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Transgenic Mice Enriched in Omega-3 Fatty Acids Are More Susceptible to Pulmonary Tuberculosis: Impaired Resistance to Tuberculosis in *fat-1* Mice

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Abstract

Background—Besides their health benefits, dietary omega-3 fatty acids (n-3 PUFAs) can impair host resistance to intracellular pathogens. Previously, we and others have showed that n-3 PUFA–treated macrophages poorly control *Mycobacterium tuberculosis* infection in vitro.

Methods—Wild-type and *fat-1* transgenic mice were infected with virulent H37Rv *M. tuberculosis* via the aerosol route. We evaluated bacteriological and histopathological changes in lungs, as well as differences in activation and antimycobacterial capacity in primary macrophages ex vivo.

Results—*fat-1* mice were more susceptible to tuberculosis, as demonstrated by higher bacterial loads and less robust inflammatory responses in lungs. Macrophages obtained from *fat-1* mice were more readily infected with *M. tuberculosis* in vitro, compared with wild-type macrophages. This impaired bacterial control in cells from *fat-1* mice correlated with reduced proinflammatory cytokine secretion, impaired oxidative metabolism, and diminished *M. tuberculosis*–lysotracker colocalization within phagosomes.

Conclusions—We showed that endogenous production of n-3 PUFAs in *fat-1* mice increases their susceptibility to tuberculosis, which could be explained in part by diminished activation and antimycobacterial responses in cells from *fat-1* mice. These data suggest that n-3 PUFA–supplemented diets might have a detrimental effect on immunity to *M. tuberculosis* and raise concerns regarding the safety of omega-3 dietary supplementation in humans.

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Tuberculosis continues to be a public health problem worldwide, with more than 1 billion people infected. An effective cellular immune response is required for resistance to tuberculosis and is characterized by the recruitment and activation of T cells and macrophages, which can be modulated by nutritional components. Protein malnutrition [1], hypercholesterolemia [2], and some dietary lipids [3] are risk factors for tuberculosis progression. It has been suggested that dietary omega-3 fatty acids (n-3 PUFAs) impair the immune response against *Mycobacterium tuberculosis* [3–5], but the mechanisms involved have not been fully elucidated.

Dietary n-3 PUFAs have anti-inflammatory properties. These lipids down-regulate immune cell activation and functionality by altering lipid membrane composition, gene expression, and generation of cytokines and other mediators [6]. Such anti-inflammatory effects make n-3 PUFAs useful in prevention and treatment of cardiovascular diseases [7] and cancer [8]. The interest in their beneficial properties arose from epidemiological studies of the Inuit people in Greenland, who have a low incidence of coronary heart disease that is associated with increased n-3 PUFA in-take [9]. However, this population also has an increased incidence of tuberculosis [10]. Indeed, we and others demonstrated that n-3 PUFA–fed guinea pigs infected with *M. tuberculosis* have poor bacterial control and more-severe disease [4, 11, 12]. However, the cellular mechanisms responsible for the detrimental n-3 PUFA effect are unknown. n-3 PUFAs interfere with immune cell activation. Diets enriched in n-3 PUFAs suppressed several functions of T cells [13–15] and macrophages [16]. We showed that incorporation of the n-3 PUFA docosahexaenoic acid reduced the ability of J774A.1 murine macrophages to kill *M. tuberculosis* by impairing cell activation, proinflammatory cytokine production, reactive oxygen intermediate generation, and phagosome maturation (D.L.B., L. Ly, Y.-Y.F., R.S.C., and D.N.M., unpublished data). Anes et al [3] and Jordao et al [5] reported that n-3 PUFAs favor mycobacterial survival by impairing phagosomal actin recruitment. Therefore, we hypothesized that n-3 PUFAs would impair host resistance to pulmonary *M. tuberculosis* infection in vivo.

Because mammals lack the desaturase enzyme needed for the synthesis of n-3 PUFAs, these fatty acids must be obtained from the diet. Most of the n-3 PUFA studies in vivo use dietary supplementation; however, many variables arise from this approach because of differences in diet composition, diet preparation, and food intake. Kang et al [17] developed a *fat-1* transgenic mouse model that endogenously produces n-3 PUFAs. *fat-1* mice express the *fat-1* gene from *Caenorhabditis elegans*, which encodes an n-3 desaturase that catalyzes n-3 PUFA synthesis from n-6 PUFA substrates. *fat-1* mice have been used to elucidate the protective effect of n-3 PUFAs in inflammatory diseases such as colitis [18], melanoma [19], and others [20– 24]. We have chosen *fat-1* mice as a relevant alternative to diet modulation to evaluate the role of n-3 PUFAs during tuberculosis infection.

Here, we demonstrate for the first time, to our knowledge, that the endogenous enrichment of n-3 PUFAs in *fat-1* mice increased their susceptibility to pulmonary tuberculosis infection, in part by impairing macrophage activation and anti-mycobacterial capacity.

MATERIALS AND METHODS

Animals

Heterozygous transgenic *fat-1* mice on a C57BL/6 background were generated as described elsewhere [21]. Nontransgenic littermate wild-type mice were used as controls. Animals were housed 5 per cage in a controlled temperature and humidity environment with a 12-h light-dark cycle and under pathogen-free conditions. Male mice, 8–10 weeks of age, were used in all experiments. Wild-type and *fat-1* mice were fed the same special AIN-76A rodent diet with 10% safflower oil (Research Diets #D03092902) [18, 21]. Mice had access to food and water ad libitum, and all procedures were approved by the Institutional Animal Care and Use committee at Texas A&M University. Genotype and phenotype were confirmed by real-time polymerase chain reaction and gas chromatography, respectively, according to protocols published elsewhere [21]. *M. tuberculosis*–infected animals were restricted to a biosafety level 3 containment facility.

Aerosol infection and necropsy

Virulent *M. tuberculosis* H37Rv (ATCC 27294) was cultured in Middlebrook 7H9 medium (Becton Dickinson), and stocks were prepared and stored at −80° C before use [25]. For infection, stocks were thawed, sonicated, and passed through a 28G needle 15 times [26]. Mice were infected via the pulmonary route in an aerosol chamber (University of Wisconsin Engineering Shops), according the protocol published elsewhere [27, 28]. Confirmation of the initial infection level was determined at 1 day postinfection. Five animals per group per time point were euthanized by intramuscular injection of sodium pentobarbital (100 mg/mL Sleepaway; Fort Dodge Laboratories).

Bacterial load determination

The right lower lung lobe and a portion of the spleen were removed aseptically, homogenized separately, diluted, and plated onto Middlebrook 7H10 agar (Becton Dickinson), as described elsewhere [29]. After 3– 4 weeks of incubation at 37°C, the number of colonies were counted and expressed as mean $\log_{10}$ colony forming units (CFUs) per tissue.

Histological evaluation

Histological sections of formalinfixed lung and spleen were stained with hematoxylin and eosin and examined by a Board-Certified pathologist in a blinded fashion. The percentage of tissue involved in the inflammatory response was determined by morphometric analysis with use of the Image J 11.4 software. The evaluation of lung sections was performed using scores of 0–3 for the following criteria: (1) the degree of inflammation (0, no inflammation, occasional inflammatory cells; 1, mild, increased number of inflammatory cells; 2, moderate, confluent inflammatory cells; 3, severe, extended infiltrate; and (2) granulomatous organization (0, tissues with no lesions; 1, infiltrates exhibiting the most organization [granuloma-like lesions, central area of macrophages surrounded by lymphocytes]; 2, cell aggregates with slight peripheralization of lymphocytes; 3, least

organized granulomatous foci [with scatterings of inflammatory cells loosely grouped]) [18, 21, 29].

Isolation and infection of elicited peritoneal cells

For ex vivo experiments, peritoneal cells were collected according to our protocol published elsewhere [28]. Contaminating erythrocytes were removed with ACK lysing buffer, and cell counts and viability were determined by trypan blue exclusion. Cells were plated and allowed to adhere. After 2 h, nonadherent cells were removed, and adherent cells were incubated in media containing 2% fetal bovine serum (Atlanta Biol). Virulent H37Rv *M. tuberculosis* (ATCC 27294) was used as described elsewhere [25]. Adherent cells were infected at a multiplicity of infection of 10 or 20, according to the type of experiment. Cell monolayers were washed with phosphate-buffered saline (Gibco) and incubated with 50-μg/mL gentamicin (Gibco) to kill extracellular bacteria [30]. Cells were spun and stained with Diff Quik (Dade Behring) for differential leukocyte count.

Fatty acid analysis

Total lipids were extracted by the method of Folch [31]. Total phospholipids were separated using chloroform, methanol, acetic acid, and water as solvents and were transesterified in presence of 6% methanolic HCl. Fatty acids methyl esters were analyzed by capillary gas chromatography as described elsewhere [32, 33]. Peaks of resolved fatty acids were identified by comparing the relative retention times with those of a known standard (GLC 463; Nu Chek Prep). Results are expressed as percentage of total fatty acids and as a ratio of n-6 (20:4n-6 + 22:4n-6 + 22:5n-6) to n-3 (20:5n-3 + 22:5n-3 + 22:6n-3).

Intracellular bacterial trafficking and survival

Cell mono-layers were infected with a green fluorescent protein (GFP)– expressing virulent strain (H37Rv) of *M. tuberculosis*, kindly provided by Dr Scott Franzblau (University of Chicago) [34]. Nuclei were counterstained with 1 mmol/L Hoescht 33342 (Fluka). Extracellular bacteria were labeled using a polyclonal antibody E193.1.11.WCL against whole cell lysate of *M. tuberculosis*, kindly provided by Dr Karen Dobos-Elder (Colorado State University). Serial sections across entire cells were taken to confirm the intracellular localization with use of a DSU1X81 Olympus microscope. The percentage of infected cells was determined by counting at least 100 cells per slide. For the CFU assay, cell lysates were plated on 7H10 Middlebrook agar (Becton Dickinson). The CFU counts were determined after 3–4 weeks of incubation at 37°C in 5% CO_2, as described elsewhere [29].

Cytokine production

Culture supernatants were harvested and assessed for tumor necrosis factor (TNF)-α, interleukin (IL)-6, monocyte chemotactic protein–1 and IL-1β by enzyme-linked immunosorbent assay (EBioscience), according to the manufacturer's protocol.

Phagolysosome maturation

Cells seeded onto #1 thickness glass coverslips were infected with GFP-expressing bacteria (multiplicity of infection, 20) and incubated with 50 nmol/L Lysotracker Red DND99

(Invitrogen) for 2 h before and during the infection. Cells were gentamycin-treated, formaldehydefixed, and mounted on glass slides with Slowfade. Nuclei were counterstained with 1 mmol/L Hoescht 33342 (Fluka). The percentage of lysotracker-positive GFP-fluorescing mycobacterial phagosomes was determined by counting at least 100 phagosomes per slide.

Reactive oxygen and nitrogen species generation

Intra-cellular reactive oxygen levels were measured by staining with 5 mmol/L dihydroethidium (Invitrogen), according to Carter et al [35]. Stained cells were fixed in 2% paraformaldehyde in phosphate-buffered saline and were analyzed using a FACSCalibur flow cytometer (Becton Dickinson). Nitric oxide (NO) levels in culture supernatants were measured using the Griess Reagent (Promega), according to the manufacturer's protocol.

Statistical analysis

Data analysis was performed with GraphPad software (GraphPad) by using the Student *t* test for samples with equal variances, considering *P* values <.05 to be significant. Values are presented as means and error bars represent standard errors (SEM). Significant correlations were determined by calculating the Pearson coefficient.

RESULTS

Nutritional status and weight gain

There were no significant differences in body weight, food intake or water consumption between *fat-1* and wild-type mice, throughout the feeding period or the course of the study. The mean starting weight (± SEM) was 30.9 ± 1.19 g for wild-type mice and 31.4 ± 2.02 g for *fat-1* mice, and the mean final weights were 31.3 ± 0.41 g and 30.3 ± 0.89 g, respectively ($P > .05$).

Mouse fatty acid profiles

Both *fat-1* and wild-type mice were fed the same diet enriched in n-6 PUFAs. Analysis of total lipids from tail snips and peritoneal macrophages showed distinct fatty acid profiles, characterized by n-3 PUFA enrichment only in transgenic mice (Table 1). In tail snips, the mean n-6/ n-3 PUFA ratio (± SEM) was 1.81 ± 0.15 in *fat-1* and 28.24 ± 0.91 in wild-type mice ($P < .001$). The mean concentration of docosahexaenoic acid (± SEM) in *fat-1* mice was 3.29% ± 0.26%, compared with 0.01% ± 0.01% in wild-type mice ($P < .001$). Conversely, there were significantly lower levels of n-6 fatty acids in *fat-1* mice. For example, the mean percentage (± SEM) of 22:4n-6 in cells from *fat-1* mice was 0.32% ± 0.29%, compared with 2.46% ± 0.17% in cells from wild-type mice ($P < .01$). Similarly, in the thioglycollate-elicited macrophages used in ex vivo experiments, the ratio of n-6 to n-3 was significantly lower in *fat-1* cells ($P < .001$).

fat-1 *mice are more susceptible to pulmonary* M. tuberculosis *infection*

To evaluate the effect of n-3 PUFA enrichment on the resistance to tuberculosis, we determined bacterial loads in lungs and spleens from aerosol-infected animals as a measure

of host resistance to the infection. The mean number (± SEM) of implanted bacteria in lung was not significantly different between *fat-1* (1.41 ± 0.02 $\log_{10}$CFU) and wild-type (1.45 ± 0.02 $\log_{10}$CFU) mice at day 1 postinfection ($P > .05$). Figure 1*A* shows that *M. tuberculosis* grew progressively until 4 weeks, after which the infection was controlled and held at a stationary level of ~4.5 $\log_{10}$CFU in lungs and 3 $\log_{10}$CFU in spleens (Figure 1*B*). *M. tuberculosis* was found in spleens only after extrapulmonary dissemination occurred about 2 weeks postinfection. We observed significantly increased mean bacterial counts (± SEM) in tissues from *fat-1* mice (4.53 ± 0.12 $\log_{10}$CFU), compared with those from wild-type animals (4.12 ± 0.12 $\log_{10}$CFU) at 8 weeks after challenge ($P < .005$) (Figure 1*A*). In spleens, the n-3 PUFA enrichment was also associated with greater bacterial loads at 4 ($P < .05$), 8 ($P < .05$), and 12 weeks ($P < .01$) (Figure 1*B*).

fat-1 *mice exhibited reduced pulmonary inflammation*

To evaluate the effect of n-3 PUFA enrichment on the cellular inflammatory response to tuberculosis in lungs, we compared the extent and organization of the inflammatory infiltrate in *fat-1* and wild-type animals in hematoxylin and eosin–stained sections. Inflammatory changes were not observed in uninfected lungs from *fat-1* and wild-type animals (Figure 2*A* and 2*B*). Two weeks following aerosol infection, histopathological changes in the lungs of both wild-type and *fat-1* mice included scattered minimal accumulations of lymphocytes within the pulmonary parenchyma, as well as perivascular recruitment of lymphocytes (Figure 2*C* and 2*D*). At 4 weeks, more focal dense accumulations of lymphocytes and macrophages were distributed randomly within the sections. At this interval, early granulomatous lesions were seen in wild-type mice, in contrast to less pronounced inflammatory responses in *fat-1* mice (Figure 2*E* and 2*F*). By 8 weeks, massive inflammation was found in the lungs of wild-type mice accompanied by perivasculatis, peribroncholitis, and thickening of parenchymal walls, with few regions organized into discrete foci of macrophages surrounded by a zone of lymphocytes (granulomas-like lesions) (Figure 2*G*). Conversely, *fat-1* mice exhibited diffused poorly-formed and less–circumscribed granulomatous accumulations with lack of granuloma-like structures (Figure 2*G* and 2*H*). The granulomatous reactions in lungs of infected wild-type mice appeared to be more cellular and better organized than those from *fat-1* animals (Figure 2*E* and 2*G*). We determined the percentage of lung tissue compromised by the inflammatory response and quantified the degree of inflammation and the organization of the granulomatous response. The analysis of the percentage of involved lung section area and the degree of inflammation confirmed that infected *fat-1* mice had a reduced inflammatory response after *M. tuberculosis* infection (Table 2). On the other hand, when the organization of granulomatous foci was scored, the nature of the lesion differed between *fat-1* and wild-type animals. *fat-1* sections were scored lower because of the diffuse nature of cell aggregate infiltration, compared with mature granuloma-like lymphoid aggregates in wild-type lungs (Table 2). A toxic effect of n-3 PUFAs was ruled out by histopatho-logical analysis of uninfected wild-type and *fat-1* tissues.

fat-1-elicited macrophages had reduced ability to control M. tuberculosis *infection in vitro*

To elucidate the potential mechanisms by which n-3 PUFAs alter the resistance to tuberculosis in *fat-1* mice, additional experiments were conducted using primary cells

infected ex vivo. Thioglycollate-elicited, adherent peritoneal cells were predominantly macrophages (>90%) as revealed by cell morphology and staining properties (data not shown). Macrophages from *fat-1* mice had a reduced ability to eliminate *M. tuberculosis* infection. Figure 3 shows representative fluorescent images of the higher percentage of infection associated with n-3 PUFA enrichment in cells from *fat-1* mice (Figure 3*A*), compared with wild-type mice (Figure 3*B*). At 1 h postinfection, cells from *fat-1* mice exhibited a significantly higher percentage of infection (mean ± SEM, 44.82% ± 2.24%), compared with wild-type macrophages (mean ± SEM, 29.83% ± 2.69%) (Figure 3*C*). In Figure 3*D*, exponential bacterial growth within macrophages was observed between 1 h and 3 days postinfection, after which infection was controlled and held at a stationary level. At 1 h postinfection, cells from *fat-1* mice had a significant 1.10-fold increase in bacterial loads, compared with wild-type cells ($P < .001$), and the n-3 PUFA levels were positively correlated with increased CFU values ($R = 0.54$; $P < .001$). Cell viability was not affected throughout the entire experiment (data not shown).

fat-1 elicited macrophages produced reduced levels of pro-inflammatory cytokines after M. tuberculosis *infection*

n-3 PUFA enrichment significantly reduced TNF-a protein levels (Figure 4*A*). Cells from *fat-1* mice produced only ~15% of the TNF-α protein levels (mean level ± SEM, 264.15 ± 22.16 pg/ mL) observed in macrophages from wild-type animals (mean level ± SEM, 1619.18 ± 298.25 pg/mL) at 6 h postinfection ($P < .05$). Furthermore, levels of IL-6 (Figure 4*B*), IL-1β (Figure 4*C*), and monocyte chemotactic protein–1 (Figure 4*D*) were also down-regulated in cells from *fat-1* mice. We observed a similar suppressive effect in cells from *fat-1* mice stimulated with lipopolysaccharide (data not shown).

Infected fat-1–elicited macrophages showed impaired co-localization of lysotracker with mycobacterial phagosomes and reduced oxidative response

The acidotropic probe lyso-tracker colocalizes with lysosomal markers in *M. tuberculosis*–containing phagosomes [36]. Figure 5*A* shows representative fluorescence images of lysotracker recruitment to mycobacterial phagosomes. At 1 h postinfection (Figure 5*B*), the percentage of lysotracker-positive *M. tuberculosis*–containing phagosomes was reduced in cells from *fat-1* mice (mean percentage ± SEM, 29.83% ± 2.69% vs 44.82% ± 2.24%; $P < .001$), suggesting a defective phagolysosome maturation. The colocalization of other late-endosomal markers was not addressed in this study.

One h postinfection, cells from *fat-1* mice had significantly ($P < .01$) lower levels of reactive oxygen (mean level ± SEM, 20.78% ± 3.12%) than those from wild-type animals (mean level ± SEM, 38.77% ± 4.09%) (Figure 5*C*). A trend toward reduced NO levels was observed at 24 and 48 h postinfection, but the differences were not statistically significant (data not shown).

DISCUSSION

Our data show that *fat-1* mice have an increased susceptibility to pulmonary *M. tuberculosis* infection, as demonstrated by higher bacillary loads (Figure 1) and poorly organized

inflammatory responses in lungs (Figure 2), compared with infected wild-type littermates. This is the first report to address the role of n-3 PUFAs in tuberculosis with use of the *fat-1* genetic model as an alternative to diet manipulation. Our observation that endogenous n-3 PUFA enrichment in mice increases susceptibility to tuberculosis is complementary to our previous study that used dietary intervention in guinea pigs [4]. We showed that n-3 PUFA–fed guinea pigs infected under conditions identical to those used in the current study had defective bacterial clearance in lungs at 3 and 6 weeks postinfection [4]. The fact that the same detrimental n-3 PUFA effect on pulmonary tuberculosis was observed in 2 different animal species (mouse and guinea pig) following fatty acid manipulation by 2 different means (genetic vs diet) increases our confidence on the validity and generalizability of the results. Indeed, it has been reported that n-3 PUFA dietary supplementation can impair the host resistance to other intracellular pathogens [37, 38].

In contrast, Jordao et al [5] recently reported a beneficial effect of n-3 PUFAs in *M. tuberculosis*–infected mice. Such contrasting results could be explained by the different genetic back- ground of the mice (C57BL/6 vs BALB/c) or by procedural differences (eg, route of infection, inoculums, or use of dietary vs genetic approach). The fatty acid levels in *fat-1* mice are comparable to those in animals fed a high n-3 PUFA diet [24], indicating that this model mimics the changes in fatty acid composition observed in dietary studies. Animals appeared to be normal and healthy with no additional phenotypic changes other than the n-3 PUFA enrichment. Differences in n-3 PUFAs that are endogenously produced and those obtained exogenously from diet in terms of acylation of signaling proteins or activation of receptor-mediated signaling pathways are unknown.

Statistically significant increased bacterial loads in *fat-1* mice were observed in both lungs and spleens; however, it remains to be determined whether the modest magnitude of the differences found in this study has any biological relevance in humans. Because well-organized tuberculous granulomas help to contain bacterial dissemination, the increase bacterial growth in *fat-1* mice could be attributed to the deficient formation of functional mature granuloma-like lesion. Reduced TNF-α levels in *fat-1* mice could be a hypothetical explanation, because this cytokine plays a pivotal role in granuloma development [40]. Changes in cytokine production in vivo in infected lungs remain to be determined.

This transgenic model gave us the opportunity to address one of the potential mechanisms by which n-3 PUFAs could alter the resistance to tuberculosis. Macrophages from *fat-1* mice infected ex vivo had defective cell activation characterized by reduced levels of proinflammatory cytokines (Figure 4). Reduced levels of TNF-α, IL-1β, and IL-6 have been reported before in *fat-1* mice [39, 41] and dietary studies [42] [5, 43]. Thioglycollate-elicited macrophages represent a population of circulating monocytes recruited and activated in response to a stimulus, which mimics the arrival of inflammatory cells to the site of infection. However, it remains to be established whether more relevant populations, such as alveolar or lung macrophages, resemble these findings.

We and other groups [3, 5, 11, 12] have shown that n-3 PUFAs favors bacterial survival in *M. tuberculosis*–infected macrophages in vitro, in agreement with our ex vivo bacterial load observations. The difference in bacterial loads ex vivo seems to be explained by differences

in the starting bacterial burden at 1 h. Our data are consistent with previous reports in which dietary n-3 PUFAs reduced phagosomal maturation [3] (D.L.B., L. Ly, Y.-Y.F., R.S.C., and D.N.M., unpublished data); however, because only one time point was evaluated, transient or delayed recruitment of lysotracker in cells from *fat-1* mice cannot be ruled out. The finding of a reduced oxidative burst in response the *M. tuberculosis* infection in cells from *fat-1* mice (Figure 5) is supported by previous studies in which fish oil decreased not only the oxidative burst in Kupffer cells infected with *Salmonella* [44] but also the nitric oxide response in *fat-1* mice [20].

Defects in activation, recruitment, and functionality of other cellular components of the immune response could account for the reduced resistance to tuberculosis observed in *fat-1* mice. n-3 PUFAs reduced T cell activation, proliferation and Th1 cytokine generation [13, 14], and antigen presentation in dendritic cells [45]. Therefore, changes in other cellular functions, including autophagy, chemokine production, and eicosanoid generation could also contribute to the observed phenotype [19, 21, 46]. It is possible that the differences observed might be explained by loss of n-6 PUFAs and their known proinflammatory effects (Table 1).

Current interest in n-3 PUFAs focuses on their protective properties against a wide range of inflammatory and autoimmune diseases. However, we observed that genetically enhanced levels of n-3 PUFAs increase the susceptibility to tuberculosis in *fat-1* mice, in conjunction with deficiency in several inflammatory macrophage functions. Additional experiments are necessary to gain better understanding of how n-3 PUFAs increase susceptibility to infection and to assess the risk of n-3 consumption in humans, especially at-risk populations in areas where tuberculosis is endemic. The long-term goal would be to provide information on which to base public dietary advisories, to prevent any detrimental effects of dietary n-3 PUFAs.

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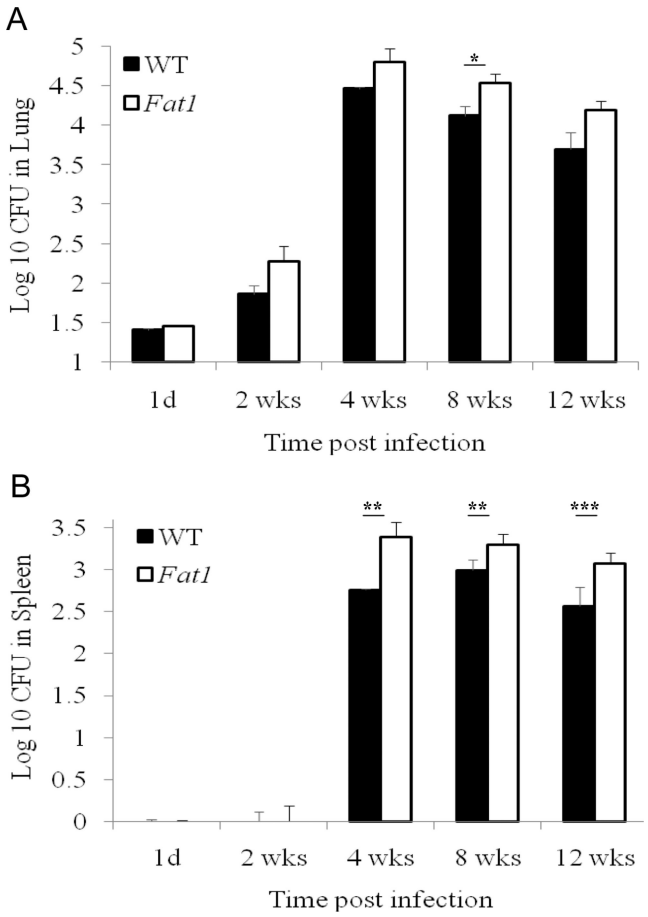

Figure 1.

Increased bacterial loads in *fat-1* mice. Animals were infected with *Mycobacterium tuberculosis* via aerosol infection. At 1 day and at 2, 4, 8, and 12 weeks, mice were euthanized, and the numbers of viable bacteria in lung and spleen were determined. Bacterial counts were estimated by the colony forming unit (CFU) assay, as described in the Materials and Methods. Data show bacterial survival as $\log_{10}$ CFU (mean ± standard error of the mean) in *fat-1* (*dashed line*) versus wild-type (WT) mice (*solid line*). **P* < .005; ***P* < .05; ****P* < .01.

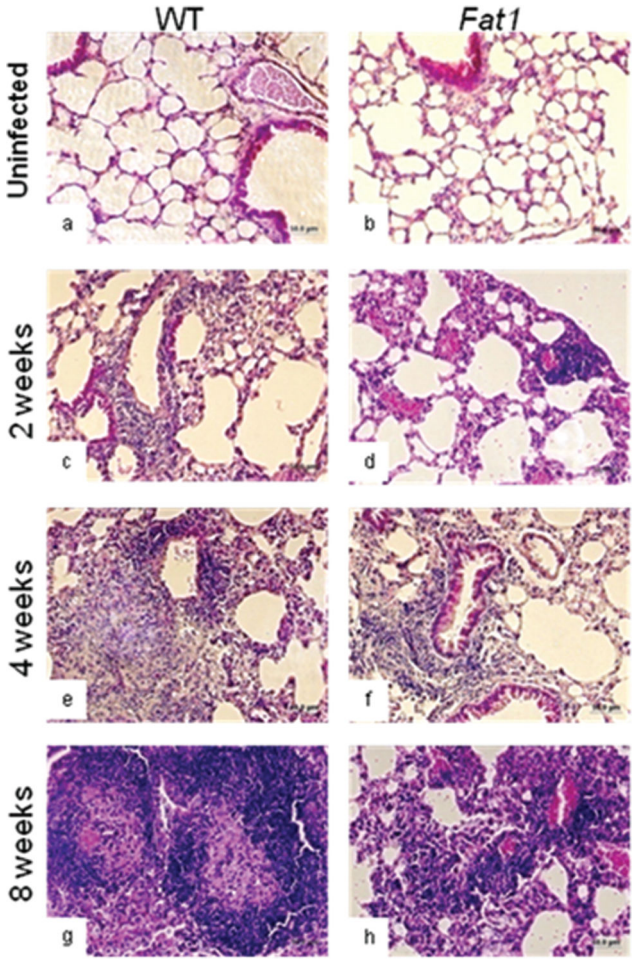

Figure 2.

Representative hematoxylin and eosin–stained lung sections from *fat-1* (*panels b*, *d*, *f*, and *h*) and wild-type (WT; panels *a*, *c*, *e*, and *g*) mice after infection with *Mycobacterium tuberculosis*. The total magnification is ×200. Animals were infected with *M. tuberculosis* via aerosol infection. At 1 day and at 2, 4, and 8 weeks mice were euthanized, and histological changes in lung and spleen were evaluated as described in the Materials and Methods.

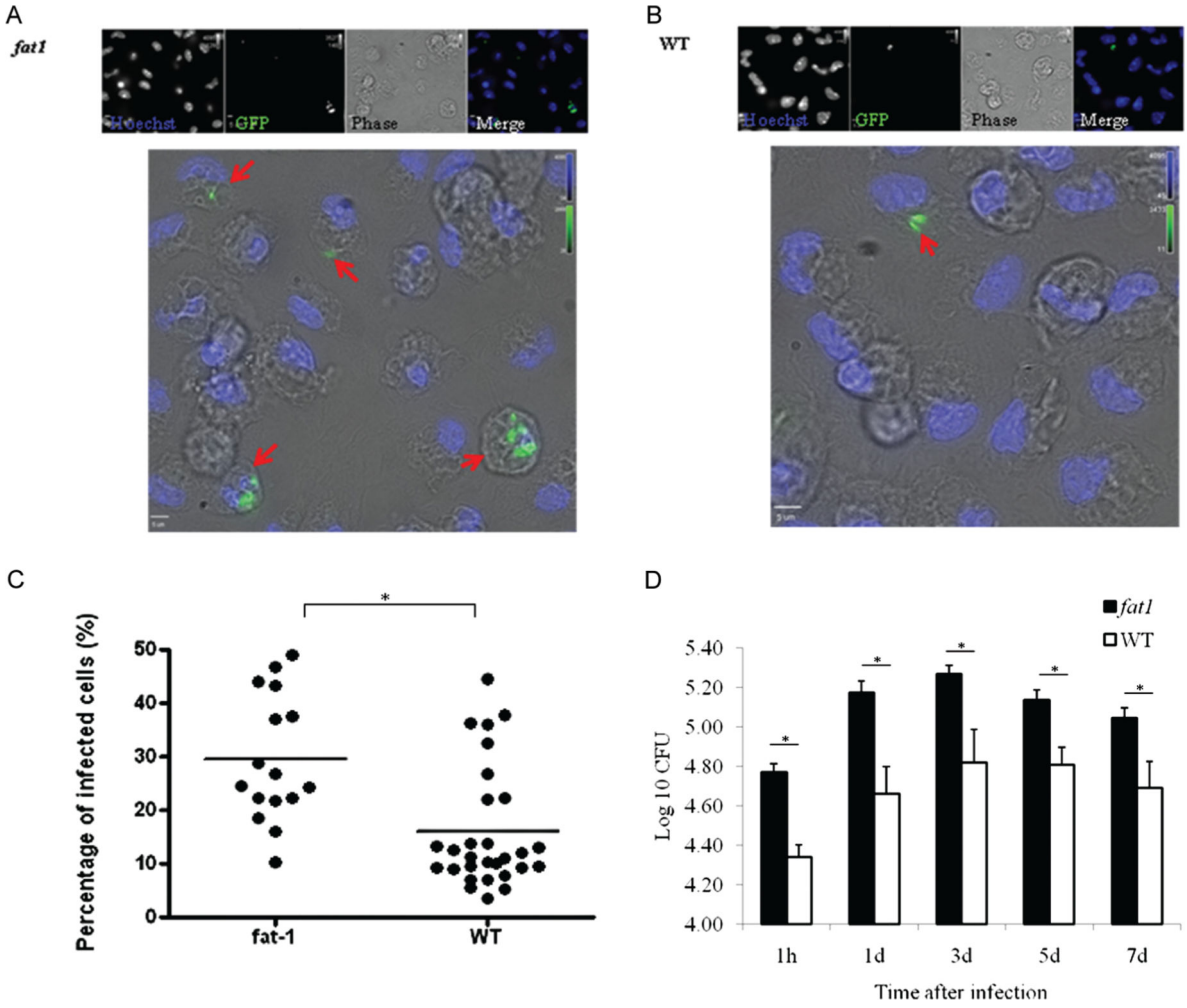

Figure 3.

Increased mycobacterial permisiveness in *fat-1* macrophages. Cells were infected with green fluorescent protein (GFP)-expressing *Mycobacterium tuberculosis* for 1 h, and the relative percentage of *M. tuberculosis*–infected cells was quantified by fluorescent microscopy, as described in the *Materials and Methods*. Representative fluorescent images of the increased bacterial accumulation in *fat-1* cells *(A)* at 1 h postinfection, compared with wild-type (WT) cells (*B*). Arrows show infected cells. Data are representative of 3 independent experiments. *C*, Quantitative data represent the percentage of infected cells. *D*, Bacterial counts were estimated at different time points by the colony forming unit (CFU) assay, as described in the Materials and Methods. Data show bacterial survival as $\log_{10}$ CFU (mean ± standard error of the mean; $n = 10$). *$P < .001$.

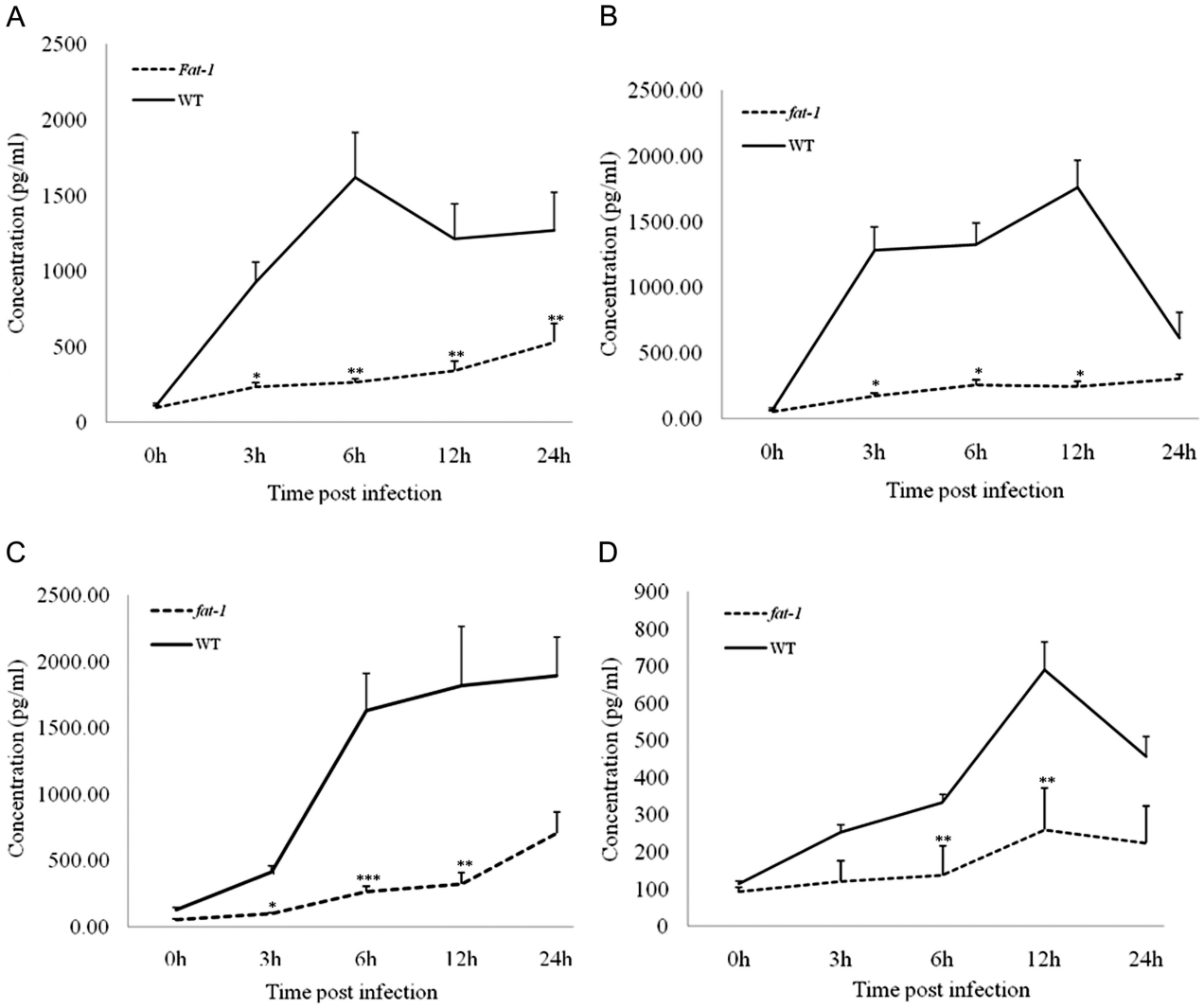

Figure 4.

Reduced cytokine production in infected *fat-1* macrophages. TNF-α (*A*), interleukin-6 (*B*), interleukin-1β (*C*), and monocyte chemotactic protein–1 (*D*) protein concentrations were quantified in culture supernatants at various times following infection with virulent *Mycobacterium tuberculosis* H37Rv by enzyme-linked immunosorbent assay, as described in the Materials and Methods. *P < .001; **P < .05; ***P < .01

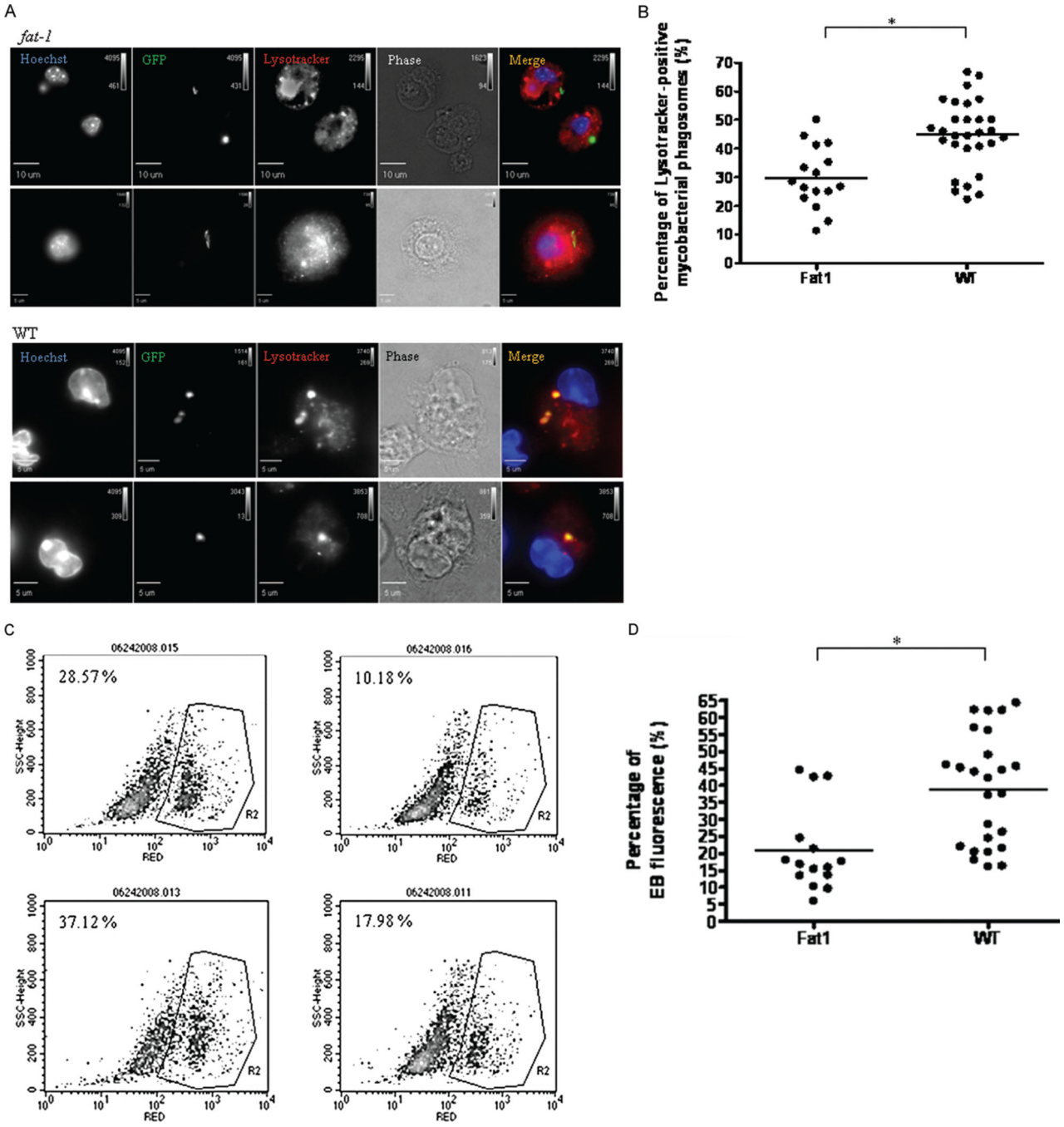

Figure 5.

Acquisition of lysotracker by green fluorescent protein (GFP) *Mycobacterium tuberculosis*–containing phagosomes and reactive oxygen generation was reduced in infected *fat-1* macrophages. Phagosomal maturation was defined based on lysotracker colocalization and visualized by fluorescent microscopy, as described in the Materials and Methods. *A*, Representative images of the reduced colocalization in *fat-1* macrophages, compared with wild-type (WT) macrophages. Data are representative of 3 independent experiments. *B*, Quantitative data show the percentage of lysotracker-positive mycobacterial phagosomes in

fat-1 and WT cells. Reactive oxygen intermediates were estimated by FACS, as described in the *Materials and Methods*. *C*, Representative density plots of reduced respiratory burst in infected *fat-1* macrophages. The analysis was restricted to the cell population in density plots and the region R2 was created for events with high intensity of red dihydroethidium (EB) fluorescence. The percentage of events in that region was determined by CellQuest. The basal level of oxidized EB due to metabolic activity of control uninfected cells was used to set gates. Data are representative of 3 independent experiments. *D*, Quantitative data show the relative percentage of EB red fluorescence. **P* < .001; ***P* < .01.

Table 1

Lipid Profiles in Tail and Peritoneal Macrophages from Wild-Type (WT) and *fat-1* Mice

	Mean mol per 100 mol total fatty acids ± SEM			
	Tail Snip		Peritoneal Macrophages	
Fatty acid	**WT**	***fat-1***	**WT**	***fat-1***
18:2n-6	17.43 ± 0.8	15.87 ± 0.9	7.4 ± 0.4	10.26 ± 0.4
18:3n-3	0.05 ± 0.1	1.70 ± 0.1[a]	1.94 ± 0.1	2.12 ± 0.2
20:4n-6	5.16 ± 0.3	0.57 ± 0.2[a]	17.66 ± 1.2	14.31 ± 1.2
20:5n-3	0.05 ± 0.1	1.93 ± 0.2[a]	0 ± 0	1.68 ± 0.6[a]
22:4n-6	2.46 ± 0.2	0.32 ± 0.3[a]	4.27 ± 0.2	0.99 ± 0.2[a]
22:5n-6	3.32 ± 0.2	0.06 ± 0.1[a]	3.00 ± 0.2	0.58 ± 0.3[a]
22:5n-3	0.05 ± 0.02	3.04 ± 0.21[a]	0 ± 0	1.89 ± 0.47[a]
22:6n-3	0.01 ± 0.01	3.29 ± 0.26[a]	0 ± 0	1.52 ± 0.17[a]
n:6/n-3 ratio	28.24 ± 0.91	1.81 ± 0.15[a]	24.93 ± 1.65	4.25 ± 2.05

NOTE. Total lipids were extracted and analyzed by gas chromatography, as described in the *Materials and Methods.* Only selected major fatty acids (>1 mol %) are reported. SEM, standard error of the mean.

[a] $P < .05$.

Table 2

Semiquantitative Analysis of the Extent and Organization of the Inflammatory Response in Hematoxylin and Eosinstained Lung Sections

Variable	WT	*fat1*
Percentage of area compromised[a]		
Time postinfection		
Uninfected	20.98 ± 1.8	22.46 ± 0.8[b]
1 day	29.28 ± 1.1	26.49 ± 2.7[b]
2 weeks	26.19 ± 1.1	22.68 ± 1.2[b]
4 weeks	32.10 ± 1.3	29.05 ± 1.0[b]
8 weeks	44.67 ± 1.2	38.91 ± 1.00[c]
Degree of inflammation[d]		
Time postinfection		
Uninfected	0.4 ± 0.1	0.30 ± 0.1
1 day	1.0 ± 0.2	0.5 ± 0.2[b]
2 weeks	1.4 ± 0.1	0.7 ± 0.1[c]
4 weeks	1.9 ± 0.2	1.4 ± 0.2[b]
8 weeks	2.5 ± 0.1	1.7 ± 0.2[c]
Granulomatous organization[e]		
Time postinfection		
Uninfected	0 ± 0	0± 0
1day	0± 0	0± 0
2 weeks	0 ± 0	0± 0
4 weeks	1.8 ± 0.1	2.5 ± 0.2[b]
8 weeks	1.8 ± 0.1	2.7 ± 0.1[b]

[a] The percentage of tissue involved in the inflammatory response was determined as described in Materials and Methods.

[b] $P < .01$.

[c] $P < .001$.

[d] The degree of inflammation was scored from 0 to 3 (0, no inflammation; 1, mild; 2, confluent inflammatory cells; 3, extended infiltrate).

[e] The granulomatous organization was scored from 0 to 3 (0, no lesions; 1, well-organized infiltrate; 2, reduced organization; 3, the least organized granulomatous foci).