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Infection of Human Dendritic Cells with a Mycobacterium tuberculosis sigE Mutant Stimulates Production of High Levels of Interleukin-10 but Low Levels of CXCL10: Impact on the T-Cell Response

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The Mycobacterium tuberculosis genome encodes 13 sigma factors. We have previously shown that mutations in some of these transcriptional activators render M. tuberculosis sensitive to various environmental stresses and can attenuate the virulence phenotype. In this work, we focused on extracytoplasmic factor σE and studied the effects induced by the deletion of its structural gene (sigE) in the infection of human monocyte-derived dendritic cells (MDDC). We found that the wild-type M. tuberculosis strain (H37Rv), the sigE mutant (ST28), and the complemented strain (ST29) were able to infect dendritic cells (DC) to similar extents, although at 4 days postinfection a reduced ability to grow inside MDDC was observed for the sigE mutant ST28. After mycobacterium capture, the majority of MDDC underwent full maturation and expressed both inflammatory cytokines, such as tumor necrosis factor alpha, and the regulatory cytokines interleukin-12 (IL-12), IL-18, and beta interferon (IFN-β). Conversely, a higher level of production of IL-10 was observed in ST28-infected MDDC compared to H37Rv- or ST29-infected cell results. However, in spite of the presence of IL-10, supernatants from ST28-infected DC induced IFN-γ production by T cells similarly to those from H37Rv-infected DC culture. On the other hand, IL-10 impaired CXCL10 production in sigE mutant-infected DC and, indeed, its neutralization restored CXCL10 secretion. In line with these results, supernatants from ST28-infected cells showed a decreased capability to recruit CXCR3+ CD4+ T cells compared to those obtained from H37Rv-infected DC culture. Thus, our findings suggest that the sigE mutant-induced secretion of IL-10 inhibits CXCL10 expression and, in turn, the recruitment of activated-effector cells involved in the formation of granulomas.

Tuberculosis remains a significant disease worldwide despite the availability of the Mycobacterium bovis bacillus Calmette-Guerin vaccine and antibiotic treatments (21). Attempts over the years to design a vaccine more protective than bacillus Calmette-Guerin have so far been unsuccessful. Thus, knowledge of Mycobacterium tuberculosis strategies to avoid destruction of host immunity and to induce a chronic disease is instrumental for setting new therapeutic or preventive interventions.

An important feature of M. tuberculosis and other pathogens is their ability to adapt to the hostile and changing environment found during infection by tuning gene transcription. Indeed, M. tuberculosis is able to regulate gene expression in response to environmental stimuli through different transcriptional regulators (19). Among these, sigma factors play a major role in controlling the expression of both essential housekeeping genes and genes induced in response to different environmental stresses. Thus, in the last few years sigma factors have become an important subject of investigation in the attempt to understand M. tuberculosis molecular physiology and mechanisms of pathogenicity. The M. tuberculosis genome encodes 13 sigma factors (3), most of them shown to be involved in virulence (19). Interestingly, σE, encoding a member of the sigma factor family of extracytoplasmic functions (ECF), is one of the first genes induced by M. tuberculosis after phagocytosis (26).

We have previously shown that sigE disruption renders M. tuberculosis more sensitive to heat shock, sodium dodecyl sulfate, and various oxidative stresses and causes an attenuation of its virulence. Indeed, the sigE mutant was defective in the ability to grow inside both human and murine nonactivated macrophages and showed a higher sensitivity to killing activity of activated murine macrophages (20). In line with these observations, the virulence of the sigE mutant was strongly attenuated in BALB/c and severe combined immunodeficient (SCID) mice (18). Based on these observations we extended our study of the ability of this mutant to infect monocyte-derived dendritic cells (MDDC) and to induce their maturation. We decided to focus on dendritic cells (DC), since they represent a bridge between the innate and adaptive immune responses.

DC are highly represented in sites of M. tuberculosis infection at the onset of the inflammatory response (10, 27, 31). We have previously shown that M. tuberculosis-infected DC are primarily involved in inducing an antimycobacterial T-cell immune response (9). After interacting with the pathogen, DC
mature and acquire the ability to stimulate T cells through surface expression of major histocompatibility complex (MHC) and costimulatory molecules as well as through secretion of immunoregulatory cytokines, such as interleukin-12 (IL-12) and type I interferon (IFN) (9). We have also shown that after *M. tuberculosis* infection, DC down-regulated CCR5 and up-regulated the CCR7 necessary for homing to secondary lymphoid organs (13). Moreover, following interaction with *M. tuberculosis*, DC showed rapid and robust production of different inflammatory chemokines that may regulate the recruitment of different leukocyte populations expressing CCR5 and/or CXCR3, such as immature DC, monocytes, macrophages, activated T lymphocytes, and natural killer cells, to infected lung tissue (13).

Given the role played by DC in initiating and regulating a protective T-cell response against *M. tuberculosis*, we sought to characterize the infection of human MDDC with the sigE mutant, comparing its uptake and growth in DC with those of the isogenic wild-type strain H37Rv or the complemented strain (ST29). A similar comparison was extended to DC maturation and cytokine and chemokine expression from infected DC and their effects on IFN-γ production and chemotaxis, which could affect the migration of T cells into infected lung tissues. Some differences in the production of IL-10 and CXCL10 were observed, suggesting that the sigE mutant and the wild-type *M. tuberculosis* strains differentially tune the host immune response.

**MATERIALS AND METHODS**

**Antibodies (Abs) and other reagents.** Monoclonal antibodies (Abs) specific for CD1a, CD14, CD54, CD86, CD83, HLA-DR, HLA-I, CCR5, CXCR3, immunoglobulin G1 (IgG1), and IgG2a (BD Pharmingen, San Diego, CA) were used as direct conjugates for fluorescein isothiocyanate or phycoerythrin. Recombinant human soluble IL-10 receptor (sIL-10R), purchased from R&D Systems (Abingdon, United Kingdom), was used at 5 μg/ml to preincubate DC for 1 h before the infection. Anti-Toll-like receptor 2 MAb (a kind gift of T. Espevik) and anti-DC-Sig MAb (a kind gift of Y. van Kooyk) and their isotype controls were used at 10 μg/ml to incubate DC for 30 min before the infection. Where indicated, lipopolysaccharide (LPS) from *Escherichia coli* (Escherichia coli O111:B4, Sigma Chemical Company, St. Louis, MO) was used at 1 μg/ml.

**MDDC and *M. tuberculosis* infection.** MDDC were prepared as previously described (9). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from freshly collected buffy coats obtained from healthy voluntary blood donors (Blood Bank of University “La Sapienza,” Rome, Italy) by density gradient centrifugation using Lympholyte-H (Cedarlane, Hornby, Ontario, Canada). Monocytes were purified by positive sorting using anti-CD14-conjugated magnetic microbeads (Miltenyi, Bergisch Gladbach, Germany). The recovered cells were >99% CD14+, as determined by flow cytometry with anti-CD14 antibody. MDDC were generated by culturing monocytes in 6-well tissue culture plates (Costar Corporation, Cambridge, MA) with 25 ng/ml granulocyte-macrophage colony-stimulating factor and 1,000 U/ml of IL-4 (R&D Systems) for 5 days at 0.5 × 10⁶ cells/ml in RPMI 1640 (BioWhittaker Europe, Verviers, Belgium) supplemented with 2 mM l-glutamine and 15% fetal calf serum (FCS; BioWhittaker Europe). No antibiotics were added to the cultures. At day 5 the cells were 70–80% CD1a, 95% CD14+ 80% CD1a, and 95% CD14+. Bacterial suspensions, at a multiplicity of infection (MOI) of 1 to 10 bacteria/cell, were added on DC, and after 16 h of incubation at 37°C, the cultures were washed (three times) with RPMI 1640 (9). MDDC were centrifuged at 150 × g for 10 min to selectively spin down cells, while extracellular bacteria remained in the supernatants. Cells were resuspended in complete medium and cultured for the times indicated in each experiment.

**Bacterial strain, media, and growth conditions.** The sigE mutant strain (ST28) and the complemented strain (ST29) used in this study were constructed as described in a previous work (20). ST28 was obtained by disrupting sigE with a hygromycin resistance cassette in H37Rv; ST29 was obtained by integrating a DNA fragment containing sigE with its upstream region on the ST28 chromo-
bacterially stimulated DC cultures were diluted 1:5 in RPMI 1640 containing 0.5% BSA and were added to the bottom chamber of the transwell, and $2 \times 10^5$ T cells were added to the top chamber (input). Plates were incubated 4 h at 37°C. The numbers of migrated cells as well as those of the input cells were evaluated by flow cytometry (FACSCalibur; Becton Dickinson) by 1 min of acquisition at a flow rate of 100 $\mu$m/min with continuous mixing. All values of cell migration were determined by subtracting the value obtained from the spontaneous migration of T cells toward RPMI 1640 containing 0.5% BSA. Results of the migration assay are presented as a percentage of the cells that migrated from the input. To block Th1 cell chemokine receptors CXCR3 and CCR5, Th1 cells were incubated 1 h at 37°C before a migration assay was performed with saturating amounts (10 pg/ml) of CXCL10 and CCL3 (Peprotech) (13), respectively. As a positive control, migration of Th1 cells was evaluated for CCL3 and CXCL10 recombinant proteins (100 ng/ml). All experimental points were performed in duplicate.

Statistical analysis. Statistical analysis was calculated using a two-tailed paired-data Student $t$ test. A $P$ value of <0.05 was considered statistically significant.

RESULTS

Infection of human DC with M. tuberculosis H37Rv or sigE mutant ST28. Initial studies were designed to compare the infectivity of the M. tuberculosis wild type (H37Rv) with those of the sigE mutant ST28 and the complemented strain ST29 in MDDC. Immature MDDC were infected at increasing MOIs, starting at 1, 5, and 10 CFU/cell (Fig. 1A). The percentage of infected cells was measured by acid-fast staining 16 h after infection. No significant differences were observed between DC cultures infected with the three strains at all tested MOIs (Fig. 1A). Cell viability was evaluated by both phase-contrast light microscopic examination and the trypan blue exclusion method (data not shown). Infection of DC with strain H37Rv, ST28, or ST29 at an MOI of 1 and 5 apparently did not strongly affect cell viability during a 4-day follow-up period, whereas DC infected at an MOI of 10 showed high mortality after 3 days of infection. From these data we chose the MOI of 5 for infection of DC in the subsequent experiments.

To determine whether sigE disruption affects the ability of M. tuberculosis to grow and survive in DC, H37Rv, ST28, and ST29 infections were studied over a 4-day period and evaluated by counting the CFU (Fig. 1B). At various times after infection (days 0, 2, and 4), DC were lysed and samples were plated to determine the number of intracellular CFU. Attempts to perform longer experiments were unsuccessful. During the first 2 days, all three strains grew similarly inside DC, as the number of CFU increased about 10-fold. Between day 2 and day 4, an arrest of ST28 growth was observed even though the differences in the number of CFU among the three strains were not statistically significant.

Up-regulation of markers specific to mature DC following H37Rv or ST28 infection. The possibility that the sigE mutant ST28 may exert a differential effect on DC maturation was also evaluated. DC were infected with H37Rv or ST28 (MOI of 5) for 24 h, and cell surface expression of CD83, B7.2 (CD86), CD54, HLA-DR, and HLA-I was examined. Both H37Rv- and ST28-infected DC showed enhanced expression of costimulatory and adhesion molecules CD86 and CD54 as well as an evident increase in CD83 levels (Fig. 2). Similarly, both strains were able to induce HLA-DR and HLA-I expression, although ST28-infected DC showed a higher number of HLA molecules than H37Rv-infected DC, as evaluated by the MFI.

Cytokine secretion by H37Rv- or ST28-infected DC. Despite the fact that the expression patterns of DC activation and maturation markers induced following infection with ST28 were similar to those obtained after infection with H37Rv, we investigated the possibility that the mutant might have a different ability to stimulate cytokine production. Cell culture supernatants were collected at 24 h after infection with H37Rv or ST28, and the expression of different cytokines, such as IL-12, IL-18, tumor necrosis factor alpha (TNF-$\alpha$), and IL-10, was analyzed by ELISA (Fig. 3).

H37Rv and ST28 infection stimulated DC to secrete comparable levels of IL-12, IL-18, and TNF-$\alpha$ (Fig. 3A). However, DC infected by ST28 produced threefold-higher amounts of IL-10 compared to the secretion from H37Rv- or ST29-infected MDDC (Fig. 3B). In all DC cultures obtained from six different donors, the levels of IL-10 induced by ST28 infection were higher than those observed following H37Rv infection (Fig. 3C).

The expression of IFN-$\beta$, another regulatory cytokine, was also investigated. This analysis was performed by real-time RT-PCR, a sensitive technique able to detect low-copy-num-
ber mRNAs. For this purpose, RNA was isolated from DC at 8 and 16 h after infection and mRNA levels were quantified by real-time RT-PCR using primers specific for sequences within the coding region of IFN-β. Both strains induced IFN-β expression 8 or 16 h after infection (Fig. 4).

FIG. 2. Analysis of cell surface phenotypes of H37Rv- or ST28-infected DC. (A) Cells were infected for 24 h at an MOI of 5, and the cell surface phenotypes were analyzed by flow cytometry. A total of 5,000 cells were analyzed per sample. "IgG1" represents the MFI values obtained from control cells (Ctr) or H37Rv- or ST28-infected DC stained with isotype control Abs. MFI from three independent experiments ± SEs are shown. (B) Representative fluorescence-activated cell sorter profiles of CD83 expression in control cells or H37Rv- or ST28-infected DC are shown. Unstimulated and infected cells stained with a control antibody are represented by the M1 bar.

Stimulation of T-cell IFN-γ production by cytokines secreted from H37Rv or ST28 infected DC. Next we investigated whether the cytokines produced by H37Rv- or ST28-infected DC were able to induce IFN-γ production from T cells. For these experiments, purified T cells were initially stimulated

FIG. 3. Cytokine production following H37Rv or ST28 infection. (A) Supernatants from H37Rv- and ST28-infected DC cultures were collected after 24 h and analyzed by ELISA for IL-12, IL-18, and TNF-α. The results represent the means ± SEs of four separate experiments. Ctr, control. (B) Supernatants from H37Rv-, ST28-, and ST29-infected DC cultures were collected after 24 h and analyzed for IL-10 by ELISA. *, P = 0.014 (H37Rv versus ST28); **, P = 0.009 (ST28 versus ST29). (C) Individual data for IL-10 production following H37Rv or ST28 infection from six donors are presented.
with plate-bound anti-CD3 MAbs and cultured in the presence of 100 U/ml IL-2 for 5 days. IL-2 was removed from T cells 16 h before stimulation with supernatants from DC cultures collected at 24 h after infection. T cells were incubated for 24 h, and secreted IFN-γ levels were analyzed by ELISA. As a positive control, T cells were also stimulated with IL-12 (20 ng/ml). In spite of the high amounts of IL-10, supernatants from ST28-infected DC induced IFN-γ synthesis in T cells at levels slightly higher than those induced by supernatants from H37Rv-infected cells (Fig. 5).

Chemokine expression by H37Rv- or ST28-infected DC.

Since we have previously shown that CCR5- and CXCR3-binding chemokine expression was induced in DC during M. tuberculosis infection (13), we extended the analysis of these chemokines in ST28-infected DC. Total cellular RNA was isolated, and chemokine gene expression was analyzed by RPA (Fig. 6A). After 20 h of stimulation, H37Rv and ST28 similarly up-regulated CCL2, CCL3, CCL4, CCL5, and CXCL8 mRNA expression. Interestingly, reduced expression of transcripts coding for CXCL10 was observed in DC infected with ST28 compared to H37Rv-infected DC results.

The concentration of CCL3 and CXCL10 was evaluated by ELISA in H37Rv- and ST28-infected DC supernatants 24 h after the infection (Fig. 6B and C). No differences in CCL3 production were found in the supernatants of DC infected with the two strains (Fig. 6B), while reduced production of CXCL10 was observed in ST28-infected DC cultures compared to those obtained from H37Rv- or ST29-infected cultures (Fig. 6C).

Since it was previously demonstrated that IL-10 is a potent inhibitor of CXCL10 production (29), we tested whether CXCL10 expression could be rescued in sigE mutant-infected MDDC blocking IL-10 activity (Fig. 6). DC cultures were pretreated for 1 h with sIL-10R before H37Rv or ST28 infection. After 20 h, total RNA was extracted and chemokine gene expression was analyzed (Fig. 6A). The addition of sIL-10R did not affect RNA expression of any analyzed chemokines in H37Rv-infected DC. Conversely, sIL10R treatment was able to rescue CXCL10 expression in ST28-infected DC. These results were also confirmed at the protein level; the pretreatment with sIL10R restored CXCL10 production in ST28-infected DC without affecting CCL3 secretion (Fig. 6B and C). A slight increase in CXCL10 production was found in DC infected with H37Rv in the presence of sIL10R (Fig. 6C).

Recruitment of CD4+ cells by chemokines induced by H37Rv or ST28 infection. CXCL10 is known to function as a chemotactic factor for CD4+ cells, which represent the major IFN-γ-producing leukocytes in granulomas (13). Since ST28-infected cells produced less CXCL10, we hypothesized that their supernatants could have a reduced capacity to recruit Th1 type cells. To verify this hypothesis, supernatants from H37Rv- or ST28-infected cells were used to stimulate Th1 cell migration. The percentage of T cells responding to chemokines present in infected DC supernatants was evaluated. For migration experiments, a Th1-specific cell line established from PBMC by using whole-cell heat-inactivated M. tuberculosis as an antigen was used as previously described (32). As expected, supernatants from DC infected with ST28 showed a significant reduction of Th1 cell migration compared to that induced by the chemokines released from DC stimulated with H37Rv (Fig. 7). The neutralization of IL-10 through the addition of sIL10R restored the chemotactic properties of the supernatants from sigE mutant ST28-infected MDDC cultures and increased that of H37Rv. The saturation of CXCR3 receptor with CXCL10 reduced Th1 cell migration nearly to the levels obtained with control supernatant, while the blocking of CCR5 receptor with CCL3 pretreatment did not affect Th1 cell migration toward supernatants from ST28-infected DC cultures. In line with these results, Th1 cells showed enhanced migration toward CXCL10, whereas a low migratory response was induced by CCL3. Together, these results indicate that the chemotactic activity in H37Rv- and ST28-infected DC supernatants is mainly attributable to CXCL10.

**DISCUSSION**

The present study was designed to characterize DC responses to infection with M. tuberculosis H37Rv wild type and the sigE-mutant ST28 with the aim of exploring the contribution of the ECF sigma factor σE to mycobacterial interactions with DC, key cells for the regulation of the immune response. The data presented here reveal that an M. tuberculosis mutant (ST28) lacking σE is still able to infect DC similarly to its isogenic wild-type parent strain (H37Rv) or its complemented strain (ST29), although its ability to multiply inside DC is reduced between day 2 and day 4 of the infection. These results...
are in line with previous data showing that this sigE mutant has a reduced ability to growth inside both human and murine nonactivated macrophages and is more sensitive than the wild type to the killing activity of activated murine macrophages (20).

Moreover, we extended our analysis to the expression of cell surface markers on infected MDDC (Fig. 2). Both H37Rv and ST28 strains induced the maturation of DC, as evaluated through the expression of CD83, CD86, and CD54 markers. Interestingly, although both M. tuberculosis strains induced a high level of MHC class II and class I, DC infected with the sigE mutant ST28 showed a higher increase of both MHC molecule levels. Thus, our results suggest that DC infection with both M. tuberculosis strains resulted in the direct maturation of DC characterized by the expression of molecules involved in antigen presentation and in T-cell stimulation.

It is well recognized that cytokine and chemokine production released from DC in response to microbial triggers is critical in modulating host immune responses (4, 22). In particular, TNF-α production is an important early event that leads to granuloma formation and a protective host immune response against tuberculosis (5). Interestingly, we observed robust TNF-α production from DC infected with both mycobacterial strains. Likewise, a typical pattern of cytokines promoting Th1 polarization was observed for DC after H37Rv or ST28 infection. Indeed, the contact between M. tuberculosis strains and MDDC resulted in the expression of IL-12, IL-18, and type I IFN. All together, these results confirmed our previous observations obtained with DC infected with H37Rv, showing that DC are engaged in inducing T cells by virtue of their production of Th1- and IFN-γ-inducing cytokines and expression of costimulatory molecules (9, 24).

The analysis of IL-10 production revealed a striking difference between the wild-type and sigE mutant strains, as the latter elicited a robust production of this cytokine when it infected DC. This increased IL-10 release could be related to the lack of ECF sigma factor σ², since the complemented strain induced IL-10 production similarly to H37Rv. The biological impact of this differential expression of IL-10 was evaluated by studying the properties of supernatants from DC infected with both strains to stimulate IFN-γ production from T cells. An interesting result was obtained indicating that the high IL-10 levels found in ST28-infected DC supernatants did not impair the production of IFN-γ from T cells. This result is in line with studies showing that under certain conditions IL-10 can exert stimulatory effects on CD4+ and CD8+ T cells, which may lead to increased IFN-γ production after their activation (14, 15). Moreover, since Fortsch and colleagues have shown that IL-10 converts DC into macrophage-like cells able to

![Fig. 6. Chemokine expression in H37Rv- or ST28-infected DC.](image)

(A) Total cellular RNA was isolated and analyzed by RPA 20 h after infection or LPS treatment. tRNA was used as an internal negative control of the RPA. Data are representative of an RPA experiment, which was repeated an additional two times with RNA extracted from different donors. Ctr, control. (B) Cell culture supernatants were col

lected 24 h after infection with H37Rv and ST28 and analyzed for CCL3 production. Where indicated, sIL-10R (5 μg/ml) was added to DC cultures 1 h before infection in order to block the effects induced by IL-10 release. The results represent the means ± SEs of four independent experiments. C) Supernatants from H37Rv-, ST28-, and ST29-infected DC were analyzed for CXCL10 production. The results represent the means ± SEs of six independent experiments. *, P = 0.005 (H37Rv versus ST28); **, P = 0.042 (ST28 versus ST28 plus sIL-10R).
inhibit the intracellular growth of \textit{M. tuberculosis} (6), it is possible that the increased release of IL-10 could also promote antibacterial activity in ST28-infected DC, leading to arrested mycobacterial growth observed 4 days after infection. Unfortunately, the inability to prolong the time of infection in DC does not allow us to investigate whether the arrested growth of ST28 observed 4 days after infection causes a significant reduction of mycobacterial replication at longer time points. However, all together these results should lead to a revaluation of the IL-10 immunosuppressive activities implicated in the development and/or reactivation of mycobacterial disease.

A complex series of interactions between various cell populations controls and contains the \textit{M. tuberculosis} infection and prevents its reactivation (21). Natural killer cells, \(\gamma/\delta\) T lymphocytes, and \(\alpha/\beta\) T lymphocytes of CD4 and CD8 phenotypes are recruited in a sequential order after \textit{M. tuberculosis} infection. All these cells share potential cytolytic activity and are able to produce IFN-\(\gamma\), which plays a central role in the host defense against \textit{M. tuberculosis}. In particular, T cells are recruited to the site of infection for granuloma formation through a gradient of CCR5- and CXCR3-binding chemokines. No significant differences were observed at the mRNA level between the expression levels of different chemokines in H37Rv- and ST28-infected DC. A significant reduction of chemotactic properties was observed in ST28-infected DC compared to those observed with the H37Rv counterpart. A complete rescue of the chemotactic properties of supernatants from ST28-infected DC was obtained by the addition of sIL-10R, indicating that CXCL10 has a major role in \textit{M. tuberculosis}-induced Th1 cell chemotaxis. Moreover, blocking of CXCR3 dramatically reduced the ability of H37Rv-stimulated DC supernatants to stimulate chemotaxis in Th1 cells, indicating that CXCL10 exerted a major role in \textit{M. tuberculosis}-induced Th1 cell chemotaxis. Thus, based on these results and given the role played by chemokines in the recruitment and selective homing of activated-effector cells into the site of \textit{M. tuberculosis} infection to form the granuloma, a reduced production of CXCL10 by ST28-infected DC might cause impaired migration of the activated T cells in the infected lung and, in turn, reduce production of IFN-\(\gamma\) in situ. Interestingly, in transgenic mice overexpressing IL-10, \textit{M. tuberculosis}-induced lung lesions were characterized by few lymphocytes and the increased presence of macrophages and neutrophils that resulted in a net reduction of IFN-\(\gamma\) levels within the lungs (30). In line with these observations, we have recently observed that lung granuloma found in ST28-infected mice contained few lymphocytes and no giant cells (18), further supporting the hypothesis that IL-10 controls the inflammation level in lung lesions and, in turn, limits collateral damage by tuning the CXCL10-induced migration of activated T cells.

In a previous work we showed that \(\sigma^{E}\) is involved in the transcriptional response to detergent-induced surface stress.
and that several genes under its regulation encode surface proteins of unknown functions, proteins involved in fatty acid degradation, and proteins involved in mycolic acid biosynthesis (20). From these data it is possible to speculate that the sigE mutant bacterial surface presents some peculiarity enabling this strain to interact with DC in a different manner than wild-type M. tuberculosis and determining the induction of a specific cytokine-chemokine profile. In agreement with this hypothesis, it has been recently observed that the different levels of virulence observed among mycobacterial strains may, at least in part, be attributable to the different lipid compositions of their surfaces, leading to subversion of the host’s protective immune response through induction of specific patterns of cytokines (16, 17, 23). Thus, it would be important to identify the molecular mechanism by which the sigE mutant induces a specific cytokine-chemokine profile. Preliminary experiments performed with heat-inactivated mycobacteria indicate that bacterial viability is not necessary to induce IL-10 expression in ST28-infected MDDC (data not shown). These observations suggest that ST28 could present modifications in cell envelope structure favoring the interaction with cellular molecules known to promote robust IL-10 production, such as DC-Sign, Toll-like receptor 2, and mannose receptor (1, 7, 8, 9). Thus, it has been recently observed that the different patterns of cytokines (16, 17, 23). Thus, it would be important to determine the induction of a specific cytokine-chemokine profile.

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**REFERENCES**


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