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Differential Monocyte Activation Underlies Strain-Specific *Mycobacterium tuberculosis* Pathogenesis

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In vitro infection of monocytes with *Mycobacterium tuberculosis* HN878 and related W/Beijing isolates preferentially induced interleukin-4 (IL-4) and IL-13, which characterize Th2 polarized immunity. In contrast, CDC1551 induced more IL-12 and other molecules associated with phagocyte activation and Th1 protective immunity. The differential cytokine-chemokine response was mediated by extracted lipids, suggesting that these molecules regulate host responses to infection.

Specific clinical isolates of *Mycobacterium tuberculosis* may be differentially pathogenic and vary in the ability to cause disease in humans. Clinical isolate CDC1551 is characterized by an unusually high rate of seroconversion but a low number of cases of active disease (20). In contrast, HN878 may be associated with an unusually high proportion of active cases of disease and a high frequency of extrapulmonary disease (18). Previously we showed that CDC1551 elicits an early and vigorous cytokine response in the infected mouse lung (10). In contrast HN878 failed to induce a prompt and effective Th1-type immune response (11).

To understand the differential activation of host immunity by CDC1551 and HN878 at the molecular level, human peripheral blood mononuclear cells isolated from healthy donors (12) were infected with either clinical isolate (9) at a multiplicity of infection (MOI) of 5:1 or stimulated at 1:50 with the total chloroform-methanol lipid extract from each strain (17). The responses to bacilli or their lipid products were compared in cells from the same donor. At 5 to 42 h, total cellular RNA was isolated with TriReagent (Molecular Research Center, Inc.), reverse transcribed into cDNA, and hybridized to gene-specific cDNA spotted on GEArray Q series membranes (SuperArray, Bethesda, Md.). Two commercial 96-gene arrays were used: human inflammatory cytokine receptors (catalog no. HS-015N-12) and common cytokines (catalog no. HS-003N12). The ScanAlyze 2 program was used for image analysis, and a GEArrayAnalyzer was used to process the raw data. Both CDC1551 and HN878 induced up-regulation of a large number of cytokines, chemokines, and their receptor genes relative to the levels observed in uninfected cells.

Host cell genes differentially induced (more than twofold) by the two strains after infection or lipid stimulation are shown in Table 1. The proinflammatory cytokines interleukin-1α (IL-1α), IL-10, and IL-12α/β, the chemokine macrophage inflammatory protein 3α (MIP-3α), and the chemokine receptor CCR7 genes were significantly and repeatedly (from donor to donor) up-regulated in cells infected with CDC1551 relative to the levels observed in HN878-infected cells. In contrast, cytokines associated with macrophage deactivation (IL-13 and IL-11) and humoral immunity (IL-4), IL-17, type I interferon genes, and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and certain growth factor genes were significantly up-regulated in HN878-infected cells compared to their levels in cells infected with CDC1551. Human monocytes stimulated with lipid extracts from the two strains showed a similar differential pattern of up-regulation of cytokine and chemokine genes. Differential up-regulation in response to HN878 lipids was noted for IL-13, TARC, CCR5, vascular endothelial growth factor (VEGF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNAs while IL-12β, MIP-3α, and FAS ligand (CD95) and other Th1 immunity-associated genes were preferentially up-regulated in CDC1551 lipid-exposed cells. A semiquantitative reverse transcription (RT)-PCR assay confirmed the differential gene expression for IL-12β (significantly [$P = 0.05$] higher in CDC1551-infected cells than in HN878-infected cells), IL-13 and VEGF (significantly [$P = 0.04$ and $P = 0.004$, respectively] higher for HN878 stimulation than for CDC1551 stimulation). The similarity of the lipid-induced gene profiles to the profiles of genes up-regulated by infection with viable bacilli suggested that the lipid components of the organisms confer the differential gene activation observed during monocyte infection.

To confirm that mRNA gene expression was associated with protein regulation, we studied MIP-3α, TRAIL, VEGF, IL-12, and TNF-α protein production in response to CDC1551 and HN878 infection. Supernatants from monocytes infected at MOIs of 1:1 and 5:1 and/or stimulated at 1:50 with total lipid extracts were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Endogen, Boston, Mass., and R&D Systems, Inc.). Bacilli or their lipid products were compared in cells from the same donor. At all of the time points analyzed, the MIP-3α concentration induced by CDC1551 was higher than that induced by HN878, with a significant ($P < 0.05$) difference at 5 h postinfection (Fig. 1A). TRAIL induc-

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TABLE 1. Gene expression profile in human monocytes infected with *M. tuberculosis* or exposed to lipid extracts

	Protein whose gene[a] is differentially elevated by:			
Gene products	CDC1551 relative to HN878		HN878 relative to CDC1551	
	Infection	Lipids	Infection	Lipids
Inflammatory cytokines/receptors	IL-12α, IL-12β[b] IL-1α, IL-1R IL-10 MIP-3α CCR7	IL-12α, IL-12β[c] IL-1α, IL-1R IL-10 MIP-3α CCR7	IL-13[b] IL-4 IL-11 IL-17 CCR5	IL-13[c] CCR5 TARC
Common cytokines	IL-12β[b] FAS ligand TNF-α	IL-12β[c] FAS ligand IL-1β IL-15 CD40L GM-CSF[e] BMP1 IGF-1, 2	IL-17 IFN-α6, -α7, -β1 TRAIL Thrombopoietin FGF, HGF VEGF[b] family	G-CSF[d] VEGF[c]

[a] Genes showing a consistent twofold or greater difference in mRNA expression between the two strains (among five [inflammatory cytokine/receptor genes] and two [common cytokine genes] donors, respectively). Underlining gene indicates products for which differences in mRNA were correlated with significant differences in protein production measured by ELISA.

[b] Gene product in which the difference in the level of expression was confirmed ($P \leq 0.05$) by RT-PCR.

[c] Gene product in which the difference in the level of expression was confirmed by RT-PCR but the experiments were carried out twice (no statistic).

[d] G, granulocyte.

[e] GM, granulocyte-macrophage.

tion, measured at 24 h after infection, was significantly ($P <$ 0.0001) elevated in HN878-infected cells (Fig. 1B). Maximal VEGF induction was detected on day 3 postinfection, and the levels induced were significantly ($P = 0.004$) higher for HN878-infected cells (Fig. 1C). HN878 induced significantly lower levels of IL-12 than did CDC1551 at 24 and 48 h ($P \leq$ 0.006) (Fig. 2A) and significantly lower levels of TNF-α at 24 h ($P = 0.01$) (Fig. 2B). We next examined the effect of total lipid extracts from HN878 and CDC1551 on cytokine induction. Maximum levels of TNF-α and IL-12 were detected at 24 and 48 h, respectively. Lipid extracts from HN878 elicited lower levels of TNF-α ($P < 0.05$) compared to levels induced by lipid extracts from CDC1551 (Fig. 2C, insert). IL-12 levels were significantly lower for lipids from HN878 than for CDC1551 at both of the time points analyzed ($P < 0.001$) (Fig. 2C). Thus, the lipid extracts appeared to impart the differential chemokines-cytokine protein response observed during monocyte infection.

The differential immune stimulatory activity of other strains was studied. Three additional clinical isolates belonging to the W/Beijing family of strains (W4, W10, and 210) (2, 5, 6, 22) and one additional non-W/Beijing isolate (NHN5) (18) were evaluated for the ability to induce cytokine release after monocyte infection or stimulation with total lipid fractions. The identical IS*6110* banding pattern observed in HN878, W10, and 210 suggested that these may be the same or very similar strains (data not shown). We infected human monocytes with HN878, W4, W10, and 210 as representatives of the W/Beijing strains and CDC1551 and NHN5 as representatives of the non-W/Beijing strains and evaluated IL-12 and TNF-α cytokine levels over 4 days of infection (Fig. 2A and B). W4, W10, and 210, similar to HN878, induced significantly lower levels of IL-12 (P ≤ 0.05) at both 24 and 48 h postinfection, and TNF-α levels were found to be significantly lower than those of CDC1551 at 24 h postinfection ($P = 0.008$ for W4). In contrast, NHN5 cytokine induction was comparable to that of CDC1551. Intracellular replication rates could not account for these differences (24 h for HN878, 210, CDC1551, and NHN5 and 30 to 33 h for W4 and W10, respectively). Rather, the strains segregated according to their relatedness to the W/Beijing family of strains as determined by restriction fragment length polymorphism. Consistent with the cytokine responses to infection, lipid extracts from the W/Beijing strains (HN878 and W4) induced significantly ($P < 0.05$) lower levels of TNF-α and IL-12 than did those from non-W/Beijing strains (CDC151 and NHN5) (Fig. 2C).

Our results suggest that certain members of the highly pathogenic W/Beijing family of *M. tuberculosis* strains produce lipids that fail to efficiently induce the cytokine-dependent Th1-type protective immune response. In the infected host this failure would ultimately result in delayed or impaired protective immunity, leading to more severe disease. These findings are consistent with previous reports associating members of the W/Beijing family of strains with multidrug-resistant outbreaks (6, 19, 21), relapse (8), and global spread (4). Our results may also explain the increased pathogenicity (reduced host survival) seen in mice infected with HN878 (11). Mycobacterial lipids have been shown to participate in both inhibitory and stimulatory signaling, as well as in granuloma formation (3, 15, 16).

Macrophage activation to a protective phenotype depends on TNF-α and gamma interferon produced by activated Th1-type lymphocytes. Less known is the alternative activation of macrophages trigged by IL-4 and IL-13. These cytokines produced during the Th2-type response (7) appear to deactivate phagocytes, inducing phenotypic changes that are different from those induced by IL-10. The alternative activation of macrophages has been studied most extensively in parasitic infection. Stimulation of macrophages with IL-4 and IL-13 contributes to the susceptibility of mice to infection with *Leish-*

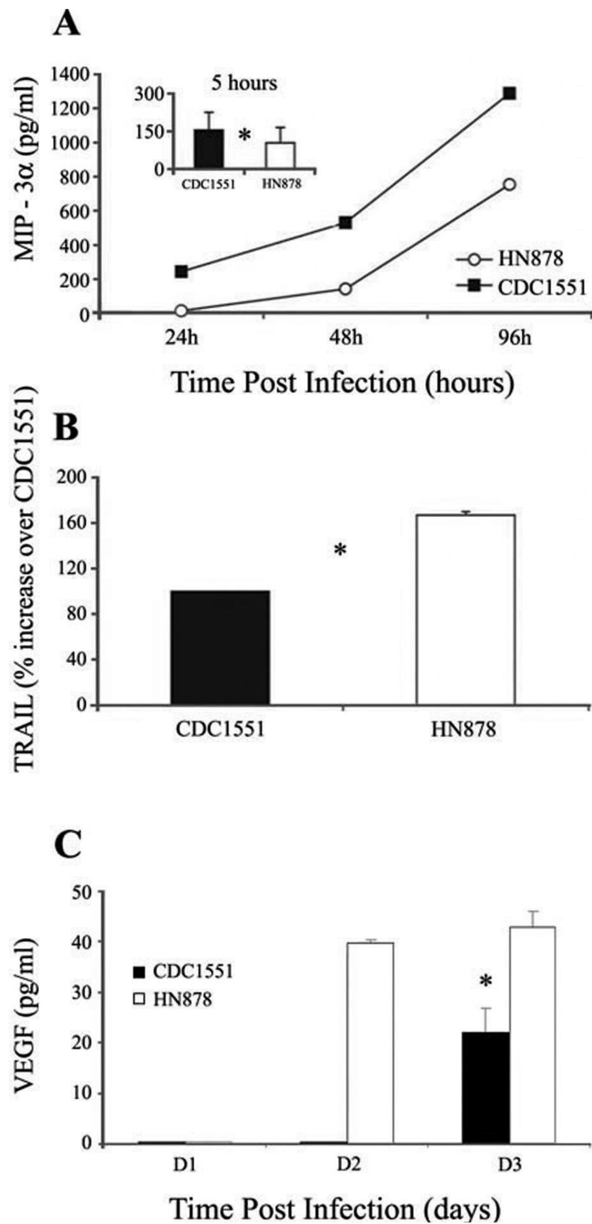

FIG. 1. Cytokine and chemokine production by human monocytes infected with clinical isolates HN878 and CDC1551. (A) Levels of MIP-3α were determined at 5, 24, 48, and 96 h postinfection with optimal concentration at 96 h. Differences in MIP-3α levels between CDC1551 and HN878 were significant at 5 h postinfection (*, $P = 0.05$). (B) Levels of TRAIL were determined at 24 h postinfection. Values are expressed as percent cytokine activity relative to that of CDC1551-infected cells; differences between cytokine levels induced by CDC1551 and HN878 were significant (*, $P = 0.0001$). (C) Levels of VEGF were detected at 24, 48, and 72 h postinfection with optimal concentrations at 72 h postinfection. A significant difference was detected at 48 and 72 h postinfection (*, $P < 0.005$). Each experiment was performed with monocytes from an individual donor. Results are the means ± standard error of three experiments done in triplicate. A paired *t* test was used for statistical analysis.

mania major by reducing classical macrophage activation and suppressing the Th1 response (7). Furthermore, it has been shown that expression of the alternative activation pathway in the later stages of infection of mice with *Trypanosoma brucei* is required for establishment of the chronic phases of the disease (1, 13).

Our observation that W/Beijing *M. tuberculosis* isolates can induce alternative macrophage activation has not been reported before, probably because most investigators have used

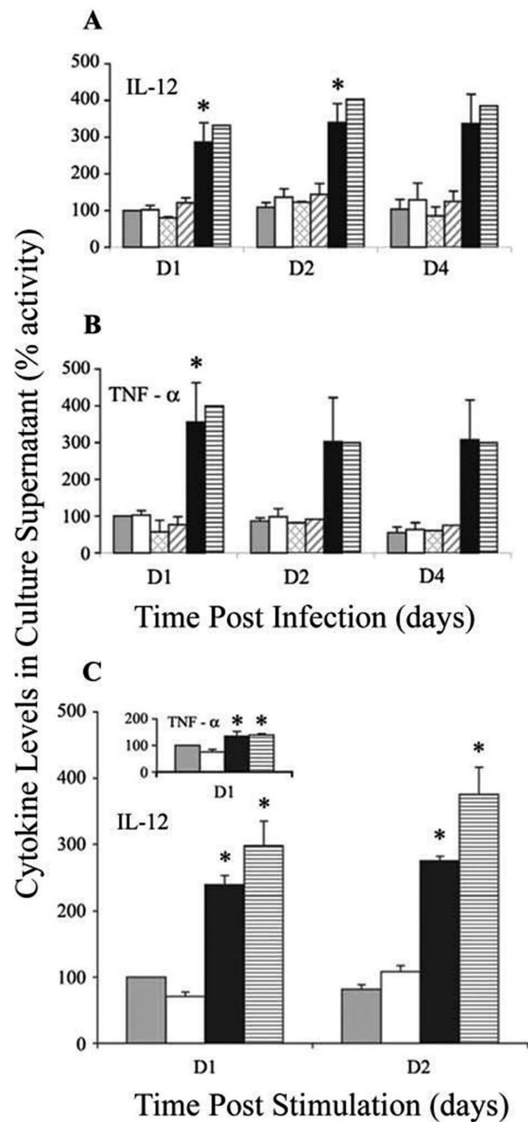

FIG. 2. Cytokine production by human monocytes infected with W4, HN878, W10, 210, CDC1551, and NHN5 (bars from left to right) (MOI of 1:1). Levels of IL-12 (A) and TNF-α (B) were evaluated by ELISA. Differences in IL-12 and TNF-α were statistically significant (*, $P \leq 0.05$ in comparison with HN878, W4, W10, and 210; $P \leq 0.05$ between CDC1551 and HN878; $P = 0.008$ between CDC1551 and W4). Results, expressed as percent activity, are means ± standard errors of two experiments for NHN5, W10, and 210, of four experiments for CDC1551, and of six experiments for W4 and HN878, all carried out in triplicate. (C) Stimulation with *M. tuberculosis* lipid extracts. Optimal cytokine levels for W4, HN878, CDC1551, and NHN5 (bars from left to right) were detected at 5 h poststimulation for TNF-α (insert) and at 24 and 48 h for IL-12. Each experiment was performed with monocytes from an individual donor. Results are the means of three experiments done in triplicate expressed as percent cytokine activity ± standard error relative to W4. A paired *t* test was used for statistical analysis (*, $P \leq 0.05$ in comparison to HN878 and W4).

H37Rv for their studies on the macrophage response to infection. Nau et al. (14) showed that *M. tuberculosis* induced little IL-12 and IL-15 relative to other organisms and also suppressed IL-12 production by cells exposed to *Escherichia coli*. Our data suggest that the virulence of the W/Beijing family of strains may, at least in part, be attributed to the ability of their lipids to subvert the host's protective immune response in association with suboptimal IL-12 production.

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