it is worth noting that mAb C1 is a unique example of a TCR V gene specific mAb generated by immunizing with a polyclonal T cell population. As discussed above, MAM is a relatively weak mitogen for human T cells, suggesting a restricted population of MAM-reactive T cells. Presumably, the highly restricted usage of Vβ genes among the MAM-reactive immunizing TCL cells allowed our immunization strategy to be successful. Consistent with this, staining data demonstrate that the TCR epitope recognized by mAb C1 is expressed not only on >60% of the immunizing TCL, which had been retriggered repetitively with MAM, but is also present on a relatively large fraction of peripheral T cells activated by MAM for only several days in primary short term cultures (Table 2). The successful application of this technique, utilizing TCL responsive to other microbial SA as immunogens, could greatly expand the available panel of mAbs against human TCR V gene products.

The mechanism which accounts for the striking capacity of MAM to induce polyclonal Ig production by unfractionated PBL remains unexplained. Most simply, perhaps, the pool of MAM-reactive T cells may be quite limited in number as compared to T cells reactive with other SA. Thus, a limited pool of potentially responsive T cells could account for a relatively weak MAM-induced T cell proliferative response, but sufficient lymphokine secretion to promote B cell activation. In this regard, Fleischer et al. (39) have reported that while 40–70% of randomly generated human T cell clones respond to one or several *Staphylococcus aureus*-derived SA, none responded to MAM. These investigators have suggested that MAM-reactive human T cells may represent only 5% of all T cells (44). Utilizing mAb C1 to address this issue, we found that on average, $C1^+$ T cells represent approximately 5% of the peripheral T cell pool. While a firm conclusion that all $C1^+$ T cells are MAM reactive must await the functional analysis of cloned $C1^+$ T cells, the marked enrichment of $C1^+$ T cells among T cell "blasts" in even short-term cultures of MAM activated PBL and marked depletion of $C1^+$ T cells from the MAM nonresponsive "small" T cell fraction provides strong, albeit indirect, evidence that this will prove the case. However, the existence of MAM-reactive T cells in addition to the $C1^+$ population is suggested by three lines of experimental evidence. First, resting peripheral blood T cells, depleted of $C1^+$ cells, show an undiminished proliferative response to MAM over a wide range of SA concentrations. Second, 40–60% of T cells which persist in long term cultures maintained by repetitive retriggering with MAM plus autologous APC are $C1^-$. Third, MAM reactive TCL which have been depleted of $C1^+$ T cells exhibit potent, SA specific lysis of MAM bearing target cells. Studies are in progress utilizing $C1^-$ MAM-reactive TCL cells to identify other Vβ genes used by MAM-reactive T cells and to generate mAbs against their products. Using such a panel of mAbs against MAM-reactive TCR V gene families, one could hope to determine if MAM's interesting functional effects on human PBL relate to: (*a*) the limited pool of MAM-responsive T cells; (*b*) unique functional properties of MAM-responsive T cells; or (*c*) structural differences among the SA themselves. In this regard, a recent report has demonstrated the capacity of TSST-1, but not SEB, to activate human B cells by binding to their MHC class II surface antigens (45).

There is considerable evidence of selective TCR Vβ gene usage among rodent T cells which mediate a number of experimental autoimmune diseases. For example, in experimental allergic encephalomyelitis (EAE), $V\beta8.2^+$ T cells play a central role. In five different strains of rat, encephalitogenic T cell clones and hybridomas, reactive against myelin basic protein (MBP) peptide fragments, are uniformly $V\beta8.2^+$ (46). Similarly, Vβ8.2 is expressed on over 85% of T cells reacting to the encephalitogenic MBP peptide in strains of mice susceptible to EAE (47, 48). Moreover, the in vivo administration of mAb specific for Vβ8.2 has been shown to both protect mice from the development of EAE induced by a subsequent challenge with MBP, and to ameliorate the clinical course of EAE in mice already affected (47). In another disease model, collagen-induced arthritis, T cells reactive against type II collagen which are capable of transferring arthritis to naive syngeneic mice are virtually all $V\beta8.2^+$ (49). These observations suggest that expression of the Vβ8.2 gene product may be associated with an autoimmune T cell pool in rodents. For instance, T cells derived from the $CD4^-$ $CD8^-$ Vβ8.2 expressing thymocyte subpopulations (50–52).

In this context, the observation that human MAM-reactive T cells identified by mAb C1 are $V\beta17^+$ is of interest. Amino acid sequence analysis has demonstrated a close relationship between the products of murine Vβ8 genes, which are expressed by MAM-reactive murine T cells (53), and several human TCR Vβ gene families, including Vβ17 (54). Moreover, there are reports implicating Vβ17 usage by autoimmune human T cells. For example, Wucherpfennig et al. (55) reported expanded populations of MBP-reactive T cells in the peripheral blood of multiple sclerosis (MS) patients, and preferential usage of $V\beta17^+$ among those T cells reactive against an encephalotogenic MBP peptide presented in association with DR2 and DR3, two MHC class II genes overrepresented in the MS patient population. In addition $V\beta17^+$ T cells have been reported to be enriched among activated T cells isolated from the synovial tissue of patients with rheumatoid arthritis (RA) (56). It should be emphasized, that these reports require confirmation as additional studies have suggested the predominant usage of other human TCR Vβ genes in both diseases (57, 58). We would suggest, however, that mAb C1 may be of considerable utility in defining a potential role for $V\beta$ 17^+ T cell participation in the pathogenesis of human autoimmune disorders, including RA and MS.

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