Human γδ T Cells Augment Antigen Presentation in Listeria Monocytogenes Infection

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Circulating γδ T cells in healthy individuals rapidly respond to bacterial and viral pathogens. Many studies have demonstrated that γδ T cells are activated and expanded by Listeria monocytogenes (L. monocytogenes), a foodborne bacterial pathogen with high fatality rates. However, the roles of γδ T cells during L. monocytogenes infection are not clear. In the present study, we characterized the morphological characteristics of phagocytosis in γδ T cells after L. monocytogenes infection using transmission electron microscopy. Results show activation markers including human leucocyte antigen DR (HLA–DR) and lymph node–homing receptor CCR7 on γδ T cells were upregulated after stimulation via L. monocytogenes. Significant proliferation and differentiation of primary γδ T cells was also observed after coculture of peripheral blood mononuclear cells with γδ T cells anteriorly stimulated by L. monocytogenes. L. monocytogenes infection decreased the percentage of γδ T cells in mouse intraepithelial lymphocytes (IELs) and increased MHC-II expression on the surface of γδ T cells in vivo. Our findings shed light on antigen presentation of γδ T cells during L. monocytogenes infection.

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INTRODUCTION

Human γδ T cells are a subset of T cells with a T cell receptor (TCR) composed of γ and δ chains (1). They constitute a small proportion (3–10%) of circulating CD3+ T-lymphocytes in peripheral blood. Compared with αβ T cells, γδ T cells recognize antigens without major histocompatibility complex (MHC) restriction and without help from antigen presenting cells (APC). They directly bind to stress-induced ligands such as heat shock proteins and mutS homolog 2 (hMSH2) (2–4). γδ T cells are believed to play import roles in innate antimicrobial and antitumor immunity defense (5). In addition to directly binding stress-induced ligand and killing target cells, γδ T cells also serve as APCs to elicit subsequent specific immune responses (6,7). Brandes et al. showed that activated human γδ T cells present protein antigens to naïve CD4+ and CD8αβ T cells (8,9). Wu et al. found that naïve peripheral blood γδ T cells phagocytized IgG opsonized Escherichia coli (E. coli), IgG opsonized latex beads and whole influenza A virus matrix (M1) protein, which produced subsequent functional effects (10).

Listeria monocytogenes (L. monocytogenes) is a Gram-positive, intracellular bacterium that causes listeriosis, primarily affecting immunocompromised individuals, pregnant women and newborns. It is the only pathogenic bacterium known to contain both mevalonate and nonmevalonate pathways of isoprenoid biosynthesis, concurrently producing metabolites such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and isopentenyl pyrophosphate (IPP) (11) which are the specific ligands of γδ TCR (12,13). Clinical experiments have confirmed that γδ T cells are overrepresented in the blood of patients during L. monocytogenes infections by up to 50% of total T cells (14). The expanded γδ T cells produce IFN-γ, TNF-α, IL-4, IL-17 or perforin to mediate inflammation or lyse L. monocytogenes-infected target cells directly (15). They also regulate the chemokine production in macrophages (16). However, it
is unknown whether γδ T cells serve as APCs during *L. monocytogenes* infection. We hypothesized that they uptake *L. monocytogenes* and process and present antigens to αβ T cells to induce specific adaptive immune responses. It is fascinating to think that γδ T cells may internalize antigens in a phagocytizing manner like phagocytes, which has been ignored for some time. Our findings from an *in vitro* experimental system prove that γδ T cells have an internalizing capability when bound to *L. monocytogenes* and induce a specific immune response to *L. monocytogenes*. This indicates that γδ T cells serve as APCs during *L. monocytogenes* infection.

**MATERIALS AND METHODS**

**Bacteria**

Toxicity strain *L. monocytogenes* ATCC 19115 (serotype 4b) was a quality control strain purchased from American Type Culture Collection (ATCC). The bacteria were cultured aerobically in brain heart infusion (BHI) at 37°C. BHI broth was obtained from BD-Biosciences.

**Human Blood Samples**

Peripheral blood samples of healthy adult donors were collected with informed consent. The study was approved by the ethical board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

**Purification of Naïve γδ T and αβ T Cells**

Peripheral blood mononuclear cells (PBMCs) from peripheral blood samples were separated by density gradient centrifugation using a Ficoll density gradient (GE Healthcare companies) as described previously (17,18). Naïve γδ T and αβ T cells were enriched from PBMCs by high-gradient magnetic cell separation (MACS) according to the manufacturer’s instructions (Miltenyi Biotechnology companies). The purity of γδ T and αβ T cells were above 90% and 95%, respectively, as analyzed by flow cytometry.

**Generation of Activated γδ T and αβ T Cells and Rested γδ T Cells**

The activation and expansion of γδ T cells was described previously (19,20). Briefly, each well of 24-well plate was coated with 0.5-μg antipan-TCRγδ mAb (Immunotech, Beckman Coulter). After solution was removed, PBMCs were added to the plates and cultured in RPMI 1640 medium (Corning, NY) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL company), 200 IU/mL recombinant human IL-2 (Beijing Read United Cross Pharmaceuticals Co., Ltd.), 100 mg/mL penicillin and 100 μg/mL streptomycin at 37°C, 5% CO₂ for five days. PBMCs were transferred to culture bottle and passaged based on growth condition until the purity was above 90%. IL-2 was removed for 24 h to obtain rested γδ T cells.

For activated αβ T cells, we followed the instructions of T Cell Activation, In Vitro from ebioscience. The culture plate was coated with 5–10 μg/mL anti-CD3e Ab for 2 h at 37°C. PBMCs were transfected to the plate and added soluble anti-CD28 at 2 μg/mL to the culture medium (RPMI 1640 with 10% FBS, 200 IU/mL IL-2 and penicillin/streptomycin). After incubation for four days, cells were harvested and processed for assays.

**Infection with L. monocytogenes**

*L. monocytogenes* was cultured in BHI broth for three to five hours, the number of CFU was calculated based on growth curve as described previously (21). Bacteria were washed twice and resuspended in phosphate-buffered saline (PBS). *L. monocytogenes* was added at the desired bacterium-to-cell ratios (ratio = 5 or 50) to γδ T cells, αβ T cells or PBMCs. They were incubated in RPMI 1640 medium with 10% fetal calf serum at 37°C. After one hour or three hours penicillin and gentamicin were added to kill extracellular bacteria.

**Coculture Experiment**

The infected γδ T cells were cultured with homologous PBMCs or αβ T cells at different ratios (1:1 or 1:10) in RPMI 1640 medium with 10% fetal bovine serum (FBS) and antibiotics at 37°C for six days. To ensure consistency of cells, some freshly isolated γδ T cells from PBMCs were cultured, the remaining were frozen in liquid nitrogen before *L. monocytogenes* infection. The total cell number was approximately 1 × 10⁶/well. After six days in coculture (9), the different group cell numbers were counted and converted to a ratio by comparison with the initial PBMC number.

**L. monocytogenes Infection Assay**

Female 10–12 wk BALb/c mice were purchased from the Laboratory Animal Research Institute of the Chinese Academy of Medical Sciences. Mice were housed at the animal facilities at the Peking Union Medical College and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences in 2002. After three hours culture, 10⁶ CFU Listeria were resuspended in 0.2 mL PBS. Mice were infected by intragastric administration, then killed after 12 h, 24 h, 36 h or 48 h. Intestinal lymphoid cells were isolated by Percoll gradient centrifugation (22). The percentage of γδ T cells and related molecular expression were detected by flow cytometry.

**Transmission Electron Microscopy (TEM)**

After *L. monocytogenes* infection, γδ T cells or αβ T cells were washed with PBS and fixed in 2.5% glutaraldehyde. Preparation for TEM was performed at the Electron Microscopy Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, as described previously (10). Briefly, after fixation, T cells were soaked and washed three times with 0.1 mol/L PBS before post fixing with 1% osmium tetroxide solution in wash buffer at room temperature (RT) for two hours. Samples were then dehydrated in graded ethanol seven times and embedded in acetone and pure Epon. Ninety nanometer ultrathin sections were stained with 8% uranyl acetate and lead citrate before observation under the electron microscope (JEOL).
Flow Cytometry (FCM)

Samples of $1 \times 10^6$ cells were harvested, washed and resuspended in 50 µL of PBS containing 1% BSA. Different fluorochrome-conjugated monoclonal antibodies were added per reaction. After incubation at 4°C for 20 min, cells were washed with PBS, resuspended in 500 µL of PBS containing 1% formaldehyde and analyzed on a BD Accuri C6 Flow Cytometer (18,23). FITC-conjugated anti-TCRγδ, PE-conjugated anti-TCRαβ and the respective isotypic control mAbs were purchased from Immunotech. FITC-conjugated anti-CD4, PE-conjugated anti-CD8a and IL-17A, PE/Cy7-conjugated anti-IL-4 and APC-conjugated anti-IFN-γ were purchased from BD Pharmingen. BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit with BD GolgiPlug was used to detect intracellular cytokines according to the manual. In short, after cell surface antigens were stained, cells were resuspended in fixation/permeabilization solution for 20 min at 4°C. Cells were washed two times in BD Perm/Wash buffer and stained for intracellular cytokines at 4°C for 30 min in the dark. Cells were washed with BD Perm/Wash buffer and resuspended in staining buffer prior to flow cytometry.

Statistical Analysis

Data are expressed as the mean ± standard error of mean (SEM). One-tailed Student’s t test (SPSS version 16.0 software) was used to determine significant differences between groups. A P value of less than 0.05 was considered statistically significant.

RESULTS

Human γδ T Cells Possess Phagocytic Capacity

Previous studies suggest γδ T cells possess antigen presenting ability (24,25). To confirm this, we first assessed the phagocytic capacity of γδ T cells. γδ T cells were expanded by culturing PBMCs in antipan-TCRγδ mAb-coated plates with RPMI 1640 medium containing IL-2 for 10 d. The purity of the γδ T cells was assessed by FCM and reached 90% (Figure 1A1). These IL-2-activated γδ T cells displayed a “hairy” appearance with a large regular round nucleus and thin cytoplasm under TEM (Figure 1B1). However, after incubation with L. monocytogenes for one hour, approximately 20% of γδ T cells resembled phagocytic cells. L. monocytogenes were surrounded by pseudopod-like protrusions extending from the cytomembrane of some activated γδ T cells (Figure 1B2). Three hours later, γδ T cells showed membrane-bound phagosomal structures containing more L. monocytogenes bacteria (Figure 1B3). After longer incubation, we observed many dead γδ T cells with L. monocytogenes bacteria (Figure 1B4). When rested by IL-2 withdrawal for 24 h before L. monocytogenes incubation, γδ T cells phagocytized the bacterium with the same percentage (Figure 1B5). The morphology of γδ T cells was similar to activated γδ T cells, but the size was slightly smaller.

Next, we determined whether naive circulating γδ T cells could also phagocytose L. monocytogenes. Freshly isolated γδ T cells (purity > 90%, Figure 1A3) were incubated with L. monocytogenes in the same conditions as activated or rested γδ T cells. We observed no phagocytized L. monocytogenes in naive γδ T cells up to three hours later (Figure 1B6). In addition, we found that γδ T cells, activated by CD3 and CD28 antibody (Figure 1A2), did not phagocytose L. monocytogenes either (Figure 1B7). These results suggest that human activated and rested γδ T cells, but not naive γδ T cells, possess the ability to phagocytose pathogenic antigens, an important phenotype of APCs.
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Infection

L. monocytogenes Infection Upregulated Expression of Antigen Presenting Related Molecules on \( \gamma \delta \) T Cells

The APC-like phenotype of \( \gamma \delta \) T cells indicates they possess the ability to process and present antigens. Therefore, we examined the expression levels of antigen presenting related molecules on \( \gamma \delta \) T cells in response to \( L. \) monocytogenes infection. The expression of HLA-DR molecules on rested \( \gamma \delta \) T cells was undetectable before the incubation with \( L. \) monocytogenes. We found the expression of HLA-DR molecules significantly increased in a dose dependent manner between six hours to 12 h after incubation with \( L. \) monocytogenes. The mean fluorescence intensities (MFI) of HLA-DR was 1.0 ± 0.20 in PBS control, and reached 1.27 ± 0.21 when infected with five-fold \( L. \) monocytogenes (R = 5, \( p = 0.188 \)). When R = 50, MFI was 1.59 ± 0.48, but \( p = 0.34 \). (Figures 3A, B).

HLA-DR expression returned to basal level after 15 h (Figures 3A, B). \( \gamma \delta \) T cells activated by IL-2 expressed high levels of HLA-DR molecules but no further increase after \( L. \) monocytogenes infection (Figures 3C, D).

CD80 and CD86 are costimulatory factors that transfer required secondary signals to active \( \alpha \beta \) T cells. In our experiments, under all conditions, we did not detect CD80 expression on \( \gamma \delta \) T cell surface from three hours to 21 h (Figures 3E, F). Similarly, although activation increased and rest reduced expression of CD86, we did not find changes after \( L. \) monocytogenes infection (Figures 3G, H).

CCR7 is an important lymph node (LN)-homing receptor for APC function (24). Therefore, we examined whether \( \gamma \delta \) T cells express CCR7 in response to \( L. \) monocytogenes infection. We found no detectable level of CCR7 in rested or activated \( \gamma \delta \) T cells in the absence of \( L. \) monocytogenes. However, CCR7 expression rapidly increased in activated \( \gamma \delta \) T cells when incubated with \( L. \) monocytogenes at six hours, MFI of CCR7 rose from 0.06 ± 0.05 (PBS control) to 0.69 ± 0.11 (R = 5) and further to 1.05 ± 0.13 (R = 50), the \( p \) values were 0.01.

**Figure 2.** \( \gamma \delta \) T cells proliferated to form colonies after incubation with \( L. \) monocytogenes. (A) Many new and small colonies were observed after rested \( \gamma \delta \) T cells were incubated with \( L. \) monocytogenes. R is the ratio of \( L. \) monocytogenes to \( \gamma \delta \) T cells. PBS was used as control. More bacteria induced more colonies of \( \gamma \delta \) T cells. (B) Quantification of total cell numbers of rested \( \gamma \delta \) T cells in different groups. (C) Activated \( \gamma \delta \) T cells gathered to many large colonies 14 d after culture. After incubation with \( L. \) monocytogenes, some small and new colonies appeared. Over 21 h the number of large colonies decreased and small new colonies grew in size and number. Scale bars = 100 μm. (D) Quantification of total cell numbers of activated \( \gamma \delta \) T cells in different groups. Data are shown as mean ± SEM. *\( p < 0.05 \). **\( p < 0.01 \). (Independent experiments: n = 5).

More \( L. \) monocytogenes (R = 50) induced larger and more numerous colonies of \( \gamma \delta \) T cells (Figures 2B, D). Activated \( \gamma \delta \) T cells grew normally to yield many large colonies after 14 d in culture in the presence of IL-2. However, when incubated with \( L. \) monocytogenes, activated \( \gamma \delta \) T cells formed new small colonies (Figure 2C). The total cell number of \( \gamma \delta \) T cells was significantly higher when incubated with \( L. \) monocytogenes (2.2 ± 0.34 × 10^6/mL in PBS versus 3.0 ± 0.28 × 10^6/mL at R = 5, \( p = 0.035 \); PBS versus 3.7 ± 0.42 × 10^6/mL at R = 50, \( p = 0.009 \), at 12 h) (Figures 2B, D). These data show that \( L. \) monocytogenes induced the proliferation of human activated or rested \( \gamma \delta \) T cells.

We observed a dramatic proliferation of \( \gamma \delta \) T cells after incubation with \( L. \) monocytogenes (Figure 2). Many small colonies were observed in rested \( \gamma \delta \) T cells 6 h after incubation (Figure 2A). The size and number of \( \gamma \delta \) T cells displayed in an \( L. \) monocytogenes dose-dependent manner. For example, at 12 h, \( \gamma \delta \) T cells in PBS control were 1.7 ± 0.24 × 10^6/mL, then increased to 2.7 ± 0.28 × 10^6/mL (\( p = 0.009 \)) when stimulated by \( L. \) monocytogenes at R = 5 (R represents ratio of the number of \( L. \) monocytogenes bacteria to \( \gamma \delta \) T cells) and to 3.5 ± 0.42 (\( p = 0.003 \)) at R = 50.

**Figure 3.** (A) The expression of HLA-DR on \( \gamma \delta \) T cells increased and rest reduced expression of \( L. \) monocytogenes, activated \( \gamma \delta \) T cells was significantly higher when incubated with \( L. \) monocytogenes (R = 50) induced larger and more numerous colonies of \( \gamma \delta \) T cells (Figures 2B, D). Activated \( \gamma \delta \) T cells grew normally to yield many large colonies after 14 d in culture in the presence of IL-2. However, when incubated with \( L. \) monocytogenes, activated \( \gamma \delta \) T cells formed new small colonies (Figure 2C). The total cell number of \( \gamma \delta \) T cells was significantly higher when incubated with \( L. \) monocytogenes (2.2 ± 0.34 × 10^6/mL in PBS versus 3.0 ± 0.28 × 10^6/mL at R = 5, \( p = 0.035 \); PBS versus 3.7 ± 0.42 × 10^6/mL at R = 50, \( p = 0.009 \), at 12 h) (Figures 2B, D). These data show that \( L. \) monocytogenes induced the proliferation of human activated or rested \( \gamma \delta \) T cells.

**Figure 4.** CCR7 is an important lymph node (LN)-homing receptor for APC function (24). Therefore, we examined whether \( \gamma \delta \) T cells express CCR7 in response to \( L. \) monocytogenes infection. We found no detectable level of CCR7 in rested or activated \( \gamma \delta \) T cells in the absence of \( L. \) monocytogenes. However, CCR7 expression rapidly increased in activated \( \gamma \delta \) T cells when incubated with \( L. \) monocytogenes at six hours, MFI of CCR7 rose from 0.06 ± 0.05 (PBS control) to 0.69 ± 0.11 (R = 5) and further to 1.05 ± 0.13 (R = 50), the \( p \) values were 0.01.
Figure 3. FCM analysis of antigen presentation related markers on γδ T cells in response to L. monocytogenes infection. (A) The level of HLA-DR expression increased on rested γδ T cells six hours after incubation with L. monocytogenes. (B) Quantification of normalized mean fluorescence intensities (MFI) of HLA-DR expression on rested γδ T cells in different groups. R represents the ratio of bacteria number to γδ T cell number. The high ratio of bacterium-to-cell of 50:1 (blue line) induced more HLA-DR expression on rested γδ T cells compared with low ratio (red line) or PBS (black line). No significant changes were observed at 12 h and 15 h time points. (C, D, E, F, G and H) No significant change was observed in the expression level of HLA-DR (C and D), CD80 (E and F) or CD86 (G and H) on activated γδ T cells either in the presence or absence of L. monocytogenes. Data are shown as mean ± SEM (Independent experiments: n = 5).

Activated γδ T Cells Induced αβ T Cell Proliferation after L. monocytogenes Incubation

To determine whether γδ T cells act as APCs to induce primary αβ T cell responses, PBMCs were cocultured for six days with either L. monocytogenes, activated γδ T cells or L. monocytogenes-infected-γδ T cells at a ratio of γδ T cells to PBMCs of 1:1 or 1:10. The proliferation of PBMCs was examined by counting the cell number after six days. We found no obvious proliferation of T cells when PBMC were cultured alone (0.42 ± 0.07) or cocultured with L. monocytogenes (0.32 ± 0.08). However, the number of T cells significantly increased when PBMCs were cocultured with γδ T cells or L. monocytogenes-infected-γδ T cells at the ratio of γδ T cells to PBMCs of 1:1 (0.32 ± 0.08 LM + PBMC versus 0.87 ± 0.15 γδ T + PBMC, p = 0.001; LM + PBMC versus 1.16 ± 0.16 γδ T + LM + PBMC, p = 0.000; LM + PBMC versus γδ T + LM + PBMC, p = 0.019) and 1:10 (0.38 ± 0.14 LM + PBMC versus 0.76 ± 0.13 γδ T + PBMC, p = 0.004; LM + PBMC versus 0.99 ± 0.16 γδ T + LM + PBMC, p = 0.000; LM + PBMC versus γδ T + LM + PBMC, p = 0.003) (Figure 5A). We also analyzed the percentages of different subsets of T cells after six days using flow cytometry. The results show that the ratios (proliferated cells of a specific subset were divided by the initial cell number of PBMCs which eliminated the bias due to different initial cell numbers) of αβ T cells (Figure 5B), CD4 + T cells (Figure 5C) and CD8 + T cells (Figure 5D) significantly increased when cocultured with L. monocytogenes-infected-γδ T cells (1.22 ± 0.21 αβ T cells, 0.73 ± 0.17 CD4 + T cells, 0.48 ± 0.08 CD8 + T cells,
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Figure 4. FCM analysis of CCR7 on activated γδ T cells after phagocytosed L. monocytogenes.
The expression of CCR7 on activated γδ T cells was upregulated in a bacteria dose dependent manner three hours after L. monocytogenes infection. The peak of CCR7 expression was at six hours and gradually decreased at nine hours. Normalized mean fluorescence intensity (MFI) values are shown as mean ± SEM (independent experiment of n = 5).

**P < 0.05.

Activated γδ T Cells Induced αβ T Cell Differentiation after L. monocytogenes Incubation

The finding that γδ T cells promoted proliferation of αβ T cells after L. monocytogenes infection led us to investigate whether γδ T cells could induce the differentiation of naïve αβ T cells. We cocultured PBMCs with L. monocytogenes, γδ T cells or L. monocytogenes-infected γδ T cells and detected the differentiation of CD4+ and CD8+ αβ T cells after stimulation with Phorbol-12-myristate-13-acetate (PMA) and ionomycin (Ion). γδ T cells induced naïve CD4+ and CD8+ αβ T cells to polarize into effector cells, especially in the presence of L. monocytogenes (Figure 6).

CD4+ αβ T cells tended to produce IFN-γ (15.75 ± 3.32 γδ T + LM + PBMC versus 4.03 ± 1.16 LM + PBMC, p = 0.009; 13 ± 1.57 γδ T + PBMC versus LM + PBMC, p = 0.001; γδ T + LM + PBMC versus γδ T + PBMC, p = 0.28; at ratio = 1:1) (Figures 6A, C) rather than IL-4 or IL-17 (data not shown). This suggests L. monocytogenes-infected γδ T cells induce CD4 + T cells to T helper 1 (Th1)-type T cells rather than Th2 or Th17 cells. In addition, we found L. monocytogenes-infected γδ T cells induced CD8+ αβ T cells to produce IFN-γ (9.73 ± 1.17 γδ T + LM + PBMC versus 4.7 ± 0.2 LM + PBMC, p = 0.002; 4.77 ± 1.15 γδ T + PBMC versus LM + PBMC, p = 0.93, at ratio = 1:1) (Figures 6B, D), indicating a direction of the differentiation to cytotoxic T lymphocytes (CTL). Interestingly, activated γδ T cells induced naïve CD4+ αβ T cells but not naïve CD8+ αβ T cells to produce IFN-γ.

These results, taken together, suggest...
Figure 5. Phagocytized L. monocytogenes, activated γδ T cells to induce CD4 cell and CD8 T cell proliferation. (A) PBMC, PBMC plus L. monocytogenes, PBMC plus γδ T cells or PBMC plus γδ T cells infected by L. monocytogenes were cultured for six days then the total cell number in each group was counted. L. monocytogenes alone did not promote PBMC proliferation. γδ T cells displayed a slight augmentation to PBMC proliferation at a high ratio of γδ T cells to PBMCs. However, the γδ T cells which phagocytized L. monocytogenes induced significant PBMC proliferation. The ratios of the numbers of proliferated (B) αβ T cells, (C) CD4+ T cells and (D) CD8+ T cells to initial PBMC numbers after six days incubation. A more significant proliferation was observed when γδ T cells were cultured at 1:1 ratio of γδ T cells to PBMCs. (E) αβ T cells were isolated from PBMC and cocultured with γδ T cells in the presence or absence of L. monocytogenes. γδ T cells could promote the proliferation of αβ T cells, especially after phagocytosed L. monocytogenes. The cell number, ratio and percentage are shown as mean ± SEM (independent experiment of n = 4). *P < 0.05. **P < 0.01. Ratios represent as the proportions of the final cell numbers after incubation over initial numbers of PBMCs.

that CD4+ αβ T cells were induced into Th1 cells and CD8+ αβ T cells into CTLs in the presence of L. monocytogenes-infected-γδ T cells.

L. monocytogenes Infection Decreased the Percentage of γδ T Cells in Mouse IELs and Increased MHC-II Expression in γδ T Cells In Vivo

To determine whether L. monocytogenes activates γδ T cells in vivo, we characterized the phenotypes of γδ T cells in the IELs from the mice intragastrically infected with L. monocytogenes. The results show that the percentage of γδ T cells in the IELs decreased in the L. monocytogenes-infected mice compared with the controls (P > 0.05, Figures 7A, B). MHC-II expression significantly increased in γδ T cells from L. monocytogenes-infected mice compared with the controls (1.65 ± 0.35 PBS versus 6.0 ± 0.9 LM, p = 0.045, at 36 h after infection; 1.9 ± 0.1 PBS versus 7.6 ± 0.4 LM, p = 0.005, at 48 h after infection; Figures 7C, D). However, no obvious changes were found in the expression levels of other antigen presentation associated molecules including CD80, CD86 and CCR7 (data not shown). These data indicate that L. monocytogenes infection induces a mild activation of γδ T cells in vivo with a significant difference in the phenotype of γδ T cells in L. monocytogenes infection between human and mouse.

DISCUSSION

Clinical cases of listerelosis provide clues to the interaction of γδ T cells and L. monocytogenes. In Bridgett’s report, L. monocytogenes bacterial infections induced multiple effector immune responses of activated γδ T cells in L. monocytogenes-infected macaques, including remarkable recall-like expansion, pulmonary or mucosal trafficking, broad effector functions producing or coproducing Th1 and Th2 or Th17 cytokines, direct lysis of L. monocytogenes-infected target cells and inhibition of intracellular L. monocytogenes bacteria (15). Recently, Romagnoli et al. reported IL-17A-producing resident
memory γδ T cells exhibited a remarkably static pattern of migration that radically changed following secondary oral L. monocytogenes infection (26).

In this study, we show a part of the activated and rested γδ T cells phagocytized L. monocytogenes bacteria. We hypothesized that it is due to different subpopulations of γδ T cells given no proliferation bias of subpopulations when activated by anti-γδ TCR antibody. Previous studies also reported that γδ T cells act as APCs including freshly isolated γδ T cells that phagocytized E. coli and 1 μm synthetic beads (10) and IPP-stimulated tonsillar γδ T cells that displayed principal characteristics of professional antigen presenting cells (9). Our findings show consistent results in activated γδ T cells and rested γδ T cells. However, we did not observe phagocytosis in the freshly isolated naïve γδ T cells. Professor Gustafsson regards CD16 as a γδ T cell phagocytic receptor (10). We know during the process of activation, γδ T cells lose CD16 expression (27,28) and upregulate the expression of MHC-II, CD80 and CD86 (9). All of these molecules are involved in antigen presentation; Gustafsson confirmed that activation increased phagocytosis and antigen presentation by γδ T cells. To further clarify these findings, we characterized phagocytized γδ T cells and the phagocytic receptor of activated γδ T cells.

We observed proliferation and colony forming in rested and activated γδ T cells after L. monocytogenes infection in vitro. In our experiments, live L. monocytogenes were added to γδ T cells to strongly activate γδ TCR and stimulate γδ T cell proliferation. After more than three hours, many γδ T cells died from necrosis, a phenomenon possibly caused by extracellular bacteria and/or their soluble products in cell culture medium or the uptake of L. monocytogenes (21,29). In addition, we found that phagocytosis triggered γδ T cells to rapidly,
Figure 7. L. monocytogenes infection activated the expression of MHC-II molecules on γδ T cells. FCM analysis of percentage of γδ T cells in mouse IEL (A) and MHC-II + γδ T in γδ T cells (C) after intragastric administration with L. monocytogenes for 48 h. (B) Quantitation of the percentages of γδ T cells in mouse IELs. (D) Quantitation of the percentages of HLA-DR + γδ T cells in γδ T cells (gated in γδ T cells). Data are shown as mean ± SEM from four independent experiments. *P < 0.05, **P < 0.01.

but transiently, increase CCR7 expression, and sustained high expression of HLA-DR and costimulatory factor CD86. The expression of CCR7 enables γδ T cells to home lymph nodes and then engage in antigen presentation. Rested γδ T cells began to increase HLA-DR expression after L. monocytogenes infection for six hours, but did not express CCR7 and showed only low expression of CD86. Neither activated nor rested γδ T cells expressed CD80 as dendritic cells (DCs) did. In many cases, the expressions of CD80 and CD86 were inconsistent. Although both CD80 and CD86 are costimulatory signals, CD86 is more important (30). These results indicate that activated γδ T cells are more effective in APCs function.

Finally, we demonstrated that activated γδ T cells induced naïve CD4+ or CD8+ αβ T cells to proliferate and differentiate after L. monocytogenes phagocytosis. Both CD4+ and CD8+ αβ T cell numbers increased, and IFN-γ production was activated. CTLs lysed infected cells directly and Th1 cells induced apoptosis, which induced the battle of cleaning L. monocytogenes. The proliferation response of CD8+ αβ T cells may be triggered by the antigen cross-presentation activity of γδ T cells as described previously (8,31).

We also note that at high incubation ratio, activated γδ T cells stimulated CD4+ and CD8+ αβ T cells to proliferate and differentiate. This phenomenon was also presented in Mao’s paper, which showed peripheral-derived γδ T cells stimulated primary CD4+ and CD8+ T cells to proliferation on day three (23). Although γδ TCR and αβ TCR recognized different ligands and required different costimulated factors, they share partial common activation signal pathways (such as extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinases (MAPK) pathway), translation factor activation and IL-2 production (32), so the bystander effect only occurs at high ratios of γδ T cells with αβ T cells or an extended incubation period.

In this study we also characterized the phenotype changes of γδ T cells from mice infected by L. monocytogenes. However, we observed only a slight decrease in the percentage of γδ T cells in the IELs and a mild elevation of MHC-II expression on γδ T cells after L. monocytogenes infection. These findings suggest that γδ T cells are activated by L. monocytogenes infection and play a role in the process of antigen presentation (33). However, we found that L. monocytogenes infected mice showed no obvious changes in the expression levels of other antigen presentation associated molecules including CD80, CD86 and CCR7. This indicates that there is a significant difference in the phenotype changes of γδ T cells in L. monocytogenes infection between human and mouse.

In summary, we show activated and rested γδ T cells are able to phagocytize L. monocytogenes. This phagocytosis leads to antigen processing and presentation. This is a helpful supplement to understanding the multiple effect functions of activated γδ T cells in L. monocytogenes infection. Furthermore, these findings suggest that γδ T cells may be potential targets for immunotherapy. Our hope is that more researchers will focus on the antigen presenting function of γδ T cells in anti-infection or antitumor immunity and translate discoveries into effective therapeutic approaches in cancer patients.

CONCLUSION

Overall, our study highlights the mechanism of human γδ T cells to serve as APCs during the infection of L. monocytogenes, which are common foodborne bacterial pathogens. The bacteria produce metabolite products recognized by γδ TCRs and results in γδ T cell overrepresentation during L. monocytogenes infection. In this study, we observed via transmission electronic microscopy that γδ T cells phagocytize L. monocytogenes. Upon stimulation with L. monocytogenes, γδ T cells increased...
surface expression of activation markers (HLA-DR and CCR 7) present antigens and induce the proliferation and differentiation of homologous αβ T cells. In vivo experiments showed that L. monocytogenes infection activated the expression of MHC-II molecules in γδ T cells. These findings indicate that human γδ T cells display APC functions during L. monocytogenes infection. These finding are beneficial to the development of γδ T cell therapeutic applications in bacterial infection or tumor development.

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DISCLOSURE
The authors declare they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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