Predominant Activation and Expansion of Vγ9-bearing γδT Cells In Vivo as well as In Vitro in Salmonella Infection

Toshiro Hara,*|| Yumi Mizuno,* Kohji Takaki,§ Hidetoshi Takada,* Hideki Akeda,§ Tomonobu Aoki,§ Mari Nagata,* Kohji Ueda,* Goro Matsuzaki,‡ Yasunobu Yoshikai,‡ and Kikuo Nomoto‡

**Department of Pediatrics, Faculty of Medicine; ‡Department of Immunology, Institute of Bioregulation, Kyushu University, Fukuoka City 812, Japan; §Fukuoka Municipal Hospital for Sick Children and Infectious Disease, Fukuoka City 812, Japan; and ||Department of Pediatrics, Saga Prefectural Hospital, Saga, 840, Japan*

Abstract

γδ T cell receptor–positive cells (γδ T cells) have recently been implicated to play a role in the protection against infectious pathogens. Serial studies on γδ T cells in 14 patients with salmonella infection have revealed that the proportions of γδ T cells (mean±SD: 17.9±13.2%) in salmonella infection were significantly increased ($P < 0.01$) compared with 35 normal controls (5.0±2.6%) and 13 patients with other bacterial infections (4.0±1.4%). Expansion of γδ T cells was more prominent in the systemic form (28.9±10.8%) than in the gastroenteritis form (10.5±7.9%) of salmonella infection ($P < 0.01$). Most in vivo–expanded γδ T cells expressed Vγ 9 gene product. Increased activated (HLA-DR+) T cells were observed in all the six patients with the systemic form and four of the seven with gastroenteritis form. Especially in the six with systemic form, γδ T cell activation was significantly higher than αβ T cell activation at the early stage of illness ($P < 0.01$). When peripheral blood lymphocytes from normal individuals were cultured with live salmonella, γδ T cells were preferentially activated and expanded and most of them expressed Vγ 9. Purified γδ T cells also responded to live salmonella in vitro. The present study suggests that human γδ T cells play a role in the protection against salmonella infection in vivo. (*J. Clin. Invest.* 1992. 90:204–210.) Key words: γδ T cell receptor • *Salmonella typhi* • mycobacterium • heat shock protein • granular lymphocyte

Introduction

Human T cell antigen receptor (TCR)[1] is composed of either α and β or γ and δ chains (1, 2). The tissue distribution and morphological characterization of γδ T cell receptor–positive cells (γδ T cells) have been studied in normal and pathological conditions (3–7). In addition, γδ T cells have been reported to produce various cytokines and to have cytolytic activity (1–3). However, physiological functions of γδ T cells remain to be elucidated.

γδ T cells appear to play a role in the defense against infectious pathogens in mice (8–12). In humans, in vitro studies have suggested a protective role of γδ T cells in various bacterial infections (13–16). However, mycobacterium species are the only human pathogens against which γδ T cells have been implicated to be involved in the host defense by both in vivo and in vitro studies (6, 17).

We report here that Vγ9-expressing γδ T cells are preferentially activated and expanded in vivo as well as in vitro associated with salmonella infection. In addition to mycobacterium species (6, 17), salmonella is the second human pathogen against which γδ T cells, especially Vγ9-expressing cells, are suggested to play a role in the protection, by in vivo and in vitro studies.

Methods

Patients

14 patients with salmonella infection were included in the present study. The diagnosis of salmonella infection was made by positive stool cultures for salmonella. In three (cases 1–3) of them, blood cultures were also positive for *Salmonella typhi*. 14 patients were classified into three groups: 6 patients with the systemic form characterized by predominant systemic symptoms (fever of over 10 d duration, malaise, lethargy); 7 patients with the gastroenteritis form; and 1 asymptomatic carrier. The mean age±SD of the 14 patients was 11.0±9.5 yr (range 0.4–28); there were 9 males and 5 females. Cases 7 and 14 were siblings. None of them had histories of recurrent infections suggestive of underlying immunological disorders. Clinical features of the patients are shown in Table I. The control population consisted of 35 normal individuals (mean age±SD: 9.3±8.7 yr, range 0.9–22) with no histories of infections with salmonella or mycobacterium and 13 patients with other bacterial infections (mean age±SD: 5.3±6.1 yr, range 0.5–23): 4 campylobacter infection; 2 shigellosis; 4 staphylococcal infection; 3 streptococcosis. Informed consent was obtained from patients or their parents.

Antibodies

TCRδ1 and δTCS1 mAbs were purchased from T Cell Sciences, Inc. (Cambridge, MA). Anti-CD3 (Leu4); anti-CD4(Leu3) anti-CD8 (Leu2); WT31, which reacts with αβTCR at low concentrations (18); anti-CD11b (Leu15); anti-CD16 (Leu11); and anti-CD57 (Leu7) mAbs were from Becton-Dickinson & Co. (Mountain View, CA). Anti-HLA-DR (OKDR), anti-CD4 (OKT4), and anti-CD8 (OKT8) mAbs were purchased from Ortho Pharmaceutical (Raritan, NJ). Anti-CD19 (B4), anti-CD20 (B1), and anti-CD56 (NKH1) mAbs were from Coulter Electronics Inc. (Hialeah, FL). TiγA mAb was kindly provided by Dr. T. Hercend, Institute Gustave-Roussy, Villejuif, France (19). FITC-conjugated goat $F(ab')_2$ anti-mouse Ig was purchased from Tago, Inc. (Burlingame, CA). For two-color analysis, FITC and phycoerythrin-conjugated mAb were employed.

Address reprint requests to Toshiro Hara, M.D., Department of Pediatrics, Saga Prefectural Hospital, 1-12-9, Mizugae, Saga City, 840, Japan.

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1. *Abbreviations used in this paper:* BCG, bacillus Calmette Guerin; γδ T cells, γδ T cell receptor-positive cells; hsp, heat-shock protein; PPD, purified protein derivative; TCR, T cell antigen receptor.

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Table I. Clinical Features of 14 Patients with Salmonella Infection

Case	Age	Sex	Salmonella serotype and form of infection	Duration	
				Fever	Diarrhea
	yr				*d*
1.	28	M	*Salmonella typhi* systemic	14	7
2.	20	M	*Salmonella typhi* systemic	12	5
3.	4	F	*Salmonella typhi* systemic	16	+
4.	22	M	*Salmonella paratyphi A* systemic	12	–
5.	13	M	*Salmonella paratyphi A* systemic	25	+
6.	1	F	*Salmonella schwarzengrund* systemic	22	+
7.	2	F	*Salmonella braenderup* gastroenteritis	3	2
8.	4	F	*Salmonella bardo/newport* gastroenteritis	7	9
9.	8	M	*Salmonella bardo/newport* gastroenteritis	9	6
10.	4	M	*Salmonella bardo/newport* gastroenteritis	4	10
11.	9	M	*Salmonella istanbul/hadar* gastroenteritis	8	5
12.	26	M	*Salmonella typhimurium* gastroenteritis	3	6
13.	13	F	*Salmonella typhimurium* gastroenteritis	10	8
14.	0.4	M	*Salmonella braenderup* asymptomatic carrier	–	–

Isolation of γδ T cells and αβ T cells

PBMC from the patients or control subjects were isolated on Ficoll Hypaque density gradient centrifugation. PBMC were depleted of monocytes by plastic adherence. T cells were purified from plastic-non-adherent PBMC by rosette formation with neuraminidase-treated sheep erythrocytes (E), as described previously (20).

Negative selection of γδ T cells was performed according to the method described by Kabelitz et al. (21) with modifications. E-rosetted T cells were first incubated with saturating amounts of anti-CD4 (OKT4A), anti-CD8 (OKT8), anti-CD20, and anti-CD57 mAbs for 30 min at 4°C. The cells were washed free of excess antibodies and resuspended in RPMI 1640 with 10% FCS (Gibco Laboratories, Grand Island, NY). The cells were incubated with rabbit baby complement (Pel-Freeze Biologicals, Rogers, AR) for 30 min at 37°C. Dead cells were removed by centrifugation on Ficoll-Hypaque gradients. Viable cells recovered from the interface were further incubated with anti-CD4 (Leu3), anti-CD8 (Leu2), anti-CD19, anti-CD20, anti-CD56, anti-CD57, and anti-TCR αβ (WT31) mAbs for 30 min at 4°C; washed; and resuspended in RPMI 1640 with 10% FCS. To this was added washed goat anti-mouse Ig-coated M-450 immunomagnetic beads (Dynabeads; Dynal A.S., Oslo, Norway) in RPMI 1640 with 10% FCS at a Dynabeads-to-target cell ratio of 40:1 (22). After incubation at room temperature for 30 min with regular gentle mixing, the mixture was suspended in 1 ml of RPMI 1640 with 10% FCS and placed on a magnetic separator. The supernatant was removed, washed, and resuspended in RPMI 1640 with 10% FCS. Depletion was repeated with anti-CD4 (Leu3), anti-CD8 (Leu2), anti-CD56, anti-CD57, and WT31 mAbs until WT31-positive cells were < 1%. When the resulting cell suspensions were > 80% TCRδ1-positive, the cells were used as purified γδ T cells. For negative selection of αβ T cells, E-rosetted T cells were incubated with anti-CD20, anti-CD16, and anti-CD57 mAbs for 30 min at 4°C, followed by complement-mediated cell lysis, as described above. The remaining cells were further incubated with anti-CD19, anti-CD20, anti-CD16, anti-CD56, anti-CD57, and anti-TCRγδ (TCR δ1) mAbs for 30 min at 4°C. After removal of mAb-coated cells with goat anti-mouse Ig-coated M-450 immunomagnetic beads (bead/cell, 40:1), depletion was repeated until TCRδ1-positive cells were < 1%. The purified αβ T cells comprised > 98% WT31+ cells.

For positive selection of γδ T cells, E-rosetted T cells were first depleted of CD4+ and CD8+ cells with complement-mediated cell lysis. The viable cells were further incubated with anti-CD4, anti-CD8, and anti-TCR αβ (WT31) mAbs, followed by wash, resuspension, and depletion with immunomagnetic beads at a Dynabeads-to-target cell ratio of 40:1. The remaining cells were incubated with TCRδ1 mAb for 15 min at 4°C. The cells were washed and resuspended in 0.5 ml of RPMI 1640 with 10% FCS. Goat anti-mouse Ig-coated M-450 immunomagnetic beads were added at a Dynabeads-to-target cell ratio of 3:1 (23). TCRδ1+ cells were recovered from bead-rosetted cells. Purified γδ T cells were > 95% TCRδ1+ and < 1% WT31+.

In vitro stimulation of T cells

Salmonellae typhi and *typhimurium* in this experiment were clinical isolate strains. These strains were cultured at 37°C on Nutrient agar plates (Eiken Chemical Co., Ltd., Tokyo, Japan), collected by scraping, and suspended in sterile PBS. The bacteria were used as live or heat-killed (autoclaved for 20 min at 120°C) condition. Bacillus Calmétte Guérin (BCG; *Mycobacterium bovis*) and purified protein derivative (PPD) were purchased from Japan BCG Laboratory (Tokyo, Japan). Recombinant 65-kD mycobacterial heat-shock protein (65-kD hsp) was kindly provided by Dr. R. van der Zee, Laboratory of Bacteriology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Recombinant human IL-2 (rIL-2) was obtained from Takeda Chemical Industries, Ltd. (Osaka, Japan). Purified T cells with 10% irradiated plastic-adherent cells or PBMC in RPMI 1640 with 15% heat-inactivated (56°C, 30 min) human AB serum, 1 mM glutamine, and no antibiotics were incubated with salmonella (bacteria/cell ratio was 10:1) or 10 μg/ml of BCG for 4 h at 37°C. Then, the cells were washed twice and resuspended in the above medium containing 100 μg/ml of streptomicin and ampicillin to kill remaining bacteria. After 4 h incubation with bacteria, or in the presence or absence of 10 μg/ml of PPD or 65-kD hsp, the cells were cultured at 5×10^5/ml. rIL-2 was added when indicated. For proliferation assay, the cells were pulsed with [^{3}H]thymidine during the last 12 h and harvested onto glass fiber filters. [^{3}H]Thymidine incorporation was measured by liquid scintillation counter, as described previously (24).

Flow cytometric analysis

Cells were stained with an appropriate mAb at 4°C for 30 min. For indirect staining, the cells were washed and further incubated with FITC-conjugated goat F(ab′)$_2$ anti-mouse Ig. After wash, single-color or two-color flow cytometric analysis was done using an Ortho Cytron (Ortho Diagnostic Systems, K.K., Tokyo, Japan) or an Epics V flow cytometer (Coulter Electronics Inc.), as described previously (24). Background fluorescence was assessed by staining with control irrelevant isotype-identical mAb.

Morphological evaluation of granular lymphocytes

Cell morphology was examined with Wright-Giemsa-stained smears. Granular lymphocytes were defined as lymphocytes with three or more azurophilic granules in the cytoplasm, according to the previous report (25). Counting of granular lymphocytes was performed by an investigator without knowledge of percentages of γδ T cells. Data were expressed as percentages of total lymphocytes.

Statistical analysis

Statistical evaluation was performed by Student's t test. Correlation coefficient was obtained by linear regression analysis.

Results

Predominant expansion and activation of γδ T cells in vivo in salmonella infection

Surface marker analysis of peripheral blood T lymphocytes from patients with salmonella infection revealed that percentages of γδ T cells (mean±SD: 17.9±13.2%) were significantly increased ($P < 0.01$) compared with normal controls (5.0±2.6%) and patients with other bacterial infections (4.0±1.4%), as shown in Fig. 1. Expansion of γδ T cells was more prominent in six patients with the systemic form (28.9±10.8%) than in seven patients with the gastroenteritis form (10.5±7.9%) ($P < 0.01$) and not observed in an asymptomatic carrier (Table II). γδ T cell expansion was not related to the age, sex, or duration of positive stool cultures for salmonella. Increases of γδ T cells were first noted on day 13.2±6.1

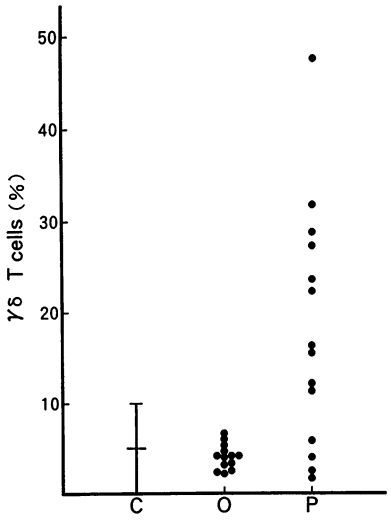

Figure 1. Proportions of peripheral blood γδ T cells in 14 patients with salmonellosis (*P*), 13 patients with other bacterial infections (*O*), and 35 normal controls (*C*). Proportions of γδ T cells in patients with salmonellosis (mean±SD: 17.9±13.2%) were significantly higher than those in patients with other infections (4.0±1.4%) and in normal controls (5.0±2.6%) ($P < 0.01$). The bar indicates the normal range (mean±2 SD: 0–10.2%) of γδ T cells.

Table II. Surface Marker Analysis of the 14 Patients

Case	Months after onset	CD3+	CD3+ TCRδ1+	HLA-DR+ TCRδ1+	HLA-DR+ WT31+	HLA-DR+ CD3+	Salmonella (stool)
1	0 (11)	82.0	24.8	18.3	15.1	37.7	+
	1	82.0	31.9	19.3	5.2	25.9	−
	2	78.1	31.1	2.8	1.5	3.7	−
	4	78.4	23.2	4.0	4.3	7.9	−
2	0 (25)	84.4	28.7	15.8	6.2	21.4	+
	1	87.9	24.2	1.7	1.8	4.8	−
3	0 (16)	70.9	18.4	12.6	3.2	15.7	+
	1	65.7	22.3	3.7	1.4	2.5	−
4	0 (13)	81.3	47.7	35.2	3.4	35.6	+
	1	89.1	43.6	3.2	1.0	3.2	−
5	0 (21)	77.8	21.3	9.2	3.1	13.1	+
	1	80.9	27.3	2.3	2.8	2.0	−
	5	79.9	25.9	1.0	1.0	1.7	−
	10	73.8	19.9	1.5	3.1	3.6	−
	17	73.8	16.0	0.8	1.8	1.4	−
6	0 (19)	74.8	15.7	4.3	2.8	7.5	+
	3	72.6	7.7	NT	NT	3.4	−
7	0 (7)	80.4	23.8	13.8	3.9	17.8	+
	1	69.0	11.4	1.8	1.2	3.7	+
8	0 (8)	73.7	5.7	2.1	21.6	24.8	+
	0 (25)	80.6	12.3	3.7	4.1	4.2	+
	1	77.6	11.1	0.8	2.7	2.7	+
	5	76.1	7.4	0.2	1.6	2.9	−
9	0 (10)	71.9	5.7	NT	NT	5.9	+
10	0 (7)	67.6	13.2	NT	NT	2.4	+
	1	80.1	15.9	1.3	3.6	4.1	+
	5	67.9	8.5	0.2	1.6	1.3	−
11	0 (6)	80.6	11.7	1.5	3.3	3.1	+
	0 (11)	78.4	10.3	0.2	0.5	1.1	−
12	0 (11)	65.4	2.4	0.3	6.0	8.4	+
13	0 (18)	70.2	1.8	NT	NT	3.7	+
14	—	72.3	3.9	3.1	3.1	4.8	+

Numbers in parentheses indicate the days of illness examined. NT, not tested.

Table III. Surface Phenotypes on Expanded γδTCR+ Cells

Case	TCRδ1+	TiγA+	δTCS1+	CD4+ TCRδ1+	CD8+ TCRδ1+	CD11+ TCRδ1+	CD16+ TCRδ1+
				%			
1	22.8	21.3	0.9	0.4	3.2	7.6	15.3
5	28.3	26.5	1.2	1.4	4.7	24.7	NT
6	15.7	NT	NT	0.3	3.7	12.2	NT
7	24.5	23.0	0.8	0.6	3.2	NT	0.9

NT, not tested.

(SD) of illness. Maximal expansion of γδ T cells was observed at the initial examinations in five patients and ~ 1 mo after onset in five patients. Strikingly, it was found that γδ T cell expansion persisted over 4 and 17 mo in cases 1 and 5. Further analysis of expanded γδ T cells was performed in cases 1, 5, 6, and 7, infected with *S. typhi, paratyphi, schwarzengrund,* and *braenderup,* respectively. All the γδ T cells examined were Tiγ A-positive and δ TCS1-negative (Table III). Other surface markers on γδ T cells included negative CD4, 3.2–4.7% dim-positive CD8, 7.6–24.7% positive CD11, and 0.9–15.3% positive CD16. In addition, simultaneous examinations of surface phenotypes and morphology of lymphocytes revealed that proportions of granular lymphocytes were significantly correlated with percentages of γδ T cells ($P < 0.02$), as shown in Fig. 2.

Increased in vivo–activated T cells (HLA-DR+CD3+ cells > 5%) were observed in 10 of the 14 patients with salmonella infection, as shown in Table II. Of the 10 with increased activated T cells, 7 demonstrated predominant activation of γδ T cells over αβ T cells, whereas 2 showed predominant αβ T cell activation. Especially in six patients with systemic form of salmonella infection, γδ T cell-activation (increases of HLA-DR+ γδ T cells) was significantly higher than αβ T cell-activation (increases of HLA-DR+ αβ T cells) at the early stage of illness ($P < 0.01$), as shown in Fig. 3. In seven patients with the gastroenteritis form, only one showed predominant γδ T cell-activation. Percentages of activated γδ T cells as well as αβ T cells were decreased below 5% within at least two months, despite continued increases of γδ T cells (Figs. 3 and 4).

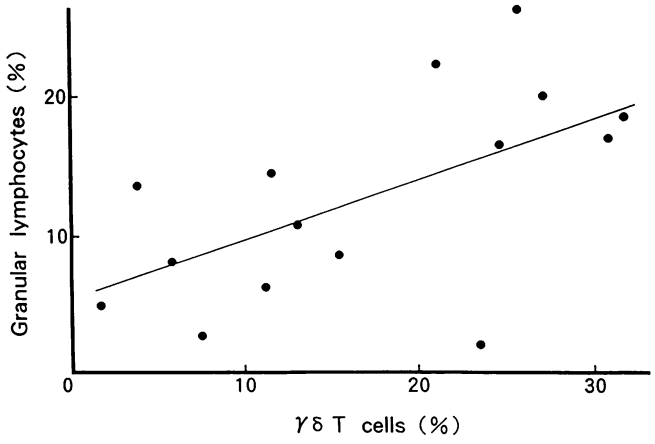

Figure 2. Correlation between γδ T cells (%) and granular lymphocytes (%). Percentages of γδ T cells were significantly correlated with proportions of granular lymphocytes ($r = 0.60$, $P < 0.02$).

Preferential activation of γδ T cells in vitro in response to live salmonella

When PBMC from normal individuals were cultured with live salmonella, γδ T cells were markedly expanded. Fig. 5 shows the representative data from four normal individuals. Proportions of γδ T cells (TCRδ1+ cells) were increased from 4.2 to 26.3% (TiγA+ cells; 25.5%, δTCS1; 0.8%) for *S. typhi* and to 20.4% (TiγA+ cells; 21.5%, δTCS1; 1.3%) for *S. typhimurium.* Slight γδ T cell-expansion (7.5%) was observed with killed *S. typhi.* Consistent with previous reports (15, 16, 26, 27), γδ T cell expansion was seen with live *Mycobacterium bovis* (BCG: 22.2%) and PPD (6.5%) and not with 65-kD hsp (2.1%). On day 8 of culture in the presence of live *S. typhi,* γδ T cells were preferentially activated (84.0% HLA-DR+) in comparison with αβ T cells (14.3% HLA-DR+) (Fig. 6). Similar results were obtained with live *S. typhimurium* and *M. bovis* (data not shown). When αβ and γδ T cells were purified by negative selection, *S. typhi* and *M. bovis* preferentially activated γδ T cells, as shown in Table IV. To further confirm the results, γδ T cells were purified from normal individuals and from patients with *S. typhi* by positive selection and cultured for 7 d in the presence of rIL-2. Purified γδ T cells showed vigorous proliferation in response to live *S. typhi* and *M. bovis.*

Discussion

Human γδ T cells have been suggested to play a role in the defense against various infections by in vitro studies (13–16).

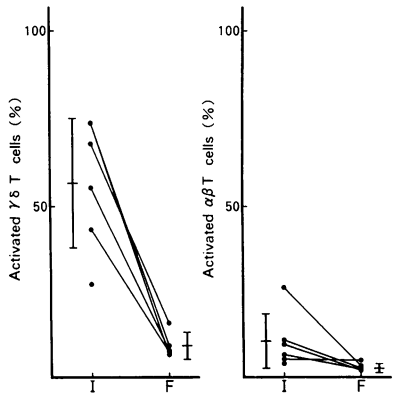

Figure 3. Activated γδ T cells and αβ T cells in six patients with the systemic form of salmonella infection at the initial (*I*) and follow-up (*F*) examinations. Activated γδ T and αβ T cells (%) were calculated by %HLA-DR+TCRδ1+ cells × 100/%TCRδ1+ cells and %HLA-DR+WT31+ cells × 100/(%CD3+ cells-%TCRδ1+ cells), respectively. Mean percentages (±SD) of activated γδ T cells (57.0±18.8%) at the initial examinations were significantly higher than those of activated αβ T cells (10.7±8.1%).

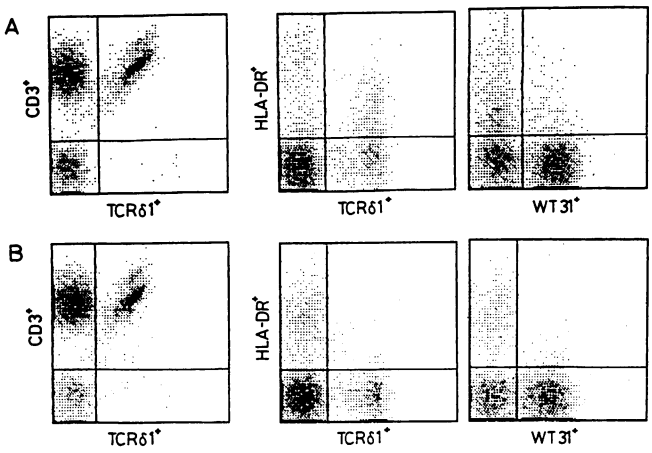

Figure 4. Surface marker analysis of the peripheral blood T cells from case 1 at the initial and follow-up examinations. The initial analysis on day 11 of illness (*A*) demonstrated 24.8% CD3+TCRδ1+ cells, 18.3% HLA-DR+TCRδ1+ cells, and 15.1% HLA-DR+WT31+ cells. The follow-up examination (*B*) showed 31.1% CD3+TCRδ1+ cells, 2.8% HLA-DR+TCRδ1+ cells, and 1.5% HLA-DR+WT31+ cells.

In vivo, human γδ T cells have been reported to be increased in the skin of *Mycobacterium leprae* infection (17), in the lymph nodes of *Mycobacterium tuberculosis* infection (6) and in the peripheral blood of *Plasmodium falciparum* infection (28, 29) or Epstein-Barr virus infection (30). However, mycobacterium species are the only pathogens that both in vivo and in vitro studies have consistently shown an involvement of γδ T cells in the immune defense.

Salmonella is one of the intracellular pathogens against which cell-mediated immunity is regarded as the most important defense mechanism (31, 32). In mice, T cells have been reported to play an important role in resistance to salmonella by T cell–transfer experiments (33–35). In humans, limited data are available on immunologic defense mechanisms against salmonella species. An importance of antibody-dependent cell-mediated cytotoxicity was suggested by in vitro studies on normal individuals and by in vivo studies on salmonella vaccinees (36).

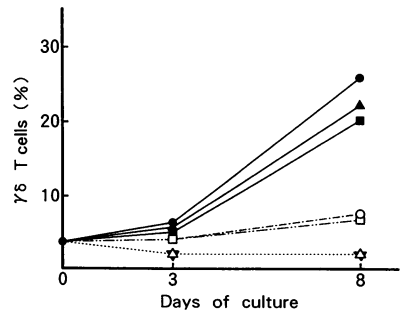

Figure 5. In vitro expansion of γδ T cells to live salmonella. For stimulation with bacteria, PBMC were incubated with live *Salmonella typhi* (●), live *Salmonella typhimurium* (■), live *Mycobacterium bovis* (▲), or killed *S. typhi* (○) for 4 h at 37°C, washed twice, and cultured at 5 × 10^5/ml in RPMI 1640 with 15% human AB serum and antibiotics. PBMC were also cultured in the absence (▽) or presence of 10 μg/ml of PPD (□) or 65-kD hsp (△). The cells were harvested and phenotypic analysis was performed at indicated days. Proliferation assay on day 8 showed 53,410 cpm for live *S. typhi*, 49,650 cpm for live *S. typhimurium*, 56,390 cpm for *M. bovis*, 35,210 cpm for killed *S. typhi*, 19,470 cpm for PPD, 1,420 cpm for 65-kD hsp, and 1,360 cpm for medium. This was representative of four normal individuals.

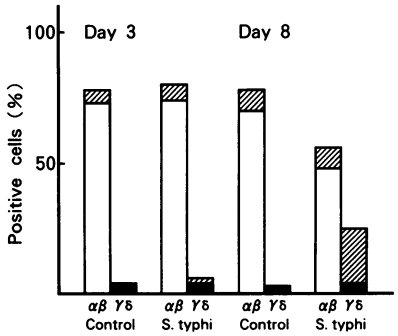

Figure 6. Preferential activation of γδ T cells by live salmonella in vitro. PBMC were incubated with or without live *S. typhi* for 4 h at 37°C, washed twice, and cultured, as described in Fig. 5. The cells were analyzed for surface phenotype on days 3 and 8. αβ T cells (WT31+ cells) (□), γδ T cells (TCRδ1+ cells) (■), activated (HLA-DR+) αβ or γδT cells (▨).

In salmonella infection, we have shown that human γδ T cells were selectively activated and expanded in vivo as well as in vitro. γδ T cell expansion in the peripheral blood was more prominent in the systemic form than in the gastroenteritis form. However, *S. typhimurium* that caused gastroenteritis without inducing γδ T cell expansion in vivo significantly activated and expanded γδ T cells in vitro in the present study and in the recent report (16). This discrepancy between in vivo and in vitro observations would be ascribed to the difference in invasion modes: salmonellae in the systemic form rapidly invade the blood stream and induce peripheral γδ T cell expansion; salmonellae that cause gastroenteritis penetrate the intestinal mucosa with no further invasion (18). It is possible that γδ T cells were locally expanded in the gut or gut-associated

Table IV. Proliferation of Purified αβ and γδ T Cells in Response to Salmonella and Other Antigens

	Nil	*S. typhi*	*M. bovis*	PPD	hsp
A. 1. αβ T cells	193	3,105	2,439	549	211
γδ T cells	215	9,815	8,231	831	178
2. αβ T cells	245	2,933	3,211	766	189
γδ T cells	392	13,619	11,007	1,245	298
B. 1. γδ T cells	1,766	40,536	48,716	NT	NT
γδ T cells	1,501	38,488	31,315	NT	NT
2. γδ T cells	1,982	53,619	35,612	NT	NT
γδ T cells	1,875	59,614	48,420	NT	NT

A. αβ or γδ T cells, purified by negative selection from normal individuals, were stimulated with *S. typhi* (bacteria/cell, 10:1), *M. bovis* (10 μg/ml), PPD (10 μg/ml), or 65-kD hsp (10 μg/ml) in the presence of 10% irradiated autologous monocytes. The cells were cultured at 2 × 10^5/ml and [^{3}H]thymidine incorporation was measured after 7 d. Mean cpm of triplicate cultures are shown. SD was < 15%. Experiment 1. αβ T cells, 98.3% WT31+; γδ T cells, 81.3% TCRδ1+. Experiment 2. αβ T cells, 98.8% WT31+; γδ T cells, 83.1% TCRδ1+. B. Purified γδ T cells by positive selection were cultured for 7 d in the presence of rIL-2 (1 U/ml). Then, the γδ T cells (2 × 10^5/ml) with 10% irradiated autologous monocytes were stimulated with bacteria in the presence of rIL-2 (0.2 U/ml), as described above. [^{3}H]Thymidine incorporation was measured after 5 d. Experiment 1. γδ T cells from normal individuals were 95.3 and 97.2% TCRδ1+. Experiment 2. γδ T cells from patients with *Salmonella typhi* and *paratyphi A* were 97.5 and 98.1% TCRδ1+. NT, not tested.

lymphoid tissue in the gastroenteritis form. Predominant expansion of in vivo–activated γδ T cells at the early phase in the systemic form of salmonella infection further suggests that γδ T cells might play a role in the defense against salmonella infection. Because salmonellosis is one of the intestinal infections, γδ T cells might play a role in intestinal immunity, as suggested in celiac disease (37). In addition, it is worthy of note that γδ T cell expansion was not observed in vivo in infection with staphylococcus or streptococcus, which has been reported to induce γδ T cell expansion in vitro (15). It suggests that in vivo, other defense mechanisms would play a primary role against such nonintracellular pathogens rather than γδ T cells.

The γδ T cells expanded in vivo and in vitro in salmonella infection showed a Tiγ A+ and δTCS1− phenotype, indicating a preferential usage of Vγ9 and probably Vδ2 (19, 38). The γδ T cells expanded in vivo and in vitro in response to various bacterial antigens such as *M. tuberculosis, M. leprae,* and *Streptococcus pyogenes* also expressed Vγ9 (26, 27, 39–41). The limited diversity of TCRδ gene expression among γδ T cells in leprosy lesions has strongly suggested that γδ T cell response to infection involves clonal selection from a diverse TCR repertoire and further expansion by antigen (42).

It remains to be determined what Vγ9-bearing cells expanded in salmonella infection recognize. There are possibilities that the salmonella-reactive Vγ9-bearing cells might be triggered by exogenous bacterial antigens or endogenous ligands expressed on human cell surface. Salmonella may have an antigenic determinant similar to a protease-resistant low molecular weight fraction of mycobacterial cell lysates that has been reported to exclusively stimulate Vγ9 T cells (43). Alternatively, Vγ9 cells in salmonella infection might have responded to an antigen displayed on host cells infected with salmonella. Delayed expansion of activated γδ T cells in cases 1 and 8 suggests a possibility that, in certain cases, γδ T cells responded to an endogenous ligand on host cells stressed by salmonella infection, as reported in murine influenza infection (44). γδ T cells have been documented to be capable of secreting various cytokines (1–3). Especially, Vγ9-bearing cells have been reported to produce high amounts of interferon-γ and tumor necrosis factor-α after mitogenic stimulation (45). Therefore, it is likely that activated Vγ9-bearing γδ T cells expanded in salmonella infection may exert effects via secretion of cytokines to activate macrophage and natural killer cells or target cell lysis, as reported in reference 46. Further study would be necessary to determine how γδ T cells are actually involved in the protection against salmonella infection.

Peripheral γδ T cells were reported to have a phenotype of granular lymphocye (7). Close correlation between increases of granular lymphocytes and percentages of γδ T cells in salmonella infection might explain a previous finding of expansion of granular lymphocytes in a patient with a history of salmonella infection (47). Granular lymphocytosis associated with other intracellular bacterial infections, including tuberculosis and brucellosis (48), might have reflected γδ T cell expansion.

Overall, the present observation that human γδ T cells were selectively activated and expanded associated with salmonella infection in vivo as well as in vitro supports a significant role of γδ T cells in intracellular infections. It might also be interesting to investigate a possible role of γδ T cells in the development of salmonella-associated immune disorders such as post-salmonella reactive arthritis (49) or Reiter disease (50).

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