今村賞受賞記念講演

結核菌感染におけるサイトカインの役割に関する研究

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要旨：結核菌感染初期に関与する種々のサイトカインの役割をマウス結核モデルを利用して追究した。その中で，IFN-γとTNF-αが感染防御に最も重要であった。この所見は，ヒト結核にも深く関与しており，たとえばIFN-γ受容体1欠損症患者やヒト型抗TNF-α中和抗体を投与された慢性リウマチやクローン病患者で結核が多発することから裏付けられる。結核感染早期の病態を免疫学的に詳細に研究することにより，新しい結核診断法や治療法の開発につながることが期待される。

キーワーズ：サイトカイン，エアロソル感染，結核菌，インターフェロン・ガンマ，腫瘍壊死因子・アルファ

1. Introduction

Tubercle bacilli that are transported aerially to alveoli are phagocytosed by alveolar macrophages and, sometimes, overt tuberculosis results. The inflammatory sequence in tuberculosis involves exudative inflammation, proliferative inflammation and, finally, productive inflammation. In a clinical setting such as an outpatients clinic, the clinician can recognize proliferative and productive inflammation. However, clinicians have difficulty in recognizing the pulmonary exudative lesions that are induced by *M. tuberculosis* because the patients lack the symptoms and signs of tuberculosis and are treated for non-specific pneumonia. At the time when a definitive diagnosis is possible, patients are in the proliferative or productive stage of tuberculosis. Therefore, from early diagnostic and therapeutic viewpoints, it is interesting to examine what is going on immunologically in the exudative stage of tuberculosis. Murine tuberculosis can be used to study the aspects of human tuberculosis, particularly the exudative stage (early-phase tuberculosis). Tuberculosis is an airborne, chronic infectious disease. Thus, it is necessary to establish an inhalation exposure system (IES) before investigating the exudative stage of murine tuberculosis immunologically and pathologically. This memorial lecture focuses first on the establishment of an inhalation exposure system and then on the roles of cytokines (IFN-γ and TNF-α) in murine tuberculosis, mainly using specific gene knockout (KO) mice.

2. Inhalation exposure system

Animal (mouse and guinea pig) pulmonary tuberculosis models have been established using an automated inhalation exposure system (IES) apparatus (Glas-Col Corp., USA, Model 099CA−4212). This system includes four steps—preheating, nebulization, cloud decay and decontamination. The optimal conditions for infection experiments with *M. tuberculosis* H37Rv and Kurono strains were as follows: 10^6 colony-forming units (CFU) of tubercle bacilli; preheating for 15 min; nebulization for 90 min; cloud decay for 15 min and decontamination for 5 min[1]. When 10^4 CFU *M. tuberculosis* H37Rv strain was introduced into the lungs of interferon (IFN)-γ knockout mice using this IES apparatus and the mice were followed up for nine months, primitive cavitary lesions were formed. This apparatus was also useful for inhalation exposure experiments in guinea pigs, and it can be used for animal inhalation experiments with allergens.

3. Roles of IFN-γ in murine tuberculosis

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IFN-γ, a cytokine secreted by activated T cells, natural killer cells and natural killer T cells, has immunomodulatory effects on several cell types. IFN-γ is one of the major cytokines responsible for the activation of macrophages that mediate non-specific, cell-mediated host defenses. To gain a better understanding of the pathological role of IFN-γ in specific mycobacterial granuloma formation, IFN-γ gene-deficient mice (BALB/c and C57BL/6) were produced. The IFN-γ gene in embryonic stem cells was disrupted by inserting the β-galactosidase gene (*lacZ*) and the neomycin resistance gene (*neo*) at the translation initiation site in exon 1 by homologous recombination[2]. Six-week-old IFN-γ-defi cient and wild-type mice were inoculated with 10^{3-7} tubercle bacilli of various strains of *M.tuberculosis* (Kurono, H37Rv, and H37Ra) and BCG Pasteur aerially. The mice were examined seven weeks later for pulmonary granuloma formation. The relatively avirulent BCG Pasteur and H37Ra strains induced granulomas in the lungs, spleen and liver of IFN-γ-deficient mice. The granulomas consisted of epithelioid macrophages and Langhans-type multinucleated giant cells with central necrosis during long-term observations (9 months). The virulent Kurono and H37Rv strains induced disseminated abscesses but not granulomas in various organs of IFN-γ-deficient mice and Mac-3-positive macrophages were not detected in the abscess lesions. These results suggest that IFN-γ may be responsible primarily for macrophage activation and that other factors may be involved in the granuloma formation mechanism[3].

4. Roles of TNF-α in murine tuberculosis

TNF-α is a cytokine with various activities that are induced by activated macrophages through signal transduction at two distinct receptors. It mediates inflammation and produces protective immunity against bacterial, parasitic, and viral infections, and is thought to play a significant role in the pathogenesis of various diseases, including cancer. Of the several cytokines associated with the pathogenesis of tuberculosis, including IL-12 and IFN-γ, TNF-α is thought to be responsible for protection against the development of the disease. Kindler et al. showed that depletion of TNF-α using polyclonal antibodies blocked granuloma formation and impaired the ability to localize infection with BCG in mice[4]. Infusion of TNF-α has been shown to increase resistance against *M. tuberculosis* and *M. avium* in mice[5]. Clearly, there are conflicting data with respect to the role of TNF-α in granuloma formation. To study the role of TNF-α in mycobacterial infection, we generated TNF-α-knockout mice, in which the third and fourth exons of the TNF-α gene were disrupted. The C57BL/6 KO mice were infected with the virulent *M. tuberculosis* strain Kurono or the relatively avirulent bacillus BCG Pasteur (10^6 CFU), by IES as described previously. The major organs were removed at weekly intervals, and morphologic observation, assay of IL-1, IL-12, IFN-γ, and inducible nitric oxide synthase mRNA expression, and colony counts in the lungs and spleen were performed. Peritoneal and alveolar macrophages from BCG- and H37Rv strain-treated mice produced significant levels of nitric oxide after stimulation *in vitro*. The formation of abscesses was seen only in the Kurono-treated groups, and these abscesses contained large numbers of mycobacteria. The administration of recombinant TNF-α significantly ameliorated the mycobacterial lesions. IFN-γ mRNA was expressed significantly in virulent H37Rv-treated groups with time, and the number of mycobacterial colonies per unit weight increased markedly with time. Nitric oxide production was not observed in H37Rv-treated groups but was seen in BCG-treated groups. We concluded that TNF-α played an important role in protective immunity against virulent mycobacteria. Because avirulent mycobacteria did not induce granulomas in TNF-α-KO mice. TNF-α played an indirect role in granuloma formation[6].

5. Other cytokines

IL-12, IL-18, IL-4, and IL-1, as well as IFN-γ and TNF-α, play important roles in protective immunity against mycobacterial infection. IL-12-, IL-18-, IL-4-, and IL-1-KO mice did not die when they were infected with the virulent Kurono strain via an airborne route in our experiments[7)~9)]. It is thought that these cytokines are not necessary for protection against mycobacterial infection or the functions of these cytokines are compensated for by other cytokines. If we rank the cytokines in terms of their roles in mycobacterial infection, we can construct a cytokine hierarchy in murine tuberculosis, as shown in Fig.

6. Clinical implications

I have presented some important findings from experimental murine tuberculosis. What is the clinical relevance of murine tuberculosis? I have reported previously that serum IFN-γ levels are significantly low in patients with advanced, and active TB[10] and it has also been reported that people with IFN-γ receptor deficiency are susceptible to *M. tuberculosis*[11]. On the other hand, humanized anti-TNF-α neutralizing monoclonal antibody is given to patients with rheumatoid

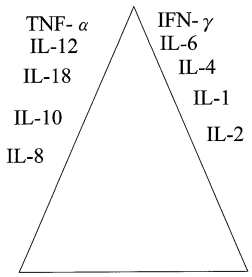

Fig. Cytokine hierarchy in tuberculosis

arthritis and Crohn's disease whose serum TNF-α levels are low [12] and these patients develop readily tuberculosis. Thus, it is meaningful to study murine tuberculosis for the insights into clinical tuberculosis.

7. Conclusion

I briefly reviewed the roles of cytokines in experimental mycobacterial infection with special emphasis on the roles of IFN-γ and TNF-α. IFN-γ and TNF-α are the 'grand champions' among the cytokines involved in mycobacterial infection. Therefore, it is very important to investigate their roles and regulatory factors for IFN-γ and TNF-α in early-phase mycobacterial infection in more detail, so that tuberculosis can be diagnosed and treated as early as possible.

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STUDY ON THE ROLES OF CYTOKINES INVOLVED IN MYCOBACTERIAL INFECTION

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Abstract The roles of various cytokines in early-phase mycobacterial infection were investigated utilizing murine tuberculosis models. Among them, IFN-γ and TNF-α are very important in protective immunity against mycobacterial infection. This finding is closely associated with human tuberculosis. It is reported that persons with IFN-γ receptor 1 deficiency and patients with rheumatoid arthritis and Crohn's disease are susceptible to *Mycobacterium tuberculosis*. It is expected that a novel immunotherapy and a diagnostic method of tuberculosis are developed by clarifying roles of various cytokines immunologically in early-phase mycobacterial infection.

Key words: Cytokine, Aerosol infection, *M. tuberculosis*, IFN-γ , TNF-α

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