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Gamma/Delta T Lymphocytes in the BCG Granulomatous Lesions

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Recent studies in man and animal models have demonstrated that TCR-γδ-bearing T cells (γδ T cells) are activated by mycobacteria and accumulate in the sites of mycobacterial infection. Although the function of γδ T cells remains unclear, some data suggest a potential role for these cells in the granulomatous immune response. To address the presence of γδ T cells within the BCG granulomas, we have characterized the TCR phenotype of T-lymphocytes present in the BCG granulomatous lesion immunohistochemically using a monoclonal antibody to TCR δ1 and others. Fairly large numbers of γδ T cells were located at the periphery of the BCG granulomas without necrosis and most of them also expressed CD8. However, γδ T cells were rarely present in the granulomas with central caseous necrosis, calcification and fibrotic changes. With these results, it might be speculated that the CD8+ γδ T lymphocytes participate in the BCG granuloma formation mainly in the early stage.

Key Words: BCG granuloma, γδ T cell

After acquiring mycobacteria, the majority of healthy individuals develop a cellular immune response, and arrest the growth and spread of the microorganisms without progressing to clinical diseases (Dannenberg, 1982: Kaufmann, 1993). CD4+ αβ T cell traditionally has been considered the major T cell subset to regulate this protective cellular immune response (Orme *et al.* 1993). However, studies in man and animal models have demonstrated that the other T cell subsets, in particular the TCR-γδ-bearing T cells (γδ T cells), are activated by mycobacteria and accumulate in the sites of mycobacterial infection (Janis *et al.* 1989: Griffin *et al.* 1991: Havlir *et al.* 1991).

In humans, γδ T cells are normally present in blood, lymphoid tissues, and a variety of nonlymphoid organs. They represent less than 10% of the total number of T lymphocytes present at these sites with the exception of spleen, which has more γδ T cells (Groh *et al.* 1989). Although the function of γδ T cells remains unclear, some data suggest a potential role for these cells in granulomatous immune response (Haas *et al.* 1990). It was reported that a large proportion of blood γδ T cells from mice and humans proliferate in response to mycobacterial antigens (O'Brien *et al.* 1989: Kabelitz *et al.* 1990). Furthermore, mice exposed to aerosoles containing extracts of *Mycobacterium tuberculosis* have an important increase in the number of activated CD3+ αβ TCR negative (presumably γδ TCR positive) T lymphocytes in the lung (Augustin *et al.* 1989) and the relative proportion of γδ T cells to αβ T cells is increased in the draining lymph nodes of mice immunized with *M. tuberculosis* (Janis *et al.* 1989). In humans, increased numbers of T cells have been described in some granulomatous lesions from patients with leprosy (Modlin *et al.* 1989), and a subgroup of patients with sarcoidosis was shown to have a

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striking increase of circulating γδ T cells (Balbi *et al.* 1990). Recently, there has been a lot of data on the response of γδ T cells to mycobacterial antigens (Balaji *et al.* 1995: Tsukaguchi *et al.* 1995).

However, little data is available concerning the presence of γδ T cells within tuberculomas and BCG granulomas. In order to address this question, we have characterized the TCR phenotype of T-lymphocytes present in the BCG granulomatous lesion.

MATERIALS AND METHODS

The lymph nodes from six patients with BCG granulomas (three boys and three girls: 5.2±2.1 months) were studied. They were admitted to the department of pediatric surgery for the excision of BCG lymphadenitis. None was receiving antimycobacterial therapy at the time of the study.

Normal mediastinal lymph nodes obtained from three patients (two boys and one girl: 5.0 ±1.9 months) at the time of thoracotomy for the repair of large ventricular septal defect were used as control.

Tissue specimens were fixed in 10% buffered formalin solution before conventional tissue processing, paraffin embedding, and preparation of hematoxylin-eosin stained histologic sections for light microscopic examinations. Tissue was also snap frozen in liquid nitrogen-isopentane and used for the preparation of frozen sections for immunohistochemical staining by the labelled streptavidin biotin method (LSAB 2 kit, Dako, Santa Barbara, CA, USA). The monoclonal antibodies used were anti-CD3 (Dako, Santa Barbara, CA, USA), anti-CD4 (Dako, Santa Barbara, CA, USA), anti-CD8 (Dako, Santa Barbara, CA, USA), anti-CD16 (Dako, Santa Barbara, CA, USA), anti-CD19 (Dako, Santa Barbara, CA, USA) and anti-TCR δ1 (T cell Sciences, Cambridge, MA, USA).

RESULTS

Lymph nodes from control subjects

Lymph nodes from control subjects demonstrated normal architecture in all cases. A small number of T cells expressing TCR δ1 were observed mainly within the interfollicular and the paracortical T-zones, with rare positive cells within the mantle zones and the germinal centers.

Lymph nodes from patients with BCG granuloma

Lymph nodes from the patients with BCG granuloma showed characteristic granulomas composed of epithelioid and giant cells surrounded by numerous lymphocytes. Some of the granulomas had central caseous necrosis, calcification and some fibrosis (Fig. 1).

The number of γδ T cells within the granulomas varied according to the morphologic findings of the granulomas. Fairly large number of γδ T cells expressing TCR δ1 were regularly located along the borders of the granulomas without necrosis (Fig. 2A). The degree and pattern of distribution of the CD8+ T cells in the granulomas without caseation necrosis were similar to those of the γδ T cells (Fig. 2B). Rare γδ T and CD8+ T cells were present in the granulomas with central caseous necrosis, calcification and fibrotic changes (Fig. 3A & 3B). A small number of γδ T cells were also observed within the T-zones in the uninvolved lymph nodes as in control. The CD16+ natural killers cells were not observed within the granulomas.

DISCUSSION

In this study, we found that increased γδ T lymphocytes were observed in the BCG granulomas. However, the granulomas with central caseous necrosis, calcification and fibrotic changes did not show the increased γδ T lymphocytes in the lesions. With these results, it might be speculated that the γδ T lymphocytes participate mainly in the early immune response of the BCG granuloma formation and that they do not participate in the immunologic process during the later stages of the granuloma formation involving caseous necrosis, calcification and fibrosis.

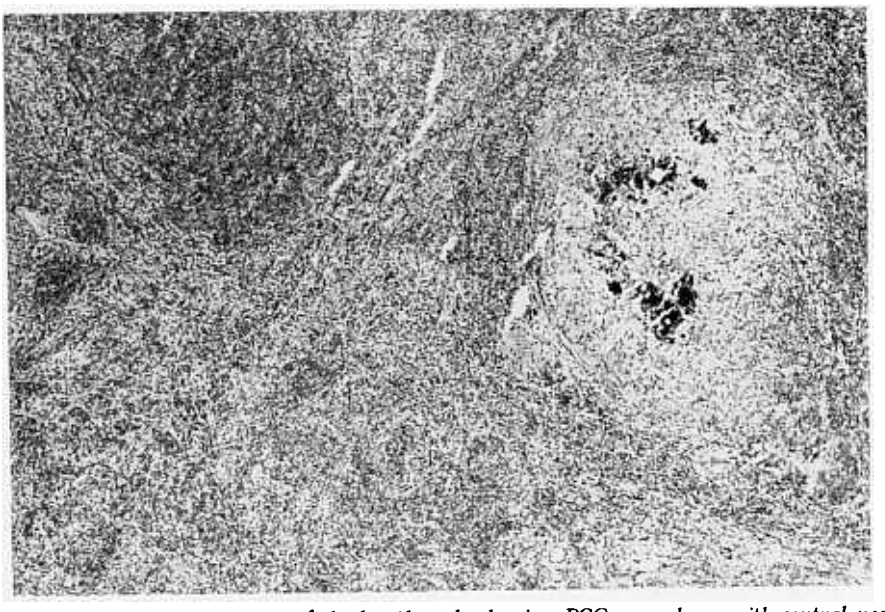

Fig. 1. Hematoxylin-eosin staining of the lymph node showing BCG granuloma with central necrosis and calcification.

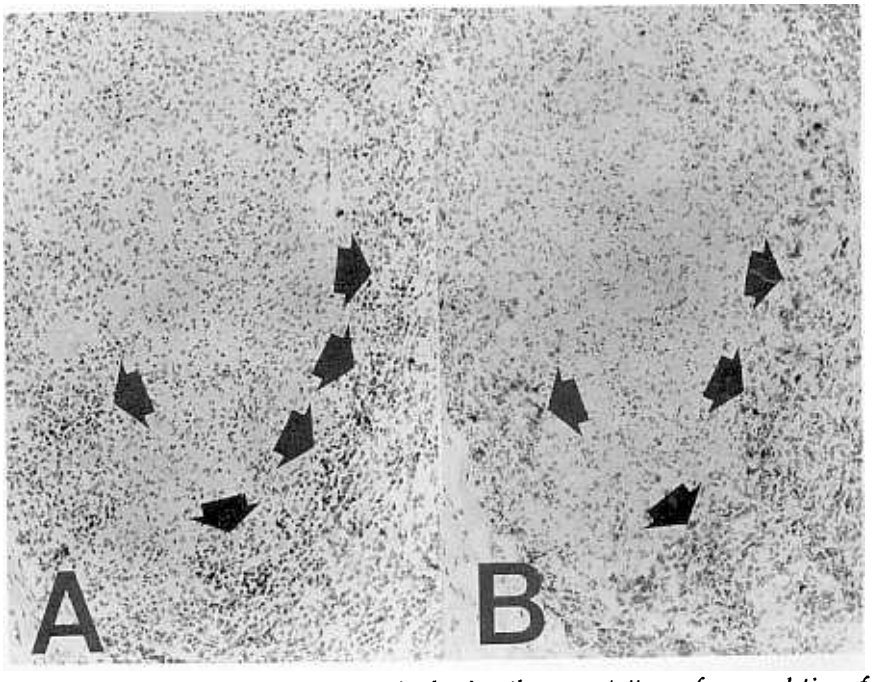

Fig. 2. The identical BCG granuloma without necrosis showing the same pattern of accumulation of the lymphoid cells expressing TCR δ1 (A) and CD8 (B) along the periphery of the granuloma (↑) (DAB chromogen with hematoxylin counterstain).

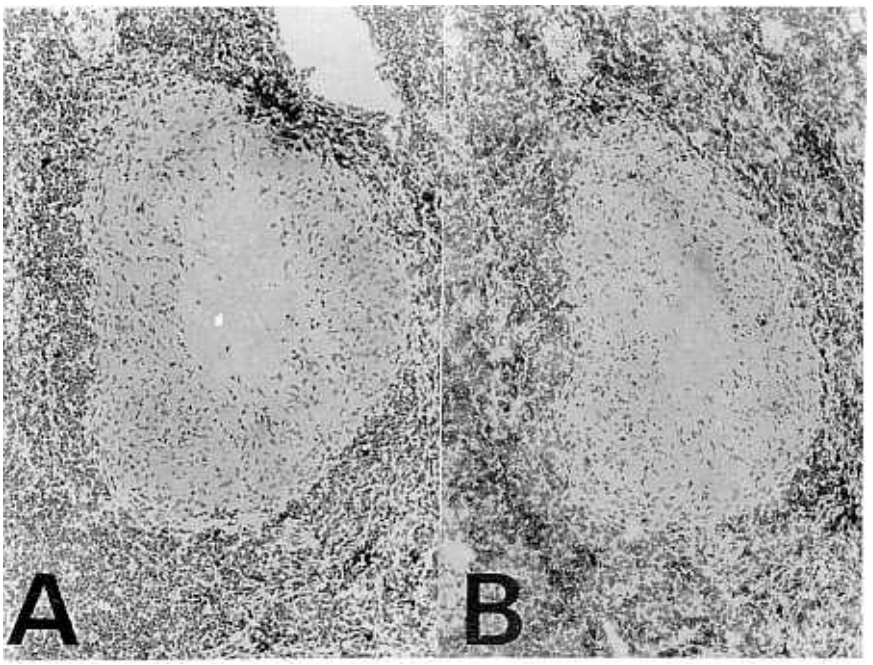

Fig. 3. *The identical BCG granuloma with necrosis showing no accumulation of the lymphoid cells expressing TCR δ1 (A) and CD8 (B) within the granuloma (DAB chromogen with hematoxylin counterstain).*

After acquiring mycobacteria, majority of healthy individuals develop a cellular immune response, and arrest the growth and spread of the microorganisms without progressing to clinical diseases (Kaufmann, 1993). The CD4+ αβ T cell traditionally has been considered the major T cell subset to regulate this protective cellular immune response (Orme *et al.* 1993). However, not only CD4+ αβ T cells but also γδ T cells are activated readily by *Mycobacterium tuberculosis*. The CD4+ and T cells differ in the range of mycobacterial antigens recognized. The CD4+ γδ T cells react to a wider molecular weight range of antigens while the γδ T cells show a restricted pattern with dominance to antigens of 10 to 15 kDa and also 65 kDa (Tsukaguchi *et al.* 1995). It is well known that these antigens are of the heat shock protein family (Kaufmann, 1993) and that the heat shock protein induces γδ T cell responses (Georgopoulos and Welch, 1993).

Tsukaguchi *et al.* (1995) studied the responses of the CD4+ αβ T cell and the γδ T cell to mycobacterial antigens in regard to antigen recognition, cytotoxic effector function, and cytokine production. They reported that the CD4+ αβ T cell and the γδ T cell subsets have similar effector functions (cytotoxicity, interferon-gamma production) in response to *M. tuberculosis*-infected macrophages, despite differences in the antigens recognized, interleukin-2 production, and the efficiency of interferon-gamma production.

However, the kinetics of the αβ T cells and the γδ T cells are thought to be different. After an initial exposure to *Listeria monocytogenes*, an early but relatively short-lived increase in the γδ T cells is observed (Ohga *et al.* 1990). Similarly, the γδ T cells are increased in lymph nodes of mice after a primary exposure to *M. tuberculosis*, but such a response is virtually absent after a second exposure to this organism (Janis *et al.* 1989). Because circulating γδ T cells have been shown to recognize a variety of bacterial and mycobacterial antigens (O' Brien *et al.* 1989; Kabelitz *et al.* 1990; Munk *et al.* 1990; Pfeffer *et al.* 1990), these findings are compatible with the idea that they represent

a first line of defense against invasion by bacterial or mycobacterial pathogens (Janeway *et al.* 1988; Haas *et al.* 1990; Munk *et al.* 1990). The sequential involvements of NK cells, γδ T cells, and αβ T cells in the cytolytic killing of bacteria at the site of intracellular bacterial growth was proposed (Kaufmann, 1993). Our results showing the presence of fairly large numbers of the γδ T cells mainly within the early granulomas are consistent with the above described kinetics of αβ T cells and γδ T cells in the immune response. The absence of NK cells detected by anti-CD16 monoclonal antibody within the granulomas in our study could be explained by time factor.

Falini *et al.* (1989) showed a high percentage of γδ T cells in the tissue of tuberculous lymphadenitis, where these cells were mainly distributed at the borders of and within necrotic areas. However, Tazi *et al.* (1991) reported that rare γδ T cells were present both inside and at the periphery of the granulomas. So, there has been some conflicting results on the presence of the γδ T cells within tuberculomas and we think this discrepancy could stem from different methods used for detecting the γδ T cells as well as from sampling problems. In our study we used the anti-TCR δ1 monoclonal antibody (T cell Sciences, Cambridge, MA, USA), which is much more sensitive and specific for detecting γδ T cells compared to the methods used in the previous studies. We don't think that there were significant differences in the pathogenesis between the tuberculous granuloma and the BCG granuloma formation since the kinetic study on the αβ and γδ T cells in the lymph node during BCG infection and *M. tuberculosis* infection showed the same results (Inoue *et al.* 1991).

Another interesting finding of this study is that the distribution pattern of CD8+ T-cells and γδ T cells within the granulomas is similar. Although we did not perform a double immunostain, mirror image examinations strongly supported that a high proportion of the γδ T cells express CD8. These results match well with the results of the recent studies using the same anti-TCR δ1 antibody, which demonstrated that nearly half of the γδ T cells expressed CD8 in contrast to the previous concept that γδ T cells are usually double negative to CD4 and CD8 (Bucy *et al.* 1989; Groh *et al.* 1989; Inghirami *et al.* 1990). The results of our study implicate that γδ T cells participate in the BCG granuloma formation mainly in the early stages and that most of them are of the CD8 positive subpopulation.

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