Haemophilus ducreyi-Induced Interleukin-10 Promotes a Mixed M1 and M2 Activation Program in Human Macrophages

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During microbial infection, macrophages are polarized to classically activated (M1) or alternatively activated (M2) cells in response to microbial components and host immune mediators. Proper polarization of macrophages is critical for bacterial clearance. To study the role of macrophage polarization during *Haemophilus ducreyi* infection, we analyzed a panel of macrophage surface markers in skin biopsy specimens of pustules obtained from experimentally infected volunteers. Lesional macrophages expressed markers characteristic of both M1 and M2 polarization. Monocyte-derived macrophages (MDM) also expressed a mixed M1 and M2 profile of surface markers and cytokines/chemokines upon infection with *H. ducreyi* *in vitro*. Endogenous interleukin 10 (IL-10) produced by infected MDM downregulated and enhanced expression of several M1 and M2 markers, respectively. Bacterial uptake, mediated mainly by class A scavenger receptors, and activation of mitogen-activated protein kinase and phosphoinositide 3-kinase signaling pathways were required for *H. ducreyi*-induced IL-10 production in MDM. Compared to M1 cells, IL-10-polarized M2 cells displayed enhanced phagocytic activity against *H. ducreyi* and similar bacterial killing. Thus, IL-10-modulated macrophage polarization may contribute to *H. ducreyi* clearance during human infection.

The Gram-negative bacterium *Haemophilus ducreyi* causes chancroid, a sexually transmitted genital ulcer disease that facilitates the acquisition and transmission of HIV-1 (45). Chancroid is rare in developed countries but is endemic in resource-poor regions of Africa and Asia. *H. ducreyi* also causes a nonsexually transmitted chronic limb ulceration syndrome throughout the South Pacific islands (29, 38, 49).

To study the immunopathogenesis of *H. ducreyi* infection, we developed a human challenge model in which the skin of the upper arm of healthy adult volunteers is inoculated with *H. ducreyi* strain 35000HP or its derivatives (21, 46, 47). Papules form within 24 h of inoculation and either spontaneously resolve or evolve into pustules within 2 to 5 days, mimicking the early stages of natural infection. The cutaneous immune response in experimental infection consists of neutrophils, macrophages, myeloid dendritic cells (DC), NK cells, effector/memory CD4 and CD8 T cells, and FOXP3⁺ regulatory T cells (5, 25, 26, 46). Experimental pustules and natural ulcers are identical histologically and represent immunological failure. In pustules and ulcers, *H. ducreyi* colocalizes with macrophages and neutrophils but resists phagocytosis and phagocytic killing and replicates extracellularly (6, 7). *H. ducreyi* secretes two antiphagocytic proteins, LspA1 and LspA2, which inhibit Fc receptor-mediated uptake by macrophage- and neutrophil-like cell lines *in vitro* (50); a mutant lacking LspA1 and LspA2 expression is attenuated in human volunteers (20). The mechanism of *H. ducreyi* clearance is unknown, but clearance is likely mediated by bacterial uptake and killing by phagocytes.

Macrophages are one of the first lines of defense against bacterial pathogens. During bacterial infections, monocytes are recruited from bloodstream into tissues, where they differentiate into macrophages. In response to tissue microenvironmental signals contributed by microbial components, the innate and adaptive immune systems, and damaged cells and tissues, macrophages become activated and acquire diverse phenotypes and functions. Classically activated (M1) and alternatively activated (M2) macrophages represent extremes of a continuum of macrophage het-

erogeneity. M1 and M2 macrophages are polarized by specific inducers, express a distinct combination of membrane receptors, cytokines, chemokines, and other immune mediators, and have specialized functions in infection, resolution of inflammation, wound repairing, and tissue remodeling (10, 27, 28, 34, 44).

M1 polarization is typically induced by gamma interferon (IFN- γ) and lipopolysaccharide (LPS) *in vitro*. M1 macrophages are characterized by high interleukin 12 (IL-12) and IL-23 and low IL-10 expression, by the production of reactive nitrogen and oxygen intermediates and proinflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF- α), and by upregulation of molecules associated with antigen presentation such as major histocompatibility complex (MHC) class II and costimulatory molecules CD40, CD80, and CD86. Thus, M1 macrophages are microbicidal and generally promote Th1-type inflammatory responses. In contrast, M2 macrophages express low levels of inflammatory cytokines such as IL-12 and high levels of the anti-inflammatory cytokine IL-10, chemokines such as CCL18, mannose receptors (MR), and scavenger receptors (SR). M2 macrophages have efficient phagocytic activity, but they are usually ineffective at killing microbial pathogens and are associated with anti-inflammatory and Th2 type responses, wound healing, and resolution of inflammation. M2 macrophages are heterogeneous and can be further classified into M2a, M2b, and M2c subsets. M2a polarization is induced by the Th2 cytokine IL-4 or IL-13, M2b by immune complexes in combination with TLR agonists,

Received 27 August 2012 Returned for modification 21 September 2012

Accepted 26 September 2012

Published ahead of print 1 October 2012

Editor: R. P. Morrison

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doi:10.1128/IAI.00912-12

and M2c by IL-10, transforming growth factor beta (TGF- β), or glucocorticoids (10, 27).

Depending on the bacterial species, M1 or M2 polarization can play a beneficial or detrimental role in disease outcomes (10, 30, 44). M1 polarization is associated with control of acute infections by many intracellular bacteria, such as *Salmonella enterica* serovar Typhi (22). In contrast, excessive M1 activation can lead to immunopathology. Enhanced M1 polarization is found in *Helicobacter pylori*-associated atrophic gastritis (41); higher levels of the M2 chemokine CCL18 in gastric tumors correlates with prolonged survival of *H. pylori*-infected patients (24). However, M2 polarization is also associated with reduced host defense and chronic infection. For example, high levels of IL-10 and enrichment of other M2-associated gene expression profiles are found in lepromatous lesions in comparison to the tuberculoid lesions of *Mycobacterium leprae* infection (32). Chronic infection by several bacteria, such as *Tropheryma whippelii*, is associated with enhanced M2c polarization and suppressed M1 activation (12, 33). Furthermore, due to their high plasticity, macrophages can switch from one polarized state to another to control infections by some pathogens or to be exploited by other microbes for immune evasion (17, 44).

The role of macrophage polarization during *H. ducreyi* infection has not been determined. Here, we characterized M1 and M2 phenotypes of macrophages obtained from *H. ducreyi*-infected sites and monocyte-derived macrophages (MDM). We showed that *H. ducreyi* infection induces a mixed M1 and M2 activation profile in lesional macrophages and MDM and that IL-10 plays a critical role in modulating phenotypes and phagocytic function of macrophages. We also showed for the first time that SR play a critical role in the phagocytosis of *H. ducreyi* by MDM.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *H. ducreyi* strain 35000HP (HP, human passaged) and *Escherichia coli* strain HB101 were grown on chocolate agar or LB plates and in GC medium broth supplemented with hemin, 1% IsoVitaleX, and 5% heat-inactivated fetal bovine serum (FBS) as described previously (4).

Flow cytometry of cells in skin lesions and blood of *H. ducreyi*-infected human subjects. Ten healthy adults (mean age $\pm$ standard deviation [SD], 33 $\pm$ 10 years), who were infected for a mean $\pm$ SD of 7.0 $\pm$ 1.3 days with 35000HP alone (this study) or 35000HP and isogenic mutants of 35000HP in two mutant-versus-parent comparison trials, contributed skin biopsy specimens of pustules (Table 1). Since the immunopathology caused by mutant strains that form pustules is indistinguishable from that caused by 35000HP (1), we analyzed biopsy specimens obtained from both parent- and mutant-infected sites. Paired samples of tissue and peripheral blood were obtained on the day of biopsy from each subject. Informed consent was obtained in compliance with the human experimental guidelines of the U.S. Department of Health and Human Services and the Institutional Review Board of Indiana University. Enrollment and exclusion criteria, preparation of *H. ducreyi*, and inoculation procedures were reported previously (2).

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque Plus gradient centrifugation. Single cell suspensions were obtained from the biopsy specimens as described previously (5). Cells were stained with the following antibodies obtained from BD Biosciences, unless indicated otherwise: peridinin chlorophyll protein (PerCP)-conjugated antibodies to CD14 (clone 61D3; eBioscience); allophycocyanin (APC)-conjugated antibodies to CCR7 (clone 3D12; eBioscience), CD40 (clone 5C3), CD206 (clone 19.2), and HLA-DR (clone LN3; eBioscience); fluorescein isothiocyanate (FITC)-conjugated antibodies to CD80 (clone

TABLE 1 Sources of tissue samples

Subject no.	Gender ^a	Site ^b	Duration of infection (days)	Reference or source ^c
376	M	P	7	This study
382	M	P	7	This study
383	M	P	8	This study
384	M	P	7	This study
385	F	P	5	This study
388	M	P	6	42
390	M	P	6	42
404	F	P	9	Unpublished
406	M	P/M	6	Unpublished
407	M	M	9	Unpublished

^a M, male; F, female.

^b Site inoculated with 35000HP (P) or an isogenic mutant (M) of 35000HP.

^c Subjects 388 and 390 were infected with 35000HP and a *sapBC* mutant (42); subjects 404, 406, and 407 were infected with 35000HP and the mutant FX548 (15).

2D10; eBioscience), CD86 (clone FUN-1), and CD163 (clone EDHu-1; AbD Serotec); and phycoerythrin (PE)-conjugated antibodies to CD163 (clone eBioGHI/61; eBioscience), HLA-DR (clone L243), and CD200R (clone OX108; Abcam). Cells were analyzed using a FACSCalibur flow cytometer and the BD CellQuest Pro version 6.0 software (BD Biosciences). To calculate the percentages and intensities of monocytes/macrophages expressing various surface markers, the monocyte/macrophage gate was set based on forward and side scatter characteristics of PBMC, followed by gating on CD14⁺ cells. CD14⁺ cells were then analyzed for expression of the surface markers. Results were expressed as percent expression and a mean fluorescent intensity ratio (MFIR) of the geometric MFI of each test antibody relative to that of its isotype-matched control antibody.

Generation, polarization, and infection of MDM from blood of uninfected donors. PBMC were isolated from leukopacks purchased from the Central Indiana Regional Blood Center from 18 anonymous donors or, after obtaining informed consent, from the peripheral blood of 5 healthy adult volunteers. CD14⁺ monocytes were purified by positive selection using magnetic CD14 microbeads (Miltenyi Biotec). To generate MDM, CD14⁺ monocytes were cultured in X-vivo 15 medium (Lonza) supplemented with 1% heat-inactivated human AB serum (Invitrogen) for 5 to 6 days at 37°C and 5% CO₂.

To polarize MDM into M1, M2a, and M2c macrophages, MDM were incubated with LPS from *E. coli* O26:B6 (200 ng/ml; Sigma-Aldrich) and IFN- γ (10 ng/ml), IL-4 (10 ng/ml), or IL-10 (10 ng/ml) for 2 days, respectively. To infect nonpolarized MDM, live bacteria were centrifuged onto wells containing MDM at an approximate multiplicity of infection (MOI) of 10:1. After 90 min of incubation at 35°C, cells were incubated at 37°C for an additional 46.5 h. Uninfected, infected, and polarized MDM were analyzed for surface expression of CD40, CD80, CD86, CD163, CD200R, CD206, HLA-DR, and CCR7. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the production of CCL18 (R&D Systems) and IL-6, IL-8, IL-10, IL-12, and TNF- α (BD Biosciences) in culture supernatants.

For antibody blocking experiments, nonpolarized MDM were preincubated with 10 μ g/ml of neutralizing anti-IL-10 or rat IgG2a isotype control antibodies (eBiosciences) for 1 h before infection. For Transwell experiments, MDM were seeded in 24-well tissue culture plates. An insert with a 0.2- μ m membrane (Nalgene Nunc) was placed in the well, and live *H. ducreyi* cells were added to the top chamber. To block *H. ducreyi* uptake, nonpolarized MDM were treated with 10 μ M cytochalasin D (Calbiochem) for 1 h prior to infection. To block the activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, MDM were incubated for 60 min at 37°C with 5 to 10 μ M MEK (extracellular signal-regulated kinase [ERK]) inhibitor U0126 (Cell Sig-

TABLE 2 Phenotypes of CD14⁺ cells from *H. ducreyi*-infected skin biopsies and autologous PBMC

Value ^a and cell source	Result ^b for:						
	CD40	CD80	CD86	HLA-DR	CD163	CD200R	CD206
% (SD)							
PBMC	5.4 (10.4)	0.3 (0.5)	88.3 (11.8)	65.4 (33.7)	69.1 (19.2)	24.8 (23.1)	0.3 (0.5)
Biopsy sample	30.4 (10.7)**	13.8 (9.2)**	67.2 (15.6)*	71.7 (16.6)	47.3 (23.1)	15.7 (12.2)	15.7 (6.3)**
MFIR (SD)							
PBMC	1.4 (0.8)	1.1 (0.5)	7.5 (3.1)	9.7 (6.5)	8.8 (8.6)	3.0 (1.3)	0.5 (0.2)
Biopsy sample	3.54 (1.9)*	1.7 (0.5)	5.2 (3.0)	22.1 (17.5)	6.5 (5.3)	2.0 (1.2)	1.0 (0.2)*

^a Percent expression and mean fluorescent intensity ratio (FMIR) of the geometric MFI of each test antibody relative to that of its isotype-matched control antibody. Values represent data for six donors for CD40, CD86, HLA-DR, and CD206, seven donors for CD80 and CD200R, and eight donors for CD163.

^b Significant difference compared to PBMC: *, $P \leq 0.05$; **, $P \leq 0.01$.

naling), 10 μ M p38 inhibitor SB203580 (Calbiochem), 10 μ M Jun N-terminal protein kinase (JNK) inhibitor SP600125 (Calbiochem), and 0.3 μ M PI3K inhibitor wortmannin (Calbiochem) prior to infection. Dimethyl sulfoxide (DMSO), which was used to dissolve cytochalasin D and the MAPK and PI3K inhibitors, served as a vehicle control for these experiments.

Phagocytosis assays with polarized MDM. MDM were harvested and seeded into wells of 24-well tissue culture plates at a density of 2.5×10^5 to 4.0×10^5 cells/well. Cells were incubated in medium or under polarizing conditions for 2 days. Nonpolarized and polarized MDM were washed once with Hanks balanced salt solution (HBSS) and infected with *H. ducreyi* at an MOI of 10:1. The plates were centrifuged at $180 \times g$ for 5 min to synchronize infection and incubated for 30 min at 35°C in 5% CO₂. Cells were then incubated in HBSS containing gentamicin (100 μ g/ml) for 30 min to kill extracellular bacteria and were washed three times with HBSS. To determine bacterial uptake, MDM were lysed with 0.2% saponin in HBSS at room temperature for 10 min and quantitatively cultured. The percentage of internalized bacteria was calculated as the ratio of the gentamicin-protected CFU count to the initial CFU count added per well. To determine the survival of the internalized bacteria, other wells were incubated in antibiotic-free medium containing 10% FBS for an additional 6 h and quantitatively cultured. The percentage of *H. ducreyi* that survived at 7 h postinfection was calculated as the ratio of the CFU count of recovered bacteria to that of internalized bacteria.

To determine the role of SR in the phagocytosis of *H. ducreyi* by macrophages, IL-10-polarized M2c and nonpolarized cells were preincubated for 30 min at 37°C with 20 μ g/ml of neutralizing anti-CD163 (clone 215927; R&D Systems) or its mouse IgG1 isotype control antibodies, 250 to 500 μ g/ml of fucoidan (Sigma), 20 to 50 μ g/ml of human acetylated low density lipoprotein (AcLDL) (Invitrogen), or 25 μ g/ml of recombinant soluble macrophage receptor with a collagenous structure (MARCO) (R&D Systems).

Statistical analysis. A paired *t* test was used to analyze comparisons between two groups. A mixed-model analysis of variance (ANOVA) with a donor as a random effect followed by Dunnett's or Tukey's procedure was used for comparisons between one group against all other groups and pairwise multiple comparisons, respectively. *P* values of ≤ 0.05 were considered to be statistically significant.

RESULTS

Phenotypic characterization of macrophages in pustules. Macrophages are rapidly recruited to *H. ducreyi*-infected skin in human inoculation experiments (19, 37). To determine the expression of surface markers associated with M1 and M2 polarization of macrophages, biopsy specimens of pustules and PBMC obtained from 10 participants who were experimentally infected were analyzed by flow cytometry. The percentage and MFIR of the markers on CD14⁺ macrophages from biopsy specimens and CD14⁺

monocytes from PBMC are shown in Table 2. Compared to peripheral blood monocytes, macrophages from infected skin significantly upregulated the M1 markers CD40 and CD80 and downregulated the M1 marker CD86. Expression of the M1 marker MHC II HLA-DR, the M2a marker CD200R, and the prototypic M2c marker CD163 (a class B SR) on macrophages was similar to that on monocytes. Only tissue CD14⁺ cells expressed the prototypic M2a marker CD206 (macrophage mannose receptor). The M1 marker CCR7 was not detected in either peripheral blood or tissue CD14⁺ cells (data not shown). Therefore, macrophages at *H. ducreyi*-infected sites were a mixed M1 and M2 population.

Analysis of *H. ducreyi*-infected MDM in comparison to M1 and M2 polarized MDM. Due to difficulties in recovering sufficient number of macrophages from biopsy specimens of infected skin, we utilized MDM to dissect the mechanisms of macrophage polarization by *H. ducreyi*. MDM were polarized to M1, M2a, and M2c with IFN- γ /LPS, IL-4, and IL-10, respectively, and were used as controls for analysis of *H. ducreyi*-infected MDM. As shown in Table 3, nonpolarized MDM constitutively expressed CD40, CD86, HLA-DR, CD163, and CD206 but not CD80 or CCR7; IFN- γ /LPS upregulated the M1 markers CD40, CD80, CD86, and CCR7 and downregulated the M2 markers CD163 and CD206; IL-4 did not affect M1 marker expression but downregulated CD163 and upregulated CD206; IL-10 greatly reduced the levels of CD86 and HLA-DR and enhanced CD163 expression.

Next, we compared the phenotypic differences between *H. ducreyi*-infected MDM and nonpolarized or M1 and M2 polarized cells (Table 3). Compared to nonpolarized MDM, *H. ducreyi*-infected cells significantly upregulated CD40, CD80, and CCR7 and downregulated CD86, HLA-DR, CD163, and CD206. In comparison to IFN- γ /LPS treatment, *H. ducreyi* infection induced similar levels of CD40 and CCR7 and significantly more CD163; however, the expression levels of CD80, CD86, HLA-DR, and CD206 were significantly lower in infected cells than the M1 cells. Relative to M2a cells, *H. ducreyi*-infected MDM expressed similar levels of CD163 and significantly less CD206. In comparison to M2c cells, *H. ducreyi*-infected MDM expressed significantly less HLA-DR and CD163 and more CD86. Thus, *H. ducreyi* infection of MDM resulted in a mixed M1 and M2 profile of cell surface markers.

We also examined cytokine and chemokine profiles of *H. ducreyi*-infected and polarized MDM (Fig. 1). Nonpolarized cells from most donors produced a low basal level of the M2-associated chemokine CCL18. Similar to M2a and M2c cells, *H. ducreyi*-infected MDM made more CCL-18 than nonpolarized MDM for

TABLE 3 Comparison of phenotypic markers on *H. ducreyi*-infected and uninfected nonpolarized and polarized MDM

Value and treatment ^a	Result ^b for:						
	CD40	CD80	CD86	HLA-DR	CD163	CD206	CCR7
% (SD)							
Medium	44.9 (30.5)***	0.4 (0.9)***	70.5 (17.0)***	88.9 (8.9)***	45.2 (23.2)**	88.8 (6.8)***	0.5 (0.8)**
IFN- γ /LPS	97.9 (1.5)	73.6 (23.2)*	82.9 (13.0)***	92.5 (7.9)***	8.5 (13.5)*	82.3 (11.3)*	8.6 (9.9)
IL-4	66.4 (28.1)***	1.1 (2.3)***	79.5 (18.2)***	87.9 (9.8)***	22.4 (14.3)	96.4 (4.8)***	0.7 (1.2)**
IL-10	69.6 (26.5)**	1.4 (2.9)***	16.9 (10.7)***	71.9 (22.9)*	88.5 (8.2)***	91.0 (6.4)***	0.2 (0.3)**
<i>H. ducreyi</i>	96.8 (5.3)	60.0 (25.2)	44.3 (27.0)	57.0 (22.0)	23.8 (18.8)	71.4 (18.9)	10.3 (10.3)
MFIR (SD)							
Medium	4.1 (2.1)***	1.1 (0.2)	5.0 (2.4)	8.8 (3.5)***	3.7 (1.9)	25.6 (19.3)**	1.2 (0.7)
IFN- γ /LPS	59.8 (32.4)***	7.7 (9.5)	10.6 (7.3)***	8.8 (2.9)***	1.4 (0.7)	12.9 (7.1)	1.6 (1.0)
IL-4	6.9 (4.1)***	1.1 (0.1)	9.3 (5.4)**	8.4 (2.6)**	2.1 (0.9)	44.2 (21.1)***	1.0 (0.5)
IL-10	5.8 (2.9)***	1.0 (0.2)	1.8 (0.4)	5.3 (1.8)	15.7 (7.6)***	20.0 (14.5)	0.9 (0.5)
<i>H. ducreyi</i>	34.3 (23.1)	5.0 (5.6)	3.5 (2.2)	5.1 (2.2)	1.9 (0.8)	7.9 (5.1)	1.5 (1.1)

^a MDM were cultured for 48 h with medium alone, LPS and IFN- γ , IL-4, IL-10, or *H. ducreyi*. Values represent the percent expression and mean fluorescent intensity ratio (MFIR) of the geometric MFI of each test antibody relative to that of its isotype-matched control antibody. The results represent data from 18 donors except for CCR7 ($n = 11$).

^b Significant difference compared to *H. ducreyi*-infected MDM: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

each donor. However, after adjustment for multiple comparisons, levels of CCL-18 produced by the infected cells were not significantly different from the levels produced by M2a, M2c, and M1 polarized or nonpolarized MDM. Nonpolarized, M1 and M2 MDM made little or no IL-6, IL-8, IL-10, IL-12, and TNF- α . IL-10 production by M2c cells was not determined due to the exogenous IL-10 added to the culture. Like the M1 stimuli, *H. ducreyi* infection induced production of the proinflammatory cytokines IL-6, IL-8, IL-12, and TNF- α . Furthermore, *H. ducreyi*-infected cells

made significantly more IL-8 than M1 cells, but the reverse was true for IL-12 production. For each donor, *H. ducreyi* infection was more potent than the M1 stimuli in inducing the anti-inflammatory cytokine IL-10, but due to donor-to-donor variations, the results were not significantly different. Taken together, our results showed that *H. ducreyi* infected-MDM primarily produced cytokines characteristic of M1 cells but also produced cytokines characteristic of M2 cells.

IL-10 produced by *H. ducreyi*-infected MDM skews MDM toward M2c phenotype. Bacterial infection of macrophages activates a core host transcriptional response, which mainly involves the upregulation of genes associated with M1 polarization (10). Several bacterial pathogens are known to suppress M1 polarization and promote M2 or a mixed M1 and M2 profile, probably through the production of IL-10 (10). Since *H. ducreyi*-infected macrophages produced IL-10, we investigated the contribution of endogenously produced IL-10 to the mixed M1 and M2 polarization during *H. ducreyi* infection. As shown in Table 4, isotype-matched control antibodies had little effect on surface marker expression on *H. ducreyi*-infected MDM, whereas anti-IL-10 neutralizing antibodies differentially modulated multiple markers. Although expression of CD40, CD80, and CCR7 was not significantly enhanced by antibody treatment, blocking IL-10 did significantly increase the expression of the M1 markers CD86 and HLA-DR. In contrast, the expression of the M2c marker CD163 was nearly completely abolished. The expression of the M2a marker CD206 was not affected by the antibodies. Neutralizing IL-10 antibodies significantly enhanced production of *H. ducreyi*-induced proinflammatory cytokines IL-6, IL-8, and IL-12 (Fig. 2). In contrast, CCL18 production was significantly reduced by the anti-IL-10 antibodies. Therefore, IL-10 produced by infected MDM plays a critical role in promoting the M2c phenotype and dampening the M1 phenotype.

Bacterial uptake and activation of MAPK and PI3K pathways are required for *H. ducreyi*-induced IL-10 production. Recognition of bacterial components by pattern recognition receptors (PRRs) on macrophages initiates multiple signaling pathways, which lead to the production of IL-10 and other effector molecules. To analyze the regulation of IL-10 expression in MDM dur-

FIG 1 Cytokine production by nonpolarized, polarized, and *H. ducreyi*-infected MDM. Differentiated MDM were incubated in culture medium (M0) or polarizing medium (M1, M2a, M2c) or infected with live *H. ducreyi* (Hd) for 2 days. The cytokine levels in the culture supernatants were quantified by ELISA. The bars represent means $\pm$ SD of results of assays done with cells from 9 donors for CCL-18, IL-6, IL-8, IL-10, and TNF- α and from 8 donors for IL-12. Nonpolarized, M2a, and M2c cells made little or no IL-6, IL-8, IL-10, and TNF- α and were not compared with *H. ducreyi*-infected cells. ND, not determined due to exogenous IL-10 in the culture supernatants. *H. ducreyi* versus all other groups for CCL18; *H. ducreyi* versus M1 for the rest of cytokines: *, $P \leq 0.05$; ***, $P \leq 0.001$.

TABLE 4 *H. ducreyi*-induced IL-10 modulates MDM phenotypes

Value and treatment ^a	Result ^b for:						
	CD40	CD80	CD86	HLA-DR	CD163	CD206	CCR7
Percentage (SD)							
Uninfected	30.4 (27.8)	0.5 (1.1)	76.1(13.7)	94.7 (3.3)	57.1 (0)	92.5 (5.1)	0.8 (1.0)
Hd	96.8 (3.2)	56.3 (23.8)	41.0 (23.2)	59.6 (23.0)	32.8 (27.2)	78.6 (19.0)	9.9 (13.8)
Hd-iso	96.6 (3.6)	54.3 (21.5)	42.3 (24.0)	63.2 (23.7)	36.5 (29.4)	84.0 (13.4)	9.5 (14.2)
Hd-αIL-10	96.8 (3.6)	71.4 (19.9)	84.5 (18.6)***	92.0 (7.0)**	5.3 (8.7)**	80.9 (14.8)	20.1(22.2)
MFIR (SD)							
Uninfected	2.7 (1.4)	1.1 (0.2)	4.9 (1.5)	9.9 (3.5)	4.4 (2.3)	27.8 (20.1)	1.1 (0.3)
Hd	19.4 (10.8)	4.6 (6.7)	3.2 (1.6)	4.8 (2.0)	2.9 (3.3)	9.9 (6.3)	1.3 (0.8)
Hd-iso	19.2 (11.5)	4.9 (7.3)	3.0 (1.6)	5.1 (2.3)	3.2 (3.2)	10.4 (6.2)	1.3 (0.9)
Hd-αIL-10	28.3 (24.5)	8.9 (15.5)	15.5 (13.1)**	10.0 (4.8)**	1.2 (0.5)	11.5 (6.7)	2.0 (1.4)

^a MDM were uninfected or infected with *H. ducreyi* in the absence (Hd) or presence of anti-IL-10 (Hd-αIL-10) or isotype antibodies (Hd-iso) for 48 h. Values represent percent expression and mean fluorescent intensity ratio (MFIR) of the geometric MFI of each test antibody relative to that of its isotype-matched control antibody. The results represent data from 10 donors except for CCR7 (*n* = 7).

^b Comparisons between Hd-αIL-10 and Hd-iso: **, *P* ≤ 0.01; ***, *P* ≤ 0.001.

ing *H. ducreyi* infection, we first determined whether physical contact between the bacteria and MDM is required. As shown in Fig. 3A (left panel), separating the bacteria from MDM with a Transwell membrane almost completely abolished IL-10 production. Cytochalasin D, an inhibitor of actin polymerization and bacterial uptake, also significantly decreased the production of *H. ducreyi*-induced IL-10 (Fig. 3A, right panel). These data suggest that bacterial adherence and phagocytosis are necessary for optimal IL-10 production.

IL-10 gene expression in macrophages is controlled by different signaling programs, including the MAPK and PI3K pathways,

that culminate in the activation of various transcription factors and chromatin remodeling at the IL-10 promoter (43). The MAPK pathways include p38, JNK, and ERK cascades. Compared to the vehicle control DMSO, specific inhibitors of p38 (SB203580), JNK (SP600125), and PI3K (wortmannin) all significantly blocked IL-10 production. The ERK inhibitor U0126 did not significantly reduce IL-10 production (*P* = 0.17). Thus, the p38, JNK, and PI3K pathways played an important role in the production of IL-10 in *H. ducreyi*-infected MDM.

FIG 2 *H. ducreyi*-induced IL-10 contributes to CCL18 production and inhibits proinflammatory cytokine production in MDM. Differentiated MDM were infected with *H. ducreyi* in the absence (control) and presence of anti-IL-10 or isotype control antibodies for 2 days. Culture supernatants were quantified for the amounts of cytokines by ELISA. Due to donor-to-donor variation in cytokine production, the cytokine level in each sample was normalized to that of MDM infected with *H. ducreyi* alone, whose value was set at 100%. The bars represent means ± SD of results of assays done with cells from 8 donors. Anti-IL-10 versus isotype antibodies: *, *P* ≤ 0.05; **, *P* ≤ 0.01; ***, *P* ≤ 0.001.

FIG 3 Bacterial uptake and the MAPK and PI3K signaling pathways are required for *H. ducreyi*-induced IL-10 production. (A) Role of bacterial uptake on IL-10 production. MDM were infected with *H. ducreyi* in the absence (–) and presence (+) of a 0.2-μm Transwell (left panel) or in medium alone (control), cytochalasin D (CD), or the vehicle control DMSO (right panel). (B) Effects of MAPK and PI3K inhibitors on IL-10 production. MDM were infected with *H. ducreyi* in medium (control) or in the presence of DMSO or inhibitors for p38, JNK, ERK, and PI3K: SB203580 (SB), SP600125 (SP), U0126, and wortmannin (WM), respectively. Culture supernatants from MDM were assessed for the accumulation of IL-10. Due to donor-to-donor variation in cytokine production, the cytokine level in each sample was normalized to that of MDM infected with *H. ducreyi* alone, whose value was set at 100%. The bars represent means ± SD of results of assays done with cells from 5 donors. (A) Cytochalasin D versus DMSO; (B) SB203580, SP600125, U0126, or wortmannin versus DMSO: *, *P* ≤ 0.05; **, *P* ≤ 0.01; ***, *P* ≤ 0.001.

FIG 4 Effect of macrophage polarization on *H. ducreyi* uptake and killing. Nonpolarized (M0) or M1 and M2 polarized macrophages were infected with *H. ducreyi* (left panels) or *E. coli* HB101 (right panels) at an MOI of 10:1 for 30 min, followed by 30 min of treatment with gentamicin. The percent bacterial uptake (A) was calculated as the ratio of counts of bacteria recovered with gentamicin treatment to initial counts of CFU added. The percent bacterial survival (B) was calculated as the ratio of counts of bacteria recovered from gentamicin-treated cultures that were incubated in antibiotic-free medium for 6 more hours to counts of internalized bacteria. The bars represent means $\pm$ SD of results of assays done with cells from 7 donors. M1 versus all other groups: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Effect of MDM polarization on phagocytosis of *H. ducreyi*.

To examine the functional consequence of M1 and M2 polarization, we used gentamicin protection assays to measure phagocytic and bactericidal activities of polarized MDM against *H. ducreyi*. Similar patterns of bacterial uptake by polarized MDM were obtained with nonopsonized *H. ducreyi* or bacteria opsonized with autologous serum (data not shown); all data presented here were generated with nonopsonized bacteria. To determine whether the effects of polarization would be specific to *H. ducreyi*, we included the nonpathogenic Gram-negative bacterium *E. coli* strain HB101 in the assays. In the phagocytic assays, M1 MDM were compared with nonpolarized and M2 MDM for their ability to ingest *H. ducreyi* and *E. coli*. At an MOI of 10:1, approximately 5 to 20% of *H. ducreyi* was taken up by the MDM (Fig. 4A, left panel). Similar percentages of *E. coli* were also ingested by the MDM (Fig. 4A, right panel). M1 macrophages tended to have a lower capacity than nonpolarized MDM to take up either bacterial species, ranging from 40 to 72% and 7 to 43% for *H. ducreyi* and *E. coli*, respectively. M2a and M2c polarization differentially affected phagocytosis of these two organisms. Compared to M1 cells, IL-4-polarized M2a cells had a significantly increased capacity to take up *E. coli*, whereas IL-10-programmed M2c cells exhibited significant higher phagocytic activities against *H. ducreyi*.

To evaluate the ability of polarized MDM to kill internalized *H. ducreyi* and *E. coli*, infected MDM were incubated in antibiotic-free medium for an additional 6 h after gentamicin removal. Only a small percentage of internalized *H. ducreyi* survived in all groups at 7 h postinfection (Fig. 4B, left panel). A much higher percentage of *E. coli* survived under similar conditions (Fig. 4B, right panel). For both *H. ducreyi* and *E. coli*, M1 cells killed significantly more bacteria than nonpolarized and M2a cells, whereas M2c cells were not significantly different from M1 cells in their killing activity (Fig. 4B). Taken together, the data indicate that in comparison to

FIG 5 Role of scavenger receptors in *H. ducreyi* uptake by M2c-polarized macrophages. Nonpolarized cells (M0), M2c cells (control), and M2c cells preincubated with neutralizing anti-CD163, isotype matched control antibodies, fucoidan, recombinant soluble MARCO (sMARCO), or AcLDL were infected with *H. ducreyi* at an MOI of 10:1 for 30 min, followed by 30 min of treatment with gentamicin. The percent bacterial uptake was calculated as the ratio of the counts of bacteria recovered with gentamicin treatment to counts of initial CFU added. The relative phagocytosis rate in each sample was normalized to that of *H. ducreyi*-infected M0 macrophages (control), whose value was set at 100%. The bars represent means $\pm$ SD of results of assays done with cells from 7 donors for the control, isotype, and anti-CD163 groups, 6 for fucoidan and sMARCO, and 4 for AcLDL. Anti-CD163 versus isotype; fucoidan, sMARCO, or AcLDL versus control M2c cells: ***, $P \leq 0.001$.

E. coli, internalized *H. ducreyi* cells are rapidly killed by macrophages. Our results also indicate that macrophage polarization plays an important role in regulating *H. ducreyi* uptake and killing, with the M2c program favoring bacterial uptake and the M1 and M2c programs leading to more efficient bactericidal activity.

Role of macrophage SR in *H. ducreyi* uptake. The class B SR CD163, which is highly induced on M2c cells, can mediate binding of both Gram-negative and Gram-positive bacteria (13). To determine whether CD163 contributes to enhanced phagocytosis of *H. ducreyi* by M2c macrophages, neutralizing anti-CD163 antibodies were used in the gentamicin protection assays. Compared to the isotype-matched control, anti-CD163 antibodies did not significantly affect bacterial uptake (Fig. 5).

In addition to CD163, IL-10-programmed M2c cells upregulate several other SR, including MARCO and SR-A, that can mediate bacterial binding and uptake (11, 27, 28, 32). MARCO and SR-A are the major class A SR (CASR) subtypes expressed on macrophages (35). Therefore, we used the general CASR inhibitor fucoidan, recombinant soluble MARCO, and the SR-A-specific ligand AcLDL to block interaction of *H. ducreyi* with CASR, MARCO, and SR-A, respectively, in M2c cells. Fucoidan significantly inhibited *H. ducreyi* uptake, whereas blocking MARCO or SR-A did not significantly reduce phagocytosis (Fig. 5). These results indicate that CASR are the major phagocytic receptors for nonopsonized *H. ducreyi* on M2c cells.

The finding that fucoidan reduced phagocytosis of *H. ducreyi* by M2c cells to a level lower than that achieved by nonpolarized M0 cells (Fig. 5) prompted us to examine the general role of CASR in *H. ducreyi* uptake by nonpolarized macrophages. Blocking CASR with fucoidan inhibited *H. ducreyi* ingestion by $75 \pm 19.8\%$ (data not shown). During the early stage of infection in humans, *H. ducreyi* cells are likely opsonized by cross-reacting antibodies and complement activation. Therefore, we examined the contribution of CASR in phagocytosis of *H. ducreyi* opsonized with

100% normal human serum. Fucoidan reduced the phagocytosis of opsonized *H. ducreyi* by $59\% \pm 26\%$ (data not shown). Taken together, our results indicate that CASR contribute to phagocytosis of opsonized and nonopsonized *H. ducreyi* by macrophages.

DISCUSSION

Macrophages are dynamic and heterogeneous cells whose phenotypes and functions are shaped by microenvironmental signals during infection. In this study, we investigated macrophage polarization in response to *H. ducreyi* infection. We showed that macrophages in endpoint pustules expressed the M1 markers CD40, CD80, CD86, and HLA-DR as well as the M2 markers CD163, CD200R, and CD206. As has been reported for other bacteria (9, 23, 41), these data indicate that macrophages in different polarization states coexist at sites of *H. ducreyi* infection.

To characterize macrophage polarization during *H. ducreyi* infection, we used MDM as a surrogate for skin macrophages. Similar to lesional macrophages, infected MDM expressed the M1 markers CD40, CD80, CD86, and HLA-DR, the M2c marker CD163, and the M2a marker CD206. Relative to uninfected nonpolarized MDM, *H. ducreyi*-infected MDM exhibited enhanced levels of CD40 and CD80 but reduced levels of CD86 and HLA-DR. The latter was mediated by IL-10, produced by infected MDM in an autocrine manner. CD163 expression also required IL-10. Unlike lesional macrophages, infected MDM did not express the M2a-associated marker CD200R, suggesting that infected MDM do not produce IL-4 or the other M2a-inducing factor, IL-13. *In vitro* differentiated MDM constitutively expressed CD206, which was further enhanced by exogenous IL-4 and dampened by *H. ducreyi* infection. The latter observation is also consistent with the lack of IL-4 or IL-13 production by infected MDM. Although not detected on lesional macrophages, the M1 marker CCR7 was induced on *H. ducreyi*-infected MDM. Therefore, as has been reported for other bacterial infections (30), macrophage polarization following *in vitro* infection with *H. ducreyi* did not completely mimic that observed *in vivo*.

In addition to direct interactions between macrophages and *H. ducreyi*, cytokines, chemokines, other inflammatory and anti-inflammatory mediators, and apoptotic and necrotic cells in the local microenvironment could influence lesional macrophage phenotypes and functions. Neutrophils are the predominant cell type in pustules (19). Phagocytosis of apoptotic neutrophils by macrophages promotes M2 polarization and the resolution of inflammation (14). NK cells are recruited to *H. ducreyi*-infected sites; lesional NK cells could produce IFN- γ (25), which could help to skew lesional macrophages toward M1 polarization. NK cells at infected sites could also promote a M1 phenotype by their ability to preferentially kill M2 macrophages (8). Effector/memory T cells in pustules have the capacity to produce the M1-inducing cytokine IFN- γ and the M2c-inducing cytokine IL-10 (16); lesional T cells could also play an important role in polarizing macrophages.

Polarized macrophages have distinct phagocytic and microbicidal activities. M2 cells are generally more phagocytic but less potent at killing than M1 cells (10, 27, 28, 34, 44). Consistent with previous findings, we showed that in comparison to M1 cells, M2a cells had a reduced capacity to kill *H. ducreyi*, whereas M2c cells had an enhanced capacity to take up the bacteria. The enhanced phagocytosis of *H. ducreyi* by M2c cells is similar to observations made with mycobacteria (32). However, M2c cells were not dif-

ferent from M1 cells in killing *H. ducreyi*. Phagocytosis of bacteria generally facilitates bacterial clearance, but it could also contribute to disease progression if internalized bacteria are not completely killed. Internalized *H. ducreyi* cells were very sensitive to phagocytic killing by all groups of macrophages, and fewer than 5% of the ingested bacteria survived in macrophages 7 h postinfection. This result is consistent with the previously published study showing that *H. ducreyi* cells associate with macrophages but are not found intracellularly during human infections (6).

During macrophage polarization of human MDM, several receptors involved in phagocytosis are differentially upregulated. M1 polarization upregulates the Fc receptor CD64, whereas M2c skewing upregulates the Fc receptors CD16 and CD32 (3). As increased uptake was observed for both unopsonized bacteria and *H. ducreyi* opsonized with autologous serum, the enhanced uptake of *H. ducreyi* by M2c cells does not appear to be mediated by Fc receptors.

Other phagocytic receptors upregulated by M2 polarization include SR and MR (27). We identified CASR as the major nonopsonic phagocytic receptors for *H. ducreyi* by M2c polarized and nonpolarized MDM. Enhanced expression of CASR on M2c polarized macrophages likely increased the efficiency of *H. ducreyi* uptake. CASR also mediated the phagocytosis of nonopsonized *E. coli*, since fucoidan blocked bacterial uptake by nonpolarized MDM (data not shown). This finding is consistent with the reported role of the CASR family members MARCO and SR-A in phagocytosis of *E. coli* by murine macrophages (39, 40, 51). Enhanced ingestion of *E. coli* by M2a cells was completely blocked by the MR antagonist mannan (data not shown). The MR CD206 binds to mannose-rich carbohydrates on the surface of *E. coli*. *H. ducreyi* does not express mannose-containing glycans (36), which likely explains the lack of enhanced phagocytosis of *H. ducreyi* by M2a-polarized macrophages. Due to differences in phagocytic receptor expression and bacterial cell surface structures, M2a and M2c polarized MDM differentially affect phagocytosis of *H. ducreyi* and *E. coli*.

H. ducreyi secretes two proteins, LspA1 and LspA2, that inhibit Fc receptor-mediated phagocytosis in polymorphonuclear leukocyte (PMN)-like and macrophage-like cell lines by blocking activation of Src family protein tyrosine kinases (31). The effect of Lsp proteins on primary macrophages has not been reported. We found that MDM uptake of nonopsonized and opsonized *H. ducreyi* was mainly mediated by SR. General inhibition of protein tyrosine kinase activity by pharmacological agents partially blocks SR-mediated phagocytosis in human MDM (48). Although enhanced expression of SR by M2c macrophages promoted *H. ducreyi* uptake, it is possible that the Lsp proteins also partially dampen SR-mediated internalization of *H. ducreyi* by interfering with Src kinase activation.

Proper polarization of macrophages may influence the ability of the host to control *H. ducreyi* infection. Differential host susceptibility has been observed in experimental *H. ducreyi* infection. When challenged twice with *H. ducreyi*, volunteers tend to resolve infection twice (resolvers) or form pustules twice (pustule formers). Microarray analysis of infected skin obtained from paired resolvers and pustule formers infected a third time for 48 h shows that both groups have a core transcript response to *H. ducreyi* (18). This core response includes transcriptional activation of many genes encoding the common cytokines/receptors, chemokines/receptors, effectors, and costimulatory molecules that are also in-

duced in macrophages in response to bacterial infections (10). Pustule formers tend to have a hyperinflammatory transcript response to *H. ducreyi* compared to the resolvers; several transcripts that are associated with M2 macrophage polarization, such as CD163, IL-1RN, and CCL-22, are differentially upregulated in infected skin from the resolvers (18). Based on this study and our current data on the effects of M1 and M2c polarization on phagocytosis and killing of *H. ducreyi* by MDM, we propose the following hypothesis. During *H. ducreyi* infection, hyperinflammation contributed in part by M1 polarization might lead to immunopathology and bacterial persistence, whereas a balanced M1 and M2 activation program or a timely switch from M1 to M2 polarization leads to resolution of inflammation and bacterial clearance. Additional studies to examine whether differential polarization of macrophages at *H. ducreyi*-infected sites correlates with disease outcomes are in progress.

ACKNOWLEDGMENTS

This study was supported by grant AI059384 to S.M.S. from the National Institutes of Allergy and Infectious Diseases (NIAID). The human challenge trials were supported by grants from the Indiana Clinical and Translational Sciences Institute and the Indiana Clinical Research Center (UL RR052761) and NIAID grant U19 AI31494.

We have no financial relationships to disclose.

We thank Carlos H. Serezani for his critical review of the manuscript, Jessica Walsh for her technical help, and the volunteers for their participation in these studies.

REFERENCES

- Al-Tawfiq JA, Spinola SM. 2002. *Haemophilus ducreyi*: clinical disease and pathogenesis. *Curr. Opin. Infect. Dis.* 15:43–47.
- Al-Tawfiq JA, et al. 1998. Standardization of the experimental model of *Haemophilus ducreyi* infection in human subjects. *J. Infect. Dis.* 178:1684–1687.
- Ambarus CA, et al. 2012. Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. *J. Immunol. Methods* 375:196–206.
- Banks KE, et al. 2008. The enterobacterial common antigen-like gene cluster of *Haemophilus ducreyi* contributes to virulence in humans. *J. Infect. Dis.* 197:1531–1536.
- Banks KE, et al. 2007. *Haemophilus ducreyi* partially activates human myeloid dendritic cells. *Infect. Immun.* 75:5678–5685.
- Bauer ME, Goheen MP, Townsend CA, Spinola SM. 2001. *Haemophilus ducreyi* associates with phagocytes, collagen, and fibrin and remains extracellular throughout infection of human volunteers. *Infect. Immun.* 69:2549–2557.
- Bauer ME, Townsend CA, Ronald AR, Spinola SM. 2006. Localization of *Haemophilus ducreyi* in naturally acquired chancroidal ulcers. *Microbes Infect.* 8:2465–2468.
- Bellora F, et al. 2010. The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes. *Proc. Natl. Acad. Sci. U. S. A.* 107:21659–21664.
- Benoit M, Barbarat B, Bernard A, Olive D, Mege JL. 2008. Coxiella burnetii, the agent of Q fever, stimulates an atypical M2 activation program in human macrophages. *Eur. J. Immunol.* 38:1065–1070.
- Benoit M, Desnues B, Mege JL. 2008. Macrophage polarization in bacterial infections. *J. Immunol.* 181:3733–3739.
- Bowdish DM, Gordon S. 2009. Conserved domains of the class A scavenger receptors: evolution and function. *Immunol. Rev.* 227:19–31.
- Desnues B, Lepidi H, Raoult D, Mege JL. 2005. Whipple disease: intestinal infiltrating cells exhibit a transcriptional pattern of M2/alternatively activated macrophages. *J. Infect. Dis.* 192:1642–1646.
- Fabrick BO, et al. 2009. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood* 113:887–892.
- Fadok VA, McDonald PP, Bratton DL, Henson PM. 1998. Regulation of macrophage cytokine production by phagocytosis of apoptotic and post-apoptotic cells. *Biochem. Soc. Trans.* 26:653–656.
- Fusco WG, et al. 2010. Immunization with the *Haemophilus ducreyi* hemoglobin receptor HgbA with adjuvant monophosphoryl lipid A protects swine from a homologous but not a heterologous challenge. *Infect. Immun.* 78:3763–3772.
- Gelfanova V, Humphreys TL, Spinola SM. 2001. Characterization of *Haemophilus ducreyi*-specific T cell lines from lesions of experimentally infected human subjects. *Infect. Immun.* 69:4224–4231.
- Gordon S, Martinez FO. 2010. Alternative activation of macrophages: mechanism and functions. *Immunity* 32:593–604.
- Humphreys T, et al. 2007. Dysregulated immune profiles for skin and dendritic cells are associated with increased host susceptibility to *Haemophilus ducreyi* infection in human volunteers. *Infect. Immun.* 75:5686–5697.
- Humphreys TL, et al. 2002. Evolution of the cutaneous immune response to experimental *Haemophilus ducreyi* infection and its relevance to HIV-1 acquisition. *J. Immunol.* 169:6316–6323.
- Janowicz DM, et al. 2004. Expression of the LspA1 and LspA2 proteins by *Haemophilus ducreyi* is required for virulence in human volunteers. *Infect. Immun.* 72:4528–4533.
- Janowicz DM, Ofner S, Katz BP, Spinola SM. 2009. Experimental infection of human volunteers with *Haemophilus ducreyi*: 15 years of clinical data and experience. *J. Infect. Dis.* 199:1671–1679.
- Jouanguy E, et al. 1999. IL-12 and IFN- γ in host defense against mycobacteria and salmonella in mice and men. *Curr. Opin. Immunol.* 11:346–351.
- Krysko O, et al. 2011. Alternatively activated macrophages and impaired phagocytosis of *S. aureus* in chronic rhinosinusitis. *Allergy* 66:396–403.
- Leung SY, et al. 2004. Expression profiling identifies chemokine (C-C motif) ligand 18 as an independent prognostic indicator in gastric cancer. *Gastroenterology* 127:457–469.
- Li W, Janowicz DM, Fortney KR, Katz BP, Spinola SM. 2009. Mechanism of human natural killer cell activation by *Haemophilus ducreyi*. *J. Infect. Dis.* 200:590–598.
- Li W, et al. 2010. Role played by CD4+FOXP3+ regulatory T cells in suppression of host responses to *Haemophilus ducreyi* during experimental infection of human volunteers. *J. Infect. Dis.* 201:1839–1848.
- Mantovani A, et al. 2004. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25:677–686.
- Martinez FO, Helming L, Gordon S. 2009. Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* 27:451–483.
- McBride WJ, Hannah RC, Le Cornec GM, Bletchly C. 2008. Cutaneous chancroid in a visitor from Vanuatu. *Australas. J. Dermatol.* 49:98–99.
- Mege JL, Mehraj V, Capo C. 2011. Macrophage polarization and bacterial infections. *Curr. Opin. Infect. Dis.* 24:230–234.
- Mock JR, et al. 2005. *Haemophilus ducreyi* targets Src family protein tyrosine kinases to inhibit phagocytic signaling. *Infect. Immun.* 73:7808–7816.
- Montoya D, et al. 2009. Divergence of macrophage phagocytic and antimicrobial programs in leprosy. *Cell Host Microbe* 6:343–353.
- Moos V, et al. 2010. Impaired immune functions of monocytes and macrophages in Whipple's disease. *Gastroenterology* 138:210–220.
- Mosser DM, Edwards JP. 2008. Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8:958–969.
- Murphy JE, Tedbury PR, Homer-Vanniasinkam S, Walker JH, Ponnambalam S. 2005. Biochemistry and cell biology of mammalian scavenger receptors. *Atherosclerosis* 182:1–15.
- Odumeru JA, Wiseman GM, Ronald AR. 1985. Role of lipopolysaccharide and complement in susceptibility of *Haemophilus ducreyi* to human serum. *Infect. Immun.* 50:495–499.
- Palmer KL, et al. 1998. The immune response to *Haemophilus ducreyi* resembles a delayed-type hypersensitivity reaction throughout experimental infection of human subjects. *J. Infect. Dis.* 178:1688–1697.
- Peel TN, Bhatti D, De Boer JC, Stratov I, Spelman DW. 2010. Chronic cutaneous ulcers secondary to *Haemophilus ducreyi* infection. *Med. J. Aust.* 192:348–350.
- Peiser L, Gough PJ, Kodama T, Gordon S. 2000. Macrophage class A scavenger receptor-mediated phagocytosis of *Escherichia coli*: role of cell heterogeneity, microbial strain, and culture conditions in vitro. *Infect. Immun.* 68:1953–1963.
- Pinheiro da Silva F, et al. 2007. CD16 promotes *Escherichia coli* sepsis through an Fc γ inhibitory pathway that prevents phagocytosis and facilitates inflammation. *Nat. Med.* 13:1368–1374.
- Quiding-Jarbrink M, Raghavan S, Sundquist M. 2010. Enhanced M1

- macrophage polarization in human helicobacter pylori-associated atrophic gastritis and in vaccinated mice. PLoS One 5:e15018. doi: 10.1371/journal.pone.0015018.
42. Rinker SD, et al. 12 September 2012. Permeases of the Sap transporter are required for cathelicidin resistance and virulence of *Haemophilus ducreyi* in humans. J. Infect. Dis. [Epub ahead of print.] doi:10.1093/infdis/jis525.
 43. Saraiva M, O'Garra A. 2010. The regulation of IL-10 production by immune cells. Nat. Rev. Immunol. 10:170–181.
 44. Sica A, Mantovani A. 2012. Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. 122:787–795.
 45. Spinola SM. 2008. Chancroid and *Haemophilus ducreyi*, p 689–699. In Holmes KK, et al (ed), Sexually transmitted diseases, 4th ed. McGraw-Hill, New York, NY.
 46. Spinola SM, Bauer ME, Munson RS Jr. 2002. Immunopathogenesis of *Haemophilus ducreyi* infection (chancroid). Infect. Immun. 70:1667–1676.
 47. Spinola SM, Wild LM, Apicella MA, Gaspari AA, Campagnari AA. 1994. Experimental human infection with *Haemophilus ducreyi*. J. Infect. Dis. 169:1146–1150.
 48. Sulahian TH, Imrich A, Deloid G, Winkler AR, Kobzik L. 2008. Signaling pathways required for macrophage scavenger receptor-mediated phagocytosis: analysis by scanning cytometry. Respir. Res. 9:59.
 49. Ussher JE, Wilson E, Campanella S, Taylor SL, Roberts SA. 2007. *Haemophilus ducreyi* causing chronic skin ulceration in children visiting Samoa. Clin. Infect. Dis. 44:e85–87.
 50. Vakevainen M, Greenberg S, Hansen EJ. 2003. Inhibition of phagocytosis by *Haemophilus ducreyi* requires expression of the LspA1 and LspA2 proteins. Infect. Immun. 71:5994–6003.
 51. van der Laan LJ, et al. 1999. Regulation and functional involvement of macrophage scavenger receptor MARCO in clearance of bacteria in vivo. J. Immunol. 162:939–947.