Article

Interleukin 17-Producing γδ T Cells Increased in Patients with Active Pulmonary Tuberculosis

Meiyu Peng[1], Zhaohua Wang[2], Chunyan Yao[1], Lina Jiang[1], Qili Jin[1], Jing Wang[1] and Baiqing Li[1, 3]

Although it has been known that γδ T cells may play an important role in the immune response to infection of *Mycobacterium tuberculosis* (*M. tb)*, the mechanisms by which the γδ T cells participate in the innate and/or acquired immunity to tuberculosis (TB) have not been full elucidated. In the present study, 27 patients with active pulmonary TB and 16 healthy donors (HD) were performed. We found that proportion of IL-17-producing cells among lymphocyte was similar between TB patients and HD, whereas the proportions of γδ T cells in IL-17-producing cells (59.2%) and IL-17-producing cells in γδ T cells (19.4%) in peripheral blood were markedly increased in TB patients when compared to those in HD (43.9% and 7.7%, respectively). In addition, the proportions of IFN-γ-producing γδ T cells in TB patients were obviously lower than that in HD. Upon re-stimulated with *M. tb* heat-treated antigen (*M. tb*-HAg) *in vitro*, fewer IL-17-producing γδ T cells were generated from HD and TB patients, whereas IFN-γ-producing γδ T cells were increased in TB patients compared to that in HD. Our findings in TB patients and healthy human were consistent with other murine investigation that the IL-17-producing γδ T cells were main source of IL-17 in mouse model of BCG infection, suggesting that γδ T cells might be involved in the formation of tubercular granuloma in pulmonary TB patients, but need further identification. *Cellular & Molecular Immunology*. 2008;5(3):203-208.

Key Words: ***Mycobacterium tuberculosis*, γδ T cell, IL-17, pulmonary tuberculosis**

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tb*), remains the leading cause of mortality among human infectious diseases in the world, estimated more than 8 millions of new cases and over one and half millions of deaths annually (1), and has been at the top position of both mortality and morbidity among legal infectious diseases in past years in China (2). Although $CD4^+$ and $CD8^+$ αβ T cells had been clearly demonstrated to be important in protective immunity against infection of *M. tb* (3, 4), several lines of evidence in past years (5-7), include our previous work (8), suggest that γδ T cells might also be important in the host immunity to the infection of *M. tb*. *M. tb* antigen reactive γδ T cell subset, Vγ9/Vδ2 T cells, apparently decreased in patients with active pulmonary TB, compared to normal healthy donors (HD). However the mechanisms by which the γδ T cells participate in the innate or acquired immunity to infection of *M. tb* have not been full elucidated. It has been found γδ T cells were main source of production of IFN-γ (9), and showed the cytotoxicity to intracellular *M. tb via* release of perforin (6). In addition, Huang recently reported that γδ T cells were also involved in the formation of tubercular granuloma in pulmonary tuberculosis (10) and suggested γδ T cells contributed to inflammatory reaction in the pathogenesis of TB.

It has been known that interleukin 17 (IL-17) is a potent inflammatory cytokine secreted by T lymphocytes (11), such as a novel CD4 subset, Th17 cells (12), and may relate to the induction of granulopoiesis (13), although the role of IL-17 in the immunity to infection of *M. tb* is not clear. Recently, Lockhart et al. reported that in the mouse model of infection

[1]Department of Immunology, Bengbu Medical College, and Anhui Key Laboratory of Infection and Immunity at Bengbu Medical College, 2600 Dong Hai Avenue, Bengbu, Anhui 233030, China;

[2]The Infectious Disease Hospital of Bengbu, 160 Third Hongqi Road, Bengbu, Anhui 233000, China;

[3]Corresponding to: Dr. Baiqing Li, Department of Immunology, Bengbu Medical College, 2600 Dong Hai Avenue, Bengbu, Anhui 233030, China. Tel/Fax: +86-552-317-1086, E-mail: baiqingli@bbmc.edu.cn

Received Feb 13, 2008. Accepted May 31, 2008.

Abbreviations: IL-17, interleukin 17; PBMC, perpherial blood mononuclear cells; IFN, interferon; HD, healthy donor; TB, tuberculosis; mAb, monoclonal antibody; BCG, bovis bacille Calmette-Guerin; PMA, Phorbo 12-myristate 13-acetate; *M.tb*, *Mucybacterium tuberculosis*; *M. tb*-HAg, *M. tb* heat treated antigen; *M. tb*-AT, *M. tb*-HAg activated T cell; NBS, newborn bovine serum.

with *M. tb*, IL-17 production was primarily from γδ T cells and other non-$CD4^+CD8^+$ cells, rather than $CD4^+$ T cells (14). Umemura et al. also reported that in mouse model of infection with *M. bovis* bacille Calmette-Guerin (BCG) γδ T cells were primary source of IL-17, which contributed to the granuloma formation in lung tissues (15). However, the source of IL-17 in TB patients and normal human subjects remains unclear. In the present study, we found main source of IL-17 were γδ T cells and IL-17-producing γδ T cells in peripheral blood of patients with TB markedly increased compared to that in the HD, measured by flow cytometry, with relative decreased IFN-γ-producing γδ T cells. Our data suggest that the IL-17 producing γδ T cells might be involved in the immunity to *M. tb* infection or pathological course of pulmonary TB.

Materials and Methods

Patients and controls

Peripheral blood samples were obtained from a total of 27 patients (average age of 48, rang 17-75 years old) with active pulmonary TB who were recruited from the Infectious Disease Hospital of Bengbu, Anhui. In TB patients, clinical presentation and chest radiographs were compatible with pulmonary TB and sputum was positive for acid fast bacilli. All healthy donors (HD, n = 16, average age of 29, rang 20-59 years old) who had been vaccinated with BCG at birth or in childhood were used as normal controls. Peripheral blood samples from each patient and healthy donor were obtained upon informed consent. This study was approved by Ethical Approval Committee of Bengbu Medical College.

Culture of M. tb and preparation of M. tb antigens

M. tb heat treated antigens (*M. tb*-HAg) were prepared according to the previous report (16). Briefly, *M. tb* (H37Ra) were cultured in Suton medium for 4 to 6 weeks to the late log phase, and the mycobacterial cells were harvested, washed three times with normal saline, and re-suspended in two volume of ultra pure water to cell pellets, followed by heated at 120°C for 30 min. Soluble *M. tb*-HAg was collected from supernatant of heat treated *M. tb* cells and concentrated to 1 mg/ml before used for predominantly stimulating γδ T cells.

Cell preparation and expansion of γδ T cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by density gradient centrifugation using Ficoll-Hypaque, lymphocyte isolation solution (Tian Jin Hao Yang Biological Manufacture Co. Ltd., Tianjin, China), and cultured at 1×10^6 cells/ml, 1 ml/well in 24-well culture plates in complete medium RPMI 1640 (GIBCO), supplemented with 10% (v/v) newborn bovine serum (NBS) (Hangzhou Sijiqing Biological Engineering Materials Co. Ltd., Hangzhou, China), 2 mmol/L L-glutamine and 50 μg/ml gentamycin at 37°C in 5% CO_2, in the presence of 5 μg/ml *M. tb*-HAg and 50 U/ml rhIL-2 (Changchun Changsheng Gene Pharmaceutical Co. Ltd., Changchun, China) for 7 to 10 days, with added rhIL-2 (50 U/ml) every 3-4 days. The *M. tb*-HAg stimulated and rhIL-2 expanded T cells were termed as *M. tb*-HAg activated T cells (*M. tb*-AT) in which γδ T cells account for 60% to 75% of total expanded T cells and used as differentiated effective γδ T cells.

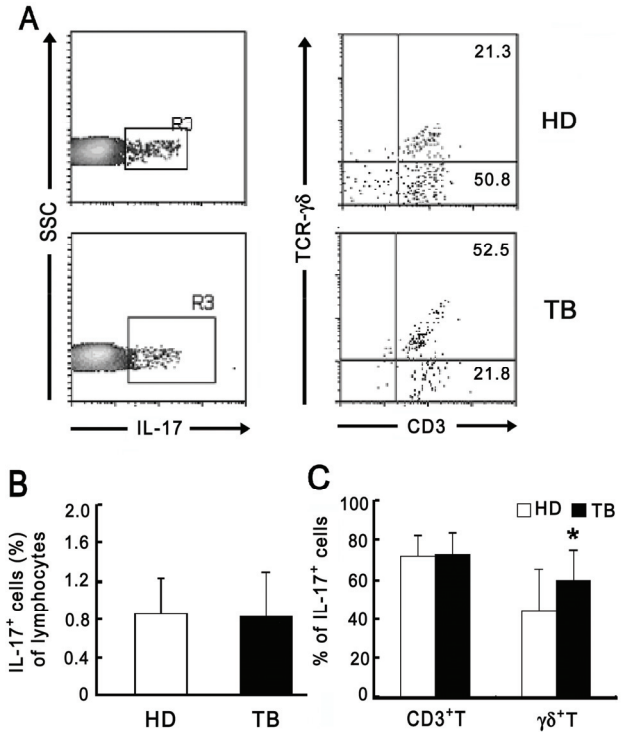

Figure 1. Flow cytometric analysis of the source of IL-17 producing cells in peripheral blood of HD and TB patients. About 100 μl whole blood was stimulated with PMA and ionomycin in the presence of monensin for 6 h, and then stained with anti-CD3-PECy5.5, anti-TCRγδ-PE and anti-IL-17A-FITC. (A) The representative dot plots showed the percentages of IL-17 producing cells in $CD3^+$ cells and γδ T cells from the gate of lymphocytes. (B) The percentages for $IL\text{-}17^+$ cells of lymphocytes. (C) The percentages for $CD3^+$ cells and γδ T cells among $IL\text{-}17^+$ cells. $^*p < 0.05$.

Induction of intracellular cytokine production in T cell subpopulations

For induction of intracellular cytokines in fresh resting T cell subpopulations, 100 μl of whole blood was stimulated with 50 ng/ml of PMA, and 1 μg/ml of ionomycin for 6 h, in the presence of 2.5 μmol/L of monensin (Sigma) during the last 3 h.

For induction of intracellular cytokines in stimulated and expanded *in vitro* T cell subpopulations, 1×10^6 cells of *M. tb*-AT were stimulated with 20 ng/ml of PMA, and 500 ng/ml of ionomycin, or re-stimulated with 5 μg/ml of *M. tb*-HAg for 6 h, in the presence of 2.5 μmol/L monensin during the last 3 h.

Detection of intracellular cytokine in T cell subpopulations by flow cytometry

The fluorochrome-conjugated anti-human mAbs used in this

study were as follows: anti-IL-17A-FITC (IgG1, clone eBio64DEC17) was purchased from eBioscience Inc.; anti-CD3-PECy5.5 (IgG2a, clone S4.1), anti-TCRγδ-PE (IgG1, clone 5A6.E9), anti-IFN-γ-biotin (IgG1, clone 4S.B3), streptavidin-Cy5 (SA-Cy5), and all isotype-matched control mAbs were from Caltag Laboratories.

For detection of intracellular cytokines by flow cytometry, 100 μl of whole blood cells that stimulated with PMA/ionomycin, or 1×10^6 cells of *M. tb*-AT that re-stimulated with *M. tb*-HAg, were stained for surface markers with anti-CD3-PECy5.5 and anti-TCRγδ-PE for 20 min at 4°C, lysing red blood cells with lysing solution (BD Biosciences) for whole blood sample, washed twice with staining buffer (5% NBS-0.1% sodium azide in PBS), and fixed with fixing solution (2% paraformaldehyde in PBS) for 30 min at 4°C. After permeabilized with permeabilizing solution (0.1% saponin-10%NBS in PBS) for 15 min at 4°C, cells were then blocked for 30 min at 4°C with 5% BSA-10% normal mouse serum in PBS, and then stained with anti-IL-17A-FITC, and anti-IFN-γ-biotin for 30 min at 4°C. After washed twice in permeabilizing solution, cells were incubated with SA-Cy5 for 30 min at 4°C, washed twice in permeabilizing solution and once with staining buffer, and finally measured on FACSCalibur (BD Biosciences). Controls for nonspecific staining were monitored with isotype-matched mAbs and nonspecific staining were always subtracted from specific staining results. Acquired data were analyzed with CellQuest software (BD Biosciences) and WinMDI 2.8.

Statistical analysis

Differences among group means were evaluated by two-group *t*-test. Values of $p < 0.05$ were considered statistically.

Results

The IL-17-producing cells in human peripheral blood was most from γδ T cells in patients with active pulmonary tuberculosis

In order to investigate the cell source of IL-17 in TB patients, we measured the proportions of IL-17-producing cells in peripheral blood of TB patients (n = 27) and HD (n = 16) by using flow cytometric intracellular cytokine detection technique. As shown in Figure 1B, the results indicated the γδ T cells were the major source of IL-17, and the proportions of IL-17-producing cells were very small part of peripheral blood lymphocytes, the average of 0.83% in TB patients was similar to that of 0.82% in HD group. The percentage for $CD3^+$ T cells of $IL\text{-}17^+$ cells in TB (72.9%) was also similar to that in HD (72.1%). However, the percentage for γδ T cells of $IL\text{-}17^+$ cells in TB (59.2%) were significant higher than that in HD group (43.9%) ($p < 0.05$, Figure 1C).

The proportion of IL-17-producing cells in γδ T cells of peripheral blood increased in TB patients, but IFN-γ-producing γδ T cells decreased

To further investigate whether the percentage of IL-17-producing cells among γδ T cells increased or not in TB patients, we analyzed and compared the proportions of $IL\text{-}17^+$ cells in γδ T cells between TB patients and HD group. As shown in Figure 2B, the percentage for $IL\text{-}17^+$ cells of γδ T cells in TB patients (19.4%) was distinctly higher than that in HD (7.7%) ($p < 0.01$). However, the percentage for $IFN\text{-}\gamma^+$ cells of γδ T cells in TB patients (30.5%) was distinctly lower than that in HD (44.4%) ($p < 0.05$). It has been known that IL-17 production was negatively regulated by IFN-γ, we calculated the ratio of percentages of $IFN\text{-}\gamma^+$ cells to $IL\text{-}17^+$ cells in γδ T cells, and found that the ratio of $IFN\text{-}\gamma^+/IL\text{-}17^+$ markedly decreased in TB patients (2.15) when compared to HD (12.21) ($p < 0.01$) (Figure 2C).

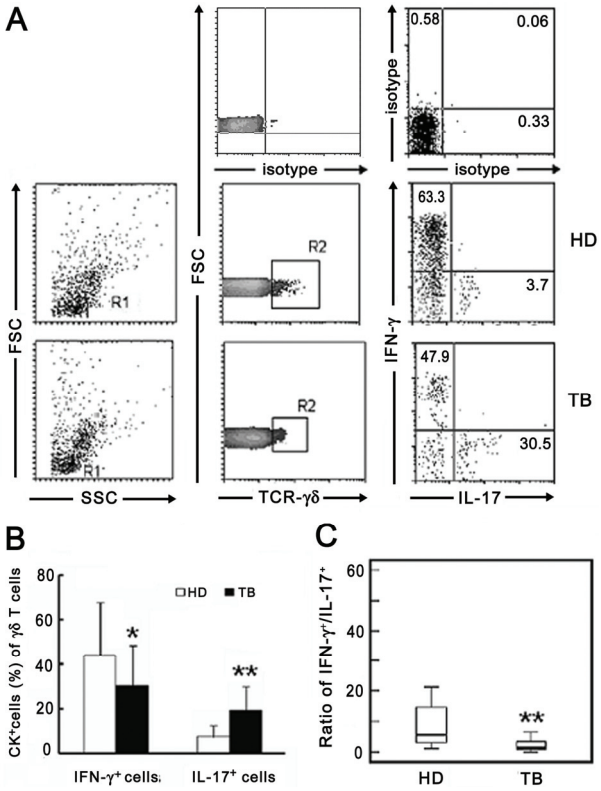

Figure 2. Flow cytometric analysis of the percentages for the $IL\text{-}17^+$ cells and $IFN\text{-}\gamma^+$ cells in γδ T cells of peripheral blood of HD and TB patients. About 100 μl whole blood was stimulated with PMA and ionomycin in the presence of monensin for 6 h, and then the cells were stained with anti-TCRγδ-PE, anti-IL-17A-FITC, and anti-IFN-γ-biotin followed by SA-Cy5. (A) The representative dot plots showed the percentages for $IL\text{-}17^+$ cells and $IFN\text{-}\gamma^+$ cells among γδ T cells in whole blood of HD and TB patients. (B) The percentages for $IFN\text{-}\gamma^+$ cells and $IL\text{-}17^+$ cells among γδ T cells from whole blood of HD and TB patients. (C) The ratio of percentages of $IFN\text{-}\gamma^+$ cells to $IL\text{-}17^+$ cells among γδ T cells in whole blood of HD and TB patients. *$p < 0.05$, **$p < 0.01$.

The capacities of IFN-γ and IL-17 production in M. tb- HAg stimulated and expanded γδ T cells

To investigate the productive capacity of IL-17 of effective γδ T cells, we cultured PBMCs with *M. tb*-HAg and IL-2 for

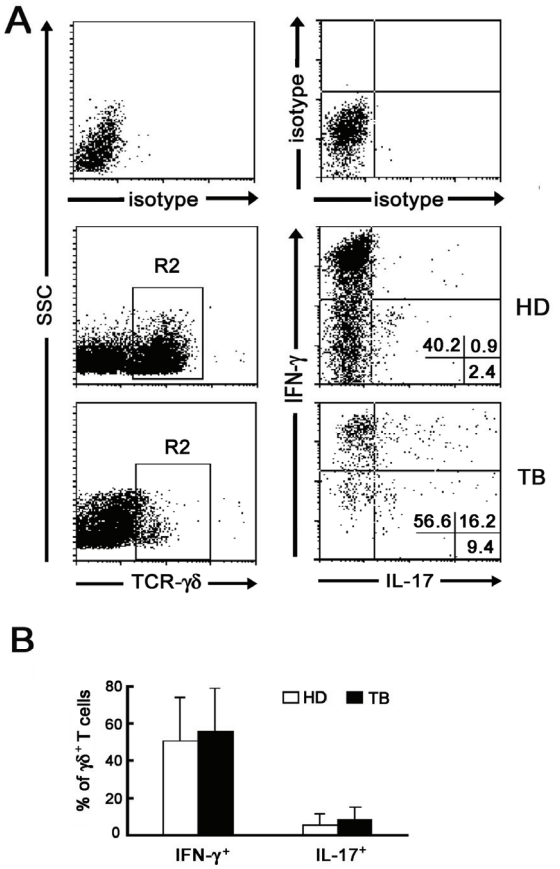

Figure 3. Flow cytometric analysis of the percentages for the IL-17$^+$ cells and IFN-γ$^+$ cells of γδ T cells from *M. tb*-AT of HD and TB patients. The PBMC were stimulated with *M. tb*-HAg in the presence of IL-2 for 7 to 9 days to generate the expanded T cell line *M. tb*-AT. About 1×10^6 cells of *M. tb*-AT were stimulated with PMA and ionomycin in the presence of monensin for 6 h, and then stained with anti-TCRγδ-PE, anti-IL-17A-FITC, and anti-IFN-γ-biotin followed by SA-Cy5. (A) The representative dot plots showed the percentages for IL-17$^+$ cells and IFN-γ$^+$ cells of γδ T cells among *M. tb*-AT of HD and TB. (B) The percentages for IFN-γ$^+$ cells and IL-17$^+$ cells among γδ T cells from *M. tb*-AT in HD and TB patients. There were no significant differences between the TB patients and HD.

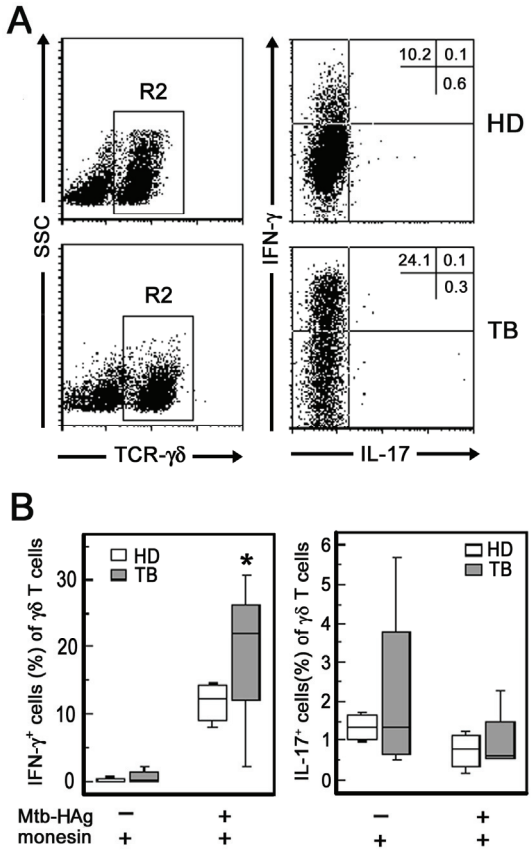

Figure 4. Flow cytometric analysis of the *M. tb*-HAg specific triggering IFN-γ and IL-17 production of γδ T cells in *M. tb*-AT of HD and TB patients. About 1×10^6 cells of *M. tb*-AT were restimulated with *M. tb*-HAg in the presence of monensin for 6 h, and then cells were stained with anti-TCRγδ-PE, anti-IL-17A-FITC, and anti-IFN-γ-biotin followed by SA-Cy5. (A) The representative flow dot plots showed the percentages for IL-17$^+$ cells and IFN-γ$^+$ cells of γδ T cells among *M. tb*-AT. (B) The percentages for IFN-γ$^+$ cells (left) and IL-17$^+$ cells (right) among γδ T cells from *M. tb*-AT stimulated with or without *M. tb*-HAg. *$p < 0.05$.

7 to 10 days to generate effective γδ T cell enriched T cell line for detection of IFN-γ- and IL-17-producing γδ T cells. The data showed that the percentages of IFN-γ$^+$ cells of effective γδ T cells in TB patients (55.0%), but not in HD (50.0%) (Figure 3B), increased when compared to that in fresh whole blood (30.5% for TB and 44.4% for HD) (Figure 2B), although the expanded amount of γδ T cells from PBMCs of TB patients that activated with *M. tb*-HAg markedly lower than that of HD (data not shown). However, the amount of IL-17$^+$ cells among these effective γδ T cells decreased in both TB patients (7.7%) and HD (5.1%) (Figure 3B) when compared to those of γδ T cells in fresh whole blood (19.4% for TB, and 7.7% for HD) (Figure 2B). Interestingly, in some individuals, in both TB patients and HD there were some γδ T cells that co-expressed both IFN-γ and IL-17 (Figure 3A).

M. tb-HAg specifically induced production of IFN-γ and IL-17 in M. tb-HAg stimulated and expanded γδ T cells

To further explore Ag specific triggering production of IFN-γ and IL-17 in effective γδ T cells, we re-stimulated the *M. tb*-AT (*M. tb*-HAg stimulated and IL-2 expanded γδ T cell enriched cell line) with the same *M. tb*-HAg and detected IFN-γ$^+$ and IL-17$^+$ γδ T cells by flow cytometry. The flow cytometry data showed that the amount of IFN-γ$^+$ γδ T cells was obviously higher in *M. tb*-AT that were re-stimulated with *M. tb*-HAg in the presence of monensin than that without *M. tb*-HAg in the presence of monensin alone. Additionally, the percentage of IFN-γ$^+$ γδ T cells in *M. tb*-AT re-stimulated with *M. tb*-HAg in TB patients (19.9%) was markedly increased when compared to that in HD (11.8%).

Contrastly, the percentages for IL-17$^+$ cells of γδ T cells in *M. tb*-AT that were re-stimulated with *M. tb*-HAg did not change in both TB patients and HD when compared to that without *M. tb*-HAg in the presence of monensin alone. Therefore, we could conclude that *M. tb*-HAg was able to specifically trigger the production of IFN-γ, but not IL-17 in effective γδ T cells.

Discussion

It has been known that, similar to CD4 αβ T cells subset Th1 and Th2 cells, γδ T cells were also able to produce Th1 and Th2 like cytokines, IL-2, IFN-γ, and IL-4, etc., which may provide the protective immunity to infection of *M. tb*, or are related to the pathogenesis of active tuberculosis (17, 18). Recently identified novel Th17 cells producing IL-17 may play an important role in the inflammation of autoimmune and infectious diseases (12). In this study, we found in peripheral blood lymphocytes the major cell source of IL-17 were γδ T cells, in normal HD (44%) and patients with active pulmonary TB (59%), and the proportion of IL-17-producing cells among γδ T cells in TB patients (19%) was markedly elevated in comparison to normal HD (8%). Our results from clinical TB cases were consistent with recently reported findings that γδ T cells were the major source of IL-17 production in the mouse model of infection with *Mt. tb* (14) or BCG (15). IL-17 has been considered to be potential to activate neutrophils, and related to formation of tubercle nodules or granuloma (15). The finding in our observation on clinical TB patients suggest the γδ T cells might be involved in the granuloma formation *via* production of IL-17, but need further identification at inflammatory focus of lung tissue of TB patients.

IFN-γ has been demonstrated to negatively regulate production of IL-17 in experimental autoimmune encephalomyelitis (19) and in mouse model of mycobacterial infection (20). On the other hand, in mouse model of infection with BCG, the absence of IL-17 decreased the production of IFN-γ and Th1 response (15). We also found that the levels of IFN-γ-producing γδ T cells were reciprocally associated with that of IL-17-producing γδ T cells in TB patients and normal HD. Moreover, the ratio of IFN-γ-producing γδ T cells to IL-17-producing γδ T cells in active TB patients was dramatically lower than that in HD (2.15 *vs* 12.21) (Figure 2). In this study we did not further explore if decreased level of IFN-γ-producing γδ T cells can induce increment of IL-17-producing γδ T cells or vice versa.

In the model of experimental autoimmune encephalomyelitis, the antigen specific Th17 cells were reported (21), and in the model of experimental autoimmune uveitis, both the antigen-specific and non-antigen specific Th17 cells were present (22). To explore whether the production of IL-17 by γδ T cells is antigen specific or not, we expanded peripheral blood γδ T cells from PBMCs cultured with *M. tb*-HAg that predominantly stimulate human γδ T cells *in vitro*, and found the expanded γδ T cells differentiated into effective cells. Upon nonspecific/polyclonal stimulation with PMA and ionomycin, among effective γδ T cells from TB patients, the IFN-γ-producing cells markedly increased, but IL-17-producing cells decreased, compared to those γδ T cells from fresh whole blood. Although the amounts of expanded γδ T cells dramatically decreased in most specimens from active TB patients (data not shown), compared to normal HD, this was consistent with our previous work (8). Our results suggest the expanded effective γδ T cells from TB patients were more antigen specific responsiveness, since after re-stimulation of expanded γδ T cells with the *M. tb*-HAg, the percentage of IFN-γ-producing γδ T cells in TB patients was significantly higher than that in HD. These results indicated that the γδ T cells from TB patients were more potential effective memory cells.

When re-stimulation with the *M. tb*-HAg, the level of IFN-γ-producing γδ T cells in active TB patients was apparently higher than that in HD, and the amount of IL-17-producing γδ T cells in antigen re-stimulation was similar to that in monensin group in both active TB patients and HD (Figure 4B). The results in this study indicated that the *M. tb*-HAg specific stimulating γδ T cells could specifically induce the production of IFN-γ, but not IL-17 among γδ T cells. There are several possibilities to explain why IL-17 production of γδ T cells was not induced by *M. tb*-HAg. Firstly, the *M. tb*-HAg used in this study, that stimulated IFN-γ production may not be suitable for stimulating IL-17 production. Secondly, there may be other antigens can induce production of IL-17 of γδ T cells. Finally, there might exist non-antigen specific IL-17-producing γδ T cells.

Interestingly, the γδ T cells that co-expressed IL-17 and IFN-γ were detected in effetive cells in both active TB patients and HD, which suggests the reciprocal suppression of IFN-γ and IL-17 might not exist in the subpopulation of γδ T cells co-expressing IFN-γ and IL-17.

Obviously, the disadvantage of the finding of this study that the amount of IL-17-producing γδ T cells in peripheral blood of patients with active pulmonary TB increased only provides indirect evidence that γδ T cells might be involved in the granuloma formation in lung tissues of pulmonary TB. We know that detection of the cytokine profile of γδ T cells in pathological focus of lung tissue of TB patients will obtain direct evidence. However, it is extremely difficult to perform this protocol in human because of medical ethical limitation in clinical practices.

In summary, our results in this study demonstrated that human γδ T cells were the main source of IL-17, and the subset of IL-17-producing γδ T cells increased in TB patients. When *M. tb*-HAg stimulated and expanded effective γδ T cells were re-stimulated with the *M. tb*-HAg, the level of IFN-γ-producing γδ T cells increased, but amount of IL-17-producing γδ T cells not change, which indicated the IFN-γ-producing γδ T cells were *M. tb*-HAg specific response cells, but IL-17-producing cells were not.

However, the precise role for the subset of IL-17-producing γδ T cells in protective immunity to infection of *M.*

tb, or in pathogenic immune reaction, either beneficial or detrimental, in pulmonary TB needs further investigation.

Acknowledgements

This work was sponsored by Key Program of Natural Science Research (No. ZD2007010-1) and Key Research Program for Key Laboratory of Universities in Anhui (No. 2004sys008) from The Anhui Provincial Education Department.

We thank the staff in the Department of Pulmonary of the Infectious Disease Hospital of Bengbu for their help in collection of specimen of TB patients, and thank our colleagues at the Anhui Key Laboratory of Infection and Immunity and Department of Immunology, Bengbu Medical College for their technical support and academic discussion in this protocol.

References

1. Global Tuberculosis Control: surveillance, planning, financing. WHO Report 2007. Geneva, World Health Organization (WHO/HTM/TB/2007.376).
2. The Ministry of Health announced the epidemic situation of legal infectious diseases in 2006. Ministry of Health of PR China. http://www.moh.gov.cn/newshtml/17829.htm
3. Kaufmann SH, Ladel CH. Role of T cell subsets in immunity against intracellular bacteria: experimental infections of knock-out mice with *Listeria* monocytogenes and *Mycobacterium* bovis BCG. Immunobiology. 1994;191:509-519.
4. Xing Z, Wang J, Croitoru K, Wakeham J. Protection by CD4 or CD8 T cells against pulmonary *Mycobacterium* bovis bacillus Calmette-Guerin infection. Infect Immun 1998; 66:5537-5542.
5. Ladel CH, Blum C, Dreher A, Reifenberg K, Kaufmann SH. Protective role of γδ T cells and αβ T cells in tuberculosis. Eur J Immunol. 1995;25:2877-2881.
6. Dieli F, Troye-Blomberg M, Ivanyi J, et al. Vγ9/Vδ2 T lymphocytes reduce the viability of intracellular *Mycobacterium tuberculosis*. Eur J Immunol. 2000;30:1512-1519.
7. Szereday L, Baliko Z, Szekeres-Bartho J. γδ T cell subsets in patients with active *Mycobacterium tuberculosis* infection and tuberculin anergy. Clin Exp Immunol. 2003;131:287-291.
8. Li B, Rossman MD, Imir T, et al. Disease-specific changes in γδ T cell repertoire and function in patients with pulmonary tuberculosis. J Immunol. 1996;157:4222-4229.
9. Tsukaguchi K, de Lange B, Boom WH. Differential regulation of IFN-γ, TNF-α, and IL-10 production by $CD4^+\alpha\beta TCR^+$ T cells and $V\delta2^+\gamma\delta$ T cells in response to monocytes infected with *Mycobacterium tuberculosis*-H37Ra. Cell Immunol. 1999;194: 12-20.
10. Huang D, Shen Y, Qiu L, et al. Immune distribution and localization of phosphoantigen-specific Vγ2Vδ2 T cells in lymphoid and nonlymphoid tissues in *Mycobacterium tuberculosis* infection. Infect Immun. 2008;76:426-436.
11. Fossiez F, Djossou O, Chomarat P, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med. 1996;183:2593-2603.
12. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235-238.
13. Forlow SB, Schurr JR, Kolls JK, et al. Increased granulopoiesis through interleukin-17 and granulocyte colony-stimulating factor in leukocyte adhesion molecule-deficient mice. Blood. 2001;98:3309-3314.
14. Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by γδ T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. J Immunol. 2006;177: 4662-4669.
15. Umemura M, Yahagi A, Hamada S, et al. IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium* bovis bacille Calmette-Guerin infection. J Immunol. 2007;178:3786-3796.
16. Boom WH, Balaji KN, Nayak R, Tsukaguchi K, Chervenak KA. Characterization of a 10- to 14-kilodalton protease-sensitive *Mycobacterium tuberculosis* H37Ra antigen that stimulates human γδ T cells. Infect Immun. 1994;62:5511-5518.
17. Follows GA, Munk ME, Gatrill AJ, Conradt P, Kaufmann SH. Gamma interferon and interleukin 2, but not interleukin 4, are detectable in γδ T-cell cultures after activation with bacteria. Infect Immun. 1992;60:1229-1231.
18. Ordway DJ, Costa L, Martins M, et al. Increased interleukin-4 production by CD8 and γδ T cells in health-care workers is associated with the subsequent development of active tuberculosis. J Infect Dis. 2004;190:756-766.
19. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6:1133-1141.
20. Cruz A, Khader SA, Torrado E, et al. Cutting edge: IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J Immunol. 2006;177: 1416-1420.
21. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med. 2006;203:1685-1691.
22. Peng Y, Han G, Shao H, Wang Y, Kaplan HJ, Sun D. Characterization of $IL\text{-}17^+$ interphotoreceptor retinoid-binding protein-specific T cells in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci. 2007;48:4153-4161.