

$\gamma\delta$ T lymphocytes and proinflammatory cytokines in bacterial meningitis

Syed Raziuddin, PhD, MRCPATH,^a Nisar A. Mir, MD, FRCP,^b
M. El-Hag El-Awad, MD,^b Abdul Wahab Telmesani, MD,^b and
Mansour Al-Janadi, MD^c Abha, Saudi Arabia

Background: Patients with bacterial meningitis have a T-cell defect and impaired cytokine production.

Methods: The phenotype and percentage of circulating $\alpha\beta$ and $\gamma\delta$ T-cell receptor-bearing lymphocytes were determined from patients with bacterial meningitis (*Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*), patients with bacterial infection but without meningitis, and healthy control subjects by a monoclonal antibody staining method. The *in vitro* production of cytokines, interleukins (IL-2, IL-6), interferon- γ and tumor necrosis factor- α was measured by the bioassay or ELISAs.

Results: The percentage of circulating $\gamma\delta$ T cells with a CD3+CD4+CD8- phenotype was significantly ($p < 0.001$) increased in all patients with bacterial meningitis compared with patients with bacterial infection and healthy control subjects. The CD3+ $\gamma\delta$ T cells from patients with meningitis produced highly elevated levels of two proinflammatory cytokines, tumor necrosis factor- α and IL-6. However, interferon- γ production was enhanced by CD3+ $\alpha\beta$ T cells.

Conclusion: The increased percentage of circulating T-cell receptor $\gamma\delta$ T cells and their *in vitro* production of tumor necrosis factor- α and IL-6 cytokines may play an important role in the pathogenesis and inflammatory response in bacterial meningitis. (*J ALLERGY CLIN IMMUNOL* 1994;93:793-8.)

Key words: Bacterial meningitis, cytokines, $\alpha\beta$ T cells, $\gamma\delta$ T cells, T-cell receptor, interleukin-6, tumor necrosis factor- α , interferon- γ

T cells contribute to immunity against bacterial, viral, and parasitic infection by recognizing foreign proteins via antigenic receptors and subsequently releasing immunoregulatory cytokines. Although most peripheral blood CD3+ T cells express an $\alpha\beta$ heterodimer as an antigenic T-cell receptor (TcR), an average of 5% to 10% expressed TcR $\gamma\delta$.^{1, 2} These cells (TcR $\gamma\delta$) usually express the CD3+CD4-CD8- surface phenotype.² Recent evidence suggests that TcR $\gamma\delta$ T cells may participate in the immune response to several infectious organisms and may function in surveillance against microbial agents and the bacteria-infected cells.³⁻⁶ The precise function of $\gamma\delta$ -bearing T cells in immune response remains to be

Abbreviations used:

IFN- γ :	Interferon- γ
IL:	Interleukin
mAb:	Monoclonal antibody
TcR:	T-cell receptor
TNF- α :	Tumor necrosis factor- α

defined. However, the elevation in $\gamma\delta$ T cells in certain pathologic situations in human disease reveals numerous distinctions between TcR $\alpha\beta$ and TcR $\gamma\delta$ T cells, suggesting that the two types of T cells ($\alpha\beta$ + and $\gamma\delta$ +) may exist for quite different reasons. Bacterial meningitis continues to be a major disease of children. *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* are the most common causes of meningitis in Saudi Arabia and elsewhere.^{7, 8} Our recent study documented a selective T-cell abnormality with increased percentages of activated CD25+ and HLA-DR+ T-cell subsets in the circulation of most patients with bacterial meningitis.⁸ In this study we show that in the circulation

From the Departments of ^aClinical Immunology, ^bPediatrics, and ^cInternal Medicine, King Saud University, College of Medicine, Abha and Asir Central Hospital, Abha, Saudi Arabia.

Reprint requests: Syed Raziuddin, PhD, MRCPATH, Department of Clinical Immunology, College of Medicine, Abha, P.O. Box 641, Abha, Saudi Arabia.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/1/52158

of patients with bacterial meningitis, increased percentages of $\gamma\delta$ T cells are present and they specifically secrete two proinflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in mitogen-stimulated cultures.

METHODS

Patients

Twenty-eight patients with bacterial meningitis in whom the diagnosis was established by clinical signs and heavy positive bacterial cultures from both cerebrospinal fluid and blood were admitted to the pediatric ward of Asir Central Hospital, Abha or Lasson Infectious Disease Hospital, Abha and were eligible for this study. Twelve children (mean age, 2.5 years; 7 boys and 5 girls) had *H. influenzae* meningitis. Cerebrospinal fluid and blood cultures from all 12 children were positive for *H. influenzae* type B microorganisms. Cerebrospinal fluid and blood cultures of eight children (mean age, 2.2 years; 5 boys and 3 girls) grew *S. pneumoniae*, and cultures of eight other children (mean age, 2.6 years; 6 boys and 2 girls) grew *N. meningitidis*. All children with meningitis had fever, irritability, and lethargy; and most patients had additional signs such as neck stiffness (14), bulging anterior fontanelle (5), and convulsions (18). There was no evidence of underlying immunodeficiency in these patients with meningitis. Thirteen patients without meningitis but proven bacterial infections (4 with *H. influenzae*, 4 with *S. pneumoniae*, 2 with *Staphylococcus aureus*, and 3 with *Klebsiella pneumoniae*) served as patient control subjects. Blood samples were drawn from patients and patient control subjects after diagnosis of disease was made and before commencement of therapy. Twelve healthy children (mean age, 2.6 years; 8 boys and 4 girls) served as healthy control subjects. Informed consent was obtained from parents of all children. Twenty-three of the 28 patients with meningitis survived after therapy.

$\alpha\beta$ and $\gamma\delta$ T-cell enumerations

Peripheral blood mononuclear cells, isolated by the Histopaque-1077 (Sigma Chemical Co., St. Louis, Mo.) density gradient centrifugation method, were analyzed with monoclonal antibodies (mAbs) specific for $\alpha\beta$ and $\gamma\delta$ TcRs and CD3 pan-T antigen by the standard indirect immunofluorescence method as described previously.^{4,8} The following mAbs were used: CD3 (OKT3, total T cells), CD4 (OKT4, helper/inducer T cells), CD8 (OKT8, suppressor/cytotoxic T cells), TcR $\alpha\beta$ (TcR-1, α/β WT31+ and BF1), and TcR $\gamma\delta$ (TcR δ 1 WT31-). The mAbs were purchased either from Ortho Diagnostics (Raritan, N.J.) or Becton Dickinson (Mountain View, Calif.). BF1 was a generous gift of Dr. M. B. Brenner (Dana Farber Cancer Institute, Boston, Mass.)

Purification of T-cell population

The T cells were purified from MNC by the 2-aminoethylisothiouonium bromide-treated sheep erythrocyte rosetting technique.^{4,8} The cells were then applied to Histopaque 1077 gradient centrifugation. The cells that formed rosettes (T cells) were collected from the bottom, and erythrocytes were removed by lysing with 0.83% ammonium chloride-Tris buffer solution. This T-cell fraction contained less than 3% of surface immunoglobulin-positive and 92% to 96% CD3+ (OKT3 mAb reactive) T cells. CD3+CD4+CD8-, CD3+CD4-CD8+, and CD3+CD4-CD8- (double negative) T-cell populations were isolated by use of OKT4 and OKT8 mAbs in an immunoabsorbent technique described in detail previously.^{4,8}

Purified CD3+ TcR $\alpha\beta$ (91% to 96% positive for both OKT3 and TcR-1 $\alpha\beta$ WT31+ mAbs) and CD3+ TcR $\gamma\delta$ (89% to 95% positive for both OKT3 and TcR δ 1 WT31- mAbs) T cells were obtained by a solid-phase immunoadsorption method as described previously.^{4,8}

Cytokine production

Highly purified CD3+ TcR $\alpha\beta$ or CD3+ TcR $\gamma\delta$ T cells (2.5×10^5 cells/ml) were cultured at 37° C in RPMI-1640 medium (Gibco, Grand Island, N.Y.) alone or with concanavalin A (5 μ g/ml) for a total period of 48 hours. Supernatants were harvested by centrifugation and stored at -70° C until they were assayed for production of cytokines.

IL-2 was measured in a standard cytotoxic assay with an IL-2-dependent murine CTLL cell line as described previously.⁴ Interferon-2 (IFN- γ) was measured in a standard ELISA in which two mAbs to IFN- γ (anti-IFN- γ) (Genzyme Corp., Boston, Mass.; Hoffman-La Roche, Basle, Switzerland) were used as catcher and tracer antibodies. IL-6 and TNF- α levels were each measured with commercially available ELISA kits (Genzyme Corp.). In each assay the appropriate recombinant human cytokines were used to generate the standard curves. Sensitivity of the various assays was as follows: IL-2, 5 U/ml; IFN- γ , 3 U/ml; IL-6, 5 U/ml; and TNF- α , 10 pg/ml.

Endotoxin assay

Serum levels of endotoxins (bacterial lipopolysaccharide) were determined by the ELISA with a chromogenic substrate, S2423, with a commercially available kit (Whittaker Bioproducts Kit 56-64OU; Whittaker Bioproducts, Walkersville, Md.) according to a previously described method.⁹

RESULTS

T-cell subsets

Patients with bacterial meningitis had almost normal percentages of total CD3+ T cells (pa-

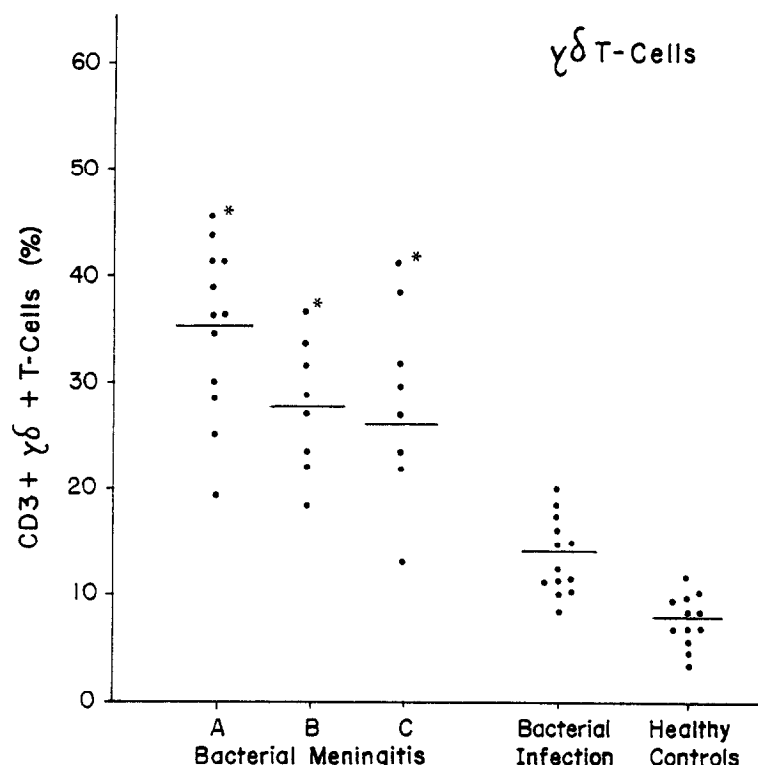


FIG. 1. CD3+ T-cell receptor $\gamma\delta$ T cells in blood of patients with bacterial meningitis (A, *H. influenzae*; B, *S. pneumoniae*; and C, *N. meningitidis*), patients with bacterial infections without meningitis (*H. influenzae*, *S. pneumoniae*, *S. aureus*, and *K. pneumoniae*), and healthy control subjects. Horizontal bar represents mean. * $p < 0.001$ versus healthy control subjects and patients with bacterial infections.

tients with meningitis, $74.4\% \pm 6\%$; patients with bacterial infection, $72.5\% \pm 8\%$; healthy control subjects, $72.8\% \pm 6\%$) but elevated percentages of CD4+ (patients with meningitis, $57.2\% \pm 5\%$; patients with bacterial infection $40.5\% \pm 4\%$; healthy control subjects, $42.7\% \pm 5\%$) and reduced percentages of CD8+ (patients with meningitis, $18.4\% \pm 3\%$; patients with bacterial infection, $25.0\% \pm 4\%$; healthy control subjects $24.2\% \pm 5\%$) T-cell subsets in circulation.

CD3+ $\gamma\delta$ T cells

The percentage distributions of CD3+ $\gamma\delta$ T cells in 28 patients with bacterial meningitis, 13 patients with bacterial infections without meningitis (patient control subjects), and 12 healthy control subjects are given in Fig. 1. The CD3+ $\gamma\delta$ T cells were significantly increased in the circulation of patients with meningitis compared with the healthy control subjects ($p < 0.001$). CD3+ $\gamma\delta$ T cells were also slightly increased in patient control subjects as compared with healthy control subjects, but these values were signifi-

cantly lower than those for patients with meningitis ($p < 0.01$).

Phenotype of CD3+ $\gamma\delta$ T cells

We next determined the phenotype of CD3+ $\alpha\beta$ and CD3+ $\gamma\delta$ T cells from six subjects each from the meningitis, patient control, and healthy control groups (Table I). CD3+ $\alpha\beta$ T cells were significantly decreased and CD3+ $\gamma\delta$ T cells were increased in the meningitis group compared with the control group ($p < 0.001$). There was no major difference in the TcR $\alpha\beta$ and TcR $\gamma\delta$ expressions on CD3+CD4-CD8+ and CD3+CD4-CD8- T cells among meningitis, patient control, and healthy control groups. T-cell phenotypes of TcR $\alpha\beta$ and TcR $\gamma\delta$ cell also did not show a major difference between patient control and healthy control groups. However, of interest is that CD3+CD4+CD8- (helper/inducer) T-cell subsets expressing TcR $\gamma\delta$ were increased significantly in the meningitis group compared with the patient control group ($p < 0.001$).

We have also determined that the CD3+

TABLE I. TcR $\alpha\beta$ and TcR $\gamma\delta$ expressing T-cell subsets in bacterial meningitis

T cells	TcR expression	Patients with bacterial meningitis (n = 6)	Patients with bacterial infection (n = 6)	Healthy control subjects (n = 6)
CD3+	TcR $\alpha\beta$	58 $\pm$ 5*	87 $\pm$ 6	89 $\pm$ 8
	TcR $\gamma\delta$	37 $\pm$ 4*	15 $\pm$ 3	9 $\pm$ 3
CD3+CD4+CD8-	TcR $\alpha\beta$	51 $\pm$ 5*	83 $\pm$ 5	91 $\pm$ 6
	TcR $\gamma\delta$	43 $\pm$ 4*	15 $\pm$ 3	6 $\pm$ 2
CD3+CD4-CD8+	TcR $\alpha\beta$	81 $\pm$ 6	88 $\pm$ 6	93 $\pm$ 5
	TcR $\gamma\delta$	13 $\pm$ 3	8 $\pm$ 3	6 $\pm$ 2
CD3+CD4-CD8-	TcR $\alpha\beta$	5 $\pm$ 1	5 $\pm$ 1	3 $\pm$ 1
	TcR $\gamma\delta$	13 $\pm$ 3	8 $\pm$ 2	11 $\pm$ 2

Percentages of positive cells are expressed as means $\pm$ SEM. Purified total CD3+ T cells and CD3+CD4+CD8-, CD3+CD4-CD8+, and CD3+CD4-CD8- T-cell subsets were stained with mAb to TcR $\alpha\beta$ and TcR $\gamma\delta$ antigens. Patients and control subjects are similar to those in Table II.

* $p < 0.001$ versus bacterial infection and healthy control groups.

TABLE II. Cytokine production by TcR $\alpha\beta$ and TcR $\gamma\delta$ T cells

Cells from	IL-2 (U/ml)	IL-6 (U/ml)	IFN- γ (U/ml)	TNF α (pg/ml)
Patients with bacterial meningitis				
CD3+ TcR $\alpha\beta$	35 $\pm$ 5	61 $\pm$ 6	138 $\pm$ 18*	198 $\pm$ 23
CD3+ TcR $\gamma\delta$	16 $\pm$ 3	94 $\pm$ 10*	18 $\pm$ 6	384 $\pm$ 36*
Patients with bacterial infection				
CD3+ TcR $\alpha\beta$	98 $\pm$ 14	45 $\pm$ 5	67 $\pm$ 8	154 $\pm$ 17
CD3+ TcR $\gamma\delta$	53 $\pm$ 7	25 $\pm$ 3	20 $\pm$ 4	98 $\pm$ 18
Healthy control subjects				
CD3+ TcR $\alpha\beta$	104 $\pm$ 16	41 $\pm$ 5	53 $\pm$ 6	164 $\pm$ 28
CD3+ TcR $\gamma\delta$	61 $\pm$ 5	18 $\pm$ 3	21 $\pm$ 4	45 $\pm$ 10

Data are means $\pm$ SEM from six patients each from the bacterial meningitis group (*H. influenzae*, *S. pneumoniae*, 3 patients each), and the bacterial infection group (*H. influenzae*, *S. pneumoniae*, 3 patients each) and six healthy control subjects. TcR $\alpha\beta$ and TcR $\gamma\delta$ purified T cells were cultured with concanavalin A (5 μ /ml) for 48 hours, and supernatants were analyzed for secretion of various cytokines.

* $p < 0.001$ versus bacterial infection and healthy control groups.

TcR $\alpha\beta$ and CD3+ TcR $\gamma\delta$ T cells measured in 13 patients with bacterial meningitis returned to almost normal levels (CD3+ TcR $\alpha\beta$, 81% $\pm$ 6%; CD3+ TcR $\gamma\delta$, 16% $\pm$ 5%) after recovery from meningitis (approximately 16 to 28 days after therapy).

Cytokine production by CD3+ $\gamma\delta$ T cells

Because our data indicated a significant increase in the CD3+ $\gamma\delta$ and a concomitant decrease in the CD3+ $\alpha\beta$ T cells in patients with meningitis, we further compared the functional capacity of these TcR $\alpha\beta$ and TcR $\gamma\delta$ T cells to secrete proinflammatory cytokines such as IL-2, IL-6, IFN- γ , and TNF- α after in vitro culture with concanavalin A (Table II). IL-2 production was lower in patients with meningitis compared with control subjects. There was no major difference in

cytokine production by these TcR $\alpha\beta$ and TcR $\gamma\delta$ T cells between patient control subjects (bacterial infection) and healthy control subjects. In contrast, highly elevated levels of TNF- α and IL-6 production were observed in the CD3+ $\gamma\delta$ T cells from patients with meningitis. Of interest, however, is that CD3+ $\alpha\beta$ T cells from patients with meningitis produced higher levels of IFN- γ as compared with their counterparts from patient control subjects and healthy control subjects ($p < 0.001$).

No spontaneous (unstimulated T cells) production of IL-2, IL-6, IFN- γ , and TNF- α was observed in patients with bacterial infection (without meningitis) or healthy control subjects. Unstimulated T cells from patients with bacterial meningitis also did not produce any IL-2. However, in patients with bacterial meningitis unstimulated

CD3+ TcR $\alpha\beta$ T cells produced IFN- γ (20 ± 4 U/ml) and TNF- α (18 ± 3 pg/ml), whereas unstimulated CD3+ TcR $\gamma\delta$ T cells produced IL-6 (15 ± 3 U/ml) and TNF- α (32 ± 6 pg/ml).

Serum levels of endotoxins

The serum levels of endotoxin in 28 patients with bacterial meningitis (mean, 186 ± 61 pg/ml) were significantly more elevated than those in 13 patients with bacterial infection (mean, 59 ± 20 pg/ml) and 20 age-matched healthy control subjects (mean, 8.7 ± 3.2 pg/ml) ($p < 0.001$).

DISCUSSION

Our study suggests that proliferation and expansion of CD3+CD4+CD8 $-\gamma\delta$ + helper/inducer T-cell subsets leads to the release of two prime proinflammatory cytokines, TNF- α and IL-6, which are important in the immunopathologic processes of bacterial meningitis. There seems to be a possible relationship between endotoxin effect, specifically on $\gamma\delta$ T cells, and release of TNF- α in this disease. This possibility is supported by our demonstration of high serum levels of endotoxins and earlier evidence of preferential release of TNF- α in serum of patients with meningitis and septicemia or both.⁹⁻¹¹

The mechanisms that determine the selection of $\gamma\delta$ T cells with CD4+CD8 $-$ phenotype and in vitro release of TNF- α and IL-6 by these cells is of considerable importance to our understanding of pathophysiology and inflammatory responses in meningitis. On the contrary, the release of IFN- γ by the other $\alpha\beta$ T cells could be a part of immune response to infection, although the full pattern of cytokine production and release in circulation remains to be elucidated. IFN- γ constitutes an important link in the cytokine network, and IFN- γ itself has little or no direct inflammatory activity.¹² Further studies are required to determine why $\alpha\beta$ and $\gamma\delta$ T cells show such variations in TNF- α , IL-6, and IFN- γ secretion. The profile of cytokine messenger RNA expression from the purified $\alpha\beta$ and $\gamma\delta$ T cells could be an appropriate first step in this direction in investigations of patients with meningitis.

Reactivity of $\gamma\delta$ T cells to stress protein and heat shock proteins has been well documented.^{6, 13} Bacterial heat shock protein might exert an effect when associated with certain genetic HLA-DR heterodimers, which are subsequently presented to the immune system.⁶ It is possible that bacterial colonization could trigger $\gamma\delta$ T-cell enrichment in genetically prone individuals with meningitis. Re-

cent evidence indicates that $\gamma\delta$ T cells specialize in higher selective ligand recognition.¹⁴ The ligands for $\gamma\delta$ T cells in meningitis may not be simple antigens of certain bacteria but rather secondary autologous antigens induced during the infectious process in genetically susceptible patients with meningitis. Moreover, the induction of $\gamma\delta$ T cells by autoantigens may not automatically result in autoimmune manifestations of infectious diseases.

TNF- α and IL-6 are now recognized as primary mediators in the pathogenesis of infection, injury, and inflammation.^{15, 16} TNF- α induces the release of a cascade of mediators, such as IL-1 and platelet activating factor, and IL-6 causes the synthesis of acute phase proteins.^{16, 17} Microvascular endothelial cells are major targets for the proinflammatory activities of TNF- α and IL-6.^{16, 17} IL-1, platelet activating factor, and acute phase proteins all act synergistically with TNF- α and IL-6 to amplify microvascular damage and deleterious inflammatory changes. The data presented here suggest a strong correlation between expansion of $\gamma\delta$ T cells and their secretion of TNF- α and IL-6 and the clinical manifestations of bacterial meningitis. Earlier production of TNF- α , IL-1, and platelet activating factor was shown to be a fundamental event in the initiation and maintenance of the inflammatory response in the central nervous system during the course of bacterial meningitis.¹⁸

Taken together, our data clearly indicate that in patients with bacterial meningitis, a selective expansion of CD3+CD4+CD8 $-$ T-cell subsets that express the TcR $\gamma\delta$ consistently occurs. In addition, increased $\gamma\delta$ T cells correlated with the enhanced production of proinflammatory cytokines, TNF α , and IL-6. However, in the healthy individuals and patients with bacterial infection but without meningitis, the CD3+CD4+CD8 $-$ T cells represent a minority of the TcR $\gamma\delta$ population, although most express the TcR $\alpha\beta$. Increased proportions of $\gamma\delta$ T cells have been found in selective infectious diseases, such as in acute Epstein-Barr virus infection,¹⁹ leprosy and other mycobacterial diseases,^{3, 6} human immunodeficiency virus-1 infections,⁵ and some parasitic diseases.^{3, 4} The elevation of $\gamma\delta$ T cells and their secretion of TNF- α and IL-6 may be more important in the pathogenetic mechanisms of inflammatory response in bacterial meningitis, because CD3+ TcR $\gamma\delta$ T cells returned to almost normal levels after meningitis had resolved in such patients. The novel features of $\gamma\delta$ T cells in bacterial

meningitis should be added to the immune system abnormalities already reported in these patients.^{8, 10, 18}

REFERENCES

1. Raulet DH. The structure, function and molecular genetics of the $\gamma\delta$ T cell receptor. *Annu Rev Immunol* 1989;7: 175-207.
2. Moretta L, Ciccone E, Ferrini S, et al. Molecular and cellular analysis of human T lymphocytes expressing $\gamma\delta$ T cell receptor. *Immunol Rev* 1991;120:117-35.
3. Born WK, O'Brien RL, Modlin RL. Antigen specificity of $\gamma\delta$ T lymphocytes. *FASEB J* 1991;5:2699-5.
4. Raziuddin S, Telmesani AW, El-Awad ME, Al-Amari O, Al-Janadi M. $\gamma\delta$ T cells and the immune response in visceral leishmaniasis. *Eur J Immunol* 1992;22:1143-8.
5. De Maria A, Ferrazin A, Ferrini S, Ciccone E, Terragna A, Moretta L. Selective increase of a subset of T cell receptor $\gamma\delta$ T lymphocytes in the peripheral blood of patients with human immunodeficiency virus type 1 infection. *J Infect Dis* 1992;165:917-9.
6. Barnes PF, Grisso CL, Abrams JS, Band H, Rea TH, Modlin RL. $\gamma\delta$ T lymphocytes in human tuberculosis. *J Infect Dis* 1992;165:506-12.
7. Azubuike JC. Childhood bacterial meningitis in Tabuk, Saudi Arabia. *Ann Saudi Med* 1990;10:145-8.
8. Raziuddin S, El-Awad ME, Mir NA. Bacterial meningitis: T cell activation and immunoregulatory CD4⁺ T cell subset alteration. *J ALLERGY CLIN IMMUNOL* 1991;87:1115-20.
9. Vinjay Kumar E, Raziuddin S, Wardle EM. Plasma endotoxin in patients with trauma, sepsis and severe haemorrhage. *Clin Intensive Care* 1991;2:4-9.
10. Waage A, Brandtzaeg P, Expevik T. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal meningitis. *Lancet* 1987;1: 355-7.
11. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Expevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. *J Exp Med* 1989; 169:333-7.
12. Tsujimoto M, Yip YK, Vilcek J. Interferon- γ enhances expression of cellular receptors for tumor necrosis factor. *J Immunol* 1986;136:2441-4.
13. Haregeoin A, Soman G, Mom RC, Finberg RW. Human gamma/delta⁺ T cells respond to mycobacterial heat-shock protein. *Nature* 1989;340:309-12.
14. Janeway CA Jr, Jones B, Hayday A. Specificity and function of T cells bearing $\gamma\delta$ receptors. *Immunol Today* 1988;9:73-6.
15. Beutler B, Cerami A. Cachetin: more than a tumor necrosis factor. *N Engl J Med* 1987;316:379-85.
16. Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990;8:253-63.
17. Cannon J, Tompkins RG, Gelfand JA, et al. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990;161:79-84.
18. Arditi M, Manogue RK, Caplan M, Yorgev R. Cerebrospinal fluid cachetin/tumor necrosis factor- α and platelet-activating factor concentrations and severity of bacterial meningitis in children. *J Infect Dis* 1990;162:139-47.
19. De Paoli P, Gennari D, Martelli P, Cavarzerani A, Comoretto R, Santini G. $\gamma\delta$ T cell receptor bearing lymphocytes during Epstein-Barr virus infection. *J Infect Dis* 1990;161: 1013-6.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY are available to subscribers (only) for the 1994 issues from the Publisher, at a cost of \$66.00 for domestic, \$88.62 for Canadian, and \$84.00 for international subscribers for Vol. 93 (January-June) and Vol. 94 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.