Latency-Associated Protein Acr1 Impairs Dendritic Cell Maturation and Functionality: A Possible Mechanism of Immune Evasion by Mycobacterium tuberculosis

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Mycobacterium tuberculosis (M. tuberculosis) in latently infected individuals survives and thwarts the attempts of eradication by the immune system. During latency, Acr1 is predominantly expressed by the bacterium. However, whether M. tuberculosis exploits its Acr1 in impairing the host immunity remains widely unexplored. Hence, currently we have investigated the role of Acr1 in influencing the differentiation and function of dendritic cells (DCs), which play a cardinal role in innate and adaptive immunity. Therefore, for the first time, we have revealed a novel mechanism of mycobacterial Acr1 in inhibiting the maturation and differentiation of DCs by inducing tolerogenic phenotype by modulating the expression of PD-L1; Tim-3; indoleamine 2, 3-dioxygenase (IDO); and interleukin 10. Furthermore, Acr1 interferes in the differentiation of DCs by targeting STAT-6 and STAT-3 pathways. Continuous activation of STAT-3 inhibited the translocation of NF-κB in Acr1-treated DCs. Furthermore, Acr1 also augmented the induction of regulatory T cells. These DCs displayed decline in their antigen uptake capacity and reduced ability to help T cells. Interestingly, M. tuberculosis exhibited better survival in Acr1-treated DCs. Thus, this study provides a crucial insight into a strategy adopted by M. tuberculosis to survive in the host by impairing the function of DCs.

Keywords. Mycobacterium tuberculosis; Acr1: alpha crystallin antigen; dendritic cells; immunosuppression.

One-third of the world’s population is infected with Mycobacterium tuberculosis (M. tuberculosis), the etiological agent of tuberculosis [1]. During latency, it tames and tunes the host immunity to successfully survive [2–4]. Consequently, it is of utmost importance to understand the intricate host–pathogen interaction and the strategies M. tuberculosis adopts to circumvent defense mechanism of the host. M. tuberculosis expresses numerous factors in the infected macrophages and DCs to systematically deteriorate the function of both macrophages and DCs [5–9]. Many studies signify the role of M. tuberculosis antigens such as CFP-10, ESAT-6, etc, expressed during active tuberculosis in modulating the function of macrophages and DCs [10–12]. However, whether M. tuberculosis employs its latency-associated antigens in manipulating the function of macrophages and DCs remains broadly undefined. M. tuberculosis small heat shock protein X (sHSPX), also known as Acr1 or HSP16.3, is predominantly expressed in the latent phase of infection and is increased in response to stress [13–16]. Whether Acr1 can affect the DC’s differentiation, maturation, cytokine secretion, and ability to stimulate T cells needs to be investigated.

Tuberculosis granuloma guards the host from dissemination of M. tuberculosis and also facilitates the survival of bacterium [17]. DCs present in granuloma exhibit downregulation of costimulatory and increased coinhibitory molecules expression. Such DCs may
induce tolerance and therefore promote \textit{M. tuberculosis} survi-
vival. Further, an augmented pool of regulatory T cells (Tregs) has
been noted in latency, which suppresses type 17 T-helper
(Th17) cells [18–21]. Th17 cells are quite crucial in providing
immunity against \textit{M. tuberculosis} [22]. Hence, enhancement in
Treg populations and decrease in Th17 cells will lead to im-
paired response against \textit{M. tuberculosis}.

DCs not only activate naive T cells to initiate effector re-
sponse but are also capable of generating tolerance [23]. It has
been demonstrated that \textit{M. tuberculosis} can hide in bone
marrow mesenchymal stem cells [24], thus possibly affecting
the differentiation of monocytes and DCs. Furthermore, DCs
can disseminate mycobacterial infection [25, 26]. For these
reasons, the role of Acr1 was investigated on DCs. We observed
that Acr1 interferes with the maturation and differentiation of
dose-dependent decline in the percentage of CD11c+/CD86+ DCs
and ESAT-6 did not substantially alter the expression of CD80,
CD40, CD86, MHC-II, or PD-L1 on DCs (Supplementary Figure 2
and ESAT-6 of \textit{M. tuberculosis} protein). DCs generated in the presence of Acr1 are depicted in text as
Acr1DCs. On day 3, cultures were replenished with media
(50%) supplemented with GM-CSF, IL-4 and respective pro-
teins. On day 6, cells were harvested and interleukin 12 (IL-12)
was measured in the supernatants.

MATERIALS AND METHODS

Dendritic Cell Culture

BMCs were collected from femurs and tibiae of C3H/HeN
mice. Cells (2 × 10^6/well) were cultured with granulocyte
macrophage colony-stimulating factor (GM-CSF; 1 ng/mL), inter-
leukin 4 (IL-4; 2 ng/mL) and in the presence/absence of either
Acr1, CFP-10, ESAT-6, and BSA (non-\textit{M. tuberculosis} protein).
DCs generated in the presence of Acr1 are depicted in text as
Acr1DCs. On day 3, cultures were replenished with media
(50%) supplemented with GM-CSF, IL-4 and respective pro-
teins. On day 6, cells were harvested and interleukin 12 (IL-12)
was measured in the supernatants.

Allogeneic T-Cell Proliferation

Acr1DCs (C3H/HeN) irradiated at 3000 rad were cocultured with
CD4 T cells (BALB/c) at a ratio of 1:20. After 72 hours, ³H-thymi-
dine (0.5 µCi/well) was added into the cultures. The plates were
harvested after 16 hours and radioactivity incorporated was mea-
sured as counts per minute (cpm) through scintillation counting.
In parallel, cultures were set for 48 hours for cytokine estimation
(interleukin 5 [IL-5] and interferon gamma [IFN-γ]).

Antigen Uptake by Acr1DCs

Acr1DCs were pulsed with horseradish peroxidase (HRP; 100
µg/mL) at 37°C and chased for 30 minutes. Antigen uptake was
arrested by adding chilled phosphate-buffered saline (PBS) at 4°
C. Cells were washed extensively with ice-cold PBS–fetal bovine
serum 1%. HRP uptake was measured by adding OPD (O-
phenylenediamine dihydrochloride) at a concentration of 10 mg/10
mL + 10 µL H_2O_2 to soluble fraction of lysed cells. Reaction
was stopped with 7% H_2SO_4. Plate was read at 492 nm.

For flow cytometry experiments, cells were pulsed with dextran-fluorescein isothiocyanate (FITC) (100 µg/mL) at 37°C
and chased for 30 minutes. Cells kept at 4°C were used as
control. Cells were acquired for the uptake of dextran-FITC
using FACS Calibur and analyzed by FACS Diva software. For
confocal analysis, Acr1DCs were placed on poly-L-lysine-
coated coverslips and imaged using the Nikon A1 Confocal
Laser Microscope system. Analysis was done using Nikon NIS-
Elements C image analysis software.

RESULTS

Acr1 Antigen of \textit{M. tuberculosis} Suppresses the Differentiation
of DCs

Latent form of \textit{M. tuberculosis} infection is one of the major
burden in developing countries [27]. \textit{M. tuberculosis} predomi-
nantly expresses Acr1 during latency [13, 15]. DCs are potent
antigen-presenting cells (APCs) capable of activating naive T cells.
However, upon infection, \textit{M. tuberculosis} can infect DCs and suc-
cessfully hamper their function [5]. Hence, we were curious to
know the impact of Acr1 on the maturation and differentiation of
DCs. We cultured BMCs with various concentrations of Acr1 (0–
9 µg/mL), in the presence of DC differentiating cytokines GM-
CSF and IL-4. We used endotoxin-free recombinant Acr1 in all
the experiments (Supplementary Figure 1, A–C). We observed
dose-dependent decline in the percentage of CD11c⁺/CD86⁺ DCs
with the increase in the concentration of Acr1 (Figure 1A). No
change was noticed in the viability of Acr1DCs (Figure 1B).

The ability of DCs to express major histocompatibility complex
(MHC), costimulatory or coinhibitory molecules, makes them potent cells to modulate T-cell immune response.
Intriguingly, Acr1 induced significant downregulation of
MHC-II (\textit{P} < .05) and costimulatory molecules, namely, CD80
(\textit{P} < .05), CD86 (\textit{P} < .005), and CD40 (\textit{P} < .005) (Figure 1C).
In contrast, significant (\textit{P} < .05) upregulation was noticed in the
coinhibitory molecules PD-L1 (Figure 1C). The ratio of costi-
mutulatory (CD86) to coinhibitory (PD-L1) molecules was also
substantially reduced (\textit{P} < .005; Figure 1D). For methodology
see Supplementary Materials and Methods.

Tim-3 is considered to be a prominent coinhibitory molecule; hence, we also checked the influence of Acr1 in influencing its
expression. CFP-10 and ESAT-6 of \textit{M. tuberculosis} and BSA (non
mycobacterial protein) were used as controls to establish the spe-
cificity of Acr1. Interestingly, Acr1DCs showed significantly
(\textit{P} < .05) higher expression of Tim-3 than did CFP-10, ESAT-6,
and BSA (Figure 1E and 1F). It was also noted that CFP-10, BSA,
and ESAT-6 did not substantially alter the expression of CD80,
CD86, MHC-II, or PD-L1 on DCs (Supplementary Figure 2A and
2B). Thus, it is quite evident from these results that the observed
effect on Acr1DCs is explicitly due to Acr1 of \textit{M. tuberculosis}.

Cytokines such as IL-12 secreted by DCs confer an instru-
mental role in skewing CD4 T cells toward Th1 phenotype [28].
Figure 1. Acr1 suppresses the expression of costimulatory molecules and secretion of interleukin 12 (IL-12) by Acr1 dendritic cells (DCs). A–D, Acr1DCs (CD11c+) generated in the presence of the indicated concentrations of Acr1 (0.3–9 µg/mL) were monitored by flow cytometry: (A) CD86; (B) PI/Annexin V; (C) MHC-II, CD86, CD80, CD40, PD-L1; (D) CD86/PD-L1; (E) Tim-3; (F) integrated mean fluorescence intensity (iMFI) of Tim-3 drawn from flow cytometry data. Data expressed as percentage in inset (A–C), ratio of CD86/PD-L1 (D), and iMFI (E and F) are of 2–3 independent experiments. G, Supernatants were collected from the DCs cultured with (+) or without (−) Acr1 (9 µg/mL) and estimated for the secretion of IL-12 by enzyme-linked immunosorbent assay. Data expressed as pg/mL are representative of 3 independent experiments. Data points show mean ± SD. Data were analyzed with 2-tailed unpaired t test. Acr1(−) and Acr1(+), signify 0 and 9 µg/mL of Acr1. *P < .05, **P < .005, ***P < .0005.
Figure 2. Acr1 impairs antigen uptake by dendritic cells (DCs) and their ability to activate T cells. Acr1DCs were generated in the presence of indicated concentrations of Acr1. Antigen uptake was monitored by (A) flow cytometry; (B and C) confocal microscopy (dextran-fluorescein isothiocyanate [FITC]); (D) spectroscopy (horseradish peroxidase). Data represent mean ± SEM (ng/mL) and are of 2 independent experiments. Statistical analysis was done with Student Newman–Keuls multiple comparison test. ***P < .001. E–G, DCs generated in the presence (9 µg/mL) or absence (0 µg/mL) of Acr1 were cultured with allogeneic T cells. E, Proliferation of T cells is represented as counts per minute (cpm). Supernatants were estimated for (F) interleukin 5 (IL-5) and (G) interferon gamma (IFN-γ). The data represent mean ± SEM (pg/mL) and are representative of 3 independent experiments. Data were analyzed with 2-tailed unpaired t test. *P < .05, **P < .005.
Interestingly, Acr1 induced a significant ($P < .0005$) decrease in the yield of IL-12 (Figure 1G), and therefore may obstruct in the generation of Th1 cells. The overall data (Figure 1A–G) signify that Acr1 debilitates the expression of key molecules that are responsible for the optimum activity of DCs.

**Acr1 Abates the Ability of DCs to Help T Cells**
Acr1DCs were quite distinct phenotypically (CD80hi/CD86hi/CD40hi/MHC-IIhi/PD-L1hi/Tim-3hi) (Figure 1). Hence, we next monitored antigen uptake capacity of such DCs and their ability to help T cells. Our flow cytometry, confocal microscopy, and spectrophotometry data exhibited a significant decrease in the antigen uptake by Acr1DCs (Figure 2A–D). Furthermore, Acr1DCs also significantly ($P < .005$) suppressed the proliferation of T cells (Figure 2E), secretion of IL-5, and IFN-γ (Figure 2F and 2G). Optimal activation of T cells was noticed in control cultures where DCs were generated in the absence of Acr1. In essence, Acr1 impedes the ability of DCs to help T cells.

**Increased Survival of *M. tuberculosis* in Acr1DCs**
DCs can engulf and kill *M. tuberculosis*. Because Acr1DCs exhibited suppressive phenotype and reduced ability to help T cells, we next assessed the killing capacity of Acr1DCs. As compared to the control DCs (-Acr1), Acr1DCs showed reduced ability to kill *M. tuberculosis*. This was evidenced by considerable increase in the *M. tuberculosis* colony-forming units in Acr1DCs (Figure 3).

**Acr1 Induces Tolerogenic Phenotype in DCs**
Immune tolerance is associated with chronic diseases such as tuberculosis and cancer. Besides DC’s crucial contribution in the activation of the immune system, their role is also surfaced in the induction of tolerance [23, 29]. Acr1DCs exhibited increased expression of PD-L1 and Tim-3 (Figure 1C and 1E). PD-L1 and Tim-3 are involved in the generation of tolerance in DCs [30–32]. The influence of tryptophan catabolizing enzyme indoleamine 2, 3-dioxygenase (IDO), IL-10, and transforming growth factor beta (TGF-β) have also been deciphered in DC’s tolerance. Amazingly, we found a significant increase ($P < .01$) in the level of IDO in the Acr1DCs (Figure 4A and 4B). The change was noticed in a dose-dependent manner. Enhancement in the yield of TGF-β and IL-10 ($P < .05$) was also observed (Figure 4C and 4D). IDO and PD-L1 are associated with the induction of Tregs. Remarkably, Acr1DCs also expanded the pool of Foxp3-expressing Tregs (Figure 4E). In conclusion, Acr1DCs expressed enhanced levels of IL-10, TGF-β, and IDO, and promoted Treg generation.

**Acr1 Modulates the Expression of SOCS Molecules**
It has been demonstrated that SOCS-1, 2, and 3 are involved in the activation and differentiation of DCs [33–35]. Interestingly, we found a significant increase of SOCS-3 ($P < .0005$), at both the messenger RNA and protein levels in Acr1DCs (Figure 5A and 5B). However, reduction ($P < .05$) was observed in SOCS-1 and SOCS-2 (Figure 5C and 5D). These results indicate that Acr1 differentially regulate the expression of SOCS molecules, ultimately modulating the JAK/STAT pathway and weakening the function of Acr1DCs.

**Acr1 Activates STAT-3 but Inhibits STAT-6**
SOCS molecules negatively regulate the JAK/STAT pathway [35]. Interestingly, we observed decreased expression and phosphorylation of STAT-6 in Acr1DCs (Figure 6A). In contrast, a substantial increase was observed in the phosphorylation of STAT-3 (Figure 6B). It has been established that STAT-3 is involved in the induction of tolerogenic phenotype of DCs by upregulating PD-L1 [30, 36]. This correlated with increased PD-L1 by Acr1 on Acr1DCs (Figure 1C). No change was seen in STAT-1 (Supplementary Figure 3). These results support substantially that Acr1 retards the activation of DCs.

**Acr1 Inhibits NF-κB Nuclear Translocation**
The DC maturation and cytokines secretion are NF-κB dependent [37]. We observed that Acr1DCs show suppression in
IL-12 secretion (Figure 1G). Hence, we hypothesize that Acr1DCs may be exhibiting reduction in the nuclear translocation of NF-κB (Figure 7). This implies a unique property of Acr1 in attenuating NF-κB activity in Acr1DCs, thereby establishing the role of this latency-associated antigen in suppressing the function of Acr1DCs.

Figure 4. Acr1 induces tolerogenic phenotype in dendritic cells (DCs). A–B, Acr1DCs generated in the presence of indicated concentrations of Acr1 were stimulated with IFN-γ, and whole cell lysate was prepared. A, Western blot analysis indicates indoleamine 2, 3-dioxygenase (IDO). β-Actin was used as housekeeping control. B, Bar diagram indicates mean ± SEM of densitometry analysis of the Western blot in terms of fold change. Data were analyzed with Tukey–Kramer multiple comparisons test. * P < .05, ** P < .01. C–D, Relative expression of transforming growth factor beta (TGF-β) and interleukin 10 (IL-10) in Acr1DCs. The comparison for cytokines by quantitative polymerase chain reaction was done with respect to control DCs (−Acr1). (−) and (+) indicate DCs cultured in the absence and presence, respectively, of Acr1 (9 µg/mL) antigen. The mRNA level was normalized with β-actin. The data represent mean ± SEM of 2 independent experiments. Data were analyzed with 2-tailed unpaired t-test. * P < .05; n.s., nonsignificant. E, Acr1DCs promote the generation of regulatory T cells. Naive CD4+ T cells were cultured with Acr1DCs. The data in the inset of contour plots indicate percentage of CD25+ and Foxp3+ CD4 T cells.
DISCUSSION

In tuberculosis-endemic areas, >90% of healthy persons are latently infected with *M. tuberculosis*, yet do not manifest disease symptoms [38]. Nonetheless, there is always an associated risk of activation of disease in 10%–15% of these individuals. *M. tuberculosis* successfully evades the immune system and can establish its persistence in the host by rapidly altering its gene expression and releasing several factors [39, 40]. Hence, latently infected people act as a reservoir of the *M. tuberculosis* and can always be a potential threat for spreading the disease. Therefore, it is quite crucial to understand the role of factors released during latency by *M. tuberculosis* and their role in impairing the immunity of the host.

Macrophages and DCs are responsible for restricting *M. tuberculosis* infection and establishment of immunity. Even though macrophages can efficiently phagocytose *M. tuberculosis*, the bacterium can methodically antagonize the activity of these cells and thrives in their inimical milieu. DCs are decisive in the initiation of immune responses due to their distinctive competence of activating naive T cells. In the case of *M. tuberculosis* infection, DCs not only fail to eliminate the mycobacterium but surprisingly, also help in spreading the infection [5, 41]. Understanding the mechanism involved in the modulation of the activity of DCs by *M. tuberculosis* antigens, particularly those induced during latency, can provide insight in understanding the immune evasion by the bacterium [42].

Acr1 is predominantly expressed during the dormant stage of *M. tuberculosis*. Whether this antigen is employed by *M. tuberculosis* in exploiting the function of DCs remains unexplored. Therefore, we deciphered the role of Acr1 in influencing the maturation and function of DCs. The following major findings emerged from the current study: Acr1 suppressed the activity of Acr1DCs as evidenced by (i) decrease in the percentage of CD11c/CD86+ cells; (ii) downregulation of MHC-II and costimulatory molecules CD80, CD86, CD40 and

![Figure 5. Acr1 modulates the expression of SOCS molecules. A–D, Acr1 dendritic cells (DCs) were monitored for the expression of SOCS molecules by quantitative polymerase chain reaction and Western blotting. A, Immunoblot of SOCS-3 and β-actin (loading control). (−) and (+) indicate CD11c+ DCs cultured in the absence and in presence, respectively, of Acr1 (9 µg/mL) antigen. Bar graph implies relative expression of (B) SOCS-3, (C) SOCS-1 and (D) SOCS-2. The comparison was done with respective to control DCs (−Acr1). mRNA level was normalized to β-actin mRNA. Data are expressed as mean ± SEM and are representative of 2 independent experiments. Data were analyzed with 2-tailed unpaired t test. *P<.05, ***P<.0005. Abbreviation: n.s., nonsignificant.](image-url)
decrease in IL-12 secretion; (iii) reduced antigen uptake ability of Acr1DCs and reduced T-cell help; (iv) induction of the tolerogenic phenotype (PD-L1hi, IDOhi, Tim-3hi, IL-10hi, TGF-βhi); (v) modulation of SOCS-2/3, STAT-3/6, and NF-κB; (vi) induction of Tregs. To best of our knowledge, this is a first report precisely demonstrating the role of Acr1 in restraining the maturation, differentiation, and functionality of DCs.

The interaction of T cells with DCs plays a fundamental role in eradicating intracellular pathogens. The optimum activation of naive T cells is accomplished by occupancy of the T-cell receptor by the peptide-MHC complex presented on the surface of DCs and delivery of costimulatory signals [43]. The extent of expression of costimulatory molecules is critical in shaping the nature and magnitude of the immune response. Thus, an encounter of peptide-MHC on the surface of DCs can result in 2 discrete events in T cells: (i) proliferation and differentiation into effector cells; and (ii) anergy, a state of unresponsiveness. The expression of costimulatory molecules may impart an imperative role during the course of acute and latent disease and its remission or relapse. Hence, modulation of these molecules by pathogens can help them to evade immune system and establish their existence in the host. We have elaborated the impact of latency-associated antigen Acr1 employed by M. tuberculosis to break the code of conduct of costimulatory molecules (CD80, CD86, and CD40) to enhance its survival and establishing infection. CD80, CD86, and CD40 are considered to be key molecules responsible for the activation of T cells. M. tuberculosis is known to impair the maturation of DCs.

Figure 6. STAT proteins are differentially activated in Acr1 dendritic cells (DCs). A–B, Whole cell lysate of Acr1DCs generated in the presence of Acr1 (0, 3, 9 µg/mL) were immunoblotted with (A) anti-pSTAT-6 and STAT-6 antibodies, and (B) anti-pSTAT-3 and STAT-3 antibodies. β-Actin was taken as a loading control. (−) and (+) indicate CD11c+ DCs cultured in the absence and presence, respectively, of Acr1 antigen. The data are representative of the 2 independent experiments. Numbers indicate normalized fold change.

Figure 7. The Acr1 inhibits the nuclear translocation of NF-κB. Acr1 dendritic cells (DCs) were stimulated with lipopolysaccharide (LPS) and interferon gamma (IFN-γ) and nuclear extract was prepared. Nuclear translocation of NF-κB was performed by electrophoretic mobility shift assay. Cells not treated with LPS and IFN-γ were taken as unstimulated control (Un). Data are represented as 1 of 2 independent experiments.
expressing DC-SIGN\textsuperscript{lo}/CD86\textsuperscript{hi} and their ability to activate T cells [44]. There are several reports signifying the role of mycobacteria in downregulating the expression of CD80, CD86, and CD40 on APCs, but we have for the first time dissected the role of Acr1, responsible for this phenomenon. Further, Acr1DCs also exhibited an augmented level of PD-L1 and Tim-3. The role of PD-L1 is correlated with the generation of tolerance. Moreover, Acr1DCs were phenotypically similar (CD80\textsuperscript{lo}/CD86\textsuperscript{hi}/CD40\textsuperscript{lo}/MHC-II\textsuperscript{lo}/PD-L1\textsuperscript{hi}) to the one present in chronic tuberculosis granulomas [20]. Such DCs might induce local tolerance and shield mycobacteria for its survival from immune cells. Furthermore, we also observed that Acr1DCs supported the generation of Tregs. The role of PD-L1\textsuperscript{hi}/Tim-3\textsuperscript{hi}/TGF-\beta\textsuperscript{hi} DCs has been implicated in the induction of Tregs. Tregs generated after \textit{M. tuberculosis} infection can inhibit the activity of Th17 cells, obstruct the early recruitment of effector T-cells at the place of infection, and, therefore, support the mycobacterial survival [45]. Acr1DCs also displayed impaired function, as evidenced by decrease in their antigen uptake capacity and reduced ability to induce the proliferation of T cells and IFN-\gamma release. The role of IFN-\gamma secreted by Th1 cells is important in imparting protection against \textit{M. tuberculosis}. Low T-cell activity might also be explained by the CD86\textsuperscript{lo}/PD-L1\textsuperscript{hi} phenotype of Acr1DCs, which is reported in chronic diseases [30, 46]. In essence, these findings suggest that \textit{M. tuberculosis} employs its Acr1 to make DCs incapable of optimally activating T cells and crafting ambient conditions for its survival.

We further substantiated the role of Acr1 in suppressing the function of Acr1DCs by dissecting its mechanism. Recently, it has been reported that SOCS protein can be upregulated in monocytes, macrophages, and DCs by \textit{M. tuberculosis} [33, 47]. SOCS molecules act as a negative regulator of the JAK/STAT pathway [35]. There was a considerable reduction in the expression of SOCS-2 whereas an increase in phosphorylation of STAT-3 was observed in Acr1DCs. Silencing of SOCS-2 inhibits the activity of DCs by stimulating hyperphosphorylation of STAT-3 [48]. Continuous activation of STAT-3 inhibits the nuclear translocation of NF-\kappaB [30, 35]. It has been reported that a decrease in the phosphorylation of STAT-6 reduces the responsiveness of IL-4 and thus inhibits the activation of DCs [49]. We also observed a significant increase in the expression of SOCS-3, which can be explained by augmented IL-10 but low IL-12 secretion (Figure 4D). DCs overexpressing SOCS-3 produce a low quantity of IL-12 but a high amount of IL-10 with tolerogenic phenotype [50]. All the pathways shown to be perturbed due to Acr1 treatment of DCs also exist in other phagocytic cells and APCs such as macrophages [23, 39]; therefore, \textit{M. tuberculosis} can also deteriorate macrophage activity. Thereby, the bug escapes intracellular destruction, multiplies, and disrupts the function of APCs.

Finally, this study suggests that Acr1 of \textit{M. tuberculosis} successfully impairs the function of DCs. Consequently, \textit{M. tuberculosis} may establish its persistence in the host. Hence, neutralizing the activity of Acr1 by either vaccine or drug can be quite crucial to circumvent the impairment of the function of the host DCs. A vaccine should evoke immunity against Acr1, whereas designing a kinase inhibitor offers an attractive drug target [16].

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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