INFECTION AND IMMUNITY, Oct. 1997, p. 4267–4272 Vol. 65, No. 10
0019-9567/97/$04.00+0
Copyright © 1997, American Society for Microbiology

In Vivo and In Vitro Activation and Expansion of γδ T Cells during *Listeria monocytogenes* Infection in Humans

FABIENNE JOUEN-BEADES,[1]* ESTELLE PARIS,[1] CORINNE DIEULOIS,[1] JEAN-FRANÇOIS LEMELAND,[2] VÉRONIQUE BARRE-DEZELUS,[3] STÉPHANE MARRET,[3] GUY HUMBERT,[4] JACKY LEROY,[5] AND FRANÇOIS TRON[1]

Groupe de Recherche en Immunopathologie, Institut Fédératif de Recherche Multidisciplinaire sur les Peptides (IFR 23),[1] Laboratoire de Bactériologie,[2] Service de Néonatologie,[3] Service des Maladies Infectieuses,[4] and Service de Réanimation Médicale,[5] Hôpital Charles Nicolle, Rouen, France

Received 28 February 1997/Returned for modification 9 May 1997/Accepted 23 July 1997

Serial flow cytometry analyses of peripheral blood mononuclear cells obtained from 8 patients infected with *Listeria monocytogenes* showed a higher percentage ($P < 0.01$) of γδ T cells (median, 11.7; range, 3.7 to 35.3) than did 16 age-matched uninfected controls (1.7, 0.4 to 13). Most in vivo-expanded γδ T cells expressed the Vγ9 and Vδ2 gene products and displayed a memory phenotype (CD45RO^{high}), and patients' γδ T cells expressed significantly more ($P < 0.01$) activation marker HLA-DR than did controls (19.8% [median] and 0.9 to 87.6% [range] versus 2.3% and 0 to 4.7%, respectively). When peripheral blood mononuclear cells from normal donors were cultured in vitro with heat-killed *Listeria* cells, analysis of CD25 and HLA-DR expression on γδ and αβ T cells indicated that a high percentage of γδ T cells was activated early compared to αβ T cells. In addition, depletion of γδ T cells before culture abrogated the early lymphocyte proliferative response induced by the pathogen. Taken together, these results argue for the involvement of γδ T cells during *L. monocytogenes* infection in humans.

Although the role of γδ T cells in the immune response has not been clearly defined, several studies of humans and experimental animals have provided evidence for the involvement of γδ T cells in defense against various pathogens (reviewed in reference 11). In humans, γδ T cells accumulate in the granulomatous lesions of patients with leprosy or leishmaniasis (24) and in the peripheral blood of patients with *Salmonella* infection (12), brucellosis (1), infectious mononucleosis (14), human immunodeficiency virus infection (7), toxoplasmosis (33), or malaria (32). In mice, γδ T cells have also been implicated in viral (17), bacterial (35, 37), and parasitic (31, 38) infections.

In this regard, experimental infection of mice with *Listeria monocytogenes* constitutes an informative model to study γδ T-cell functions and has shown that (i) γδ T cells accumulate within the peritoneum 3 days after intraperitoneal injection of *Listeria* and prior to the αβ T-cell response (28, 35); (ii) γδ T cells are able in vitro to produce gamma interferon in response to *Listeria* antigens (39); (iii) in vivo depletion of γδ T cells exacerbates murine listeriosis at the early phase of infection (15); and (iv) in αβ T-cell receptor (TCR)-deficient mice, γδ T cells can confer early immunity against listeriosis (25). In addition to this protective role, other studies have suggested an immunoregulatory function for γδ T cells during primary listeriosis (9, 16, 22, 25).

These observations prompted us to determine whether γδ T cells also play a role in immunity against *L. monocytogenes* in humans. Herewith, we report that γδ T cells were expanded and activated in the peripheral blood of eight patients infected with *L. monocytogenes* and that, in vitro, a high percentage of γδ, but not αβ, T cells are activated early in response to heat-killed *L. monocytogenes*. These results suggest that, in humans, γδ T cells are involved in immunity against *L. monocytogenes*.

* Corresponding author. Mailing address: Laboratoire d'Immunopathologie Clinique et Expérimentale, Hôpital Charles Nicolle, 1, rue de Germont, 76031 Rouen, France. Phone: 33 2 32 88 80 71. Fax: 33 2 32 88 81 86.

MATERIALS AND METHODS

Patients. Eight patients (three males, five females) with *L. monocytogenes* infection were included in the study. Listeriosis was diagnosed based on the isolation of *L. monocytogenes* from cultures of ear and gastric fluids from three newborns with neonatal listeriosis (patients 1 through 3), amniotic fluid and placenta from a patient who had listeriosis during pregnancy (patient 4), blood from patients who were septicemic (patients 5 and 6), and cerebral fluid from patients who had meningitis (patients 7 and 8). The clinical and other relevant features of these patients are shown in Table 1. Because the age range of infected patients was broad, and neonatal and elderly blood T-lymphocyte subpopulations differ significantly, each patient was matched with two uninfected individuals of the same age. This study received local ethics committee approval. All *L. monocytogenes*-infected individuals received appropriate antibiotic therapy and recovered without complications.

Antibodies. A two- or three-color cytofluorometric analysis was performed with the following monoclonal antibodies (MAb): phycoerythrin (PE) cyanine 5-conjugated anti-CD3 (Immunotech, Marseille, France), PE-conjugated anti-CD25 and anti-CD45RA (Coulter Immunology, Hialeah, Fla.), fluorescein isothiocyanate (FITC)-conjugated anti-HLA-DR and anti-CD45RO (Immunotech), anti-Vγ9 (TiγA; Coulter), anti-Vδ2 (Tiδ2; Immunotech), anti-Vδ1 (δ TCS1; T Cell Diagnostics Inc., Woburn, Mass.), and PE- or FITC-conjugated anti-pan γδ (TCR-γδ-1; Becton Dickinson, Pont-de-Claix, France).

Bacteria. *L. monocytogenes* was a clinically isolated strain. Heat-killed *L. monocytogenes* (HKLM) cells were prepared as follows: bacteria were grown at 37°C on blood agar plates and collected by scraping. The concentration was determined by plating serial 10-fold dilutions of *L. monocytogenes* suspension on blood agar plates. Colonies were counted after 24 h of incubation at 37°C. Bacteria were washed twice in sterile phosphate-buffered saline (PBS) and then autoclaved for 20 min at 100°C. The sterile material was stored at −80°C until use.

PBMC isolation and culture. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation. The cells were washed twice in PBS and suspended in RPMI GLUTAMAX II 1640 culture medium (Gibco BRL Life Technologies, Paisley, United Kingdom) supplemented with 10% human AB serum (Sigma-Aldrich, St. Quentin-Fallavier, France) and 1% penicillin-streptomycin-amphotericin B (Gibco BRL). PBMC (2.5×10^6/ml) were plated in triplicate in 96-well round-bottomed microtiter plates (Costar, Cambridge, Mass.) and incubated with either 10 μg of phytohemagglutinin (PHA) (Sigma) per ml or 5×10^7 HKLM cells/ml for 7 days at 37°C in 5% CO_2.

Immunophenotyping and flow cytometry analysis. Whole blood samples were incubated for 20 min at room temperature with the appropriate fluorescent MAb dilutions and treated with erythrocyte-lysing solution (Q-Prep; Coulter). Cultured cells were incubated for 20 min at room temperature with MAbs in PBS–10% fetal calf serum (FCS) (D. Dutcher, Brumath, France)–0.1% sodium azide, washed twice, and fixed in PBS–1% formaldehyde. Two- or three-color

TABLE 1. Clinical and other relevant features of eight patients with *L. monocytogenes* infection

Patient no.	Age (yr)	Sex[a]	Clinical manifestation
1	0	F	Neonatal listeriosis
2	0	F	Neonatal listeriosis
3	0	F	Neonatal listeriosis
4	31	F	Listeriosis during pregnancy
5	89	M	Septicemia
6	69	M	Septicemia
7	63	M	Meningitis
8	1.5	F	Meningitis

[a] F, female; M, male.

cytofluorometry was performed with an EPICS-ELITE flow cytometer (Coulter Electronics). Analysis of cell surface markers continued until 1,000 γδ T cells had been counted. Based on previous experiments carried out in our laboratory (18) $CD3^+$ $\gamma\delta^-$ T lymphocytes are $CD3^+$ $\alpha\beta^+$ T cells. Negative controls utilized irrelevant MAbs of the corresponding isotype.

Selective depletion of γδ T cells and proliferation assay. PBMC were suspended in cold PBS–5% FCS–5 mM EDTA (Sigma) and incubated with anti-TCR γδ MAb for 30 min at room temperature. After being washed, the cells were incubated for 20 min at 4°C with immunomagnetic beads coated with goat anti-mouse immunoglobulin G (Miltenyi Biotech Inc., Sunnyvale, Calif.). The cells were washed and placed on a magnetic separation column (MiniMACS; Miltenyi), and the effluent collected contained the negative fraction. The depletion procedure was repeated on successive negative fractions until the concentration of γδ T cells was <0.1%. Triplicate cultures of 200 μl each were set up with 1.5×10^5 PBMC or γδ T-cell-depleted PBMC in 96-well round-bottomed microtiter plates in the presence of HKLM (bacterium-to-cell ratio, 20:1) or in medium alone for 7 days. In the proliferation assay, the cells were pulsed for 18 h with [^{3}H]thymidine, (1 μCi/well; Amersham Life Science, Bucks, Great Britain). The cells were harvested onto glass fiber filters, and [^{3}H]thymidine incorporation was measured in a β-scintillation counter according to standard procedures.

Statistical analysis. The nonparametric two-sided Mann-Whitney rank test was applied. P values of <0.05 were considered significant.

RESULTS

Longitudinal study of in vivo γδ T-cell percentages and the γδ T-cell repertoire in patients with *L. monocytogenes* infection. Surface marker analysis of peripheral blood T lymphocytes from eight patients with *Listeria* infection (Table 1) indicated that their percentages of γδ T cells (median, 11.7; range, 3.7 to 35.3) were significantly higher ($P < 0.01$) than those of age-matched controls (1.7, 0.4 to 13) (Fig. 1).

Seven patients were studied longitudinally, and changes in γδ T-cell representation with time were detected in four of them (Table 2). At the first sampling, 0.2, 0.8, and 2.5% of the total $CD3^+$ T cells of patients 1, 3, and 7, respectively, expressed the γδ TCR. Four days later, γδ T cells had increased to 27% in patient 1 and, 13 days later, to 14.6 and 11% in patients 3 and 7, respectively. For patient 4, who was studied over a 1-year period, γδ T cells represented 23% of $CD3^+$ T cells 4 days after the onset of infection, increased to 35.3% 38 days later, and then returned to 15.5% at 6 months with only a slight decrease (to 12%) after 1 year.

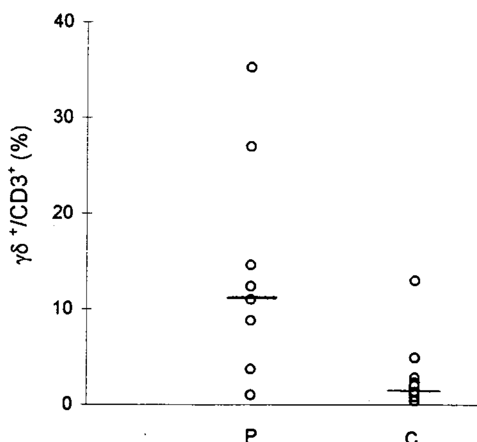

FIG. 1. The percentages of peripheral blood γδ T cells in patients with *L. monocytogenes* infection (P) and 16 age-matched uninfected controls (C) were determined by cytofluorometry analysis. Results are expressed as percentages of CD3 T cells bearing the γδ TCR. Horizontal bars indicate the median values.

TABLE 2. Analysis of γδ T-cell percentages and γδ T-cell repertoire in eight patients as a function of time after the onset of *L. monocytogenes* infection

Patient no.	No. of days after onset	% $\gamma\delta^+$ $CD3^+$ T cells	No. of γδ T cells/mm^3	% γδ T cells		
				TiγA$^+$	Tiδ2$^+$	δTCS1$^+$
1	1	0.2	8	NT[a]	NT	NT
	5	27	876	100	92	3
	60	12.5	369	100	83	3.3
2	4	10.5	297	99	99	1
	15	12.3	457	99	80	15
3	2	0.8	9	99	80	20
	15	14.6	633	99	91	2.5
	30	14.6	753	90	98	2
4	4	23	324	95	87	2.3
	8	20.4	564	84	100	0
	42	35.3	596	96	75	0
	180	15.5	311	100	85	1.5
	365	12	265	100	NT	1
5	4	1	11	99	90	8
6	24	8	109	95	NT	8
	31	8.8	122	90	NT	1.4
	42	7	111	84	NT	10
7	2	2.5	13	20	27	20
	7	8.3	132	90	83	16
	15	11	133	85	90	10
8	3	3.3	111	54	66	NT
	9	3.3	146	68	60	NT
	16	3.7	135	71	50	NT

[a] NT, not tested.

Most γδ T cells from *Listeria*-infected patients reacted with TiγA$^+$ and Tiδ2$^+$ MAbs, which recognize, respectively, Vγ9 and Vδ2 gene products (Table 2). The very strong pressure exerted by this antigenic stimulation is particularly well illustrated in the newborns. In agreement with a previous report (4), approximately half of our control newborns had γδ T cells that reacted with the δTCS1 MAb (Table 3), which recognizes Vδ1 gene products. During neonatal listeriosis (patients 1, 2,

TABLE 3. γδ T-cell repertoire and CD45RA and CD45RO expression during neonatal listeriosis

Newborns (n[a])	% γδ T cells				
	TiγA$^+$	Tiδ2$^+$	δTCS1$^+$	CD45RA$^+$	CD45RO$^+$
Infected (3)	90–100	80–99	1–20	22–46	43–75
Controls (6)	33–75	33–47	33–57	60–92.2	7.9–34

[a] n, number investigated.

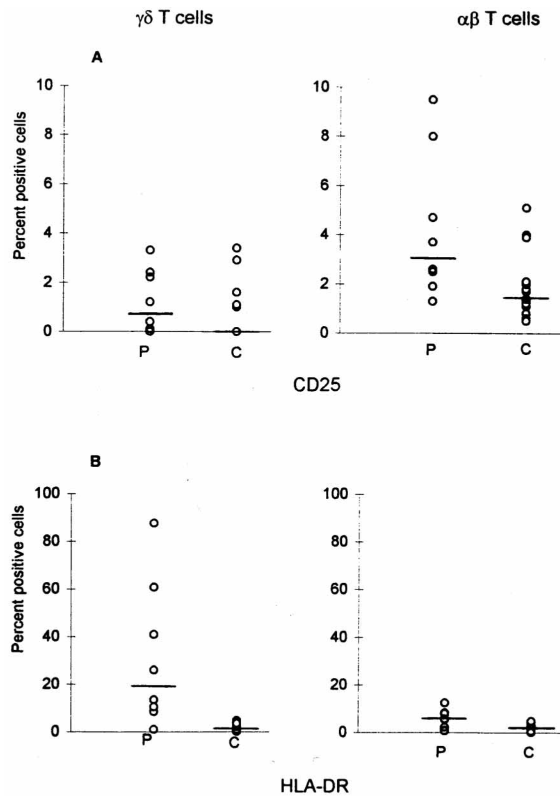

FIG. 2. Percentages of peripheral blood γδ T-cell activation markers in patients with *L. monocytogenes* infection (P) and 16 age-matched uninfected controls (C). Expression of CD25 (A) and HLA-DR (B) on peripheral blood γδ and αβ ($CD3^+$ $γδ^-$) T cells was studied cytofluorometrically. Results are expressed as the percentages of γδ and αβ T cells positive for each marker. Bars indicate the median values.

and 3), the γδ T-cell repertoire was dramatically modified, with 80 to 99% of γδ T cells reacting with the Tiδ2 MAb. This reactivity was also observed in one adult (patient 7), whose γδ T-cell expansion was associated with repertoire changes as time elapsed (Table 2).

In vivo preferential activation of γδ T cells during *L. monocytogenes* infection. Expression of CD25 and HLA-DR activation markers was studied on γδ and αβ T cells obtained from infected and uninfected individuals. CD25 expression (Fig. 2A) on both types of T cells from controls and patients was unchanged. In contrast, HLA-DR expression (Fig. 2B) on γδ T cells from infected patients was significantly higher than from controls (19.8% [median] and 0.9 to 87.6% [range] versus 2.3% and 0 to 4.7% [$P < 0.01$]), while no significant change was found on αβ T cells. This enhanced HLA-DR expression on γδ T cells occurred before the 15th day after the onset of the acute phase of the infection (Table 4).

Moreover, analysis of CD45RA (naive cells) and CD45RO (memory cells) indicated that the majority of γδ T cells from infected patients were $CD45RO^{high}$ (Table 4). Especially in *Listeria*-infected newborns, most γδ T cells expressed CD45RO, while control newborns, as reported in the literature (4), had only a minority of $CD45RO^+$ γδ T cells (Table 3). These results argue for in vivo preferential activation of γδ T cells during *Listeria* infection.

In vitro activation of γδ T cells in response to HKLM. An early γδ T-cell response has been reported during murine listeriosis (14). To determine whether different kinetics of activation were at play between γδ and αβ T cells in response to HKLM, every day for 7 days we measured the expression of CD25 and HLA-DR molecules on γδ and αβ T cells obtained from three different healthy donors and cultured in the presence of HKLM (Fig. 3A) or PHA (Fig. 3B). On γδ T cells, CD25 expression in response to HKLM peaked on day 3 and

TABLE 4. In vivo expression of activation markers on γδ and αβ T cells during *L. monocytogenes* infection

Patient no.	No. of days after onset	% γδ T cells				% αβ T cells			
		$CD25^+$	$HLA\text{-}DR^+$	$CD45RA^+$	$CD45RO^+$	$CD25^+$	$HLA\text{-}DR^+$	$CD45RA^+$	$CD45RO^+$
1	5	0.4	60.8	37	75	8	5.5	57	21
	60	0.8	0.4	46	72.5	4.5	0.8	68	36
2	4	NT[a]	26	NT	NT	NT	8	NT	NT
	15	0.1	8	32	61	3.7	5.4	79	13
3	2	2.2	8.4	22	43	1.6	0.6	76.2	7.9
	15	0.5	0.8	26	74	4.7	0.6	82	13
	30	2	0.2	35	52	0	0.3	80.3	8.2
4	4	0.1	12.5	NT	80.8	1.9	1.3	NT	28
	8	0.1	13.5	NT	86.5	1.5	2.5	NT	30.9
	42	0.1	5.6	52	80	0.9	5.6	68	33
	180	0	0	44	NT	1	1.1	71	NT
	365	NT	0	44	NT	NT	0.1	73	NT
5	4	2.4	41	30.5	83	2.6	2.2	53	31.5
6	24	0	0.1	31	83	0	0	54	58
	31	0	0.9	27	62	1	0	58	50
	42	0	0	37	77	1.3	0.8	58	50
7	2	1.2	9.6	65	32.9	3.5	2	36.4	43.4
	7	0.5	87.6	14	57	9.5	12.5	28	47
	15	0.5	69.6	18.3	50.4	2.7	6.3	33.5	46.5
8	3	0.4	2.7	55	50	1	4.1	60	37
	9	3.3	10.5	44	56	1.2	8.2	60	41
	16	1	1.2	50	49	2.5	2.1	70	33

[a] NT, not tested.

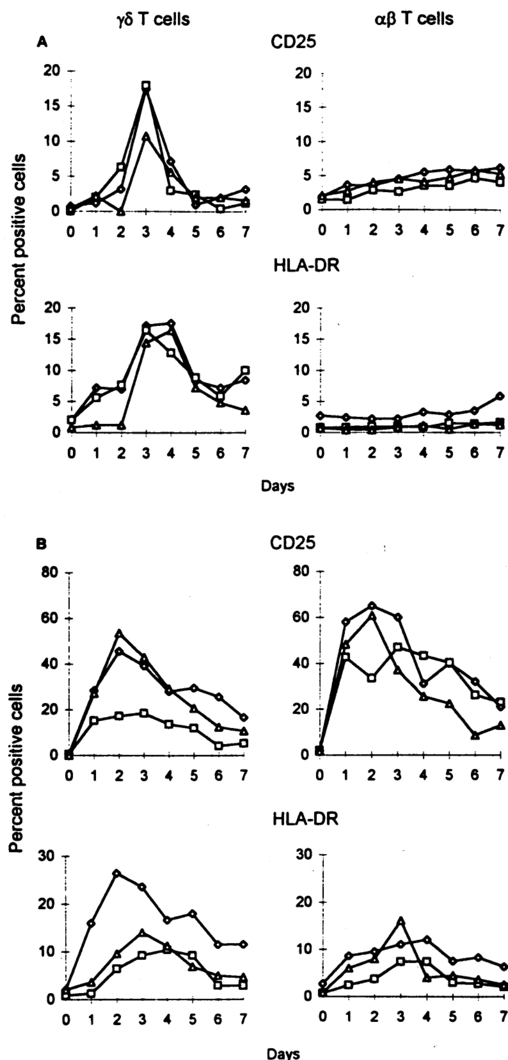

FIG. 3. Kinetics of CD25 and HLA-DR expression on γδ and αβ T cells in response to HKLM. PBMC (2.5×10^6 cells/ml) from three healthy donors (△, □, ◇) were cultured in the presence of either HKLM (5×10^7 cells/ml) (A) or PHA (10 μg/ml) (B) for 7 days. Three-color cytofluorometric analysis was performed daily with a MAb specific to CD3, γδ TCR, CD25, or HLA-DR, and the percentages of positive γδ T cells and αβ ($CD3^+$ $\gamma\delta^-$) T cells were determined.

HLA-DR expression peaked on days 3 and 4. On αβ T cells, the only reaction to HKLM was a regular but very slight rise in CD25 expression. In contrast, PHA induced simultaneous increases of CD25 and HLA-DR expression on γδ and αβ T cells. These results indicate that a high percentage of γδ T cells respond rapidly to HKLM stimulation.

Effect of γδ T-cell depletion on the cell proliferative response to HKLM. To investigate whether the early in vitro activation of γδ T cells induced by HKLM was associated with proliferation of γδ T cells, we studied the proliferative capacities of PBMC and γδ T-cell-depleted PBMC (residual γδ T cells, <0.1%) obtained from three different healthy donors in response to HKLM. Figure 4 shows that γδ T-cell depletion reduced the lymphocyte proliferative response to HKLM while γδ T-cell-depleted PBMC still had a high proliferative response to PHA (data not shown). These results confirm the involvement of γδ T cells in early lymphocyte proliferation in response to HKLM.

DISCUSSION

Several experimental models have indicated that γδ T cells play an important role during the early phase of listeriosis (9, 15, 25, 28). However, only a few studies have been performed with humans (2, 6, 8, 26). The present study, which included both adults and children, demonstrated that γδ T cells were expanded in vivo during *L. monocytogenes* infection and confirmed results obtained in the only previous in vivo analysis carried out with newborns and children (2).

Expanded γδ T cells were activated, as demonstrated by their increased expression of HLA-DR, expressed a memory phenotype, and used the Vγ9 and Vδ2 gene products predominantly. These changes were observed in all patients tested, regardless of age. Thus, the immaturity of neonatal immune systems and the alteration of immune function present in elderly individuals do not prevent expansion and activation of γδ T cells. That these γδ T-cell modifications occurred early, within the first few days of infection, strongly suggests the participation of γδ T cells. This was confirmed by in vitro analyses, which showed that HKLM induced early activation of a high percentage of γδ T cells and that γδ T-cell depletion reduced this early proliferative response of PBMC to the bacteria. Interestingly, proliferative response to HKLM by Vγ9 and Vδ2 mycobacterium-specific clones has been reported previously (6). These results bolster those previously obtained by

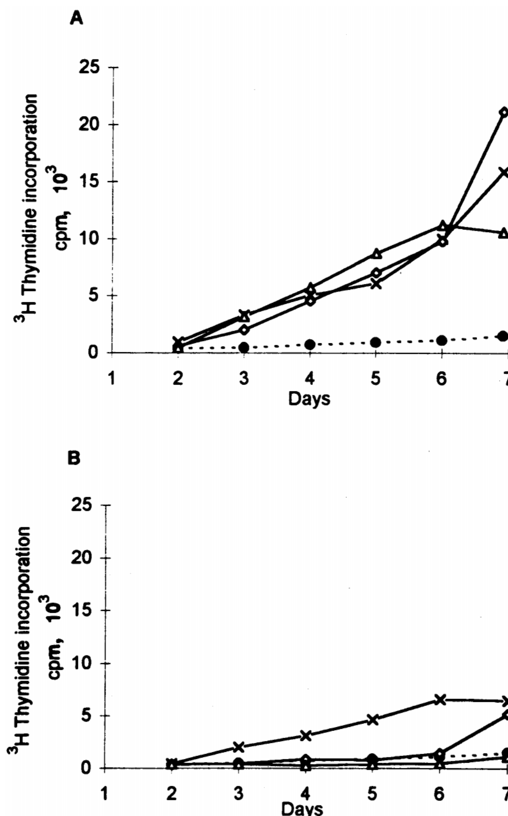

FIG. 4. Effect of γδ T-cell depletion on the PBMC proliferative response to HKLM. PBMC (A) and PBMC depleted of γδ T cells with magnetic beads (B) from three healthy donors (×, ◇, △) were cultured (1.5×10^5 cells per well) in the absence (----) or presence (—) of HKLM (bacterium-to-cell ratio, 20:1) for 7 days. [^{3}H]Thymidine incorporation was measured from day 2 to 7. On day 0, the percentages of γδ T cells in the three different blood samples were 1.7 (donor 1), 2.7 (donor 2), and 5.2 (donor 3). The residual percentage of γδ T cells in depleted PBMC was <0.1%. Mean counts per minute of triplicate cultures are shown.

others (8, 10, 26) and further support the role of γδ T cells during human *Listeria* infection.

In particular, the kinetic study of activation markers expressed by γδ T cells clearly may indicate the early response of a high number of γδ T-cell precursors compared to αβ T cells. This result should be viewed in light of data obtained from mouse models of *Listeria* infection which showed that the γδ T-cell response preceded that of αβ T cells and contributed to host defense at the early stage of infection (25, 28). Interestingly, in the present study, the γδ T-cell population was still expanded in some patients 4 to 8 weeks after the onset of infection (Table 2) even though listerial antigenemia had disappeared, an observation which is not unique to *Listeriosis* since it has been previously reported for *Salmonella* spp. (12), *Brucella melitensis* (1), and *Plasmodium falciparum* (32) infections. This observation suggests that γδ T cells, in addition to their participation in host defense, may have immunoregulatory functions (9, 16, 22, 25) and thus may have two different functional properties.

The molecular structures responsible for the early activation of γδ T cells are still unknown. The only *Listeria* antigen reported to induce γδ T-cell response is the exotoxin listeriolysin O. This virulence factor is an antigen common to γδ and αβ T cells. However, the anti-listeriolysin O response was selectively obtained with live *Listeria* but not with HKLM (10), suggesting that the γδ T-cell response observed in our study involved molecules different from listeriolysin O. Among the known ligands of γδ T cells, two are of particular interest because of their wide distribution in prokaryotic and eukaryotic cells: heat shock proteins and phospholigands (3, 5, 19–21, 23, 29, 30, 34). In humans, both types induce a response limited to γδ T cells expressing the Vγ9 and Vδ2 gene products (20). However, some previously reported data indicate that the response to protease-resistant phospholigands in human infection seems to be more important than that mediated by heat shock proteins (19, 20, 29). Further studies are needed to determine the structures involved in the γδ T-cell response during *Listeria* infection.

Alternatively, γδ T-cell activation could be obtained via non-TCR-dependent stimulation. In this regard, a cytokine-dependent response has been shown to contribute to early γδ T-cell response. For example, during murine *Listeria* infection, macrophage cytokines, interleukins 12 and 1, synergize to trigger proliferation and gamma interferon production by γδ T cells in response to HKLM (36); during murine *Salmonella* infection, interleukin 15 synthesized by activated monocytes and/or macrophages serves as a growth factor for γδ T cells (27). However, such unique cytokine-dependent stimuli cannot explain the selective Vγ9 and Vδ2 repertoire expression during the response to *Listeria* or other bacterial infections.

Regardless of the mechanism(s) of activation, these data indicate that *L. monocytogenes* should be added to the list of pathogens (1, 12, 32, 33) that induce a γδ T-cell response in humans. In this context, the antigenic pressure exerted by various microorganisms may be responsible for the preferential expression of the Vγ9 and Vδ2 repertoire in adults (4), as strongly suggested by the data obtained from our three newborn patients, in whom marked increases of γδ T-cell percentages and shifts towards the γδ T-cell repertoire were observed during the first days of life.

Finally, one can propose that γδ T cells are an important component of the early immune response to *Listeria*. The presence of γδ T cells in the intestinal mucosa and the peroral route of *Listeria* infection suggest that these T cells may constitute a first line of defense against the pathogen. The rapid response of numerous reactive γδ T cells induced by *Listeria* antigen(s) may fill the gap between the innate response mediated by NK cells and inflammatory phagocytes and the highly specific immune response mediated by few αβ T-cell-reactive precursors (13).

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Santé et de la Recherche Scientifique (INSERM) CRI 950602.

We thank Isabelle Duval for typing the manuscript and Janet Jacobson for correcting the manuscript.

REFERENCES

1. **Bertotto, A., R. Gerli, F. Spinozzi, C. Muscat, F. Scalise, G. Castellucci, M. Sposito, F. Candio, and R. Vaccaro.** 1993. Lymphocytes bearing the γδ T cell receptor in acute *Brucella melitensis* infection. Eur. J. Immunol. **23:**1177–1180.
2. **Bertotto, A., F. Spinozzi, R. Gerli, G. Bassotti, N. Forenza, C. Vagliasindi, and R. Vaccaro.** 1995. Peripheral blood γδ T cells in human listeriosis. Acta Paediatr. **84:**1434–1435.
3. **Biehr, C., R. Poupot, M. A. Peyrat, Y. Poquet, P. Constant, P. Dubois, M. Bonneville, and J. J. Fournié.** 1996. *Plasmodium falciparum* stimuli for human γδ T cells are related to phosphorylated antigens of mycobacteria. Infect. Immun. **64:**2892–2896.
4. **Christina, M., M. Parker, V. Groh, H. Band, S. A. Porcelli, C. Morita, M. Fabbe, D. Glass, J. L. Strominger, and M. B. Brenner.** 1990. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. J. Exp. Med. **171:**1597–1612.
5. **Constant, P., F. Davodeau, M. A. Peyrat, Y. Poquet, G. Puzo, M. Bonneville, and J. J. Fournié.** 1994. Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands. Science **264:**267–270.
6. **De Libero, G., G. Casorati, C. Giachino, C. Carbonara, N. Migone, P. Matzinger, A. Lanzavecchia.** 1991. Selection by two powerful antigens may account for the presence of the major population of human peripheral blood gamma/delta T cells. J. Exp. Med. **173:**1311–1322.
7. **De Maria, A., A. Ferrazin, S. Ferrini, E. Ciccone, A. Terragna, and L. Moretta.** 1992. Selective increase of a subset of T cell receptor γδ T lymphocytes in the peripheral blood of patients with human immunodeficiency virus type 1 infection. J. Infect. Dis. **165:**917–919.
8. **Follows, G. A., M. E. Munk, A. J. Gatrill, P. Conradt, and S. H. E. Kaufmann.** 1992. Gamma interferon and interleukin 2, but not interleukin 4, are detectable in γ/δ T-cell cultures after activation with bacteria. Infect. Immun. **60:**1229–1231.
9. **Fu, Y. X., C. E. Roark, K. Kelly, D. Drevets, P. Campbell, R. O'Brien, and W. Born.** 1994. Immune protection and control of inflammatory tissue necrosis by γδ T cells. J. Immunol. **153:**3101–3115.
10. **Guo, Y., H. K. Ziegler, S. A. Safley, D. W. Niesel, S. Vaidya, and G. R. Klimpel.** 1995. Human T-cell recognition of *Listeria monocytogenes*: recognition of listeriolysin O by TcRαβ$^+$ and TcRγδ$^+$ T cells. Infect. Immun. **63:**2288–2294.
11. **Haas, W., P. Pereira, and S. Tonegawa.** 1993. Gamma/delta cells. Annu. Rev. Immunol. **11:**637–685.
12. **Hara, T., Y. Mizuno, K. Takaki, H. Takada, H. Akeda, T. Aoki, M. Nagata, K. Ueda, G. Matsuzaki, Y. Yoshikai, and K. Nomoto.** 1992. Predominant activation and expansion of Vγ9-bearing γδ T cells in vivo as well as in vitro in Salmonella infection. J. Clin. Invest. **90:**204–210.
13. **Harty, J. T., L. L. Lenz, and M. J. Bevan.** 1996. Primary and secondary immune response to *Listeria monocytogenes*. Curr. Opin. Immunol. **8:**526–530.
14. **Hassan, J., C. Feighery, B. Bresnihan, and A. Whelan.** 1991. Elevated T cell receptor γδ$^+$ T cells in patients with infectious mononucleosis. Br. J. Haematol. **77:**255–258.
15. **Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, S. Ohga, K. Muramori, K. Matsumoto, J. A. Bluestone, and K. Nomoto.** 1992. A protective role of γδ T cells in primary infection with *Listeria monocytogenes* in mice. J. Exp. Med. **175:**49–56.
16. **Hsieh, B., M. D. Schrenzel, T. Mulvania, H. D. Lepper, L. DiMolfetto-Landon, and D. A. Ferrick.** 1996. In vivo cytokine production in murine listeriosis. Evidence for immunoregulation by γδ$^+$ T cells. J. Immunol. **156:**232–237.
17. **Johnson, R. M., D. W. Lancki, A. I. Sperling, R. F. Dick, P. G. Spear, F. W. Fitch, and J. A. Bluestone.** 1992. A murine CD4$^-$, CD8$^-$ T cell receptor γδ T lymphocyte clone specific for herpes simplex virus glycoprotein I. J. Immunol. **148:**983–988.
18. **Jouen-Beades, F., D. Gilbert, S. Ramzaoui, F. Borsa-Lebas, G. Humbert, and F. Tron.** 1996. Similarity of expression of activation markers and CD28 on γδ and αβ-receptor T cells in HIV infection. Clin. Immunol. Immunopathol. **79:**189–193.
19. **Kabelitz, D., A. Bender, S. Schondelmaier, B. Schoel, and S. H. E. Kaufmann.** 1990. A large fraction of human peripheral blood γ/δ$^+$ T cells is

activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. J. Exp. Med. **171:**667–679.
20. **Kaufmann, S. H. E.** 1996. γ/δ and other unconventional T lymphocytes: what do they see and what do they do? Proc. Natl. Acad. Sci. USA **93:**2272–2279.
21. **Kaur, I., S. D. Voss, R. S. Gupta, K. Schell, P. Fisch, and P. M. Sondel.** 1993. Human peripheral γδ T cells recognize hsp60 molecules on Daudi Burkitt's lymphoma cells. J. Immunol. **150:**2046–2055.
22. **Ladel, C. H., C. Blum, and S. H. E. Kaufmann.** 1996. Control of natural killer cell-mediated innate resistance against the intracellular pathogen *Listeria monocytogenes* by γ/δ T lymphocytes. Infect. Immun. **64:**1744–1749.
23. **Lang, F., M. A. Peyrat, P. Constant, F. Davodeau, J. David-Ameline, Y. Poquet, H. Vié, J. J. Fournié, and M. Bonneville.** 1995. Early activation of human Vγ9/Vδ2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. J. Immunol. **154:**5986–5994.
24. **Modlin, R. L., C. Pirmez, F. M. Hofman, V. Torigian, K. Uyemura, T. H. Rea, B. R. Bloom, and M. B. Brenner.** 1989. Lymphocytes bearing antigen specific γδ T cell receptor accumulate in human infectious disease lesions. Nature **339:**544–548.
25. **Mombarts, P., J. Arnoldi, F. Russ, S. Tonegawa, and S. H. E. Kaufmann.** 1993. Different roles of αβ and γδ T cells against an intracellular bacterial pathogen. Nature **365:**53–56.
26. **Munk, M. E., A. J. Gatrill, and S. H. E. Kaufmann.** 1990. Target cell-lysis and IL-2 secretion by γ/δ lymphocytes after activation with bacteria. J. Immunol. **145:**2434–2439.
27. **Nishimura, H., K. Hiromatsu, N. Kobayashi, K. H. Grabstein, R. Paxton, K. Sugamura, J. A. Bluestone, and Y. Yoshikai.** 1996. IL-15 is a novel growth factor for murine γδ T cells induced by *Salmonella* infection. J. Immunol. **156:**663–669.
28. **Ohga, S., Y. Yoshikai, Y. Takeda, K. Hiromatsu, and K. Nomoto.** 1990. Sequential appearance of γδ and αβ bearing T cells in peritoneal cavity during an ip infection with *Listeria monocytogenes*. Eur. J. Immunol. **20:**533–538.
29. **Pfeffer, K., B. Schoel, H. Gulle, S. H. E. Kaufmann, and H. Wagner.** 1990. Primary responses of human T cells to mycobacteria: a frequent set of gamma/delta T cells are stimulated by protease-resistant ligands. Eur. J. Immunol. **20:**1175–1179.
30. **Pfeffer, K., B. Schoel, N. Plesnila, G. B. Lipford, S. Kromer, K. Deusch, and H. Wagner.** 1992. Lectin-binding, protease-resistant mycobacterial ligand specifically activates $V\gamma9^+$ human γδ T cells. J. Immunol. **148:**575–583.
31. **Rosat, J. P., H. R. MacDonald, and J. A. Louis.** 1993. A role of $\gamma\delta^+$ T cells during experimental infection of mice with *Leishmania major*. J. Immunol. **150:**550–555.
32. **Roussilhon, C., M. Agrapart, P. Guglielmi, P. Brasseur, and J. J. Ballet.** 1994. Human $TcR\gamma\delta^+$ lymphocyte response on primary exposure to *Plasmodium falciparum*. Clin. Exp. Immunol. **95:**91–97.
33. **Scalise, F., R. Gerli, F. Castellucci, F. Spinozzi, M. Fabietti, S. Crupi, L. Sensi, R. Britta, A. Vaccaro, and A. Bertotto.** 1992. Lymphocytes bearing the γδ T cell receptor in acute toxoplasmosis. Immunology **76:**668–670.
34. **Schoel, B., S. Sprenger, and S. H. E. Kaufmann.** 1994. Phosphate is essential for stimulation of Vγ9/Vδ2 T lymphocytes by mycobacterial low molecular weight ligand. Eur. J. Immunol. **24:**1886–1892.
35. **Skeen, M. J., and H. K. Ziegler.** 1993. Induction of murine peritoneal γδ T cells and their role in resistance to bacterial infection. J. Exp. Med. **178:**971–984.
36. **Skeen, M. J., and H. K. Ziegler.** 1995. Activation of γδ T cells for production of IFN-γ is mediated by bacteria via macrophage-derived cytokines IL-1 and IL-12. J. Immunol. **154:**5832–5841.
37. **Takada, H., K. Hiromatsu, G. Matsuzaki, K. Muramori, and K. Nomoto.** 1993. Peritoneal γδ T cells induced by *Escherichia coli* infection in mice. J. Immunol. **151:**2062–2069.
38. **Tsuji, M., P. Mombaert, L. Lefrançois, R. S. Nussenzweig, F. Zavala, and S. Tonegawa.** 1994. γδ T cells contribute to immunity against the liver stages of malaria in αβ T cell-deficient mice. Proc. Natl. Acad. Sci. USA **91:**345–349.
39. **Yamamoto, S., R. Friedemann, H. C. Teixeira, P. Conradt, and S. H. E. Kaufmann.** 1993. *Listeria monocytogenes*-induced gamma interferon secretion by intestinal intraepithelial γ/δ T lymphocytes. Infect. Immun. **61:**2154–2161.

Editor: S. H. E. Kaufmann