A Macrophage Invasion Mechanism for Mycobacteria Implicating the Extracellular Domain of CD43

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Abstract

We studied the role of CD43 (leukosialin/sialophorin), the negatively charged sialoglycoprotein of leukocytes, in the binding of mycobacteria to host cells. CD43-transfected HeLa cells bound *Mycobacterium avium*, but not *Salmonella typhimurium* or *Shigella flexneri*. Quantitative bacteriology showed that macrophages (Mf) from wild-type mice (CD43+/+) bound M. avium, *M. tuberculosis* (strain H37Rv), whereas Mf from CD43 knockout mice (CD43-/-) did not. The inability of CD43-/- Mf to bind M. avium could be restored by addition of galactoglycoprotein (Galgp), the extracellular mucin portion of CD43. The effect of Galgp is not due to opsonization of the bacteria, but required its interaction with the Mf; other mucins had no effect. CD43 expression by the Mf was also required for optimal induction of tumor necrosis factor-α (TNF-α) production, which likewise could be reconstituted by Galgp. In contrast, interleukin (IL)-10 production by M. avium-infected Mf was CD43 independent, demonstrating discordant regulation of TNF-α and IL-10. These findings describe a novel role of CD43 in promoting stable interaction of mycobacteria with receptors on the Mf enabling the cells to respond specifically with TNF-α production.

Key words: mycobacteria • CD43 • macrophages • tumor necrosis factor α • interleukin 10

Introduction

Mycobacteria are among the most important infectious agents in the world. *M. tuberculosis* infection, which is spread by airborne transmission, causes more deaths globally than any other infectious agent. Estimates indicate 90 million people infected with *M. tuberculosis* during the current decade, 30 million of whom will die as a result of infection (1). In the United States, *M. tuberculosis* complex has emerged as the most prevalent opportunistic infection among patients with advanced HIV-1 infection. *M. avium* infections in patients with AIDS and other immunodeficiencies typically manifest with widespread visceral organ involvement and high-grade bacteremia; the patients suffer considerable morbidity and shortened life span (2–5).

Macrophages (Mφ),1 the predominant host cells, are the first line defense against spread of mycobacterial infection. In the successful process, Mφ eliminate mycobacterial in-
fection by a complex network of events involving TNF-α production (6) and leading to apoptosis and elimination of the host Mφ containing endocytosed microorganisms (7, 8). Pathogenic mycobacteria have evolved mechanisms to survive and replicate within endosomes of Mφ (9) after entering the cells by one of several Mφ receptor pathways. These include complement receptors CR1, CR3, and CR4, mannose receptor, CD14, Fc receptors, scavenger receptors, and one or more receptors for pulmonary surfactant protein A (SP-A) (10, 11). To some extent, the outcome of mycobacterial infection is influenced by the receptor, in that uptake via Fc receptors, but not complement receptors, induces reactive oxygen metabolites, a process believed to decrease survival of endocytosed mycobacteria (12).

This study aims to understand the role of nonreceptor surface molecules in regulating binding and uptake of mycobacteria by Mφ. CD43, a cell surface mucin on macrophages, was considered as a candidate regulatory molecule because of its known ability to interfere with adhesion processes. For example, CD43-transfected HeLa cells, unlike wild-type HeLa cells, display decreased intracellular adhesion molecule (ICAM)-mediated adhesion (13), and targeted disruption of the CD43 gene in mice increased adhesiveness of splenic T cells (14). Therefore, we used CD43-transfected and wild-type HeLa cells as well as Mφ from CD43 knockout and wild-type mice to determine whether the presence of CD43 diminishes binding of mycobacteria to Mφ and, by doing so, diminishes or abrogates important downstream events such as cytokine production.

Materials and Methods

Animals. CD43 knockout mice were generated by homologous recombination via embryonic stem cell chimeras (14). The CD43−/− mice and control wild-type mice (in C57 × SJL 129 background) were maintained under specific pathogen-free conditions at the Tufts University School of Medicine. For all experiments, 6–8 wk-old mice matched for sex were used. In select experiments, gene status was verified by flow cytometric analysis of cells stained with PE-conjugated S7 rat anti–mouse CD43 mAb (BD Pharmingen).

Microorganisms. Shigella flexneri and Salmonella typhimurium were provided by Dr. J. Cohen, Imperial College of Science, Technology and Medicine (London, UK). BCG (M. bovis strain bacillus Calmette-Guérin) was obtained from the Tufts University School of Medicine. For all experiments, 6–8 wk-old mice matched for sex were used. In select experiments, gene status was verified by flow cytometric analysis of cells stained with PE-conjugated S7 rat anti–mouse CD43 mAb (BD Pharmingen).

Live M. avium, S. flexneri, and S. typhimurium were stained with carboxyfluorescein diacetate (CFDA; Molecular Probes). The bacteria at 104/ml were incubated with 4 μM CFDA in DM EM at 37°C for 30 min. After extensive washing, the CFDA-stained microorganisms were used to challenge confluent cultures of CD43-transfected, wild-type, and vector-transfected HeLa cells by coculture at 1, 5, or 10 microorganisms/cell in 2–4 h. The cells were washed three times in HBSS and analyzed by fluorescence microscopy.

Murine Spleen Mφ. Murine spleens were removed aseptically and teased, and the released cells were washed three times in HBSS and suspended in RPMI 1640 with 10% heat-treated (56°C for 30 min) FCS, 25 mM Hepes, and 2 × 10−5 M 2- M E (Sigma-Aldrich). All media tested negative for LPS (<0.05 ng/ml) in the Limulus amebocyte lysate assay (BioWhittaker). Spleen cells were plated at 2 × 106 cells/well in 24-well plates (Costar) for 4–6 d in 5% CO2 at 37°C. Immediately before infection, nonadherent cells were discarded, and the Mφ populations, which were 97–99% pure by esterase stain, were washed four times in HBSS, and fresh medium was added.

Triplicate wells of adherent Mφ (~1.0 × 106 cells/well) were inoculated with M. avium, BCG, or M. tuberculosis strain H37Rv at 2, 10, or 20 microorganisms/cell and cultured for 1–4 h for mycobacteria binding assays or 4–48 h for cytokine measurements. To quantify bound mycobacteria, the adherent Mφ were washed four times with HBSS, lysed by addition of SDS (500 μM of 0.2%), and the effect of SDS was determined with FCS (500 μM of 50%). Cell lysates (100 μl) were cultured at 37°C in 5-ml vials containing culture broth and [14C]palmitic acid (Amersham). All media tested negative for LPS (<0.05 ng/ml) in the Limulus amebocyte lysate assay (BioWhittaker). Spleen cells were plated at 2 × 106 cells/well in 24-well plates (Costar) for 4–6 d in 5% CO2 at 37°C. Immediately before infection, nonadherent cells were discarded, and the Mφ populations, which were 97–99% pure by esterase stain, were washed four times in HBSS, and fresh medium was added.

Light Microscopy. M onolayers of adherent Mφ were prepared by plating 2 × 106 murine spleen cells on 13-mm-diameter plastic coverslips (Thermanox™, no. 174950; Nunc); these were incubated with M. avium, harvested and washed as described above, and dried. The coverslips were stained with TB carbon-labeled KF and counterstained with TB brilliant green K following the manufacturer’s instructions (Kinyoun acid-fast staining procedure for mycobacteria; Difco). Mφ containing >10 detectable bacteria were scored as positive. Quantitation is based on visual scoring of 3–5 fields with 30–80 cells each.

Fluorescence Microscopy. M onolayers of adherent Mφ on plastic coverslips as described above were incubated with M. avium, harvested, washed, and fixed with 4% paraformaldehyde in PBS.
The mycobacteria in the fixed monolayers were stained with the rhodamine-auramine TB Fluorescent stain kit (no. 4312521; Becton Dickinson) according to the manufacturer's directions. The stained cells were examined by phase-contrast and fluorescent microscopy with a Nikon microscope.

Galactoglycoprotein (Galgp; provided by Dr. Karin Schmidt, Department of Biochemistry, Boston University School of Medicine, and Dr. H. Gerhard Schwick, Behringwerke AG, Marburg/Lahn, Germany), was purified from pooled normal human plasma by anion exchange chromatography of the supernatant of Cohn fraction V followed by gel filtration (17) with the additional step of solid-phase immunosorobption using antiserum raised in a rabbit that had been immunized with Galgp but that produced antibodies only against the trace contaminants (18). Galgp appeared as a single ~120-kD band on SDS gels stained with Alcian blue, a glycoprotein stain (19), and no component was detected when overloaded gels (50 μg samples) were stained with the protein stain Coomassie blue (data not shown).

Antifreeze fish glycoprotein (AFGP) (20) isolated from Northern cod (mol wt of ~3,000) was provided by A/F Protein, Inc. (Waltham, MA). Purified porcine submaxillary gland mucin (PSM) was reduced, carboxymethylated, and treated with trypsin to generate a monomeric mucin species (PSM-R T) (21). The mucins were added to Mφ cultures at the same time as M. avium, unless otherwise noted.

Measurement of TNF-α and IL-10. Murine spleen Mφ were isolated, cultured in 24-well plates, and inoculated with M. avium as described above. At 4–48 h after infection, supernatants were harvested and the concentrations of TNF-α and IL-10 were determined by ELISA using matched antibody pairs and cytokine standards (Endogen).

Phagocytosis. Monolayers of murine spleen Mφ were prepared by plating 2 × 10⁶ murine spleen cells on plastic coverslips as described for microscopy experiments. 4–6 d later, the adherent monolayers were washed and polystyrene (latex) fluorescent microspheres of 1-μm diameter (Fluoresbrite YG Microspheres; Polysciences, Inc.) were added to triplicate wells at a ratio of 200 beads/Mφ and incubated for 30 min. The coverslips were washed five times in HBSS at 37°C and air-dried. The Mφ were examined in a Nikon microscope; cells with at least two phagocytized beads were scored as positive.

Statistical Analysis. The results are expressed as the mean ± SEM. Statistical differences were determined using SigmaStat Statistical Software (Jandel Scientific), using the t test for normally distributed data with equal variances and the Mann-Whitney rank sum test for data populations with nonnormal distributions and/or unequal variances.

Results

CD43 Is Required for Optimal Binding of Mycobacteria to Mφ. As our initial approach, we incubated CD43-transfected HeLa cells and control HeLa cells with M. avium, S. typhimurium, and S. flexneri. After 4 h exposure to the pathogen, we quantified the cells that had bound bacteria. Contrary to expectations, M. avium were found to be stably associated with the CD43-transfected HeLa cells (Fig. 1, left) and were not associated with control HeLa cells, both wild-type (data not shown) and vector-transfected cells (Fig. 1, right). The binding of M. avium to CD43-expressing HeLa cells appeared to be specific, since neither of the other tested bacterial species, S. typhimurium and S. flexneri, bound to CD43-transfected (or control) HeLa cells (Fig. 1).

The surprising finding that M. avium bind to CD43+ HeLa cells prompted us to examine the role of CD43 in

![Figure 1](image1.png)

**Figure 1.** Specific association of M. avium with CD43-transfected HeLa cells. HeLa cells transfected with CD43 (HeLa-CD43) and vector-transfected cells (HeLa) were incubated as indicated with stained live M. avium, S. typhimurium, and S. flexneri at 10 organisms/cell for 4 h. Cells were washed, and the infected cells were counted using fluorescence microscopy (see Materials and Methods). Shown is a representative experiment. The number of CD43-transfected HeLa cells that scored positive for M. avium binding was significantly different than the number of vector-transfected cells (n = 3, P < 0.0001).

![Figure 2](image2.png)

**Figure 2.** Binding of M. avium and M. tuberculosis to murine CD43+/+ and CD43−/− Mφ examined by quantitative bacteriology. (A) M. avium serovar 4 were incubated with adherent splenic Mφ (≈1 × 10⁶) at a bacterial cell ratio of 2:1 for the indicated time; the Mφ were extensively washed, and the adherent bacteria were quantified. (B) M. tuberculosis strain H37Rv were similarly analyzed. Shown are mean values ± SEM for three mice of each group. The number of M. avium and M. tuberculosis associated with CD43+/+ and CD43−/− Mφ are significantly different at all time points (P < 0.0001). Although the absolute numbers of bound mycobacteria varied, similar differences between CD43+/+ and CD43−/− Mφ were observed in two additional independent experiments.
binding of mycobacteria by Mφ, their normal host cell. Monolayers of splenic Mφ were prepared from wild-type (CD43+/+) and CD43 gene-deleted (CD43−/−) mice. When these were challenged with M. avium, quantitative bacteriology showed that the CD43+/+ Mφ bound significant numbers of M. avium (Fig. 2 A). In contrast, CD43−/− Mφ failed to bind M. avium or bound minimal numbers of the bacteria. The binding of M. avium to CD43+/+ Mφ was dependent on bacterial dose (shown below) and time of coincubation (Fig. 2 A). An exposure time of 4 h was chosen for further experiments because, at this time, binding of the M. avium to the Mφ was substantial and bacterial growth was not yet observed.

To examine whether other mycobacterial species are also dependent on CD43 for uptake, splenic mouse Mφ were challenged with M. tuberculosis H37Rv, a virulent strain. Whereas substantial binding was seen for CD43+/+ Mφ, CD43−/− Mφ failed to bind M. tuberculosis (Fig. 2 B). Similar results, i.e., binding to CD43+/+ Mφ and minimal or absent binding to CD43−/− Mφ, were obtained also for BCG (data not shown). Collectively, these findings strongly indicate a positive role for CD43 in mycobacterial binding.

In an independent approach, Mφ incubated with M. avium were evaluated by light microscopy after acid-fast staining of the mycobacteria. On infection with M. avium at a 20:1 ratio, 57 ± 6% of CD43+/+ Mφ had >10 bacteria detectable by staining compared with 8 ± 3% of CD43−/− Mφ (n = 4).

In other experiments, Mφ incubated with M. avium were examined by fluorescence microscopy after staining of the mycobacteria with rhodamine-auramine. When incubated at a 20:1 ratio, fluorescent micrographs showed multiple mycobacteria associated with all or most of the CD43+/+ Mφ (Fig. 3 A, left) and negligible association of mycobacteria with CD43−/− Mφ (right). Higher magnification micrographs showed that the mycobacteria associated with CD43+/+ Mφ are localized predominantly...
within the cells (Fig. 3 B, left). In contrast, in the rare cases where mycobacteria were found associated with CD43−/− Mφ, the microorganisms were localized to the periphery of the cells (Fig. 3 B, right).

To test whether the diminished association of mycobacteria with CD43−/− Mφ is due to a global defect in phagocytosis, monolayers of wild-type and CD43−/− Mφ were incubated with nonopsonized inert latex fluorospheres (200 spheres/cell) for 30 min (22). Uptake of the latex beads examined by fluorescence microscopy showed that 76 ± 5% of CD43+/+ and 73 ± 5% of CD43−/− Mφ contained fluorescent beads. The finding that CD43−/− Mφ do not take up less beads than CD43+/+ Mφ (n = 3, P < 0.6) suggests that the cells are not globally defective in phagocytosis.

Galgp Restores Binding to CD43−/− Mφ. The extracellular mucin region of CD43 was identified as a normal component of human plasma, given the name Galgp, and isolated (17). We reasoned that if CD43 functions as a receptor for mycobacteria, addition of soluble extracellular CD43 to wild-type Mφ might abrogate mycobacterial binding by competing with cell surface CD43. However, addition of Galgp to CD43−/− Mφ resulted in enhanced mycobacterial binding (not shown). More importantly, addition of 100 μg/ml Galgp to CD43−/− Mφ at the time of infection restored the time-dependent (Fig. 4 A) and bacterial dose-dependent (Fig. 4 B) association of M. avium with CD43−/− Mφ. Restoration of M. avium binding to CD43−/− Mφ by Galgp (100 μg/ml) was also seen when the cells were evaluated by acid-fast staining (data not shown). These findings suggest that Mφ CD43, rather than serving as a receptor for mycobacteria, functions by promoting or stabilizing binding or uptake of mycobacteria.

The proposed collaborative role of CD43 in enabling mycobacterial association with Mφ was further tested by preincubating M. avium with Galgp for 4 h, washing the bacteria extensively, and adding them to CD43−/− Mφ for an additional 4-h incubation. Whereas coincubation of Galgp, M. avium, and Mφ enhanced the binding of mycobacteria to Mφ, no enhancement was observed when mycobacteria were preincubated with Galgp, washed, and then added to the cells (Fig. 5), indicating that Galgp does not opsonize the mycobacteria.

We next determined whether other mucins are also able to restore the association of M. avium with CD43−/− Mφ. M. avium were added to CD43−/− Mφ together with 100 μg/ml of Galgp or AFGP (20) or PSM that had been reduced, carboxymethylated, and trypsin-treated to generate linear monomeric mucin molecules (PSM-R-T) (21). After 4 h, association of bacteria with Mφ was assessed. M. avium binding to CD43−/− Mφ was enhanced only by the presence of Galgp. AFGP and PSM-R-T failed to enhance association of M. avium with Mφ (Fig. 6).

In contrast, addition of Galgp did not enhance association of M. avium with wild-type (CD43+) Hela cells. On infection with a 1:1 ratio, the number of bacteria associated per 100 cells was 5, 7, and 40, respectively, for vector-transfected Hela, vector-transfected Hela with Galgp (100 μg/ml), and CD43-transfected Hela. At an infection ratio of 5:1, the corresponding results were 10, 10, and 70 bacteria bound per 100 cells. Similar results were obtained when M. avium-challenged Hela cells (infection ratio of 15:1)

![Figure 4](image-url)

**Figure 4.** Addition of Galgp restores binding of M. avium by CD43−/− Mφ. (A) M. avium were incubated for varying times with CD43−/− Mφ in the absence (○) and presence (●) of 100 μg/ml Galgp. Bacterial cell ratio was 2:1. The Mφ were harvested and extensively washed, and adherent bacteria were quantified. The binding of M. avium to CD43−/− Mφ in the absence of Galgp is shown for comparison (●, dashed line). (B) M. avium at varying bacteria/cell ratios as indicated were incubated with CD43−/− Mφ for 4 h in the absence (○) and presence (●) of 100 μg/ml Galgp. Shown are mean values ± SEM for three mice of each group. The number of mycobacteria associated with CD43−/− Mφ in the presence and absence of Galgp was significantly different in all conditions (P < 0.0001). Comparable effects of Galgp addition were observed in two additional experiments. Galgp at 200 μg/ml produced similar results (data not shown).

![Figure 5](image-url)

**Figure 5.** Preincubation of M. avium with Galgp does not enhance their binding to CD43−/− Mφ. M. avium were preincubated for 4 h with 100 μg/ml Galgp or without additive, as indicated, and then extensively washed. The bacteria were used to infect CD43−/− Mφ (infection ratio of two organisms per cell). Galgp (100 μg/ml) or saline was added, as indicated, at the time of infection. After extensive washing, M. avium associated with Mφ were quantified. Shown are mean values ± SEM for cells from three mice. The number of M. avium associated with Mφ was significantly different for mycobacteria preincubated with Galgp compared with M. avium infected in the presence of Galgp (P < 0.0001). Similar effects of Galgp treatment were seen in one additional experiment.
were evaluated by acid-fast staining and light microscopy. 70% of CD43-transfected HeLa cells, but <10% of vector-transfected HeLa and vector-transfected HeLa with Galgp, scored positive for bacterial association (≥5 bacteria/cell). In the case of CD43-transfected HeLa cells, the associated mycobacteria were clearly located outside the cell perimeter as anticipated for these nonphagocytic cells (23).

In contrast to CD43/−/− Mφ, CD43/−/− Mφ fail to produce TNF-α in response to M. avium and this deficiency is corrected by Galgp. Early after inoculation with mycobacteria, wild-type Mφ respond by producing the proinflammatory cytokine TNF-α (24), which plays a central role in defense against microbial organisms including mycobacteria (25, 26). To investigate whether M. avium-dependent TNF-α production is altered in CD43/−/− Mφ, we measured TNF-α levels produced by CD43/−/− and CD43/−/− Mφ over the 4-48 h period after inoculation. M. avium inoculation of CD43/−/− Mφ resulted in the production of 1,150 ± 200 ng/ml TNF-α at 8 h. In contrast, CD43/−/− Mφ inoculated with the same M. avium/cell ratio produced <200 ng/ml TNF-α (Fig. 7 A). The possibility of a TNF-α expression defect in CD43/−/− Mφ was ruled out by finding that CD43/−/− and wild-type Mφ produced comparable levels of TNF-α when stimulated for 8 h with LPS (100 ng/ml; data not shown), suggesting that the defective induction of TNF-α production is M. avium specific. Importantly, the inability of CD43/−/− Mφ to produce TNF-α in response to M. avium was overcome by the addition of Galgp during inoculation (Fig. 7 B), and the extent of the restorative effect was dependent on Galgp concentration.

Incubation of wild-type Mφ with mycobacteria also induces production of the immunosuppressive cytokine IL-10, which counteracts the effects of TNF-α (27). Incubation with M. avium of CD43/−/− and CD43/−/− Mφ induced comparable levels of IL-10 (Fig. 7 C), indicating that the lesser interaction of mycobacteria with CD43/−/− Mφ is sufficient to induce IL-10 production. The discordant regulation of TNF-α and IL-10 demonstrates that the failure
to produce TNF-α in response to mycobacterial challenge is a specific defect of CD43−/− macrophages.

Discussion

Mycobacterial diseases, some of which are spread by airborne transmission, are a serious global public health problem (3). In this communication, we provide the first evidence identifying a new component of apparent importance for the uptake of mycobacteria by their primary host cell, the Mφ. This component, CD43, is the predominant sialoglycoprotein on leukocytes. We show that the presence of CD43 is obligatory for firm association of mycobacteria with Mφ. Mφ from wild-type mice and CD43-transfected HELa cells bound high numbers of M. avium. In contrast, negligible numbers of M. avium associated with untransfected HELa cells and with Mφ from CD43 gene-deleted mice. The role of CD43 in the binding of mycobacteria is specific because other bacteria, S. typhimurium and S. flexneri, did not bind to CD43+ HELa cells. Also, Mφ from CD43−/− mice bound two other species of mycobacteria, M. tuberculosis and M. bovis, and these also failed to bind to CD43− Mφ. Fluorescence microscopy of Mφ incubated with M. avium showed that the associated mycobacteria had been ingested by the CD43− Mφ. CD43 was also required for the production of TNF-α, but not of IL-10, by Mφ challenged with M. avium, strongly suggesting a functional link between the action of CD43 in mycobacterial binding and/or uptake and induction of TNF-α production.

CD43, also called sialophorin or leukosialin, is a prevalent sialoglycoprotein on monocyes, neutrophils, and T lymphocytes (28, 29). On T cells, CD43 extends 45 nm from the phospholipid bilayer, making it the largest glycoprotein on the cell surface (30). One function of CD43 is that of a repulsive or barrier molecule restricting cell to cell contact (13, 14, 31) due to its negative charge, prevalence, size, and rigidity. In addition, in vitro binding of CD43 mAb transduces intracellular signals that lead to T lymphocyte proliferation (e.g., 32) and activation of macrophages, increasing homotypic adhesion and hydrogen peroxide production (32, 33). At the mechanistic level, evidence suggests that ligation of CD43 induces tyrosine phosphorylation reactions (34, 35), and ligated CD43 itself binds the actin filament linker proteins, moesin and ezrin, which associate with the IL-6R coreceptor gp130, thereby mediating signaling in IL-6R-negative cells (39). Similar behavior is observed for the membrane and soluble forms of CD43. On binding LPS, soluble CD43, like cell surface CD43, can trigger intracellular signaling by interacting with its coreceptor toll-like receptor 2 (TLR-2) (40, 41).

Previous studies have shown that binding and phagocytosis of mycobacteria by Mφ can be mediated by several surface receptors, including complement receptors CR1, CR3, and CR4, Fc receptors, CD14, mannose receptor, receptors for SP-A, and scavenger receptors (for a review, see reference 11). In light of this plethora of receptors, the near absence of mycobacterial uptake in CD43−/− Mφ suggests that CD43 functions as a coreceptor or facilitator and/or cofactor of the mycobacterial uptake process. The finding that soluble Galgp restores M. avium binding to CD43− Mφ but not to CD43− HEla cells also attests to the contribution of Mφ components other than CD43/Galgp in the interaction with mycobacteria.
The action of Galgp to enhance mycobacteria binding and/or uptake resembles the action of SP-A. Addition of SP-A, a member of the lectin family of multimeric innate defense glycoproteins, increases the adhesion and phagocytosis of M. tuberculosis by Mphi (42). Like Galgp, SP-A must be present during the interaction of mycobacteria with Mphi. Indeed, reference to earlier studies suggests a candidate Mphi molecule that may functionally interact with both CD43/Galgp and SP-A. This molecule, a surface glycoprotein called C1qRp, binds SP-A (43), and the binding of SP-A to C1qRp enhances the Mphi capacity for phagocytosis of opsonized targets (44). Since it was also shown that CD43 and C1qRp copurify and coimmunoprecipitate (45), Galgp/CD43 and C1qRp may be components of a multimeric Mphi complex that enhances mycobacteria binding and uptake.

Inoculation of wild-type M. phi with mycobacteria induces the proinflammatory cytokine TNF-α (46), which is known to decrease the survival of M. avium (6) by promoting apoptosis of the infected host Mphi (47), an apparent innate defense mechanism that prevents systemic spread of infection (8). M. avium-induced TNF-α production failed to occur in CD43-/- Mphi and was restored by addition of Galgp, strongly suggesting that the action of CD43 in mycobacterial binding and/or uptake and TNF-α induction are functionally linked.

On the other hand, M. avium-dependent induction of IL-10, an antiinflammatory cytokine and TNF-α antagonist (27), was not impeded in CD43-/- Mphi (47), an apparent innate defense mechanism that prevents systemic spread of infection (8). M. avium-induced TNF-α production failed to occur in CD43-/- Mphi and was restored by addition of Galgp, strongly suggesting that the action of CD43 in mycobacterial binding and/or uptake and TNF-α induction are functionally linked.

In summary, this study identifies the surface mucin CD43 as a component essential for robust binding and/or uptake of mycobacteria by Mphi and for mycobacteria-induced TNF-α production, but not for production of IL-10. Further studies are warranted to identify the Mphi molecules that cooperate with CD43 and the mechanism that enhances mycobacterial binding.

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