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Adjuvant treatment increases the resistance to *Mycobacterium avium* infection of Mycobacteria-susceptible BALB/c mice

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SUMMARY

We have investigated the effect of inflammation on host resistance against infection by *Mycobacterium avium*, an atypical mycobacteria species that is responsible for life-threatening opportunistic infections in AIDS patients. Inflammation was induced in BALB/c mice by two intraperitoneal injections of mineral oil (Freund's incomplete adjuvant, FIA). The BALB/c strain was chosen because it is naturally susceptible to *Myco. avium* infection. One week after the second FIA injection, the BALB/c mice were infected intravenously with $2{\cdot}6 \times 10^6$ *Myco. avium* bacilli; at this time, the mice showed systemic granulocytosis because of the FIA injections. The kinetics of the murine infection was determined during 3 months by quantification of *Myco. avium* loads in the major target organs (liver and spleen) of the mycobacteria. The FIA treatment resulted in a significant decrease in the growth of *Myco. avium* in the infected BALB/c mice. This enhancement in host resistance to *Myco. avium* infection lasted for 2–3 months. In contrast with BALB/c animals, C3H mice (naturally resistant to *Myco. avium* infection) did not show an increased anti-*Myco. avium* action in association with the FIA treatment. The antimycobacterial effect of the FIA injections in BALB/c mice was compared with that produced by the injection of mycobacterial antigens (heat-killed *Myco. tuberculosis*) added to the mineral oil (i.e. Freund's complete adjuvant, FCA). The FCA treatment resulted in strong and sustained enhancements in the microbicidal capacities of BALB/c, and also of C3H mice. Data obtained with mutant athymic BALB/c mice revealed that the anti-*Myco. avium* effect of the FCA treatment was T cell-dependent. Our results indicate that: (i) non-immune inflammatory stimulation (FIA) of *Myco. avium*-susceptible hosts is able to cause a significant, albeit transient, increase in the resistance to *Myco. avium* infection; (ii) this protective effect is enhanced if heat-killed mycobacteria are added to the phlogistic agent (FCA), i.e. if a T cell-dependent response is induced; and (iii) systemic increase in the number of circulating granulocytes may help host defence against *Myco. avium* infection.

Keywords mycobacteria infection granulocytes inflammation neutrophils

INTRODUCTION

The number of pathogenic infections caused by *Mycobacterium avium* has steadily increased during the last decade, mainly because *Myco. avium* is one of the most common agents identified in the opportunistic infections that plague AIDS patients [1–5]. In addition, *Myco. avium* has also been identified as the cause of severe respiratory disorders in elderly patients showing no signs of conditions that are known to predispose to infections by atypical mycobacteria [6]. In contrast with tubercle and leprosy bacilli, no drug therapy has shown a reasonably good effectiveness in stopping the growth of *Myco. avium* in susceptible hosts. It is therefore important to test new experimental strategies aimed at enhancing the resistance to *Myco. avium* of hosts that are susceptible to this infectious agent.

Recent reports from this and other laboratories have documented that granulocytes may participate in host defence against mycobacterial infections [7–13], namely through the transfer of their antimicrobial armoury to macrophages [9], the cells that are parasitized by the mycobacteria. This concept is consistent with the finding that the more severe infections caused by intracellular parasites in AIDS patients are associated with impaired function or decreased numbers of circulating granulocytes [14].

We decided, therefore, to investigate whether a systemic increase in the number of granulocytes induced by non-immune inflammation had any effect on the resistance of mice to *Myco.*

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avium infection. For that, we have triggered granulocytosis in the *Myco. avium*-susceptible BALB/c mice by intraperitoneal injection of mineral oil (Freund's incomplete adjuvant, FIA) before the animals were intravenously inoculated with *Myco. avium*. We followed the murine infections for 3 months and compared the action of the FIA inflammation with that produced by injection of mineral oil containing mycobacterial antigens (Freund's complete adjuvant, FCA). The effect on *Myco. avium* infection of the FIA or FCA treatments was also studied in the *Myco. avium*-resistant C3H strain of mice. Our experiments showed that the FIA inflammation caused a significant increase in the resistance against *Myco. avium* infection in BALB/c but not in C3H mice. The antimicrobial activity induced by the FIA inflammation was less pronounced and shorter lived than that produced by the T cell-dependent response triggered by the FCA treatment.

MATERIALS AND METHODS

Mice

Female C3H, euthymic and athymic (*nu/nu*) BALB/c mice were supplied by Harlan Olac Limited (Bicester, UK) and kept in the animal facilities of this research institute under standard housing conditions, fed commercial chow and acidified water.

Mycobacteria

Bacilli of *Myco. avium* strain ATCC 25291 (serotype 2) were grown in Middlebrook 7H9 medium with ADC supplement (Difco) and 0·04% Tween 80 (Sigma). Inocula were prepared as previously described [15] and stored frozen at −70°C before use.

Experimental groups

Seventy-two 10–12 weeks old mice of each of the two strains of euthymic BALB/c and C3H animals were divided into three experimental groups of 24 mice. The animals of Group I were twice injected intraperitoneally with 100 μl of mineral oil (FIA, Sigma Chemical Co., St. Louis, MO, cat. no. F-5506); the two i.p. injections were performed with an interval of 1 week from each other. The mice of Group II were treated similarly to the animals of Group I, with the difference that heat-killed *Myco. tuberculosis* were added to the mineral oil (i.e. FCA, Sigma, cat. no. F-4258). The mice of Group III (controls) were treated as the animals of Groups I and II, but the i.p. injections were of 100 μl of PBS. Additional groups of mice were used to study the effect of FIA, FCA and PBS treatments on the number of leucocytes of blood and peritoneal cavities of the animals. Fifteen athymic BALB/c (*nu/nu*) mice were divided in three groups of five mice that were pretreated with PBS, FIA or FCA as described above.

Mycobacterium avium *infection of BALB/c and C3H mice*

One week after the second i. p. injection of adjuvants or PBS, all of the euthymic and athymic BALB/c mice and C3H mice were inoculated intravenously, through a tail vein, with $2{\cdot}6 \times 10^6$ colony forming units (CFU) of *Myco. avium*. The inocula were prepared from frozen-stored *Myco. avium* aliquots that were quickly thawed at 37°C and diluted in saline with 0·04% Tween 80. The 24 euthymic mice of each of the three experimental groups and of both BALB/c and C3H strains were divided into three subpopulations of six animals that were killed at 18 h, 1, 2 and 3 months of infection. The three groups of athymic BALB/c mice were killed 3 months after the *Myco. avium* inoculation. The number of viable *Myco. avium* bacilli was determined in homogenates of liver and spleen by serial dilution and plating onto 7H10 agar media (Difco). Because liver and spleen are the major target organs of mycobacteria inoculated by the i.v. route, indices of liver and spleen enlargement were calculated using the formula: square root of organ weight/body weight × 100.

Leucocyte counts

Blood for cell counts was obtained by puncture of the orbital plexus of the mice. Heparin was added to the blood to prevent coagulation and the cells were counted in a cell counter (Sysmex CC-110) to determine the number of leucocytes. Differential leucocyte counts were performed on Wright stained blood smears. Peritoneal exudates were obtained by washing the peritoneal cavities of the mice with 3 ml of PBS. The number of collected cells was determined with a haemocytometer. The cell suspensions were spun down in a cytocentrifuge onto glass slides. The preparations were fixed with 10% formol in ethanol for 1 min and stained with Wright's stain. Light microscopic observation was done to determine the percentage of each subpopulation of leucocytes.

Statistical analysis

Statistical comparison of the numerical data was done using Student's *t*-test with a two-tailed analysis.

RESULTS

We first compared adjuvant-treated and control mice regarding the number and cell type of leucocytes present in the peripheral blood and in the peritoneal cavity of the animals at the time of the *Myco. avium* infection (i.e. 1 week after the second injection of adjuvants or PBS). This comparison is illustrated in Fig. 1. We found that the adjuvant-induced inflammations caused marked enhancement in the concentration of circulating leucocytes, and also in their numbers in the peritoneal cavities of the animals. The blood leucocytosis was, in both cases, derived mostly from marked increases in the number of granulocytes; this systemic enhancement in inflammatory cells was higher in FCA-treated mice than in FIA-injected animals. At the site where the adjuvant inflammations were induced, i.e. the peritoneal cavity, the leucocytosis was predominantly of the granulocytic type in FIA-injected mice and of the monocytic type in the FCA-treated animals (Fig. 1).

Eighteen hours after the *Myco. avium* i.v. injection, we calculated the relative loads of live mycobacteria in the liver and spleen of the mice from the different experimental groups (Fig. 2a, b). This early timing was chosen for two reasons: (i) to quantify the precise number of live bacilli in liver and spleen at the beginning of the murine infections (in order to compare these values with those obtained after 1, 2 and 3 months of infection); and (ii) to search for inflammation-associated differences in the relative sorting into liver and spleen of the i.v. injected *Myco. avium* bacilli. On the latter issue, we observed that the three groups of C3H mice (FIA- or FCA-treated and controls) showed no statistical differences between their mycobacterial organ loads. In contrast, both groups of adjuvant-treated BALB/c mice showed significantly higher ($P < 0{\cdot}05$)

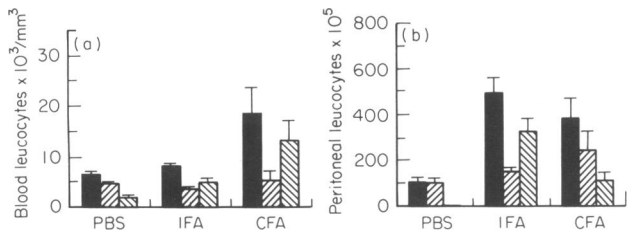

Fig. 1. Effect of intraperitoneal injections of Freund's incomplete adjuvant (FIA), i.e. mineral oil, or of Freund's complete adjuvant (FCA) i.e. FIA plus heat-killed *Mycobacterium tuberculosis*, on the number and cell type of leucocytes of blood (a) and of peritoneal cavity (b) of BALB/c mice. Controls were injected with PBS instead of adjuvants. The animals were killed 1 week after the second adjuvant injection. The FIA and FCA treatments markedly enhanced the concentration of leucocytes in the blood and their number in the peritoneal cavities of the mice. In the blood, these enhancements in leucocytes were due primarily to increase in granulocytes. The values for total leucocytes and granulocytes of FIA- and FCA-treated mice were significantly higher ($P < 0{\cdot}01$) than those of controls (PBS). ■, Total; ▨, mononuclears; ▧, granulocytes.

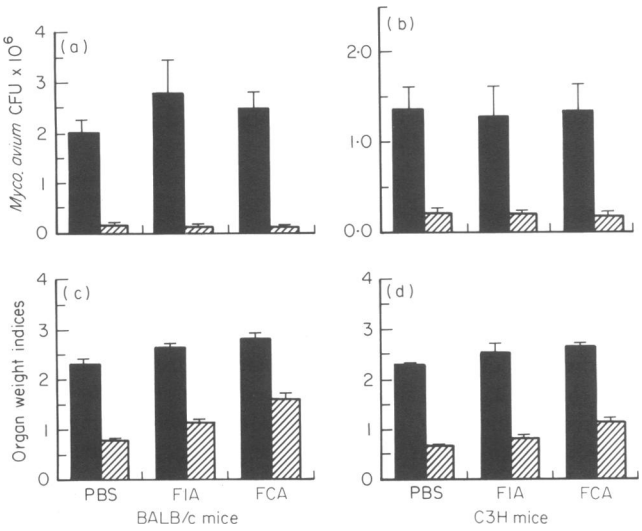

Fig. 2. Effect of Freund's incomplete adjuvant (FIA) and Freund's complete adjuvant (FCA) treatments on the *Mycobacterium avium* sorting into liver and spleen (a) and (c) and organ weight indices (c) and (d) of BALB/c and C3H mice. Controls were injected with PBS instead of adjuvant. The animals were intravenously inoculated with $2{\cdot}6 \times 10^6$ CFU of *Myco. avium* 1 week after the FIA, FCA or PBS treatments. The mice were killed 18 h after the i.v. *Myco. avium* injection. The FIA and FCA pretreatments induced no changes in mycobacterial loads in livers and spleens of C3H mice, whereas the same treatments significantly increased the hepatic *Myco. avium* loads of BALB/c mice. Both adjuvant treatments significantly increased the relative weights of liver and spleen of BALB/c and C3H mice. ■, Liver; ▨, spleen.

Myco. avium loads in the liver (but not in the spleen) than the animals of the control group (Fig. 2 a, b).

We found that both FIA and FCA treatments induced significant enhancement in the relative weight of both liver and spleen (measured as weight indices, i.e. square root of organ/body weight × 100) of BALB/c and C3H mice determined at the time of infection (Fig. 2c, d). Of the two inflammatory

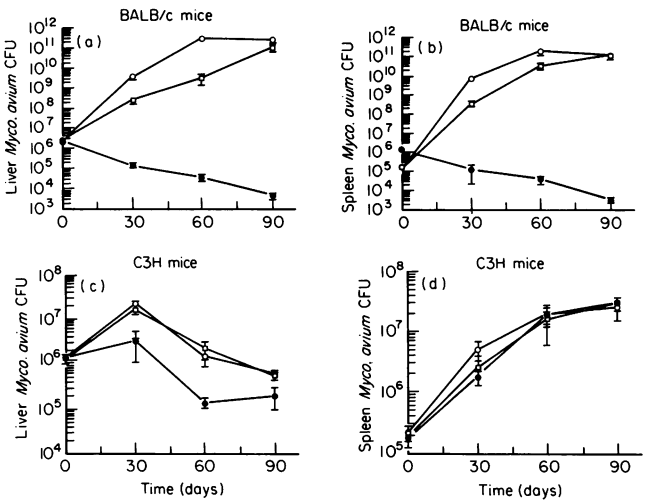

Fig. 3. Effect of Freund's incomplete adjuvant (FIA) and Freund's complete adjuvant (FCA) treatments of BALB/c and C3H mice on the kinetics of *Mycobacterium avium* loads in liver (a) and (c) and spleen (b) and (d) of the infected mice. Controls were injected with PBS instead of adjuvants. The mice were intravenously inoculated with $2{\cdot}6 \times 10^6$ CFU of *Myco. avium* 1 week after the FIA, FCA or PBS treatments; the infections were studied for 3 months. In BALB/c mice, the FIA treatment induced significant reductions in mycobacterial loads that were, however, restricted to the first 2 months of infection, whereas the FCA injections had strong and sustained antimycobacterial effect that was maintained throughout the 3-months-long period of infection. In C3H mice, only the FCA treatment was able to cause significant reduction of the *Myco. avium* loading measured in the liver. ○, PBS; □, FIA; ●, FCA.

treatments, FCA induced a higher increase in organ weight. The enhancement in liver and spleen weight was higher in BALB/c than in C3H mice.

The FIA pretreatment of BALB/c mice caused a statistically significant reduction in the number of viable *Myco. avium* bacilli during the first 2 months of the infection (Fig. 3). At the second month of infection, the FIA-induced reduction in bacillar growth was higher in the liver (about 2 logs) than in the spleen (about 1 log). The antimycobacterial effect of FIA was no longer observed after 3 months of infection. The prodromic FCA injections of BALB/c mice resulted in a higher decrease in the number of viable bacilli detected in liver and spleen of the animals than that triggered by the FIA treatment. In contrast with the FIA treatment, the FCA-induced mycobactericidal action was sustained throughout the 3-months-long period of infection. When compared with controls, the FCA-treated BALB/c mice showed a massive reduction in the number of bacilli found in liver and spleen (i.e. 7 logs after 3 months of infection; see Fig. 3). The anti-*Myco. avium* effect of the FCA immunization was equally strong in liver and spleen of the BALB/c mice.

The differences in *Myco. avium* loading of spleen and liver in the three groups of mice were reflected in the weights of the two organs, and, therefore, in the liver and spleen indices (Fig. 4a, b). Thus, the FCA-treated mice, the group that showed the lowest values of *Myco. avium* loading, also presented the lowest organ indices. It must be stressed that, because of inflammation-induced enlargement of spleen and liver, the organ indices of the

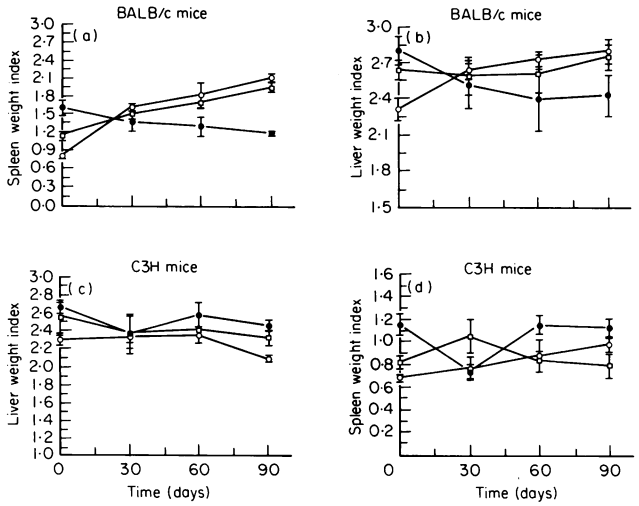

Fig. 4. Effect of Freund's incomplete adjuvant (FIA) and Freund's complete adjuvant (FCA) treatments of *Mycobacterium avium*-infected BALB/c and C3H mice on the kinetics of weight indices of liver and spleen. Controls were injected with PBS instead of adjuvant. The animals were intravenously inoculated with $2{\cdot}6 \times 10^6$ CFU of *Myco. avium* 1 week after the FIA, FCA or PBS treatments and studied for the 3 months thereafter. The FCA injections caused significant reductions ($P < 0{\cdot}05$) in the hepatic and splenic weight indices of BALB/c mice at 2 and 3 months of *Myco. avium* infection. In C3H mice, the variations in organ weight indices were distinct in liver and spleen: the liver values were not very different from each other, whereas in spleens the changes induced by FIA and FCA made a mirror image of each other. ○, PBS; □, FIA; ●, FCA.

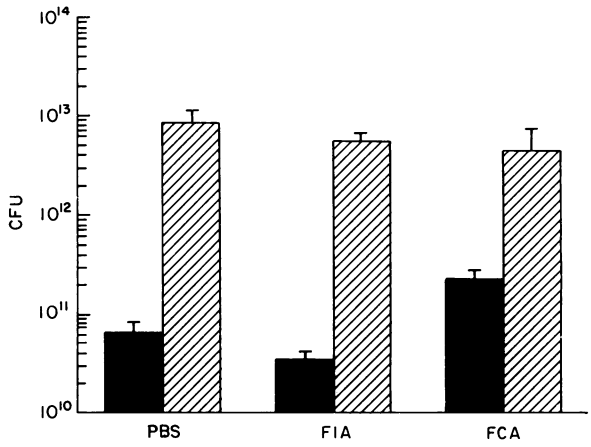

Fig. 5. Comparison of the effect of Freund's incomplete adjuvant (FIA), Freund's complete adjuvant (FCA)and PBS treatments on *Mycobacterium avium* infection of athymic BALB/c mice as represented by mycobacterial loads in liver and spleen of the animals 3 months after being inoculated with $2{\cdot}6 \times 10^6$ CFU of *Myco. avium*. No statistically significant differences were observed among the three groups of BALB/c (*nu/nu*) mice regarding the mycobacterial loads detected in the liver (the major target organ of the infection) of the animals. The FCA pretreatment of the mice induced an increase in the *Myco. avium* loading of the spleen of the mice.■, Spleen; ▨, liver.

three groups of mice were already different when we performed the *Myco. avium* inoculation (compare Figs 2 and 4). Interestingly, while the pre-infection treatment with FCA provoked the highest organ indices observed at the time of the *Myco. avium* inoculation, the same group of mice showed later, that is during infection, the lowest organ indices of the three experimental groups. This suggested that, from 1 month of infection on, the degree of mycobacterial loading was a more important factor for the increase in spleen and liver weight in the three groups of mice than the prodromic inflammatory stimulation that was produced by the FIA or FCA injections.

The action of the pre-infection FIA or FCA injections was also studied in a murine strain (C3H) that is naturally resistant to *Myco. avium* infection. Our goal was to compare the response of C3H mice with that of the *Myco. avium*-susceptible BALB/c mice (reported in the previous paragraphs). In the control group of *Myco. avium*-infected C3H mice we found that the bacilli showed some initial growth in the liver and spleen of the animals, followed, after the first 1–2 months of infection, by a clear bactericidal response in the liver, the major target organ of the i. v. infection (Fig. 3 c, d). In contrast, *Myco. avium* was able to continue to grow in the spleen of C3H mice, an organ that captured only 10% of the total inoculum of the i.v. injected mycobacteria.

We found that the FIA treatment caused no significant changes in the resistance of C3H mice to *Myco. avium* infection. The decrease in mycobacterial loading induced by the FCA injections was also less pronounced in C3H than in BALB/c mice. It should be noted that the reason for this difference will have to take into account that the innate antimycobacterial capacities of the C3H mice will not allow *Myco. avium* bacilli to grow up to the elevated numbers that are observed in the susceptible BALB/c mice; consequently, an equally effective anti-*Myco. avium* action of the FCA treatment may result in higher reduction of mycobacterial loads in BALB/c than in C3H mice.

The FCA injections of C3H mice resulted in a significant decrease in the number of *Myco. avium* bacilli counted in the liver of the animals during the whole 3-month period of infection; this antimycobacterial action of FCA immunization increased from the first to the second month of infection. The anti-*Myco. avium* action of FCA was shorter in the spleen than in the liver of the C3H mice: the only significant reduction in spleen *Myco. avium* loading caused by the FCA treatment was observed in the group of mice that were killed after 1 month of infection (Fig. 3c, d).

Adjuvant-injected C3H mice showed different variations in weight indices of liver and spleen during the *M. avium* infection (Fig. 4c, d). The relative effects of FIA and FCA treatments on the spleen weight indices were quite variable. The values for the two groups of mice were almost the mirror image of each other: FCA-treated mice showed the lowest splenic indices at 1 month of infection, and the highest at 2 and 3 months of infection, and FIA-treated mice presented the highest values at 1 month and the lowest at 2 and 3 months (Fig. 4c, d). Regarding the liver weight indices, the values for the three experimental groups were indistinguishable after 1 month of infection, and from then on (2 and 3 months of infection) the FCA-treated mice presented the highest values.

In order to investigate whether T lymphocytes participated in the expression of the strong anti-*Myco. avium* effect of adjuvant treatments that was found in the *Myco. avium*-susceptible BALB/c mice, we have studied the infection in

mutant athymic BALB/c mice. The antimicrobial consequences of FCA, FIA and PBS treatments in the T cell-deficient BALB/c mice were, thus, compared with the data obtained in euthymic congeneic mice. After 3 months of infection, no significant difference in compound (liver plus spleen) mycobacterial loading was seen in athymic mice between FCA,FIA or PBS-treated animals (Fig. 5). The values of mycobacterial loading of the athymic mice were slightly higher than those of PBS-treated euthymic BALB/c animals.

The major difference between the euthymic and athymic BALB/c mice was in the effect the FCA treatment on the *Myco. avium* infection: the adjuvant treatment failed to induce any significant increase in resistance against *Myco. avium* in the T cell-deficient mice, a finding that was in contrast with the strong antimycobacterial action triggered by FCA immunization in the euthymic mice. Interestingly, the FCA treatment of athymic BALB/c animals induced a significant enhancement in *Myco. avium* loading in the spleen of the T cell-deficient mice (Fig. 5).

DISCUSSION

We report here that non-antigenic inflammation triggered by FIA in *Myco. avium*-susceptible BALB/c mice resulted in a significant enhancement in the resistance of the animals to a subsequent infection by *Myco. avium* bacilli. In contrast, the same FIA-triggered inflammation failed to increase the anti-*Myco. avium* response of C3H mice, a murine strain that is naturally resistant to *Myco. avium*. Addition of heat-killed *M. tuberculosis* to FIA (i.e. FCA) enhanced the effectiveness and duration of the antimycobacterial effect of the FIA treatment in both BALB/c and C3H mice.

Intraperitoneal injection of mice with FIA is known to cause inflammatory phenomena that include local and systemic granulocytosis (as documented here) and stimulation of phagocytes [16–18]; it does not, however, enhance cellular immunity [19]. Thus, our data on FIA-treated BALB/c mice indicate that non-specific stimulation of phagocyte function and number is by itself (i.e. in absence of an enhanced T cell response) an effective mechanism to increase the resistance against *Myco. avium* infection in naturally susceptible hosts. The inflammatory changes produced by FIA i.p. injection were found to be transient [16–19]; this may explain the also transient nature of the anti-*Myco. avium* effect of FIA in BALB/c mice.

A recent study showed that infusion of the adjuvant muramyl dypeptide (MDP, N-acetyl muramyl-L alanyl-D-isoglutamine) in a gel delivery system (hypromellose), performed at the onset of *Myco. avium* i. v. infection of BALB/c mice, led to a significant increase in the resistance of the mice to the mycobacteria [20]. This enhanced resistance was expressed by a 2 log reduction (as compared with controls) in mycobacterial loads after 80 days of infection. MDP had also been reported to enhance the resistance of mice against *Myco. intracellulare* infection [21]. The MDP-hypromellose effect was found to be T cell-independent and associated with increased bacteriostactic activity of macrophages [20]. Interestingly, treatment of mice with MDP alone failed to induce any significant anti-*Myco. avium* change in the mice [20]. In contrast with this finding, we observed that FIA was able to trigger by itself a significant reduction in *Myco. avium* loads in BALB/c mice, an effect that lasted up to the second month of infection. This difference between the antimycobacterial actions of MDP and FIA indicates that, of the two adjuvants, FIA may produce the stronger anti-*Myco. avium* action.

We found that i.p. treatments with FCA resulted in the acquisition of a strong and sustained mycobactericidal activity by the *Myco. avium*-susceptible BALB/c mice; this effect was present throughout the 3-month period of infection. The intensity of the FCA antimycobacterial action was expressed by a 7 log reduction in *Myco. avium* loads observed in the treated BALB/c mice. In contrast with FIA, i.p. injection of mice with FCA is known to induce strong B and T cell responses, i.e. FCA triggers an immune response, whereas FIA causes a non-immune stimulation of phagocytes [16–19]. We also found that the FCA treatment triggered higher systemic granulocytosis than the FIA injections. This is important, because several studies have shown that granulocytes may increase host defence against mycobacteria [7–12].

The two murine inbred strains used in this study were previously reported to be naturally resistant (C3H) or naturally susceptible (BALB/c mice) to *Myco. avium* infection; this innate resistance/susceptibility trait is known to be associated with the expression of the *Bcg* gene [22–26]. We found that the anti-*Myco. avium* action of FCA in BALB/c mice is T cell-dependent, since it was not observed in athymic mice of the same strain. Our data can not, however, define the relative contribution of specific and non-specific resistance to mycobacteria involved in this T cell-dependent anti-*Myco. avium* effect produced by FCA. Further studies will be necessary to address this question, namely the comparison of our data with those obtained with an intracellular bacterium unrelated to mycobacteria.

Our finding that, in comparison with FIA, the FCA treatment caused a marked increase in BALB/c resistance to *Myco. avium* indicates that a previous contact with mycobacterial antigens, even from non-living bacilli, may result in a significant enhancement in the resistance to *Myco. avium* in hosts that are susceptible to this infectious agent. Earlier reports have documented significant augmentations in host resistance against tubercle bacilli upon immunization of animals with oil-saline emulsions containing mycobacterial cell walls (CW) [27–33].

In recent years, Orme [33] has reinvestigated the nature of the increased anti-*Myco. tuberculosis* resistance induced by the CW-containing emulsions in *Bcg*^{r} mice. He found that the treatment did not result in protective immunity, since the antimycobacterial resistance could not be transferred to naive mice through the transfusion of T lymphocytes, the cells that are known to mediate protective immunity against mycobacteriosis [34–36]. He also showed that the protection produced by the CW-containing emulsions was a transient phenomenon lasting no more than a few weeks [33]. Our data can not, however, be readily compared with Orme's findings [33] because of the quite different experimental protocols that the two studies have adopted. In fact, he tested host resistance to other mycobacterial species (*Myco. tuberculosis*) and used different time courses between vaccination and challenge. In addition, Orme also chose a distinct adjuvant: an oil-in-water emulsion that is cleared faster by the animals than the oil adjuvants that we have used here.

In C3H mice killed 18 h after the *Myco. avium* inoculation we found no statistical difference of mycobacterial loads of target organs (liver and spleen) between adjuvant-treated (FIA

and FCA) and control animals. In contrast, in BALB/c mice the *Myco. avium* loads of liver and spleen were higher in the adjuvant-treated mice than in control animals. These higher mycobacterial loads may be related to augmentation of number and/or phagocytic activity of liver and spleen macrophages induced by the prodromic adjuvant inflammations, an interpretation that is derived from previous reports of increased phagocytic activity of macrophages from mice submitted to injections of phlogistic agents [37–39]. The dissimilarity between the phagocytic responses of the two murine strains suggests that their macrophages respond differently to inflammatory stimulation.

In the *Myco. avium*-resistant (Bcg^r) C3H mice, the *Myco. avium* bacilli showed a moderate growth in the spleen of the animals throughout the 3 months of infection, whereas the opposite phenomenon was found in the liver of the same mice. In addition, in the spleen of C3H mice, the FCA injections had a weak anti-*Myco. avium* effect that was restricted to the group of mice killed after 1 month of infection. In contrast, the same FCA treatment caused a marked increase in antimycobacterial activity in the liver of the same animals. These differences suggest that, in C3H mice, macrophages from liver and spleen have different anti-*Myco. avium* capacities.

Because adjuvant treatment of mice caused systemic granulocytosis, it is reasonable to consider that the increase in granulocyte number may be one of the factors that participates in the enhancement of the anti-*Myco. avium* resistance caused by the prodromic adjuvant injections. This interpretation is supported by previous evidence showing that granulocytes can kill mycobacteria *in vitro* [7,8] and that they can also indirectly enhance host resistance against mycobacteria by the transfer of their antimicrobial molecules to macrophages [9].

Our findings are of particular importance because, in contrast with the currently available pharmacological therapy of *M. tuberculosis* infections, there is no reliable drug treatment of pathogenic *Myco. avium* infections. In conclusion, our study shows that non-specific stimulation of inflammatory cells is a useful strategy to increase the resistance to *Myco. avium* in hosts that are susceptible to the infection, and also that the addition of heat-killed mycobacteria to the phlogistic agents may further augment the effectiveness of host response against *Myco. avium*.

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