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Anaplasma phagocytophilum Infects Mast Cells via α 1,3-Fucosylated but Not Sialylated Glycans and Inhibits IgE-Mediated Cytokine Production and Histamine Release[▽]

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Mast cells are sentinels for infection. Upon exposure to pathogens, they release their stores of proinflammatory cytokines, chemokines, and histamine. Mast cells are also important for the control of certain tick-borne infections. *Anaplasma phagocytophilum* is an obligate intracellular tick-transmitted bacterium that infects neutrophils to cause the emerging disease granulocytic anaplasmosis. *A. phagocytophilum* adhesion to and infection of neutrophils depend on sialylated and α 1,3-fucosylated glycans. We investigated the hypotheses that *A. phagocytophilum* invades mast cells and inhibits mast cell activation. We demonstrate that *A. phagocytophilum* binds and/or infects murine bone marrow-derived mast cells (BMMCs), murine peritoneal mast cells, and human skin-derived mast cells. *A. phagocytophilum* infection of BMMCs depends on α 1,3-fucosylated, but not sialylated, glycans. *A. phagocytophilum* binding to and invasion of BMMCs do not elicit proinflammatory cytokine secretion. Moreover, *A. phagocytophilum*-infected cells are inhibited in the release of tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), IL-13, and histamine following stimulation with IgE or antigen. Thus, *A. phagocytophilum* mitigates mast cell activation. These findings potentially represent a novel means by which *A. phagocytophilum* usurps host defense mechanisms and shed light on the interplay between mast cells and vector-borne bacterial pathogens.

Mast cells are inflammatory cells strategically located at sites that are exposed to the external environment such as the skin, intestines, and airways. Their location, coupled with the presence of preformed mediators in their cytoplasmic granules, makes them well suited to be first responders against pathogen invasion. The significance of mast cells in host defense has become increasingly recognized in recent years (7, 22). Classic mast cell degranulation is induced in response to antigen-specific IgE, which cross-links Fc ϵ receptor I (Fc ϵ RI) on the mast cell surface in the presence of antigen (1, 22). This activation results in rapid exocytosis of preformed granules containing histamine, proteoglycans, and serine proteases followed by synthesis of other mediators, including cytokines and chemokines (1, 18, 20, 26, 27). Pathogen activation of mast cells prompts release of preformed and newly synthesized mediators that are critical for inflammatory cell recruitment and resolution of infection (1, 27). For instance, mast cell-derived tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6) are important for bacterial clearance and improved host survival (6, 19, 21, 35).

Mast cells have been documented at tick feeding sites in the skin and have been shown to respond to arthropods that are acquiring blood meals at the dermis (4, 5, 44). Mast cell-released mediators are implicated in the development of im-

munity to molecules in the saliva of feeding ticks and in imparting resistance to tick feeding (24, 25, 40). For instance, mast cell-deficient mice acquire resistance to feeding ticks only after being reconstituted with cultured mast cells or bone marrow-derived mast cells from wild-type mice. Acquisition of tick immunity is linked to reduction in pathogen transmission from infected ticks (14, 43, 45). Furthermore, mast cells play crucial roles in responding to tick-borne pathogens, as *Borrelia burgdorferi* and *Francisella tularensis* stimulate mast cell production of TNF- α and IL-4, respectively, each of which limits the spread of infection (17, 36). The interplay between mast cells and other tick-borne pathogens is unknown. Since mast cells are critical for mounting an immune response to tick-derived antigens during tick feeding and for combating the transmission of tick-borne infections, it stands to reason that tick-transmitted pathogens may have evolved strategies for avoiding and/or directly inhibiting mast cell activation.

Anaplasma phagocytophilum is the etiologic agent of human granulocytic anaplasmosis (HGA), which is a potentially fatal disease that is transmitted by *Ixodes* ticks. HGA is the second most common tick-transmitted infection in the United States and is also found in Europe and Asia where it is endemic (38). *A. phagocytophilum* is an obligate intracellular, vacuole-adapted bacterium that displays an unusual tropism for neutrophils. The bacterium exhibits a biphasic developmental cycle, transitioning between an adherent and invasive dense-cored cell (DC) and a noninfectious reticulate cell (RC) that divides by binary fission (39). *A. phagocytophilum* infection of neutrophils requires sialylated and α 1,3-fucosylated receptors (3, 9, 12, 47). Whether the pathogen infects host cell types other

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than neutrophils upon tick inoculation is unknown. *A. phagocytophilum* infects myeloid cell lines, such as HL-60, as well as endothelial, megakaryocytic, and tick embryonic cell lines (10, 11, 46).

Given that mast cells are present at the ixodid tick bite site and because *A. phagocytophilum* is able to infect a variety of host cell types in addition to neutrophils *in vitro* (10, 11, 28), we investigated whether the bacterium is capable of infecting mast cells. Our findings reveal that *A. phagocytophilum* binds to and invades murine bone marrow-derived mast cells (BMMCs) and human skin-derived mast cells. *A. phagocytophilum* binding to and entry into mast cells involve α 1,3-fucosylated, but not sialylated, receptors. *A. phagocytophilum* infection of mast cells does not elicit a cytokine response or degranulation. Moreover, it suppresses IgE- or antigen (Ag)-mediated activation of cytokine, chemokine, and histamine release. Our findings reveal a novel means by which *A. phagocytophilum* modulates the host cell response.

MATERIALS AND METHODS

Cultivation of uninfected and *A. phagocytophilum*-infected myeloid cell lines.

The human promyelocytic cell line HL-60 and HL-60 cells infected with *A. phagocytophilum* (NCH-1 strain) were cultivated in Iscove's modified Dulbecco's Eagle medium (IMDM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (IMDM-10) as described previously (2, 8). *A. phagocytophilum* NCH-1A2 was cultured in sialyl transferase- and α 1,3-fucosyltransferase-defective HL-60 A2 cells as described previously (9, 32).

Mouse and human mast cell cultures. Murine bone marrow-derived mast cells (BMMCs) were harvested from the tibias and fibulas of C57BL/6, C57BL/6 $\times$ 129, and Fuc-TIV^{-/-}/Fuc-TVII^{-/-} mice [Fuc-TIV stands for α (1,3)-fucosyltransferase IV] (13). BMMCs were harvested from both C57BL/6 and C57BL/6 $\times$ 129 mice to assess whether BMMCs from different genetic backgrounds are susceptible to *A. phagocytophilum* infection. BMMCs were cultured in complete RPMI 1640 medium (cRPMI; Invitrogen, Carlsbad, CA) containing 10% FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1 mM HEPES (Biofluids, Rockville, MD) supplemented with IL-3-containing supernatant from WEHI-3 cells and stem cell factor (SCF)-containing supernatant from BHK-MHL cells. The mast cell phenotype was confirmed by flow cytometric staining with fluorescein isothiocyanate (FITC)-conjugated anti-c-Kit antibody (BD Biosciences, San Jose, CA) and phycoerythrin (PE)-conjugated Fc ϵ RI (eBiosciences, San Diego, CA), analyzed with a FACSCalibur (BD Biosciences, San Jose, CA). At the time of use, BMMC cultures were more than 90% mast cells. Mouse peritoneal mast cells were obtained by lavage of the peritoneal cavity with 6 ml of cRPMI. Fluid was recovered with a Pasteur pipette after vigorous massage of the peritoneum to loosen peritoneal cells. Surgical skin samples were obtained from the Cooperative Human Tissue Network of the National Cancer Institute or from the National Disease Research Interchange. Skin mast cells (Sk-MCs) were prepared as previously described (48) and were 100% mast cells, as determined by staining with toluidine blue. All protocols involving mouse and human tissues were approved by the Virginia Commonwealth University (VCU) Institutional Animal Care and Use Committee and the human studies Internal Review Board, respectively.

Assessment of *A. phagocytophilum* binding and infection. Host cells were incubated with host cell-free *Anaplasma* organisms that had been obtained from 4 times the number of infected HL-60 cells as those to be infected. The amounts of host cell-free NCH-1 and NCH-1A2 organisms were normalized according to spectrophotometric measurements at an optical density at 600 nm (OD₆₀₀). After 40 min, unbound bacteria were removed by washing the cells with phosphate-buffered saline (PBS). *A. phagocytophilum* organisms that were observed adhering to the host cell surface at 40 min were classified as bound bacteria. Host cells harboring intracellular colonies of *A. phagocytophilum* in the cytoplasm (morulae) were classified as infected (39). The numbers of bound *A. phagocytophilum* per cell and morulae per cell were recorded using indirect immunofluorescence microscopy at 40 min and at various postinfection time points, respectively, as described previously (2). Primary antibodies used to assess infection were rabbit polyclonal antiserum and mouse monoclonal antibody (MAb) 20B4 (29) (a gift from J. Stephen Dumler, Johns Hopkins University, Baltimore, MD) targeting the *A. phagocytophilum* major surface protein Msp2 (P44). In some

cases, host cells were treated with sialidases or mock treated with Hanks buffered salt solution (HBSS) and 1% FBS prior to incubation with *A. phagocytophilum* (3). Bacterial binding to and infection of BMMCs were also analyzed by transmission electron microscopy as described previously (39). For assessment of bacterial binding to peritoneal mast cells *ex vivo*, host cell-free NCH-1 organisms were fluoresceinated using CellTracker green (Invitrogen) as described previously (3). CellTracker green-labeled *A. phagocytophilum* organisms were incubated with 10⁶ peritoneal cells for 30 min at room temperature. Unbound bacteria were removed by centrifugation at 300 $\times$ g for 5 min, and *A. phagocytophilum* binding to mast cells was detected by flow cytometry using PE-conjugated anti-c-Kit antibody for mast cell detection.

Measurement of cytokine and/or histamine release. To determine whether *A. phagocytophilum* infection elicits cytokine release from mast cells, BMMCs were incubated with host cell-free *A. phagocytophilum* organisms or *Escherichia coli* or were mock infected for 3, 6, or 12 h at 37°C. The mock-infected control sample that was added to BMMCs corresponds to the minute amount of contaminating HL-60 cellular debris that remains associated with each sample of *A. phagocytophilum* organisms following release from HL-60 cells by mechanical lysis and the washing steps. To generate the mock-infected sample, the same number of uninfected HL-60 cells as infected cells from which *A. phagocytophilum* bacteria were obtained was processed in parallel with infected HL-60 cells. The barely discernible pellet of HL-60 cellular debris that remained after the washing steps was resuspended in medium and added to BMMCs in the exact same manner as *A. phagocytophilum* organisms that had been obtained from infected HL-60 cells. The amounts of *E. coli* and host cell-free *A. phagocytophilum* organisms were normalized according to spectrophotometric measurements at OD₆₀₀. Supernatants from BMMCs that had been incubated with *A. phagocytophilum*, *E. coli*, or mock-infected control were collected at each time point and measured for levels of tumor necrosis factor alpha (TNF- α), interleukin 1 β (IL-1 β), IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant peptide 1 (MCP-1), macrophage inflammatory protein 1 α (MIP-1 α), and MIP-1 β using the Bio-Plex 200 system (Bio-Rad, Hercules, CA). To determine whether *A. phagocytophilum* infection inhibits IgE-induced histamine and/or cytokine release in an infection load-dependent manner, BMMCs were incubated with *A. phagocytophilum* NCH-1A2 organisms or various concentrations of NCH-1 organisms for 3 h, after which unbound bacteria were removed by washing with PBS. The host cells were sensitized with 0.5 μ g/ml mouse anti-dinitrophenol (anti-DNP) IgE (BD Pharmingen, San Diego, CA) overnight at 37°C in a 5% CO₂ atmosphere, washed, and resuspended at a concentration of 10⁶ cells/ml. The cells were then activated with 50 ng/ml DNP-human serum albumin (HSA) at 37°C for 1 h or 24 h for subsequent analysis of histamine or cytokine release, respectively. Cells incubated in the absence of DNP-HSA served as negative controls. Histamine levels in the supernatant were measured using a histamine enzyme-linked immunosorbent assay (ELISA) kit (Neogen, Lansing, MI). Supernatant levels of TNF- α , IL-6, and IL-13 were measured using OptEIA ELISA kits (Peprotech, Rocky Hill, NJ).

Statistical analyses. Statistical analyses were performed using the Prism 4.0 software package (Graphpad; San Diego, CA). If one-way analysis of variance (ANOVA) indicated a group difference ($p < 0.05$), then Dunnett's posthoc test was used to test for a significant difference among groups. In some instances, the Student *t* test was used to assess statistical significance. Statistical significance was set at $p < 0.05$.

RESULTS

***A. phagocytophilum* binds, invades, and replicates within mast cells.** To investigate the ability of *A. phagocytophilum* to bind to mast cells, host cell-free *A. phagocytophilum* organisms (NCH-1 strain) were incubated with bone marrow-derived mast cells (BMMCs) for 40 min. After removal of unbound bacteria, host cells were assessed by transmission electron microscopy. Dense-cored cell (DC) organisms were observed adhering to the host cell surface, and some were in the process of being enveloped by filopodia (Fig. 1A). Immunofluorescence microscopic analysis further confirmed the susceptibility of BMMCs to NCH-1 binding and entry (Fig. 1B and C). The percentages of BMMCs from C57BL/6 and C57BL/6 $\times$ 129 mice with bound NCH-1 organisms were comparable, as were the mean number of bound bacteria per cell (data not shown).

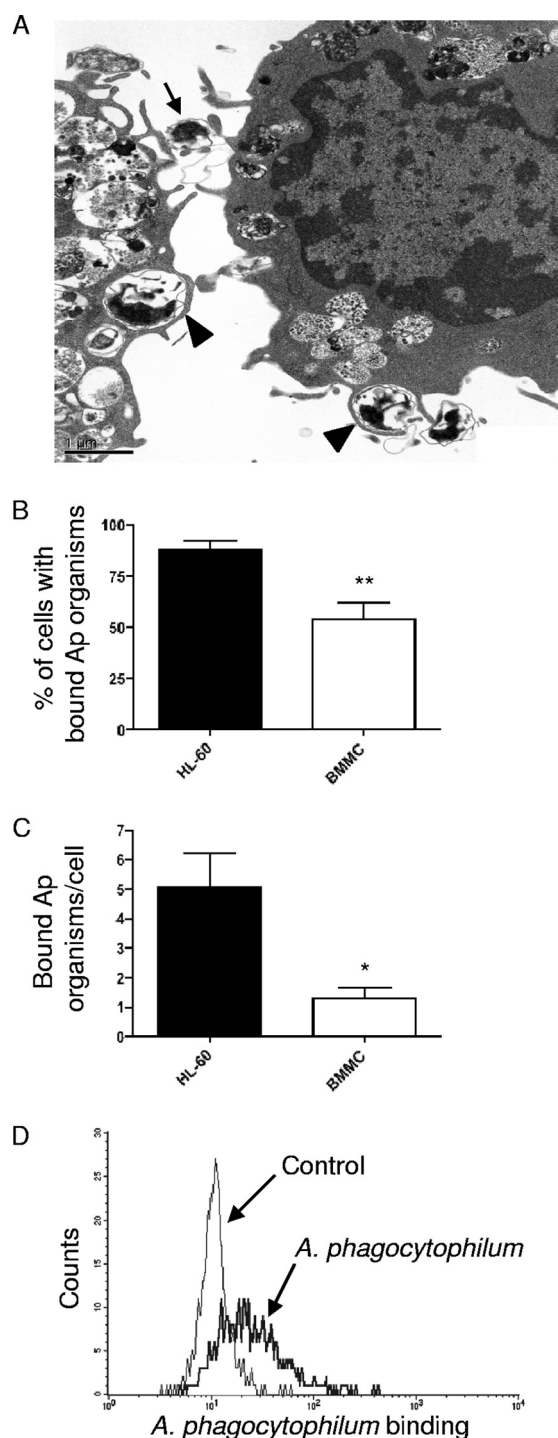


FIG. 1. *A. phagocytophilum* adheres to bone marrow-derived mast cells (BMMCs). (A) Host cell-free NCH-1 organisms were incubated with BMMCs. After 40 min, unbound bacteria were removed, and BMMCs were analyzed by transmission electron microscopy. NCH-1 dense-cored cell (DC) organisms were observed binding to the surface of and being enveloped by BMMCs. The black arrow denotes a DC organism simultaneously interacting with the surfaces of two BMMCs. The black arrowheads denote DC organisms that have been internalized into a vacuole or are in the process of being engulfed. Note the presence of intact cytoplasmic granules in the BMMCs. Bar, 1 μ m. (B and C) Binding of NCH-1 DC organisms to HL-60 cells or BMMCs was assessed by indirect immunofluorescence microscopy. Panels B and C show the percentages of HL-60 cells or BMMCs with bound

The percentage of infected BMMCs and the mean number of bacteria bound to BMMCs were significantly lower than to the human promyelocytic cell line HL-60 (Fig. 1B and C). To extend our studies *ex vivo* using mature tissue resident mast cells, CellTracker green-labeled NCH-1 organisms were incubated with mast cells obtained from the peritoneal cavities of mice. Fluoresceinated bacterial binding to c-Kit-positive mast cells was assessed by flow cytometry. Supernatant from CellTracker green-labeled NCH-1 was incubated with peritoneal mast cells as a background control. *A. phagocytophilum* bound efficiently to peritoneal mast cells, as the mean fluorescence intensities of peritoneal mast cells incubated with NCH-1 and supernatant control were 25.9 and 10.8, respectively (Fig. 1D).

We next assessed whether NCH-1 replicates within mast cells. BMMCs that had been incubated with *A. phagocytophilum* were examined by electron microscopy for morulae at 24 h postinfection. Detection of multiple intravacuolar reticulate cell (RC) organisms within individual vacuoles indicated that the DC-to-RC transition and bacterial replication had occurred (Fig. 2A). As revealed by immunofluorescence microscopy, NCH-1 infection of BMMCs is less productive than infection of HL-60 cells. Whereas $40\% \pm 9.8\%$ of HL-60 cells was infected with 4.6 ± 0.7 morulae per infected cell at 24 h, only $14.3\% \pm 3.2\%$ of BMMCs was infected with 1.5 ± 0.1 morulae per infected cell (Fig. 2B and C). By 48 h, NCH-1 infection of BMMCs had declined, as $6.5\% \pm 2.2\%$ BMMCs contained bacterial inclusions with 1.6 ± 0.1 morulae per cell (Fig. 2D and E). The degrees of *A. phagocytophilum* binding to and entry of BMMCs derived from C57BL/6 and C57BL/6 $\times$ 129 mice were comparable (data not shown). In contrast, the percentage of NCH-1-infected HL60 cells and the number of morulae per infected cell had risen to 70 ± 6.0 and 4.8 ± 0.7 , respectively, by 48 h.

***A. phagocytophilum* NCH-1 binding to BMMCs is sialic acid independent.** To determine whether sialic acid residues are important for *A. phagocytophilum* adhesion to BMMCs, we incubated NCH-1 organisms with BMMCs that had been treated with sialidases and determined the percentage of host cells with bound bacteria and the mean number of bound bacteria per cell. Consistent with earlier observations (3, 9, 12, 33, 47), sialidase treatment of control HL-60 cells significantly diminished NCH-1 binding (Fig. 3A and B). In contrast, sialidase treatment of BMMCs had no effect on NCH-1 binding. *A. phagocytophilum* bound equally well to sialidase-treated BMMCs derived from C57BL/6 and C57BL/6 $\times$ 129 mice (data not shown).

NCH-1 bacteria and the mean numbers plus standard deviations (SDs) (error bars) of bound NCH-1 organisms per cell, respectively. Data shown are the mean of three independent experiments, each of which was performed using mast cell populations derived from C57BL/6 and C57BL/6 $\times$ 129 mice. Ap, *A. phagocytophilum*. *, $P < 0.05$; **, $P < 0.005$. (D) Binding of NCH-1 to freshly isolated peritoneal mast cells. CellTracker green-labeled NCH-1 organisms were incubated with murine peritoneal cells. Bacterial binding was directly correlated to CellTracker green fluorescence using flow cytometry. Peritoneal mast cells incubated with supernatant from CellTracker green-labeled NCH-1 organisms served as a control for background fluorescence. Results shown are representative of two independent experiments.

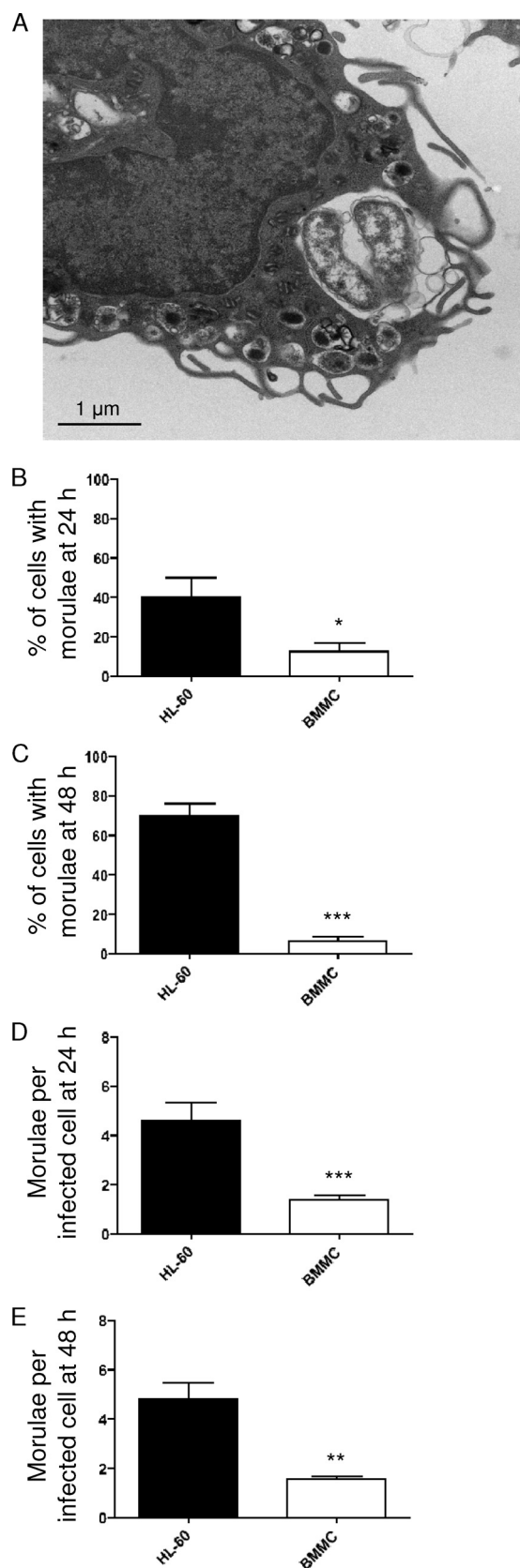


FIG. 2. *A. phagocytophilum* invades and replicates within bone marrow-derived mast cells. Host cell-free NCH-1 organisms were incubated with BMMCs for 40 min, after which unbound bacteria were

A. phagocytophilum NCH-1 is dependent on α 1,3-fucosylated glycans for adhesion, while NCH-1A2, which productively infects BMMCs and human skin-derived mast cells, is considerably less dependent. To investigate the importance of α 1,3-fucosylated receptors to *A. phagocytophilum* binding to murine mast cells, host cell-free NCH-1 organisms were incubated with Fuc-TIV^{-/-}/Fuc-TVII^{-/-} BMMCs. In contrast to wild-type BMMCs, NCH-1 binding to Fuc-TIV^{-/-}/Fuc-TVII^{-/-} BMMCs was reduced 2.4-fold (Fig. 3C and D). Despite the overall critical importance of sialylated and α 1,3-fucosylated glycans to *A. phagocytophilum* adherence, subpopulations of *A. phagocytophilum* organisms that do not depend on these determinants for adhesion exist (31–33). Such organisms can be selected for by repeated passage in the clonal cell line HL-60 A2, which is devoid of sialylated and α 1,3-fucosylated glycans (9, 32, 33). NCH-1A2 is an enriched subpopulation that was obtained by cultivating NCH-1 in HL-60 A2 cells (32). NCH-1A2 bacteria bound to a percentage of Fuc-TIV^{-/-}/Fuc-TVII^{-/-} BMMCs that was comparable to that of wild-type BMMCs and bound to considerably higher percentages of both Fuc-TIV^{-/-}/Fuc-TVII^{-/-} and wild-type BMMCs than NCH-1 organisms (Fig. 3C and D). Significantly fewer NCH-1A2 bacteria bound to Fuc-TIV^{-/-}/Fuc-TVII^{-/-} BMMCs than to wild-type BMMCs (Fig. 3D). However, greater numbers of NCH-1A2 organisms bound to both Fuc-TIV^{-/-}/Fuc-TVII^{-/-} and wild-type BMMCs than NCH-1 organisms (Fig. 3D).

We next sought to directly compare NCH-1A2 binding and infection kinetics of BMMCs with those of NCH-1. BMMCs were incubated with normalized amounts of either NCH-1 or NCH-1A2 organisms, and host cells were monitored for bacterial adherence and morula development. NCH-1A2 organisms bound to a slightly greater percentage of BMMCs with a significantly greater number of bacteria binding per BMMC than NCH-1 (Fig. 4A and B). The efficacy of NCH-1A2 infection of BMMCs far exceeded that observed for NCH-1, as significantly higher percentages of BMMCs with NCH-1A2 morulae were observed at both 24 and 48 h postinfection (Fig. 4C). Moreover, by 48 h postinfection, the number of NCH-1A2 morulae exceeded the number of NCH-1 morulae by approximately 5-fold (Fig. 4D). We next extended our analyses to human mast cells. NCH-1 and NCH-1A2 binding and infection kinetics of human skin-derived mast cells exhibit trends similar to those observed for murine BMMCs (Fig. 5).

***A. phagocytophilum* infection of mast cells does not elicit the release of cytokines or chemokines.** After observing that *A. phagocytophilum* adheres to and infects mast cells, we investi-

removed. (A) At 24 h postinfection, host cells were examined by transmission electron microscopy. A representative BMMC harboring two NCH-1 RC bacteria within a vacuole is presented. Note the presence of cytoplasmic granules throughout the BMMC. Additional experiments were performed in which *A. phagocytophilum* infection was assessed using indirect immunofluorescence microscopy. *A. phagocytophilum* infection was measured by the presence of morulae in the mast cells at 24 and 48 h. Panels B and C present the percentages of HL-60 cells or BMMCs with morulae at 24 h and 48 h, respectively. Panels D and E present the mean numbers plus SDs of morulae per infected HL-60 cell or BMMC at 24 h and 48 h, respectively. Data are means of three experiments. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

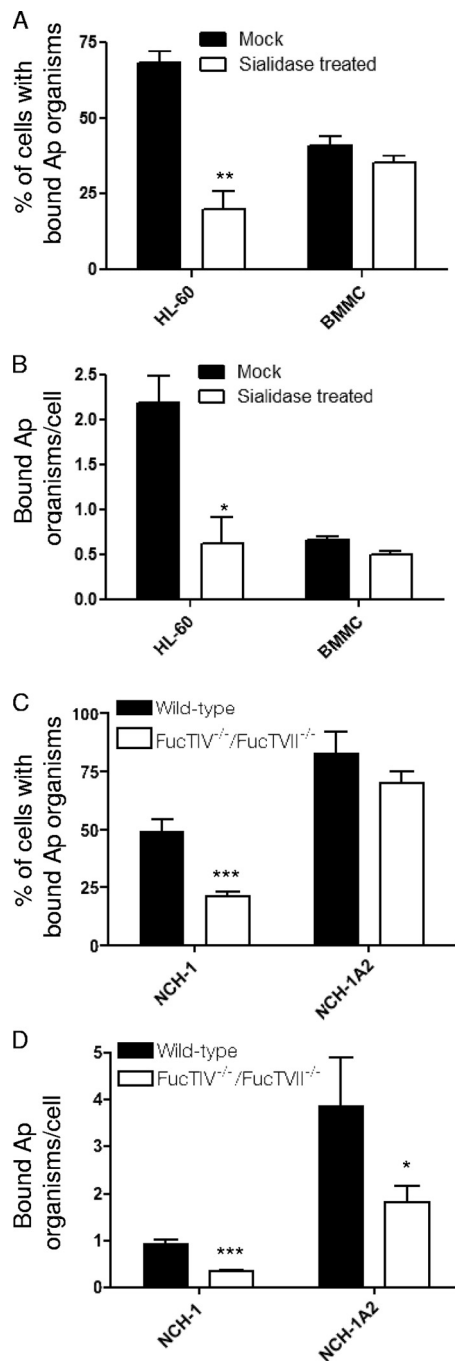


FIG. 3. *A. phagocytophilum* (Ap) NCH-1 binding to BMMCs does not depend on sialic acid, but it does depend on α 1,3-fucose. HL-60 cells and BMMCs were mock treated or treated with sialidase prior to incubation with host cell-free NCH-1 organisms. Unbound bacteria were removed after 40 min. Panels A and B present the percentages of HL-60 or BMMCs with bound NCH-1 organisms and the mean numbers plus SDs of bound NCH-1 bacteria per cell, respectively, as determined by indirect immunofluorescence microscopy. (C and D) Binding of NCH-1 and NCH-1A2 to wild-type and FucTIV^{-/-}/FucTVII^{-/-} BMMCs was analyzed by indirect immunofluorescence microscopy. Panels C and D present the percentages of BMMCs with bound *A. phagocytophilum* bacteria and the mean numbers plus SDs of bound *A. phagocytophilum* organisms per cell, respectively. Data are representative of 2 independent experiments. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

gated whether these events stimulate release of cytokines and chemokines that are associated with the mast cell antibacterial response. BMMCs were infected with NCH-1 or NCH-1A2 organisms, and the supernatant was assessed over a 24-h time course for a panel of 12 cytokines and chemokines that are typically released by mast cells in response to exposure to pathogens. Aliquots of BMMCs were confirmed for bound and/or internalized *A. phagocytophilum* organisms by indirect immunofluorescence microscopy (data not shown). As a positive control, BMMCs were incubated with equivalent amounts of *E. coli*. Of the 12 cytokines/chemokines measured, 5 (IL-1 β , IL-6, IL-13, MIP-1 α , and MIP-1 β) were detected in the supernatants (Fig. 6). Of note, there were no detectable levels of TNF- α , IL-4, and IL-5 released in response to *A. phagocytophilum* infection. Moreover, neither NCH-1 nor NCH-1A2 elicited a significant increase in the level of IL-1 β , IL-6, IL-13, MIP-1 α , or MIP-1 β compared to mock-infected cells (Fig. 6). In contrast, exposure to *E. coli* stimulated higher levels of all chemokines and cytokines examined compared to mock-infected cells. Incubating BMMCs with fivefold-less *E. coli* than *A. phagocytophilum* organisms yielded similar results (data not shown).

***A. phagocytophilum* infection inhibits IgE-mediated mast cell cytokine and histamine release in a bacterial dose-dependent manner.** After observing that *A. phagocytophilum* infection does not elicit the release of inflammatory cytokines or chemokines, we investigated whether infection alters IgE/Ag-mediated mast cell activation. NCH-1, NCH-1A2, or mock-infected IgE-sensitized BMMCs were activated with IgE plus dinitrophenol (DNP)-human serum albumin (HSA) for 24 h, after which the supernatants were assessed for the presence of TNF- α , IL-6, and IL-13, which are classical proinflammatory cytokines released by mast cells that promote inflammation. As expected, NCH-1A2 achieved a considerably higher bacterial burden than NCH-1 did (Fig. 7). Mock-infected mast cells released large amounts of TNF- α , IL-6, and IL-13 upon activation (Fig. 7A to C). NCH-1-infected BMMCs released slightly, but not statistically significantly, lower levels of cytokines than mock-infected cells. Cytokine production in NCH-1A2-infected cells was significantly reduced compared to mock-infected cells. Mast cell viability and cell numbers were unaffected by NCH-1 or NCH-1A2 infection (data not shown). Surface expression of c-Kit and Fc ϵ RI were comparable in mock-infected and *A. phagocytophilum*-infected cells, as determined by flow cytometry (data not shown), which suggests that the disruption of cytokine/chemokine release in *A. phagocytophilum*-infected mast cells is not due to altered expression of these receptors.

Since the BMMCs infected with NCH-1A2 had a significantly higher bacterial load than those infected with NCH-1, we reasoned that the observed inhibition of mast cell cytokine release is *A. phagocytophilum* dose dependent. To test our hypothesis, we infected BMMCs with increasing numbers of NCH-1 organisms to approximate the bacterial load achieved by NCH-1A2 and subsequently activated the cells with IgE/DNP-HSA for 24 h. IgE/Ag-stimulated cytokine release from BMMC populations of which 21.0% $\pm$ 5.0%, but not 15.5% $\pm$ 0.5% of cells were infected was significantly inhibited compared to mock-infected cells (Fig. 7D to F). We next investigated whether *A. phagocytophilum* infection also alters the

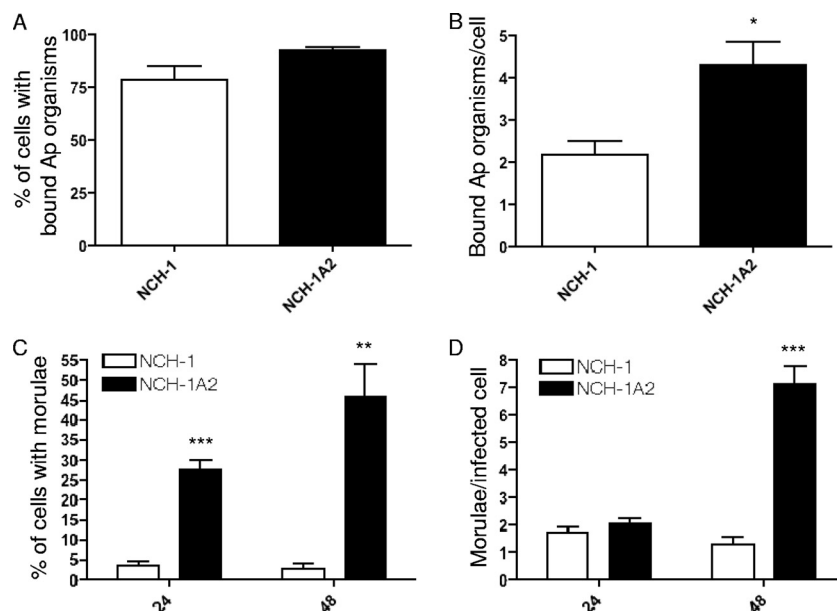


FIG. 4. *A. phagocytophilum* NCH-1A2 binds and invades murine BMMCs more efficiently than NCH-1. Host cell-free NCH-1 and NCH-1A2 organisms were incubated with murine BMMCs for 40 min. (A and B) Following removal of unbound bacteria, mast cells were examined for *A. phagocytophilum* (Ap) binding by indirect immunofluorescence microscopy. (C and D) The infections were allowed to proceed for 48 h, during which aliquots were assayed for bacterial load at 24 and 48 h. (A) Percentages of BMMC with bound NCH-1 or NCH-1A2 organisms. (B) Mean numbers plus SDs of bound NCH-1 or NCH-1A2 organisms per BMMC. (C) Percentages of BMMC with morulae. (D) Mean numbers plus SDs of morulae per infected BMMC. Results presented are the mean values plus SDs of three independent experiments. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

early phase of mast cell activation by assessing bacterial load and histamine release. Culture supernatants of NCH-1-, NCH-1A2-, and mock-infected BMMCs activated with IgE/DNP-HSA for 1 h were tested for the presence of histamine. Only

6.8% $\pm$ 1.9% of BMMCs were infected with NCH-1 with 1.4 $\pm$ 0.2 morulae per infected cell at the time at which histamine release was assessed (Fig. 8). This low infection load did not yield a detectable inhibition of IgE-stimulated histamine re-

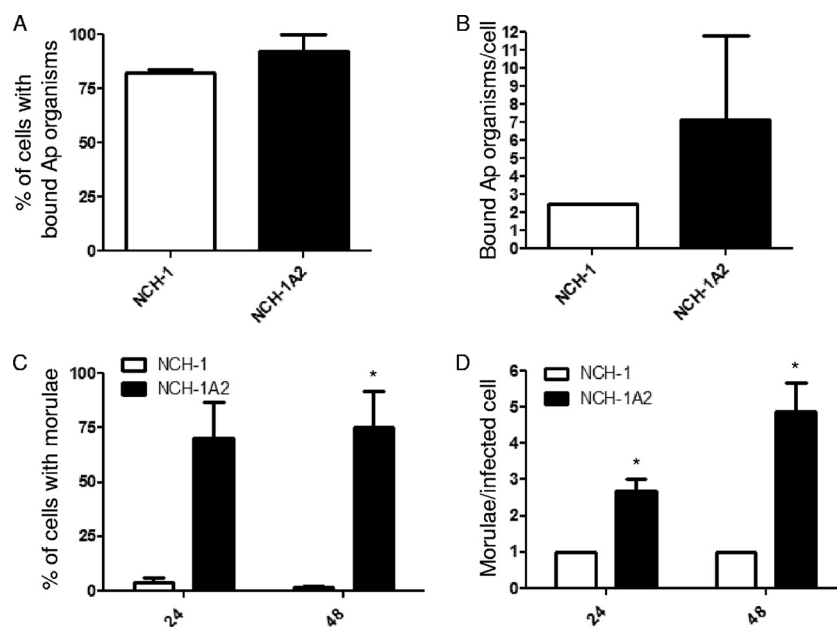


FIG. 5. *A. phagocytophilum* NCH-1A2 binds and invades human skin-derived mast cells more efficiently than NCH-1 does. Host cell-free NCH-1 and NCH-1A2 organisms were incubated with human skin-derived mast cells for 40 min. (A and B) Following removal of unbound bacteria, mast cells were examined for *A. phagocytophilum* (Ap) binding by indirect immunofluorescence microscopy. (C and D) The infections were allowed to proceed for 48 h, during which aliquots were assayed for bacterial load at 24 and 48 h. (A) Percentages of skin-derived mast cells with bound NCH-1 or NCH-1A2 organisms. (B) Mean numbers plus SDs of bound NCH-1 or NCH-1A2 organisms per skin-derived mast cell. (C) Percentages of skin-derived mast cells with morulae. (D) Mean numbers plus SDs of morulae per infected skin-derived mast cell. Results presented are the mean values $\pm$ SDs of three independent experiments. *, $P < 0.05$.

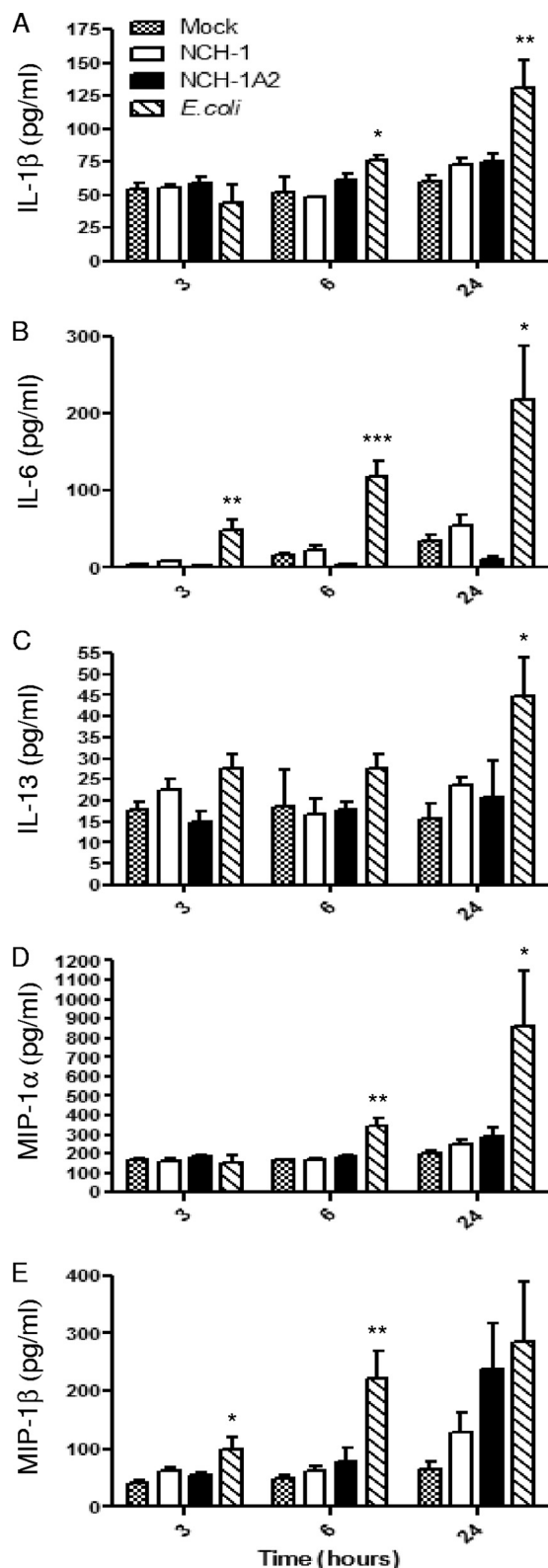


FIG. 6. Mast cells do not secrete cytokines or chemokines following *A. phagocytophilum* infection. BMMCs were incubated for 3, 6, or 24 h with NCH-1 or NCH-1A2 bacteria, mock control, or *E. coli*. Cell-free supernatants were collected and assessed for 12 cytokines and chemokines that are typically released from mast cells upon stimulation using the Bio-Plex 200 system assay, as described in Materials

and Methods. Of these 12 cytokines and chemokines, only IL-1β (A), IL-6 (B), IL-13 (C), MIP-1α (D), and MIP-1β (E) were detectable. Results are means plus SDs of triplicate determinations. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

DISCUSSION

Mast cells are crucial for initiating innate and adaptive immune responses that are critical for preventing bacterial dissemination and clearing bacterial infections (6, 15, 16, 34, 37). Mast cells' proximity to the vasculature and the rapid kinetics by which their mediators are released upon encounter with pathogens distinguishes them from other immune cells (1, 23). Given their abundant expression in the skin and their ability to recruit effector cells (41, 42), mast cells are likely critical in mounting immune resistance to arthropod vector-borne diseases. This study provides the first evidence that *A. phagocytophilum* infects mast cells. DC organisms adhere to BMMCs and are internalized by murine BMMCs from different genetic backgrounds. The bacterium also binds fully differentiated murine peritoneal mast cells and human skin-derived mast cells.

Sialic acid is dispensable for *A. phagocytophilum* binding to murine BMMCs, while α1,3-fucosylation is critical. The ability of *A. phagocytophilum* to infect human neutrophils and HL-60 cells is linked to P-selectin glycoprotein ligand 1 (PSGL-1) and other sialylated and α1,3-fucosylated receptors (3, 9, 12, 47). Sialylated glycans are important and α1,3-fucosylated glycans are essential, while PSGL-1 is not involved in *A. phagocytophilum* infection of murine neutrophils (3, 47). α1,3-Fucose is important for the bacterium to colonize its ixodid tick vector (30). Thus, while *A. phagocytophilum* differentially utilizes PSGL-1 and sialic acid to infect different cell types, its targeting of α1,3-fucosylated receptors is conserved among susceptible host cells.

The percentage of BMMCs with NCH-1 morulae and bacterial load per infected BMMC declined over the course of infection, whereas infection of control HL-60 cells increased throughout the time course. This is likely because NCH-1 does not bind as efficiently to BMMCs as to HL-60 cells, perhaps because the α1,3-fucosylated receptor is in relatively low abundance on the BMMC surface. Support for this premise comes from our observations that significantly fewer bacteria bound to BMMCs than to HL-60 cells, which translated to significantly less morulae per infected BMMC than per infected HL-60 cell. This, in turn, results in significantly fewer infectious DC progeny being released from BMMCs than HL-60 cells following each round of NCH-1 RC to DC conversion. Over the course of the experiment, BMMC division outpaces the release of DC, which dilutes the infection. In contrast, the

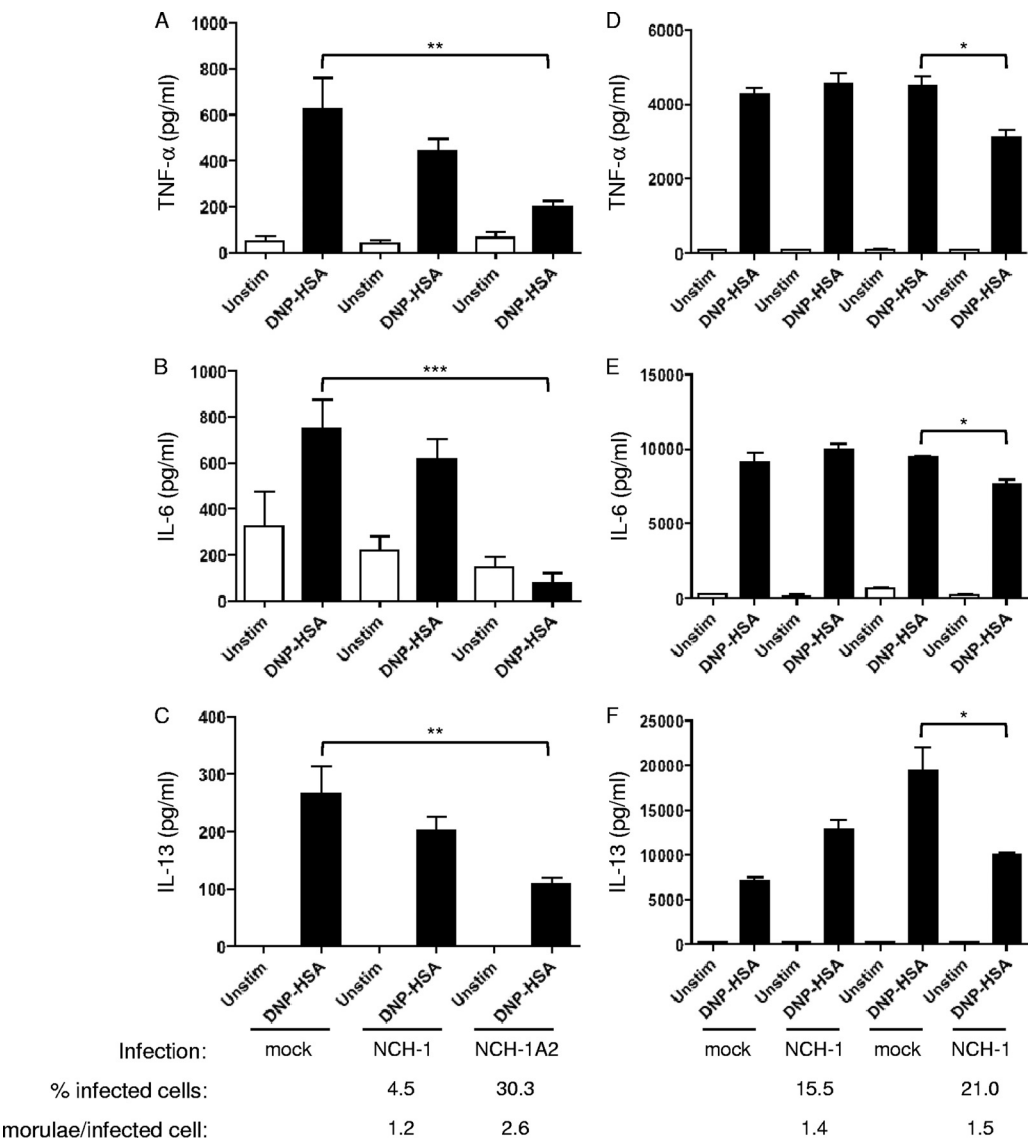


FIG. 7. *A. phagocytophilum* inhibits IgE-mediated cytokine release from bone marrow-derived mast cells. (A to C) NCH-1-, NCH-1A2-, or mock-infected BMMCs were sensitized with IgE overnight and subsequently activated with dinitrophenol (DNP)-human serum albumin (HSA) for 24 h. Duplicate samples that were not primed with IgE served as unstimulated (unstim) controls. Supernatant levels of TNF- α (A), IL-6 (B), and IL-13 (C) were measured by ELISAs. (D to F) To determine whether a greater degree of NCH-1 infection than that achieved in panels A to C is necessary for detecting a statistically significant decline in IgE-mediated release of cytokines, BMMCs were infected with increasing amounts of NCH-1 organisms followed by IgE priming, incubation with DNP-HSA, and assessment of TNF- α (D), IL-6 (E), and IL-13 (F) supernatant levels by ELISAs. Mock infection controls were performed for each dosage of NCH-1 organisms. Statistical significance in panels D to F was determined by comparing the cytokine levels released from NCH-1-infected BMMCs and their corresponding mock-infected controls. Please note that the overall increases in cytokine levels in panels D to F are due to the fact that considerably more BMMCs were assayed in this set of experiments than in the experiments presented in panels A to C. The bacterial burdens for panels A to C and panels D to F are listed below panels C and F, respectively. Data are representative of 3 individual mast cell populations that were assayed in triplicate. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

amount of infectious DC released from HL-60 cells increases by approximately 5-fold following each round of RC-to-DC conversion, which yields a higher bacterial load as the time course progresses. Further support for our argument comes from the fact that NCH-1A2 organisms, which do not depend on α 1,3-fucosylated receptors (32), were able to productively infect BMMCs, as the percentage of BMMCs with NCH-1A2 morulae and NCH-1A2 load per infected BMMC increased throughout the time course.

Since mast cells are critical for mounting an immune response to tick feeding and inhibiting tick transmission of pathogens (14, 24, 25, 40, 43, 45), we rationalized that *A. phagocytophilum* may have evolved strategies for avoiding and/or inhibiting mast cell activation. Consistent with our hypothesis, we observed that *A. phagocytophilum*-infected BMMCs failed to produce cytokines or chemokines. TNF- α , which recruits inflammatory cells to sites of bacterial infection (1, 21), was undetectable in the supernatants of infected cells. This is

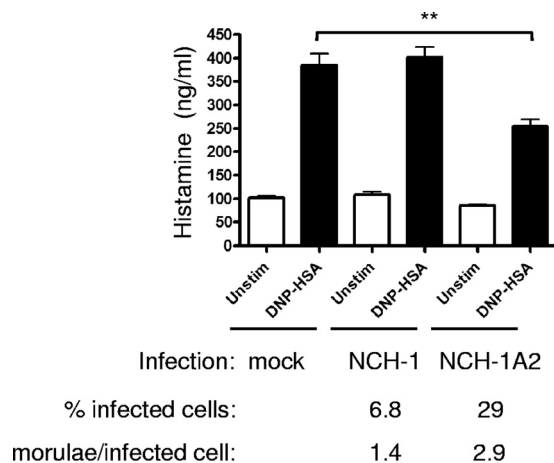


FIG. 8. *A. phagocytophilum* infection of bone marrow-derived mast cells inhibits IgE-mediated histamine release. A total of 10^6 BMMCs were infected with host cell-free NCH-1 or NCH-1A2 organisms or were mock infected, after which they were sensitized overnight with IgE and subsequently activated with DNP-HSA for 1 h. Duplicate samples that were not primed with IgE served as unstimulated (unstim) controls. ELISA was used to assess cell-free supernatants for histamine concentrations. Data presented are the means plus SDs of 3 individual mast cell populations. **, $P < 0.005$.

supported by our electron micrographs, which revealed that mast cells having bound or internalized *A. phagocytophilum* organisms do not degranulate. NCH-1- and NCH-1A2-infected BMMCs were significantly inhibited in their abilities to release TNF- α , IL-6, and IL-13 upon IgE stimulation. The fact that this phenomenon could be detected when only 21 to 30% of BMMCs were infected suggests that *A. phagocytophilum* pronouncedly blocks IgE-mediated mast cell cytokine release. Exposure to *E. coli* elicited an inflammatory response, which ruled out the possibility of an inherent defect in chemokine and cytokine release from BMMCs. NCH-1A2, but not NCH-1, inhibited IgE-prompted histamine release. We presume that this differential result is due to the fact that nearly one-third of BMMCs had detectable NCH-1A2 infection at the time of assay, whereas only $6.8\% \pm 1.9\%$ of BMMCs were infected with NCH-1. These data demonstrate the potential of *A. phagocytophilum* to not only invade mast cells in a manner that does not elicit cytokine production but to also actively inhibit IgE-mediated mast cell activation.

Our findings present the first description of direct interaction between *A. phagocytophilum* and mast cells and underscore the proinflammatory inhibitory potential of this pathogen. Moreover, our data promote the hypothesis that *A. phagocytophilum* would be able to infect mast cells present at the tick bite site. A complete *A. phagocytophilum* transmission cycle in the reservoir mammalian host requires a minimum of two tick feedings. The first blood meal results in tick acquisition of the bacterium, while the second transmits the pathogen to a subsequent reservoir host. In the first blood meal, the host could conceivably be immunologically naïve and thus have not mounted resistance to tick feeding, which would allow for prolonged tick feeding required for transmission. However, there would be expected to be some level of resistance for the second feeding, which would be at least partially mast cell

dependent. *A. phagocytophilum* infection of dermal mast cells could dampen this resistance to minimize tick rejection and promote successful transmission feeding, a phenomenon that would benefit both vector and pathogen. The findings of the current study set the stage for assessing whether *A. phagocytophilum* infects mast cells in the dermis and whether such an infection translates to biologically relevant inhibition of mast cell-mediated resistance.

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REFERENCES

- Abraham, S. N., and A. L. St. John. 2010. Mast cell-orchestrated immunity to pathogens. *Nat. Rev. Immunol.* **10**:440–452.
- Carlyon, J. A. 2005. Laboratory maintenance of *Anaplasma phagocytophilum*. *Curr. Protoc. Microbiol.* Chapter 3, Unit 3A.2.
- Carlyon, J. A., et al. 2003. Murine neutrophils require alpha1,3-fucosylation but not PSGL-1 for productive infection with *Anaplasma phagocytophilum*. *Blood* **102**:3387–3395.
- Demeure, C. E., et al. 2005. Anopheles mosquito bites activate cutaneous mast cells leading to a local inflammatory response and lymph node hyperplasia. *J. Immunol.* **174**:3932–3940.
- Depina, N., F. Hacin, W. Beghdadi, R. Peronet, and S. Mecheri. 2006. Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites. *J. Immunol.* **176**:4141–4146.
- Echtenacher, B., D. N. Mannel, and L. Hultner. 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* **381**:75–77.
- Enerback, L., and G. B. Lowhagen. 1979. Long term increase of mucosal mast cells in the rat induced by administration of compound 48/80. *Cell Tissue Res.* **198**:209–215.
- Goodman, J. L., et al. 1996. Direct cultivation of the causative agent of human granulocytic ehrlichiosis. *N. Engl. J. Med.* **334**:209–215.
- Goodman, J. L., C. M. Nelson, M. B. Klein, S. F. Hayes, and B. W. Weston. 1999. Leukocyte infection by the granulocytic ehrlichiosis agent is linked to expression of a selectin ligand. *J. Clin. Invest.* **103**:407–412.
- Granick, J. L., D. V. Reneer, J. A. Carlyon, and D. L. Borjesson. 2008. *Anaplasma phagocytophilum* infects cells of the megakaryocytic lineage through sialylated ligands but fails to alter platelet production. *J. Med. Microbiol.* **57**:416–423.
- Herron, M. J., M. E. Ericson, T. J. Kurti, and U. G. Munderloh. 2005. The interactions of *Anaplasma phagocytophilum*, endothelial cells, and human neutrophils. *Ann. N. Y. Acad. Sci.* **1063**:374–382.
- Herron, M. J., et al. 2000. Intracellular parasitism by the human granulocytic ehrlichiosis bacterium through the P-selectin ligand, PSGL-1. *Science* **288**:1653–1656.
- Homeister, J. W., et al. 2001. The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity* **15**:115–126.
- Jones, L. D., and P. A. Nuttall. 1990. The effect of host resistance to tick infestation on the transmission of Thogoto virus by ticks. *J. Gen. Virol.* **71**(Part 5):1039–1043.
- Jutel, M., et al. 2001. Histamine upregulates Th1 and downregulates Th2 responses due to different patterns of surface histamine 1 and 2 receptor expression. *Int. Arch. Allergy Immunol.* **124**:190–192.
- Jutel, M., et al. 2001. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* **413**:420–425.
- Ketavarapu, J. M., et al. 2008. Mast cells inhibit intramacrophage *Francisella tularensis* replication via contact and secreted products including IL-4. *Proc. Natl. Acad. Sci. U. S. A.* **105**:9313–9318.
- Kobayashi, H., T. Ishizuka, and Y. Okayama. 2000. Human mast cells and basophils as sources of cytokines. *Clin. Exp. Allergy* **30**:1205–1212.
- Malaviya, R., and S. N. Abraham. 2000. Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J. Leukoc. Biol.* **67**:841–846.

20. Malaviya, R., and A. Georges. 2002. Regulation of mast cell-mediated innate immunity during early response to bacterial infection. *Clin. Rev. Allergy Immunol.* **22**:189–204.
21. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham. 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF- α . *Nature* **381**:77–80.
22. Marshall, J. S. 2004. Mast-cell responses to pathogens. *Nat. Rev. Immunol.* **4**:787–799.
23. Marshall, J. S., and D. M. Jawdat. 2004. Mast cells in innate immunity. *J. Allergy Clin. Immunol.* **114**:21–27.
24. Matsuda, H., K. Fukui, Y. Kiso, and Y. Kitamura. 1985. Inability of genetically mast cell-deficient W/W^v mice to acquire resistance against larval *Haemaphysalis longicornis* ticks. *J. Parasitol.* **71**:443–448.
25. Matsuda, H., T. Nakano, Y. Kiso, and Y. Kitamura. 1987. Normalization of anti-tick response of mast cell-deficient W/W^v mice by intracutaneous injection of cultured mast cells. *J. Parasitol.* **73**:155–160.
26. McLachlan, J. B., and S. N. Abraham. 2001. Studies of the multifaceted mast cell response to bacteria. *Curr. Opin. Microbiol.* **4**:260–266.
27. Metz, M., and M. Maurer. 2007. Mast cells—key effector cells in immune responses. *Trends Immunol.* **28**:234–241.
28. Munderloh, U. G., et al. 2004. Infection of endothelial cells with *Anaplasma marginale* and *A. phagocytophilum*. *Vet. Microbiol.* **101**:53–64.
29. Park, J., K. S. Choi, and J. S. Dumler. 2003. Major surface protein 2 of *Anaplasma phagocytophilum* facilitates adherence to granulocytes. *Infect. Immun.* **71**:4018–4025.
30. Pedra, J. H., et al. 2010. Fucosylation enhances colonization of ticks by *Anaplasma phagocytophilum*. *Cell. Microbiol.* **12**:1222–1234.
31. Reneer, D. V., et al. 2006. Characterization of a sialic acid- and P-selectin glycoprotein ligand-1-independent adhesin activity in the granulocytotropic bacterium *Anaplasma phagocytophilum*. *Cell. Microbiol.* **8**:1972–1984.
32. Reneer, D. V., M. J. Troese, B. Huang, S. A. Kearns, and J. A. Carlyon. 2008. *Anaplasma phagocytophilum* PSGL-1-independent infection does not require Syk and leads to less efficient AnkA delivery. *Cell. Microbiol.* **10**:1827–1838.
33. Sarkar, M., D. V. Reneer, and J. A. Carlyon. 2007. Sialyl-Lewis x-independent infection of human myeloid cells by *Anaplasma phagocytophilum* strains HZ and HGE1. *Infect. Immun.* **75**:5720–5725.
34. Siebenhaar, F., et al. 2007. Control of *Pseudomonas aeruginosa* skin infections in mice is mast cell-dependent. *Am. J. Pathol.* **170**:1910–1916.
35. Sutherland, R. E., J. S. Olsen, A. McKinstry, S. A. Villalta, and P. J. Wolters. 2008. Mast cell IL-6 improves survival from *Klebsiella pneumoniae* and sepsis by enhancing neutrophil killing. *J. Immunol.* **181**:5598–5605.
36. Talkington, J., and S. P. Nickell. 1999. *Borrelia burgdorferi* spirochetes induce mast cell activation and cytokine release. *Infect. Immun.* **67**:1107–1115.
37. Thakurdas, S. M., et al. 2007. The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J. Biol. Chem.* **282**:20809–20815.
38. Thomas, R. J., J. S. Dumler, and J. A. Carlyon. 2009. Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and Ehrlichia ewingii ehrlichiosis. *Expert Rev. Anti Infect. Ther.* **7**:709–722.
39. Troese, M. J., and J. A. Carlyon. 2009. *Anaplasma phagocytophilum* dense-core organisms mediate cellular adherence through recognition of human P-selectin glycoprotein ligand 1. *Infect. Immun.* **77**:4018–4027.
40. Wada, T., et al. 2010. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *J. Clin. Invest.* **120**:2867–2875.
41. Wasserman, S. I. 1989. Mast cell-mediated inflammation in asthma. *Ann. Allergy* **63**:546–550.
42. Weber, A., J. Knop, and M. Maurer. 2003. Pattern analysis of human cutaneous mast cell populations by total body surface mapping. *Br. J. Dermatol.* **148**:224–228.
43. Wikel, S. K. 1980. Host resistance to tick-borne pathogens by virtue of resistance to tick infestation. *Ann. Trop. Med. Parasitol.* **74**:103–104.
44. Wikel, S. K. 1982. Immune responses to arthropods and their products. *Annu. Rev. Entomol.* **27**:21–48.
45. Wikel, S. K., R. N. Ramachandra, D. K. Bergman, T. R. Burkot, and J. Piesman. 1997. Infestation with pathogen-free nymphs of the tick *Ixodes scapularis* induces host resistance to transmission of *Borrelia burgdorferi* by ticks. *Infect. Immun.* **65**:335–338.
46. Woldehiwet, Z., et al. 2002. Cultivation of an ovine strain of *Ehrlichia phagocytophila* in tick cell cultures. *J. Comp. Pathol.* **127**:142–149.
47. Yago, T., et al. 2003. Structurally distinct requirements for binding of P-selectin glycoprotein ligand-1 and sialyl Lewis x to *Anaplasma phagocytophilum* and P-selectin. *J. Biol. Chem.* **278**:37987–37997.
48. Zhao, W., C. A. Oskeritzian, A. L. Pozez, and L. B. Schwartz. 2005. Cytokine production by skin-derived mast cells: endogenous proteases are responsible for degradation of cytokines. *J. Immunol.* **175**:2635–2642.

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