MICROBIAL PATHOGENICITY

Role of T cells in granuloma formation induced by *Rhodococcus aurantiacus* is independent of their interferon-gamma production

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Intravenous injection of *Rhodococcus aurantiacus* into mice causes granulomatous inflammation dependent on endogenous interferon- γ (IFN- γ). This study investigated the mechanism of granuloma formation with an adoptive transfer system in IFN- γ knockout (IFN- $\gamma^{-/-}$) mice. IFN- $\gamma^{-/-}$ mice infected with *R. aurantiacus* did not develop granulomas, and high titres of endogenous interleukin-10 (IL-10) were detected in spleen extracts at 2 weeks after infection. The adoptive transfer of splenocytes from infected wild-type (IFN- $\gamma^{+/+}$) mice did not restore granuloma formation, although this treatment diminished IL-10 production in IFN- $\gamma^{-/-}$ mice. Adoptive transfer of splenocytes from infected IFN- $\gamma^{-/-}$ mice into infected IFN- $\gamma^{+/+}$ reduced granuloma formation. These results suggest that splenocytes of IFN- $\gamma^{-/-}$ mice suppress granuloma formation. On the other hand, although IFN- γ production induced by *R. aurantiacus* infection was detected in nude mice, which are deficient in T cells, granuloma formation was not induced in them. However, adoptive transfer of immune splenocytes from either IFN- $\gamma^{+/+}$ mice or IFN- $\gamma^{-/-}$ mice could induce granuloma formation. This means that splenocytes of IFN- $\gamma^{-/-}$ mice have the ability to both induce and suppress granuloma formation. Induction of granuloma is probably dependent on both T cells and IFN- γ produced by non-T cells. It is suggested that the role of T cells in granuloma formation is not dependent on their IFN- γ production.

Introduction

Granulomatous inflammation can be classified on the basis of cytokine profiles into type 1 characterised by interferon- γ (IFN- γ) dominance and type 2 showing interleukin (IL)-4, IL-5 and IL-10 involvement [1, 2]. Studies in mice with targeted knockout of cytokines help to clarify the role of cytokines in immune and inflammatory conditions. It has been reported that for type 1 granulomatous responses, IFN- γ knockout (IFN- $\gamma^{-/-}$) mice infected with *Mycobacterium tuberculosis* exhibit impaired resistance [3, 4], and type 1 granulomatous inflammation in IFN- $\gamma^{-/-}$ mice administered purified protein derivative (PPD) shifts to an exacerbated type 2 pattern [2].

Infection of wild-type (IFN- $\gamma^{+/+}$) mice with *Rhodococcus aurantiacus* induces the formation of non-necrotic granuloma resembling sarcoidosis [5, 6],

characterised by the infiltration of mononuclear phagocytes and the maturation of these cells into epithelioid cells [7–9]. A previous study reported that biphasic production of endogenous IFN- γ appeared within 24 h and at 3 weeks after *R. aurantiacus* infection in mice, the former attributable to NK cells and the latter dependent on CD8⁺ T cells, and that granuloma formation is regulated by secretion of endogenous IFN- γ [7, 8, 10]. Therefore, granulomatous inflammation in *R. aurantiacus* infection is classified as type 1. It is not clear whether IFN- γ produced by both NK cells and T cells is important. To clarify the different roles of IFN- γ produced by NK cells and T cells on granuloma formation, an adoptive transfer experiment was performed in IFN- $\gamma^{-/-}$ mice.

Materials and methods

Animals and infection

C57BL/6 and BALB/c IFN- $\gamma^{-/-}$ mice have been described previously [11]. Female C57BL/6 IFN- $\gamma^{-/-}$ mice, C57BL/6 IFN- $\gamma^{+/+}$ mice, BALB/c nude mice,

BALB/c IFN- $\gamma^{-/-}$ mice and BALB/c IFN- $\gamma^{+/+}$ mice, aged 6–8 weeks, were used. IFN- $\gamma^{-/-}$ mice were phenotypically confirmed by PCR and fertility analysis [11]. IFN- $\gamma^{+/+}$ mice were purchased from SLC (Hamamatsu, Shizuoka, Japan).

Mice were infected intravenously with 10^8 cfu of viable *R. aurantiacus* (strain 80005) suspended in 0.2 ml of phosphate-buffered saline (PBS).

Determination of the number of bacteria in the organs

The numbers of viable *R. aurantiacus* in the spleens and livers of infected mice were counted by plating serial 10-fold dilutions of organ homogenates in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY, USA) on Nutrient Agar (Nissui Pharmaceutical, Tokyo, Japan). Colonies that developed on the plates were counted 48 h later.

Adoptive transfer of splenocytes

Spleens of mice killed at 1 week post infection (p.i.) were disrupted by gentle teasing and repeated suction and expulsion through a 1-ml syringe in PBS. The single cells were resuspended in ammonium chloride 0.83% solution containing Tris buffer 10% v/v (pH 7.65) to lyse erythrocytes, then were washed three times in RPMI 1640 medium. Cell viability was determined by the trypan blue exclusion test. Then 10^8 splenocytes in 0.5 ml of RPMI were injected intravenously into mice that had been infected with *R. aurantiacus* 1 day earlier.

Preparation of organ extracts for cytokine assays

The spleen homogenates for cytokine assays were prepared as follows. The spleens were removed aseptically from the mice and suspended in RPMI 1640 medium containing CHAPS (Wako Pure Chemical, Kyoto, Japan) 1% w/v and homogenates (10% w/v) were prepared with a Dounce grinder. The homogenates were clarified by centrifugation at 2000 g for 20 min. The organ extracts were stored at -70°C until cytokine assay.

Cytokine concentrations in supernates were measured in triplicate by two sandwich ELISAs. To measure IFN- γ , plates were coated with a monoclonal antibody (MAb) produced by hybridoma R4-6A2 and incubated with supernates of organ extracts. IFN- γ was detected with polyclonal rabbit anti-IFN- γ followed by biotinylated goat anti-rabbit IgG, streptavidin-horseradish peroxidase. IL-10 was captured with a rat anti-mouse IL-10 MAb (JES5-2A5; PharMingen) and detected with a biotinylated rat anti-mouse IL-10 MAb (SXC-1; PharMingen). The sensitivities of the ELISAs were 0.1 U/ml for IFN- γ and 500 pg/ml for IL-10.

Histological studies

Granulomas were measured from a separate set of formalin-inflated livers that were paraffin-embedded, sectioned, then stained with haematoxylin and eosin. In each experiment, the sections were made from three-to-six pieces of each organ. The mean granuloma area was measured from the number of granulomas in all fields of each section.

Statistical analysis

Friedman's test was used to compare control with experimental groups; $p < 0.05$ indicated significance.

Results

R. aurantiacus-induced granuloma formation

A histological technique was used to investigate granuloma formation in the livers of IFN- $\gamma^{-/-}$ mice and IFN- $\gamma^{+/+}$ mice inoculated with *R. aurantiacus*. In the control group, granulomas in the livers of C57BL/6 and BALB/c IFN- $\gamma^{+/+}$ mice were composed primarily of mononuclear cells and lymphocytes at 1 week p.i. Non-necrotic and epithelioid granulomas grew to maximum size at 2 weeks p.i. (C57BL/6 mice, 34 SD 0.05%; BALB/c mice, 38 SD 0.05%). Thereafter, the number of granulomas decreased and at 4 or 5 weeks p.i. the granulomas had regressed completely. In the livers of the two strains of IFN- $\gamma^{-/-}$ mice, clusters of mononuclear cells and lymphocytes appeared, but no granuloma developed (Figs. 1 and 2).

Next, the effects of adoptive transfer of splenocytes from IFN- $\gamma^{-/-}$ mice on granuloma formation were examined. Transfer of 10^8 splenocytes from infected C57BL/6 IFN- $\gamma^{-/-}$ mice into infected C57BL/6 IFN- $\gamma^{+/+}$ mice reduced the area of granuloma in the tissues at 2 weeks p.i. (C57BL/6 mice, 26 SD 0.05%). In contrast, the transfer of 10^8 splenocytes from infected C57BL/6 IFN- $\gamma^{+/+}$ mice was not effective in inducing granuloma formation in the C57BL/6 IFN- $\gamma^{-/-}$ mice (Fig. 2).

The following experiment was performed with nude mice. The intravenous injection of 10^8 cfu of *R. aurantiacus* into nude mice did not cause granuloma formation in the liver. However, the transfer of 10^8 splenocytes from either infected BALB/c IFN- $\gamma^{+/+}$ mice or infected BALB/c IFN- $\gamma^{-/-}$ mice induced granuloma formation in the livers of the infected nude mice. The area of the granuloma induced by the latter was smaller than that induced by the former (former, 40 SD 0.05%; latter, 28 SD 0.05%) (Fig. 2).

Kinetics of bacterial growth

After C57BL/6 IFN- $\gamma^{+/+}$ mice and IFN- $\gamma^{-/-}$ mice were challenged with 10^8 cfu of *R. aurantiacus*, the

Fig. 1. Histological appearance of livers of a C57BL/6 IFN- $\gamma^{+/+}$ mouse (**a**) and a C57BL/6 IFN- $\gamma^{-/-}$ mouse (**b**) with *R. aurantiacus* infection at 2 weeks p.i. A granulomatous lesion is seen in the liver of the C57BL/6 IFN- $\gamma^{+/+}$ mouse; clusters of mononuclear cells and lymphocytes appeared, but no granuloma developed in the tissue of the C57BL/6 IFN- $\gamma^{-/-}$ mouse (magnifications: a, 400 $\times$; b, 200 $\times$).

numbers of bacteria in the livers and spleens were determined. Bacterial counts in these organs in C57BL/6 IFN- $\gamma^{+/+}$ mice fell to an undetectable level by 2 weeks p.i., while those of IFN- $\gamma^{-/-}$ mice were detectable at 2 weeks p.i. (Fig. 3).

Endogenous cytokine production

The study examined whether adoptive transfer of immune splenic cells from infected IFN- $\gamma^{+/+}$ mice could restore IFN- γ production and granuloma forma-

tion in IFN- $\gamma^{-/-}$ mice. The titres of IFN- γ in the extracts of the spleens from either C57BL/6 IFN- $\gamma^{-/-}$ mice or C57BL/6 IFN- $\gamma^{-/-}$ mice into which splenocytes of C57BL/6 IFN- $\gamma^{+/+}$ mice were transferred were determined at 2 and 3 weeks p.i. Endogenous IFN- γ was not produced in either group of mice (Fig. 4a). The titres of IFN- γ in the extracts of spleens from C57BL/6 IFN- $\gamma^{+/+}$ mice and from C57BL/6 IFN- $\gamma^{+/+}$ mice into which splenocytes of either C57BL/6 IFN- $\gamma^{-/-}$ mice or C57BL/6 IFN- $\gamma^{+/+}$ mice were transferred were also determined until 3 weeks

Fig. 2. Granuloma formation in the livers of various groups of mice at 2 weeks p.i. The area of granulomas in a field was determined in the sections from all areas of each of the livers of the mice. (a) C57BL/6 IFN- $\gamma^{+/+}$ mice (■), C57BL/6 IFN- $\gamma^{-/-}$ mice (□), C57BL/6 IFN- $\gamma^{+/+}$ mice with splenocytes transferred from C57BL/6 IFN- $\gamma^{-/-}$ mice (■), C57BL/6 IFN- $\gamma^{-/-}$ mice with splenocytes transferred from C57BL/6 IFN- $\gamma^{+/+}$ mice (□). (b) BALB/c IFN- $\gamma^{+/+}$ mice (■), BALB/c IFN- $\gamma^{-/-}$ mice (□), BALB/c nude mice (■), BALB/c nude mice with splenocytes transferred from BALB/c IFN- $\gamma^{+/+}$ mice (□), BALB/c nude mice with splenocytes transferred from BALB/c IFN- $\gamma^{-/-}$ mice (▨).

p.i. There was no significant difference in IFN- γ production among the various groups and the peak of IFN- γ production was seen at 2 weeks p.i. (Fig. 4b).

IFN- γ was detected quantitatively in the extracts of spleens from infected nude mice into which splenocytes of BALB/c IFN- $\gamma^{+/+}$ mice and BALB/c IFN- $\gamma^{-/-}$ mice, respectively, were transferred and from control nude mice by ELISA at 2 weeks p.i. IFN- γ was detected in the spleens of the infected nude mice, and the titres of IFN- γ from the infected nude mice that received splenocytes of BALB/c IFN- $\gamma^{+/+}$ and BALB/c IFN- $\gamma^{-/-}$ mice were twice as high as those of the control group (Fig. 5).

Fig. 3. Kinetics of bacterial number ($\log_{10}$ cfu) in the livers and spleens of *R. aurantiacus*-infected C57BL/6 IFN- $\gamma^{+/+}$ mice (○) and C57BL/6 IFN- $\gamma^{-/-}$ mice (●). Each point represents the mean and SD for 10 mice.

Furthermore, the spleen extracts obtained from the *R. aurantiacus*-infected mice were used for detection of IL-10 by ELISA. In the C57BL/6 IFN- $\gamma^{-/-}$ mice, the level of endogenous IL-10 reached a peak at 2 weeks p.i. Adoptive transfer of splenocytes from C57BL/6 IFN- $\gamma^{+/+}$ mice impeded IL-10 secretion in C57BL/6 IFN- $\gamma^{-/-}$ mice (Fig. 6a). In the infected IFN- $\gamma^{+/+}$ mice and those that received spleen cells from infected IFN- $\gamma^{+/+}$ mice, endogenous IL-10 was low at 2 weeks p.i. However, adoptive transfer of splenocytes from C57BL/6 IFN- $\gamma^{-/-}$ mice into IFN- $\gamma^{+/+}$ mice caused the elevation of IL-10 (Fig. 6b).

Discussion

It is recognised that NK cells and T cells produce IFN- γ [12–14]. A previous study demonstrated that biphasic IFN- γ production, primarily by NK cells and secondarily by T cells, might contribute to granuloma formation in *R. aurantiacus*-infected mice [8]. IFN- γ is also secreted by NK cells and T cells in *Listeria*

Fig. 4. Effects of adoptive transfer of splenocytes on endogenous IFN- γ production. (a) in the spleen extracts, as in the IFN- $\gamma^{-/-}$ mice infected only (■), IFN- γ production could not be observed in the IFN- $\gamma^{-/-}$ mice with splenocytes transferred from IFN- $\gamma^{+/+}$ mice (□). (b) There was no difference in endogenous IFN- γ production between infected-only IFN- $\gamma^{-/-}$ mice (■), and IFN- $\gamma^{+/+}$ mice with splenocytes transferred from either IFN- $\gamma^{+/+}$ mice (▨) or IFN- $\gamma^{-/-}$ mice (□) at 1–3 weeks p.i. Data are the means and SDs of multiple determinations from each of three separate experiments.

monocytogenes and *Salmonella enterica* serotype Typhimurium infection [15–18]. It is not clear whether the role of IFN- γ produced by T cells is different from that produced by non-T cells. Although IFN- γ -producing cells existed in IFN- $\gamma^{-/-}$ mice that received transfer from infected IFN- $\gamma^{+/+}$ mice, IFN- γ production and granuloma formation were not restored (Fig. 2 and Fig. 4a). This indicated that IFN- γ produced by T cells was not important in inducing granuloma. On the other hand, granuloma formation was induced in T-cell-deficient nude mice that received splenocytes from IFN- $\gamma^{+/+}$ or IFN- $\gamma^{-/-}$ mice (Figs. 2 and 5). This suggested that T cells were important in inducing granuloma *via* an IFN- γ -independent mechanism. In nude mice, IFN- γ -producing cells like NK cells exist. However, IFN- $\gamma^{-/-}$ mice into which splenocytes from

Fig. 5. Endogenous IFN- γ production from splenocytes of infected-only BALB/c nude mice (■), and BALB/c nude mice with splenocytes transferred from either BALB/c IFN- $\gamma^{+/+}$ mice (▨) or BALB/c IFN- $\gamma^{-/-}$ mice (□) at 2 weeks p.i. Data are the means and SDs of multiple determinations from each of three separate experiments.

IFN- $\gamma^{+/+}$ mice were transferred were deficient in NK cells producing IFN- γ because the number of NK cells in the splenocytes was smaller than that of T cells. Therefore, IFN- γ produced by non-T cells, probably in the early phase of the infection, is crucial in inducing granuloma. It is reported that IFN- γ is important in inducing granuloma in other infectious diseases [19]. However, it is not clear that IFN- γ directly induces granuloma formation. We speculate that IFN- γ produced by non-T cells in the early phase of the infection stimulates T cells, and that they produce some factors for inducing granuloma formation directly. For example, it is reported that nitric oxide directly induces granuloma formation [20]. It is possible that some factor(s) such as nitric oxide is directly important in inducing granuloma in *R. aurantiacus* infection [20].

Granulomas were not induced in IFN- $\gamma^{-/-}$ mice infected with *R. aurantiacus* because of the lack of non-T cells producing IFN- γ . However, it is possible that there is another mechanism that suppresses granuloma formation. It is reported that Th2 cytokines such as IL-4 and IL-10 are antagonistic for the activities of Th1 cytokines and decrease the size of granulomas [10]. This preferential expression of Th1 cytokines *versus* Th2 cytokines may be driven by various mechanisms. Transfer of splenocytes from IFN- $\gamma^{+/+}$ mice into IFN- $\gamma^{-/-}$ mice was not effective in restoring granuloma formation (Fig. 2). We speculate that splenocytes of IFN- $\gamma^{-/-}$ mice have a suppressive function against granuloma formation. It was thought that the number of Th2-type cells increased in splenocytes of IFN- $\gamma^{-/-}$ mice, and that these cells inhibited granuloma formation and Th1 cytokine

Fig. 6. Effects of adoptive transfer of C57BL/6 mouse splenocytes on endogenous IL-10 production at 2 weeks p.i. (a) IL-10 production in spleens of the infected-only IFN- $\gamma^{-/-}$ mice (■) and IFN- $\gamma^{-/-}$ mice with splenocytes transferred from IFN- $\gamma^{+/+}$ mice (□). (b) IL-10 production in the spleens of infected-only IFN- $\gamma^{+/+}$ mice (■), and IFN- $\gamma^{+/+}$ mice with splenocytes transferred from either IFN- $\gamma^{+/+}$ mice (▨) or IFN- $\gamma^{-/-}$ mice (□). * $p < 0.01$.

production by T cells of IFN- $\gamma^{+/+}$ mice. It has been reported that Th2 cytokines in IFN- $\gamma^{-/-}$ mice are increased by stimulation with mycobacterial antigens compared with IFN- $\gamma^{+/+}$ mice [2]. In the present study, increases of IL-4 were not observed in the IFN- $\gamma^{-/-}$ mice (unpublished data). However, the titre of IL-10 in the extracts of the spleen at 2 weeks after infection was greater than in IFN- $\gamma^{+/+}$ mice (Fig. 6a). Furthermore, the adoptive transfer of splenocytes of IFN- $\gamma^{-/-}$ mice to IFN- $\gamma^{+/+}$ mice caused the elevation of IL-10 in the spleen (Fig. 6b) and the number of granulomas was diminished in the liver (Fig. 2). IL-10 is a cytokine mainly derived from macrophages, and it

has been reported that neutralisation of endogenous IL-10 restores in-vitro production of IFN- γ by spleen cells and enhances host resistance against infection with *M. avium* [21, 22]. The present study indicated that mice infected with *R. aurantiacus* were able to produce IL-10, but its production was normally inhibited by IFN- γ during the granuloma formation phase in IFN- $\gamma^{+/+}$ mice.

References

- Chensue SW, Warmington KS, Ruth JH, Lincoln P, Kunkel SL. Cytokine function during mycobacterial and schistosomal antigen-induced pulmonary granuloma formation. Local and regional participation of IFN- γ , IL-10 and TNF. *J Immunol* 1995; **154**: 5969–5976.
- Chensue SW, Warmington K, Ruth JH, Lukacs N, Kunkel SL. Mycobacterial and schistosomal antigen-elicited granuloma formation in IFN- γ and IL-4 knockout mice. Analysis of local and regional cytokine and chemokine networks. *J Immunol* 1997; **159**: 3565–3573.
- Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon γ gene-disrupted mice. *J Exp Med* 1993; **178**: 2243–2247.
- Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* 1993; **178**: 2249–2254.
- Sugiyama Y. Macrophage activation in sarcoidosis. *Clin Immunol* 1998; **30**: 199–206.
- Asano M, Minagawa T, Ohmichi M, Hiraga Y. Detection of endogenous cytokines in sera or in lymph nodes obtained from patients with sarcoidosis. *Clin Exp Immunol* 1991; **84**: 92–96.
- Asano M, Nakane A, Minagawa T. Endogenous gamma interferon is essential in granuloma formation induced by glycolipid-containing mycolic acid in mice. *Infect Immun* 1993; **61**: 2872–2878.
- Asano M, Nakane A, Kohanawa M, Minagawa T. Sequential involvement of NK cells and CD8 $^{+}$ T cells in granuloma formation of *Rhodococcus aurantiacus*-infected mice. *Microbiol Immunol* 1995; **39**: 499–507.
- Kaneda K, Sumi Y, Kurano Y, Kato Y, Yano I. Granuloma formation and hemopoiesis induced by C36-48-mycolic acid-containing glycolipids from *Nocardia rubra*. *Infect Immun* 1986; **54**: 869–875.
- Asano M, Kohanawa M, Minagawa T, Nakane A. Reciprocal action of interferon- γ and interleukin-4 promotes granulomatous inflammation induced by *Rhodococcus aurantiacus* in mice. *Immunology* 1996; **88**: 394–399.
- Tagawa Y, Sekikawa K, Iwakura Y. Suppression of concanavalin A-induced hepatitis in IFN- $\gamma^{-/-}$ mice, but not in TNF- $\alpha^{-/-}$ mice. Role for IFN- γ in activating apoptosis of hepatocytes. *J Immunol* 1997; **159**: 1418–1428.
- Dunn PL, North RJ. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect Immun* 1991; **59**: 2892–2900.
- Squires KE, Schreiber RD, McElrath MJ, Rubin BY, Anderson SL, Murray HW. Experimental visceral leishmaniasis: role of endogenous IFN- γ in host defense and tissue granulomatous response. *J Immunol* 1989; **143**: 4244–4249.
- Bogen SA, Fogelman I, Abbas AK. Analysis of IL-2, IL-4, and IFN- γ producing cells in situ during immune responses to protein antigens. *J Immunol* 1993; **150**: 4197–4205.
- Sacco RE, Jensen RJ, Thoen CO *et al.* Cytokine secretion and adhesion molecule expression by granuloma T lymphocytes in *Mycobacterium avium* infection. *Am J Pathol* 1996; **148**: 1935–1948.
- Nakane A, Numata A, Chen Y, Minagawa T. Endogenous gamma interferon-independent host resistance against *Listeria monocytogenes* infection in CD4 $^{+}$ T cell- and asialo GM1 $^{+}$ cell-depleted mice. *Infect Immun* 1991; **59**: 3439–3445.
- Nakane A, Numata A, Asano M, Kohanawa M, Chen Y, Minagawa T. Evidence that endogenous gamma interferon is produced early in *Listeria monocytogenes* infection. *Infect Immun* 1990; **58**: 2386–2388.

18. Ramarathinam L, Niesel DW, Klimpel GR. Ity influences the production of IFN- γ by murine splenocytes stimulated in vitro with *Salmonella typhimurium*. *J Immunol* 1993; **150**: 3965–3972.
19. Jouanguy E, Döffinger R, Dupuis S, Pallier A, Altare F, Casanova J-L. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 1999; **11**: 346–351.
20. Ehlers S, Kutsch S, Benini J *et al*. NOS₂-derived nitric oxide regulates the size, quantity and quality of granuloma formation in *Mycobacterium avium*-infected mice without affecting bacterial loads. *Immunology* 1999; **98**: 313–323.
21. Bermudez LE, Champs J. Infection with *Mycobacterium avium* induces production of interleukin-10 (IL-10), and administration of anti-IL-10 antibody is associated with enhanced resistance to infection in mice. *Infect Immun* 1993; **61**: 3093–3097.
22. Denis M, Ghadirian E. IL-10 neutralization augments mouse resistance to systemic *Mycobacterium avium* infections. *J Immunol* 1993; **151**: 5425–5430.