CD43 Is Required for Optimal Growth Inhibition of *Mycobacterium tuberculosis* in Macrophages and in Mice

April K. Randhawa, Hermann J. Ziltener, Jasmeen S. Merzaban and Richard W. Stokes

*J Immunol* 2005; 175:1805-1812; doi: 10.4049/jimmunol.175.3.1805

http://www.jimmunol.org/content/175/3/1805

References  This article cites 56 articles, 38 of which you can access for free at: http://www.jimmunol.org/content/175/3/1805.full#ref-list-1

Subscription  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CD43 Is Required for Optimal Growth Inhibition of Mycobacterium tuberculosis in Macrophages and in Mice

April K. Randhawa,*† Hermann J. Ziltener,†‡ Jasmeen S. Merzaban,*§ and Richard W. Stokes‡*†¶

We explored the role of macrophage (Mφ) CD43, a transmembrane glycoprotein, in the pathogenesis of Mycobacterium tuberculosis. Using gene-deleted mice (CD43−/−), we assessed the association of the bacterium with distinct populations of Mφ and found that CD43−/− Mφ bound less M. tuberculosis than CD43+/+ Mφ. Increased infective doses did not abrogate this difference. However, reduced association due to the absence of CD43 could be overcome by serum components. Mφ from heterozygote mice, which express 50% of wild-type CD43, bound more bacteria than CD43−/− but less than CD43+/+, proving that the gene dose of CD43 correlates with binding of M. tuberculosis. Furthermore, the reduced ability of CD43−/− Mφ to bind bacteria was restricted to mycobacterial cells. We also found that the survival and replication of M. tuberculosis within Mφ was enhanced significantly in the absence of CD43, making this the first demonstration that the mechanism of mycobacterial entry influences its subsequent growth. Most importantly, we show here that the absence of CD43 in mice aerogenically infected with M. tuberculosis results in an increased bacterial load during both the acute and chronic stages of infection and more rapid development of granulomas, with greater lung involvement and distinctive cellularity. The Journal of Immunology, 2005, 175: 1805–1812.

Mycobacterium tuberculosis infects >8 million people and causes ~2 million deaths annually, making it the deadliest human pathogen. The World Health Organization estimates that between 2002 and 2020, 1 billion people will become infected with M. tuberculosis and >36 million people will die of tuberculosis if the rise in incidence is not controlled.

A critical step in the pathogenesis of M. tuberculosis is the initial interaction of the pathogen with the host macrophage (Mφ). This interaction is mediated by several Mφ receptors in association with ligands on the bacterium, including the complement receptors CR1, CR3, and CR4, (1–6), FcγRs (7), mannose/glucan receptors (1, 8), scavenger receptors (4), and surfactant protein receptors A (11, 12) and D (13). It has also been shown that CD43 (leukosialin; sialophorin) may be important in promoting a stable interaction of mycobacteria with Mφ (14).

CD43 is a negatively charged transmembrane sialoglycoprotein expressed on most hemopoietic cells (15). The function of this molecule has been the subject of debate; it has been shown that CD43 on T and B cells acts as a barrier molecule restricting cell-cell contact (16–19) but that it can also have a proadhesive quality (20–22). Thus, it has been proposed that CD43 may play a dual role in intercellular contact (23, 24). Involvement of CD43 in leukocyte homing and tissue infiltration, possibly due to its adhesive or anti-adhesive properties, has been shown in several studies (19, 25, 26). It has also been demonstrated that CD43 can regulate cell survival (27, 28) and is involved in the apoptosis of T cells and hemopoietic progenitor cells (29–32). Fratuzzi et al. (14) first described a role for CD43 in mycobacterial pathogenesis when they found that splenic Mφ (SpMφ) from CD43−/− mice could not bind M. tuberculosis or Mycobacterium avium in vitro but that the ability to bind M. avium could be restored by addition of the extracellular region of CD43. They also found that CD43-transfected HeLa cells bound M. avium but not other bacteria and that CD43 was required for TNF-α production by Mφ in response to infection with M. avium (14).

In this study, we further explore the role of CD43 in the binding and uptake of M. tuberculosis by Mφ to determine the role of CD43 in M. tuberculosis pathogenesis using a gene-deleted mouse model that lacks expression of CD43 (33).

Materials and Methods

Bacteria

M. tuberculosis (strain Erdman, TMC no. 107; ATCC no. 35801), M. tuberculosis (strain H37Rv, TMC no. 102, ATCC no. 27294), and M. avium (TMC no. 724, ATCC no. 25291) were grown to late log phase in Proskauer and Beck medium supplemented with 0.05% Tween 80. Cultures were stored and tested for viability as described previously (2). Salmonella enterica serovar Typhimurium (S. typhimurium) and Listeria monocytogenes were grown to mid-log phase in tryptic soy broth (Difco) and washed in PBS before use.

Mice

Wild-type (WT) control mice (CD43+/+), CD43−/−, and CD43+/− mice backcrossed seven generations on C57BL/6 background (33) were housed in a specific-pathogen-free animal facility in micro isolator cages. Experiments were done in accordance with the standards set by the Canadian Council on Animal Care. For all experiments, mice were age- and sex-matched and controls were littermates.
Macrophage monolayers

Resident alveolar, peritoneal, and bone marrow-derived Mφ (AMφ, PMφ, and BMMφ, respectively) were obtained from CD43+/− and WT mice as described previously (2, 34, 35). PMφ were obtained by gently disrupting spleens into single-cell suspensions, washing in PBS, and resuspending cells in supplemented RPMI 1640 (RPMI 1640 medium with 10% FCS, 10 mM L-glutamine, and 10 mM sodium pyruvate; all from Invitrogen Life Technologies) at a concentration of 2 × 10⁶ cells/ml. Cells were plated in 1-ml aliquots onto 13-mm acid-washed, sterile glass coverslips in 24-well plates and incubated at 37°C/5% CO₂ for 24 h, at which point the nonadherent cells were removed, and 1 ml of fresh medium was added to each well. The cells were incubated for an additional 4 days before use.

Particles for probing macrophage receptors

The function of FcγRs was examined using SRBC coated with IgG (ElgG), complement receptors were investigated using SRBC coated with IgM and iC3b (ElgMC), whereas lectin-like receptors were probed using zymosan particles, all as described previously (2). Latex beads (diameter 1.07 μm, Polybead polystyrene microspheres; Polysciences) were used to investigate nonspecific phagocytosis.

Flow cytometry

AMφ, PMφ, BMMφ, and SpMφ from CD43+/− and control mice were isolated as described above and plated onto sterile petri dishes (bacteriologic plastic was used to facilitate subsequent removal of adherent cells). Following incubation at 37°C/5% CO₂, nonadherent cells were removed by washing with RPMI 1640 medium. Adherent cells were removed by cooling and scraping, washed with DMEM (Invitrogen Life Technologies), and processed for flow cytometry. Cells were stained with mAb S11-FITC (rat-anti-mouse CD43/kly5, kindly supplied by Dr. J. Kemp, University of Iowa, Iowa City, Iowa) (36, 37) and/or rat anti-mouse Mφ F4/80 (Caltag Laboratories) at 2 mg/ml or with secondary Ab alone (streptavidin-Cychrome). After staining, cells were washed twice with HBSS (Invitrogen Life Technologies) and analyzed on a FACScan IV flow cytometer (BD Biosciences).

In vitro assay for binding of particles to macrophages

Mφ monolayers on coverslips in 24-well plates were twice washed with binding media (138 mM NaCl, 8.1 mM Na₂HPO₄·12H₂O, 1.5 mM KH₂PO₄, 2.7 mM KCl, 0.6 mM CaCl₂, 1 mM MgCl₂, and 5.5 mM d-glucose) (38). A 500-μl aliquot of binding medium was added to each well, and the cells were acclimatized for 10 min at 37°C/5% CO₂. For nonopsonic studies, the particles to be tested were diluted to the desired concentration in binding medium and added to monolayers. In studies of opsonic binding, 1% normal or 1% heat-inactivated CD43+/− or WT mouse serum was added before addition of bacteria. For experiments with mycobacteria or particles, monolayers were infected for 1 h of rocking (Nutator; BD Biosciences) followed by 2 h stationary at 37°C/5% CO₂, whereas in experiments with S. typhimurium and L. monocytogenes, monolayers were infected for 40 min at 4°C. Monolayers were then washed three times, fixed, and stained with Kinyoun’s Carbol Fuchsin and malachite green for mycobacteria-infected Mφ or Giemsa for other bacteria and control particles. Binding was quantitated microscopically by counting 100 Mφ/coverslip and assessing the percentage of Mφ that bound at least one bacterium and the average number of bacteria that were associated with each infected Mφ.

In vitro survival and replication of M. tuberculosis following phagocytosis by CD43+/− and CD43+/+ Mφ

Intracellular growth assays were conducted in BMMφ from CD43+/− and WT CD43+/+ mice. Monolayers were infected with M. tuberculosis Erdman at a multiplicity of infection (MOI) of 20:1 (bacteria:Mφ). Because preliminary studies showed that fewer bacteria were able to infect CD43+/− Mφ, these were also infected at 30:1. Monolayers were infected according to the procedures described above. To eliminate extracellular bacteria from the infected Mφ monolayer, coverslips were washed three times in binding medium after infection and transferred to new 24-well plates containing 1 ml of supplemented RPMI 1640 medium/well. At this time (day 0) and on days 1, 4, and 7 postinfection, coverslips and supernatants were processed to assess the CFU, as described previously (39).

Growth and pathogenesis of M. tuberculosis in mice

CD43+/− and WT mice were infected with a low dose of M. tuberculosis Erdman (50–100 CFU) using an inhalation exposure chamber (GlaxoCol). On specific days postinfection, four to six mice in each group were euthanized, and lungs, livers, and spleens were aseptically removed and weighed. A portion of each tissue was removed and fixed in 10% buffered formalin for histopathological examination, while the remainder (lungs and spleens only) was homogenized in PBS to assess bacterial loads. Serial dilutions of tissue homogenates were plated on 7H10 agar supplemented with Oleic Acid Dextrose Complex. Plates were incubated at 37°C and CFU counted after 21–25 days. Another group of mice was infected i.v. with M. tuberculosis by injecting 1 × 10⁶ bacteria into the tail vein. Bacterial loads were determined as described above.

Histopathology

Livers, spleens, and lungs from mice infected with M. tuberculosis (as described above) were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with H&E for histopathological examination using standard techniques. The population of cells in areas of pulmonary inflammation was quantitated by counting cells as either 1) typical Mφ (including epithelioid cells), 2) “foamy” Mφ—cells having highly vacuolated cytoplasm, which gave it a foamy appearance, or 3) lymphocytes. The proportion of each cell type in representative areas of inflammation was determined by counting 100 cells, and the results expressed to the nearest 5% to allow for a degree of variation between areas.

Statistics

Data are expressed as mean ± SEM. Student’s t-test for independent means was used; a value of p < 0.05 was considered significant unless otherwise noted.

Results

CD43+/− Mφ of different origins bind M. tuberculosis less readily than CD43+/+ Mφ at various multiplicities of infection

We first determined whether CD43 affected the nonopsonic binding of M. tuberculosis by using SpMφ, PMφ, BMMφ, or AMφ from CD43+/− and WT CD43+/+ mice. SpMφ, PMφ, and BMMφ were infected at an MOI of 20:1, whereas AMφ were infected at a higher MOI of 500:1 as it has been shown that these cells do not bind M. tuberculosis very well in vitro (35). After a 3-h infection, we found that SpMφ and PMφ bound bacteria more efficiently than did BMMφ and AMφ, as assessed by both the percentage of the Mφ population infected (data not shown) and the number of bacteria binding to individual infected Mφ (Fig. 1A). All CD43+/− Mφ phenotypes bound significantly less bacteria than WT CD43+/+ Mφ (p < 0.01); WT SpMφ, PMφ, BMMφ, and AMφ bound, respectively, 117, 31, 162, and 55% more bacilli than did CD43+/−-derived Mφ (Fig. 1A). We chose to use BMMφ as a model in subsequent studies because they can be obtained in large numbers and represent a recently differentiated Mφ, such as those that may be found entering sites of infection during the course of pathogenesis of M. tuberculosis.

Infection of BMMφ with increasing numbers of M. tuberculosis demonstrated that reduced association of the bacteria with CD43+/− Mφ was consistent over a wide range of MOI. At ratios of 20:1, 40:1, 60:1, 100:1, and 200:1 (Fig. 1B) bacteria:Mφ, CD43+/− Mφ associated with significantly less bacteria than did CD43+/+ Mφ (p < 0.05). The reduction in nonopsonic binding of M. tuberculosis by CD43+/− BMMφ is manifested not only as a reduction in the number of bacteria binding to the Mφ population but also a reduction in the percentage of Mφ binding at least one bacillus (data not shown).

Opsonization of bacteria overcomes the impaired ability of CD43+/− Mφ to bind M. tuberculosis

CD43+/+ and CD43+/− BMMφ were infected with M. tuberculosis in the absence or presence of 1% normal or heat-inactivated (56°C for 30 min) mouse serum at a MOI of 40:1 (Fig. 2). For these experiments, only serum analogous to the Mφ source was used. In the unopsonized control, 27.8 ± 1.4% of CD43+/+ Mφ had associated with bacteria while only 9.3 ± 1.3% of CD43+/− Mφ were infected (p < 0.001). In the presence of serum opsonins, a higher percentage of both Mφ populations bound bacteria, and
FIGURE 1. *M. tuberculosis* has a reduced ability to associate with CD43-deficient Mφ. A, SpMφ, PMφ, and BMMφ from CD43+/− and WT mice were infected with *M. tuberculosis* Erdman at 20:1 bacteria:Mφ, whereas AMφ were infected at 500:1. The average number of bacteria per infected Mφ was assessed microscopically for 100 randomly chosen Mφ. B, CD43+/− and WT BMMφ were infected with *M. tuberculosis* Erdman at MOIs of 20:1, 40:1, 60:1, 100:1, and 200:1, and associated bacteria were quantified as above. A and B, The mean ± SEM from two independent experiments, each with three coverslips, is shown. All CD43−/− values are statistically less than WT values (p < 0.05).

there was no significant difference between them (51.0 ± 1.6% of CD43+/− and 47.3 ± 4.8% of CD43−/− Mφ-bound bacteria). However, when monolayers were infected with *M. tuberculosis* in the presence of heat-inactivated serum, the difference in binding of bacteria to the two Mφ populations was restored (32.5 ± 2.5% of CD43+/− Mφ; 20.8 ± 1.9% of CD43−/− Mφ associated with bacteria, p < 0.01). Interestingly, the level of binding observed with CD43−/− Mφ in the presence of heat-inactivated serum was higher than that seen in the absence of any serum.

CD43 is involved in binding other mycobacteria, but its absence does not abrogate binding of *S. typhimurium* or *L. monocytogenes* by BMMφ

To determine whether the reduced ability of CD43−/− Mφ to bind *M. tuberculosis* strain Erdman was unique, we infected BMMφ from CD43−/− and WT mice with the virulent strain, H37Rv, with the opportunistic pathogen *M. avium* and with representative Gram-positive (*L. monocytogenes*) or -negative (*S. typhimurium*) intracellular bacteria (Fig. 3). For *M. tuberculosis* and *M. avium*, BMMφ were infected at MOIs of 40:1 and 20:1, respectively, for 3 h. For nonmycobacteria, monolayers were infected at a MOI of 10 bacteria/Mφ for 40 min at 4°C. Less CD43−/− Mφ were able to bind the mycobacterial species than could CD43+/− Mφ. However, there was no difference between CD43−/− and CD43+/− Mφ in their ability to bind the other bacteria.

CD43 deficiency does not affect Mφ-nonspecific uptake or phagocytosis via FcγRs and complement receptors but does enhance binding via lectin-like receptors

To investigate whether CD43 deficiency affects the function of other Mφ receptors, we measured binding of several different control particles that are commonly used to study receptor-ligand interactions of phagocytes (Fig. 4). ElG were used to investigate the function of FcγRs, and ElGMC′ were used to examine complement receptors. Zymosan, a yeast cell wall preparation containing polysaccharides, was used to probe for lectin-like receptors, while latex beads were used to examine nonspecific interactions. When assessing the actual number of particles associated with Mφ, there was no difference in the ability of the CD43−/− Mφ to phagocytose ElG, ElGMC′, or latex spheres when compared with the WT controls (Fig. 4). However, CD43−/− Mφ bound significantly more zymosan particles than WT Mφ (20.4 ± 0.8 vs 14.13 ± 1.1, p < 0.01, respectively).

The level of CD43 surface expression differs between Mφ phenotypes

CD43 cell surface expression was monitored using mAb S11 (37). The epitope recognized by anti-CD43 mAb S11 is not affected by changes in CD43 glycosylation (40); thus, mAb S11 cell surface binding reflects the level of CD43 protein expression. As expected, all Mφ populations from CD43−/− mice did not express CD43 above background. Although all cell types from WT mice expressed low levels of CD43 compared with T cells (41), levels on BMMφ were higher than those on SpMφ, PMφ, or AMφ (Fig. 5). The mean fluorescence intensity for AMφ was 20% of that seen in

FIGURE 2. Heat-labile serum opsonins overcome the reduced binding of *M. tuberculosis* to CD43−/− Mφ. BMMφ from CD43+/− and CD43−/− mice were infected with *M. tuberculosis* Erdman in the absence (serum-free) or presence of 1% normal (+ serum) or heat-inactivated (+ heat-inactivated [HI]-serum) mouse serum at a MOI of 40:1. The percentage of Mφ binding at least one bacillus is shown. Values represent the mean ± SEM from three independent experiments, each with three coverslips. **, p < 0.001 and *, p < 0.01 when compared with WT control.

FIGURE 3. CD43 is involved in Mφ binding of other Mycobacterial species but not other intracellular bacteria. CD43−/− and WT BMMφ were infected with *Mycobacterium tuberculosis* H37Rv (H37Rv) or *M. avium* (M. av) at MOI of 40:1 and 20:1, respectively, for 3 h or with *S. typhimurium* (S. ty) or *L. monocytogenes* (L. mo) at 10:1 for 40 min. The mean ± SEM percentage of Mφ binding at least one bacillus is shown for three independent experiments, each with three coverslips. *, p < 0.01 when compared with control.

FIGURE 3. CD43 is involved in binding other mycobacteria, but its absence does not abrogate binding of *S. typhimurium* or *L. monocytogenes* by BMMφ

To determine whether the reduced ability of CD43−/− Mφ to bind *M. tuberculosis* strain Erdman was unique, we infected BMMφ from CD43−/− and WT mice with the virulent strain, H37Rv, with the opportunistic pathogen *M. avium* and with representative Gram-positive (*L. monocytogenes*) or -negative (*S. typhimurium*) intracellular bacteria (Fig. 3). For *M. tuberculosis* and *M. avium*, BMMφ were infected at MOIs of 40:1 and 20:1, respectively, for 3 h. For nonmycobacteria, monolayers were infected at a MOI of 10 bacteria/Mφ for 40 min at 4°C. Less CD43−/− Mφ were able to bind the mycobacterial species than could CD43+/− Mφ. However, there was no difference between CD43−/− and CD43+/− Mφ in their ability to bind the other bacteria.

CD43 deficiency does not affect Mφ-nonspecific uptake or phagocytosis via FcγRs and complement receptors but does enhance binding via lectin-like receptors

To investigate whether CD43 deficiency affects the function of other Mφ receptors, we measured binding of several different control particles that are commonly used to study receptor-ligand interactions of phagocytes (Fig. 4). ElG were used to investigate...
BMMφ. In addition, a much lower percentage of the AMφ population expressed CD43.

CD43 gene dose correlates to the ability of BMMφ to bind M. tuberculosis

To ascertain whether the amount of CD43 expressed by Mφ affects their ability to bind M. tuberculosis, we compared the binding of the bacterium to Mφ that were heterozygous (+/−) for the CD43 gene (and express 50% less CD43 than do CD43+/+ (33)) with CD43+/− and WT CD43+/+ Mφ (Fig. 6). We found that the CD43+/− Mφ population associated less with bacteria than did the WT Mφ (19.0 ± 2.5 and 37.3 ± 2.1%, respectively). In contrast, only 11.8 ± 1.6% CD43−/− Mφ had associated bacilli (Fig. 6A). When the actual numbers of bacteria per Mφ were assessed, the CD43 gene dose again correlated with the amount of associated bacteria as WT cells bound 2.07 ± 0.24, whereas CD43+/− bound 1.08 ± 0.24 bacilli/Mφ, and CD43−/− bound 0.56 ± 0.11 bacilli/Mφ (Fig. 6B).

The survival and replication of M. tuberculosis within CD43+/− Mφ is enhanced

BMMφ from WT and CD43−/− mice were infected with M. tuberculosis, and the subsequent growth of the bacteria was measured by determining CFU over 7 days. As shown in Table I, at a MOI of 20:1, CD43+/+ Mφ phagocytosed more bacteria than CD43−/− Mφ on day 0 (p < 0.001), but by day 7, there were comparable amounts in the two populations. However, when CD43−/− Mφ were infected at a MOI of 30:1, the same amount of bacteria was taken up as in CD43+/+ Mφ at 20:1 on day 0, and by day 7 postinfection, there were twice as many bacteria in the CD43−/− population (p < 0.001). Moreover, the doubling times of M. tuberculosis in CD43−/− Mφ were significantly less than in WT Mφ, where it took 27.69 ± 0.26 h for one doubling compared with 24.04 ± 0.18 and 24.12 ± 0.39 h in CD43+/− Mφ infected at 20:1 and 30:1, respectively (p < 0.001).

CD43-deficient mice have a reduced ability to control M. tuberculosis growth during the acute and chronic phases of infection following aerosol inhalation of bacteria

To determine the role of CD43 on the in vivo growth and pathogenesis of M. tuberculosis, we infected CD43−/− and WT mice aerogenically with a low dose of the bacterium (50–100 bacilli). There appeared to be no differences in bacterial load in either the lung or spleen during the first 2 wk of infection (Fig. 7). However, by day 28, there was a significantly higher bacterial load in both the lungs and spleens of mice lacking CD43. After this initial peak, there was a period of host control of growth in the CD43+/− mice, resulting in the bacterial load being reduced to levels similar to those in the WT. Subsequently, bacterial loads remained relatively constant in WT mice, whereas in CD43−/− mice, bacterial loads steadily increased until the termination of the experiment. There were no differences in the survival of mice (data not shown). A comparable experiment following infection over a shorter time period (84 days) gave similar results (data not shown).

To determine whether the greater bacterial load in the spleens of CD43−/− mice during the acute phase of infection (up to day 28) was due to a greater susceptibility of splenic Mφ in CD43−/− mice or to greater seeding of the spleen with bacteria from the lung, we
infected CD43−/− and WT mice with *M. tuberculosis* via the i.v. route. This ensured equal numbers of bacteria were deposited into the spleens and lungs of both mouse strains. No differences were seen in the growth rate of *M. tuberculosis* over 6 wk (Fig. 8).

**Organ pathology is exacerbated in CD43-deficient mice**

Histopathological assessment of organs from *M. tuberculosis*-infected mice revealed that pathology in CD43−/− mice was more severe and developed more rapidly than in WT mice. By day 1 postinfection, lymphoid hyperplasia was evident in the spleens of CD43−/− mice, and by day 56, these mice displayed multifocal granulomatous inflammation affecting ~50% of the lung (Table II). This level of pathology did not appear in the WT mice until day 85. By the final experimental time point, CD43−/− mice had granulomatous inflammation affecting >50% of the lung sections, severe lymphoid hyperplasia in the spleen, and vascular, perivascular, and interstitial infiltrates of lymphocytes and neutrophils in the liver. At various time points, CD43−/− mice also showed an increased number of foamy Mφ in lung sections. Although granulomas in WT mice also contained some foamy Mφ, these were only seen during the chronic stages of infection and decreased in numbers toward the end of the experiment, whereas in CD43−/− mice, foamy Mφ were present from day 56 onward and in greater numbers (Fig. 9). Overall granuloma formation in CD43−/− mice occurred more rapidly and more extensively, affected a greater proportion of the lung, and included more foamy Mφ.

**Discussion**

Although recent studies have shown that the interaction of *M. tuberculosis* with Mφ does not necessarily result in uptake of the bacteria, certain Mφ populations can and do ingest them (2, 35, 38). Understanding how *M. tuberculosis* enters, survives, and establishes an infection in these Mφ populations is crucial to comprehending the pathogenesis of mycobacterial infections.

Recently, Fratazzi et al. (14) described a role for CD43 in mycobacteria-Mφ interactions. Their results suggested that CD43 may play a role in promoting a stable interaction of mycobacteria with receptors on host cells and that this interaction regulated TNF-α production by the Mφ. To advance our understanding of this interaction, we further characterized the role of CD43 in mycobacterial infections by analyzing the association of *M. tuberculosis* with different Mφ phenotypes, by using other bacterial species and control particles, by studying the relationship between CD43 expression and mycobacterial binding, and by monitoring the growth of *M. tuberculosis* in Mφ monolayers and in CD43-knockout mice.

We confirm that CD43 is involved in the ability of Mφ to bind and engulf mycobacteria because Mφ from CD43-knockout mice were less able to phagocytose *M. tuberculosis*. Our studies extend this observation and show that this reduction of *M. tuberculosis* binding depends upon various factors. Firstly, Mφ of distinct origins varied in their ability to bind *M. tuberculosis* and showed different levels of reduction in binding when CD43 was absent.

### Table I. Intracellular survival and replication of *M. tuberculosis* is enhanced in CD43−/− BMMφ

<table>
<thead>
<tr>
<th>Mφ Type (MOI)</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Doubling Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD43−/− (20:1)</td>
<td>4.76 × 10^4 (7.85 × 10^3)</td>
<td>4.01 × 10^4 (9.35 × 10^3)</td>
<td>1.63 × 10^5 (2.85 × 10^5)</td>
<td>3.19 × 10^6 (5.46 × 10^6)</td>
<td>27.69 (0.256)</td>
</tr>
<tr>
<td>CD43−/− (20:1)</td>
<td>2.58 × 10^4 (4.42 × 10^3)</td>
<td>2.31 × 10^4 (7.38 × 10^3)</td>
<td>1.68 × 10^5 (3.85 × 10^5)</td>
<td>3.31 × 10^6 (4.91 × 10^6)</td>
<td>24.04* (0.177)</td>
</tr>
<tr>
<td>CD43−/− (30:1)</td>
<td>4.81 × 10^4 (8.08 × 10^3)</td>
<td>3.96 × 10^4 (9.30 × 10^3)</td>
<td>2.20 × 10^5 (4.25 × 10^5)</td>
<td>6.13 × 10^6 (9.59 × 10^6)</td>
<td>24.12* (0.387)</td>
</tr>
</tbody>
</table>

* CD43−/− and WT BMMφ were incubated with *M. tuberculosis* Erdman at 20:1 bacteria: Mφ, and CD43−/− Mφ were also infected at 30:1. Average CFU/ml and doubling times are shown for day 0 and days 1, 4, and 7 postinfection. Results are expressed as mean ± SEM for three independent experiments, each with three coverslips, plated in duplicate at each time point.

**FIGURE 7.** CD43 is necessary for the control of *M. tuberculosis* growth during both the acute and chronic phase of infection in mice. CD43−/− (○) and WT (●) mice were infected aerogenically with a low dose of *M. tuberculosis*. The bacterial loads in the lung (A) and spleen (B) are shown as the mean CFU/organ × 10^2 ± SEM for four to six mice per experimental group at each time point. *, p < 0.05 and **, p < 0.01 when compared with control.

**FIGURE 8.** CD43-deficient mice infected i.v. with *M. tuberculosis* do not show impaired control of bacterial growth during the acute phase of infection. CD43−/− (○) and WT (●) mice were infected i.v. with *M. tuberculosis*. The bacterial loads in the lung (A) and spleen (B) are shown as the mean CFU/organ × 10^2 ± SEM for five mice per group at each time point. No significant difference was found between the experimental groups.
Although the previous study of CD43-tuberculosis interactions focused on SpMφ (14), we show here that BMMφ, PMφ, and AMφ also had an impaired association with \textit{M. tuberculosis} in the absence of CD43. Although AMφ are the cell that first encounters \textit{M. tuberculosis} in vivo, new mononuclear phagocytes arrive at the site of infection during the course of the disease, where they may differentiate and encounter bacteria. BMMφ are an acceptable model for these elicited Mφ and are commonly used in studies of \textit{M. tuberculosis}. Therefore, we used these cells as a model for our studies.

Within a single population of Mφ (BMMφ), the level of expression of CD43 directly correlated with binding of \textit{M. tuberculosis} (Fig. 6). However, flow cytometry of different Mφ populations showed that expression of CD43 did not directly correlate with binding of \textit{M. tuberculosis}. AMφ expressed the lowest level of CD43 and bound \textit{M. tuberculosis} poorly. However, BMMφ bound \textit{M. tuberculosis} at levels lower than did SpMφ and PMφ yet expressed the highest levels of CD43 (Fig. 5). It is possible that the expression of CD43 on the surface of Mφ may not reflect its functional state, as has been seen with other Mφ receptors (2). Alternatively, CD43 may act in conjunction with other Mφ receptors to mediate uptake of \textit{M. tuberculosis}. Thus, variation in expression of these other receptors would explain differences in binding capacity of the various Mφ populations. This contention is supported by the observation that binding of mycobacteria to CD43 is restored by the addition of the extracellular portion of CD43 (14). Therefore, it appears, in agreement with previous findings, that soluble CD43 present in serum may potentiate mycobacteria-Mφ interactions. It is also very likely that complement is responsible for at least some of the enhanced binding of the bacterium in the presence of serum, as it has been implicated in facilitating uptake of mycobacteria by Mφ (1, 3, 5, 6), and heat-inactivated serum lacks the capacity for complement activation (43). However, other heat-resistant opsonins may contribute to enhanced binding. Even in the presence of heat-inactivated serum, both CD43\textsuperscript{+/+} and WT Mφ showed increased binding of \textit{M. tuberculosis} compared with nonopsonic binding. This suggests that some component of serum that is heat stable can also mediate binding to Mφ. Thus, our studies show that CD43-mediated binding of \textit{M. tuberculosis} depends upon the Mφ phenotype, the number of infecting bacteria, the presence of serum opsonins, and the amount of CD43 expression.

Binding studies with control particles demonstrated that the absence of CD43 does not affect nonspecific phagocytosis by Mφ or the function of complement receptors and FcyRs. Interestingly, CD43\textsuperscript{+/−} Mφ had an increased affinity for zymosan. This could be due to the removal of factors impeding the interaction of zymosan binding, such as the large negative charge of CD43 sialic acid residues or steric hindrance created by the large size of CD43. The effect of CD43 on bacterial binding to Mφ also had a level of specificity, as representative Gram-negative and -positive bacteria did not require the presence of CD43 to bind to Mφ. However, three strains of mycobacteria all required the presence of CD43 for

\begin{table}[h]
\centering
\caption{Granuloma formation in CD43\textsuperscript{−/−} mice is more severe and has altered morphology\textsuperscript{a}}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Time Postinfection & Mouse Type & Granuloma Formation & Area of Lung Affected (%) & Infiltrating Cell Types (%) \\
& & & & Normal Mφ & Foamy Mφ & Lymphocytes \\
\hline
Day 56 & CD43\textsuperscript{+/+} & Single area & \textless 25\% & 50 & 0 & 50 \\
& CD43\textsuperscript{−/−} & Multifocal & \textless 50\% & 20 & 50 & 50 \\
Day 85 & CD43\textsuperscript{+/+} & Single area & \textless 25\% & 25 & 25 & 50 \\
& CD43\textsuperscript{−/−} & Multifocal & \textless 50\% & 0 & 50 & 50 \\
Day 127 & CD43\textsuperscript{+/+} & Multifocal & \textless 25\% & 20 & 30 & 50 \\
& CD43\textsuperscript{−/−} & Multifocal & \textless 50\% & 0 & 25 & 75 \\
Day 168 & CD43\textsuperscript{+/+} & Multifocal & \textless 50\% & 25 & 25 & 50 \\
& CD43\textsuperscript{−/−} & Multifocal & \textgreater 50\% & 0 & 25 & 75 \\
Day 210 & CD43\textsuperscript{+/+} & Multifocal & \textless 50\% & 5 & 5 & 70 \\
& CD43\textsuperscript{−/−} & Multifocal & \textgreater 50\% & 0 & 25 & 75 \\
\hline
\end{tabular}
\textsuperscript{a}At the indicated times postinfection, H&E-stained sections of lung from CD43\textsuperscript{−/−} and WT mice infected with \textit{M. tuberculosis} were evaluated for the number and type of granulomas present (multifocal = numerous granulomas throughout the lung), the amount of the section affected (%), and the dominant cell types present in the granulomatous region (%), assessed as described in \textit{Materials and Methods}. Organ sections are from the same mice for which bacterial loads were assessed in Fig. 7.
\end{table}
optimal binding. This supports the contention that CD43 binds specifically to a mycobacterial moiety.

The intracellular growth of M. tuberculosis was significantly enhanced in CD43\(^{-/-}\) M\(\phi\), even though the bacteria are less readily phagocytosed by the M\(\phi\). This increased growth rate was independent of the number of bacteria initially ingested. The higher rate of growth could be due to the fact that CD43\(^{-/-}\) M\(\phi\) have an impaired ability to initiate TNF-\(\alpha\) production (14), which is known to be involved in controlling intracellular growth of M. tuberculosis (44–47). Other cytokines have also been shown to be involved in stimulating the release of the chemokines RANTES and M\(\phi\) inflammatory protein-1 (48), which could also affect the intracellular growth of M. tuberculosis. Additionally, CD43\(^{-/-}\) M\(\phi\) could be selectively phagocytosing the more virulent bacteria within the inoculum, or uptake of the bacterium in the absence of CD43 may lead to altered phagosome maturation, which is known to be associated with the survival of intracellular mycobacteria (49–53). Moreover, the induction of killing mechanisms by M\(\phi\) may differ in CD43\(^{-/-}\) and CD43\(^{+/+}\) cells. For example, it has been demonstrated that CD43 is involved in apoptotic signaling pathways that would affect the fate of mycobacteria-infected M\(\phi\) (27, 29–31).

CD43 appears to have a significant role in controlling the growth of M. tuberculosis in the murine host. When infected via aerosol, CD43\(^{-/-}\) mice had increased bacterial loads in the lung and spleen during the acute phase of infection up to day 28. This increased growth of M. tuberculosis in CD43\(^{-/-}\) mice may be attributed to the enhanced growth in CD43\(^{-/-}\) M\(\phi\) we demonstrated in vitro. Alternatively, it may be due to differences in the type and/or number of cells recruited to sites of infection. In H&E-stained tissue samples, lymphoid hyperplasia was seen in the spleens of CD43\(^{-/-}\) mice as early as day 1 postinfection, and there were more granulomas in the lungs by day 28 compared with WT. The difference in bacterial growth during the acute phase of infection in CD43\(^{+/+}\) and CD43\(^{-/-}\) mice was more pronounced in the spleens. Because this effect was not seen in infected i.v. with M. tuberculosis, we can conclude that this is not just because of enhanced bacterial growth in CD43\(^{-/-}\) SpM\(\phi\) but is more likely due to increased dissemination from the lungs of CD43\(^{-/-}\) mice. Following the development of the adaptive immune response (around day 28), the mice were able to control the infection for a period of time. However, following this period the CD43\(^{-/-}\) mice also failed to control bacterial growth during the chronic stage of infection. It is possible this difference can also be ascribed to the increased susceptibility of CD43\(^{-/-}\) M\(\phi\). However, the adaptive immune response was able to reduce the bacterial load in CD43\(^{-/-}\) mice between days 28 and 56. This suggested that CD43\(^{-/-}\) M\(\phi\) were capable of being activated to kill intracellular M. tuberculosis just as effectively as CD43\(^{+/+}\) M\(\phi\) during this stage of the infection. During the chronic stage of the infection, an effective immune response must be maintained with the corresponding maintenance of granulomata to contain the bacteria. We have shown that during this late stage of infection, bacterial growth is not controlled in CD43\(^{-/-}\) mice, and histological findings show that normal granuloma formation is impaired in these mice and may account for increased bacterial loads. Other published roles of CD43, including involvement in T cell activation and differentiation, and the recruitment of lymphocytes to sites of infection (19, 25, 26) could also explain the inadequacies in the immune response against M. tuberculosis.

It is critical to note that the ability of CD43\(^{-/-}\) mice to control infection with M. tuberculosis depended upon the route of infection. Although significant differences were seen between CD43\(^{-/-}\) and WT mice infected aerogenically with M. tuberculosis, there was no significant difference when mice were infected via an i.v. injection with the same bacterium. It has been shown previously that M. tuberculosis may have increased virulence when administered aerogenically as opposed to i.v. (54) and that the pathogenesis of the organism is affected by route of delivery (55). This emphasizes the importance of using experimental infection procedures that most closely mimic natural exposure to obtain results that are most physiologically relevant.

In summary, this study establishes that CD43 is involved in the binding, uptake, and subsequent growth of M. tuberculosis in murine M\(\phi\) and in vivo. These results support the theory that CD43 has a dual function in cell-cell interactions (23, 24, 56) and that the nature of particles interacting with CD43 can dictate its function. Additional studies are necessary to determine the biology of M. tuberculosis-CD43 interactions, to identify potential mycobacterial ligands for CD43, and to understand mechanisms of cell recruitment in CD43-knockout mice.

**Acknowledgments**

We are grateful to Lisa Thorson for technical assistance, to Amanda Rooyackers for her assistance with in vitro growth assays, and to Dr. Douglas Carlow for help with the FACS analysis. We also thank Dr. Brett Finlay for providing Salmonella and Listeria strains, and Dr. P. N. Nation at the University of Alberta, Edmonton, Alberta, Canada, for his/hersopathological analysis.

**Disclosures**

The authors have no financial conflict of interest.

**References**


