Correspondence

Mycobacterium tuberculosis Cell Wall–Associated Rv3812 Protein Induces Strong Dendritic Cell–Mediated Interferon γ Responses and Exhibits Vaccine Potential

To the Editor—*Mycobacterium tuberculosis*–associated tuberculosis is the leading cause of mortality due to any single infectious agent. Although BCG vaccine is widely used in many parts of the world to protect against severe childhood forms of tuberculosis, this vaccine is unreliable against the highly infectious pulmonary form of tuberculosis found in adults. These situations stress the need to develop better intervention strategies to combat tuberculosis [1].

The new vaccines that are in clinical trials have mostly exploited secreted antigens, such as Ag85 and ESAT-6 [1, 2]. Pinto et al recently reported that host cell–induced components of the sulfate assimilation pathway (SAP) are the major protective antigens of *M. tuberculosis* and suggested that SAP components are promising candidates for inclusion in new vaccines [3]. Collectively, these reports imply that secreted antigens are major vaccine antigens for *M. tuberculosis*, as they are readily seen by the immune system. Alternatively, *M. tuberculosis* cell wall is also a major source of immunogenic antigens, and these antigens come in direct contact with innate cells when *M. tuberculosis* encounters these immune cells. Therefore, we propose that immunogenic cell wall antigens could also be considered for vaccine trials.

The resurgence in the efforts to understand the pathogenesis of tuberculosis and to develop effective vaccines was evoked by the completion of the *M. tuberculosis* genome sequence and the identification of many new tuberculosis antigens, such as proteins expressed by members of the proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) gene family, which have characteristic PE and PPE motifs near the N-terminus. PE and PPE gene family members represent about 10% of the coding capacity of the *M. tuberculosis* genome. The genes encoding several members of PE family proteins also exhibit polymorphic GC-rich repetitive-sequences (PGRS) coding for glycine-alanine repetitive sequences at the C-terminus [4]. There is growing evidence from signature-tagged mutagenesis and microarray studies that several PE_PGRS proteins are localized in the cell wall of bacteria and influence the interactions of bacteria with immune cells. PE_PGRS family proteins have been shown to have a critical role in the pathogenesis of tuberculosis and in the innate and adaptive immune responses to bacilli. In this report, we demonstrate that one such cell wall–associated PE_PGRS protein, Rv3812 (PE_PGRS 62), binds to Toll-like receptor 2 (TLR2) and induces interferon γ (IFN-γ) responses in T cells by inducing activation of human dendritic cells (DCs).

Recombinant Rv3812 was expressed in *Escherichia coli* and purified as previously described [5]. The protein was further passed through a polymyxin B agarose column to remove endotoxins. As shown in Figure 1*A*, Rv3812 selectively recognized TLR2, whereas other TLRs, particularly TLR4, were not recognized, thus confirming the endotoxin-free status of the protein. Rv3812 induced maturation of DCs, as determined by the significantly increased expression of the costimulatory molecules CD80 and CD86 and the antigen-presenting molecule HLA-DR (Figure 1*B*). Rv3812 also induced the activation of DCs, as shown by the secretion of significantly increased amounts of the immunoregulatory cytokines interleukin 6, interleukin 12p70 (IL-12p70), and tumor necrosis factor α (Figure 1*C*) and early activation of the ERK1/2, p38 MAPK, and NF-κB pathways (Figure 1*D*).

Protective immunity against *M. tuberculosis* essentially depends on cell-mediated immunity, T-helper cell 1 (Th1) polarization (via IL-12), and Th1 cytokines (ie, IFN-γ and IL-2). In this context, we analyzed whether activation of DCs mediated by Rv3812 also translated into a functional response—the ability to stimulate $CD4^+$ T cells. Interestingly, Rv3812-educated DCs induced high amounts of pro-Th1 cytokines, such as IL-2 and IFN-γ, in T cells, while IL-4 production was minimal (Figure 1*E*). Together, these data implicate the potential of Rv3812 to stimulate efficient Th1 cell responses via DCs.

By in silico and in vitro approaches, it has been demonstrated that Rv3812 is one of 3 proteins, among all 167 proteins of the PE/PPE family, that harbor the highest number of major histocompatibility complex (MHC) class I– and MHC class II–restricted peptides [6, 7]. Full-length Rv3812, as well as peptides derived from this protein, induced significant cytotoxic T-cell responses in peripheral blood mononuclear cells of healthy subjects from a tuberculosis-endemic country, indicating that cytotoxic T-cell precursor populations against this mycobacterial protein exist at a higher frequency within the T-cell repertoire [5]. Together, these data therefore suggest that Rv3812 is highly immunogenic and could be considered as fusion protein subunit vaccine to boost BCG (improved/modified)–induced immune responses, either alone

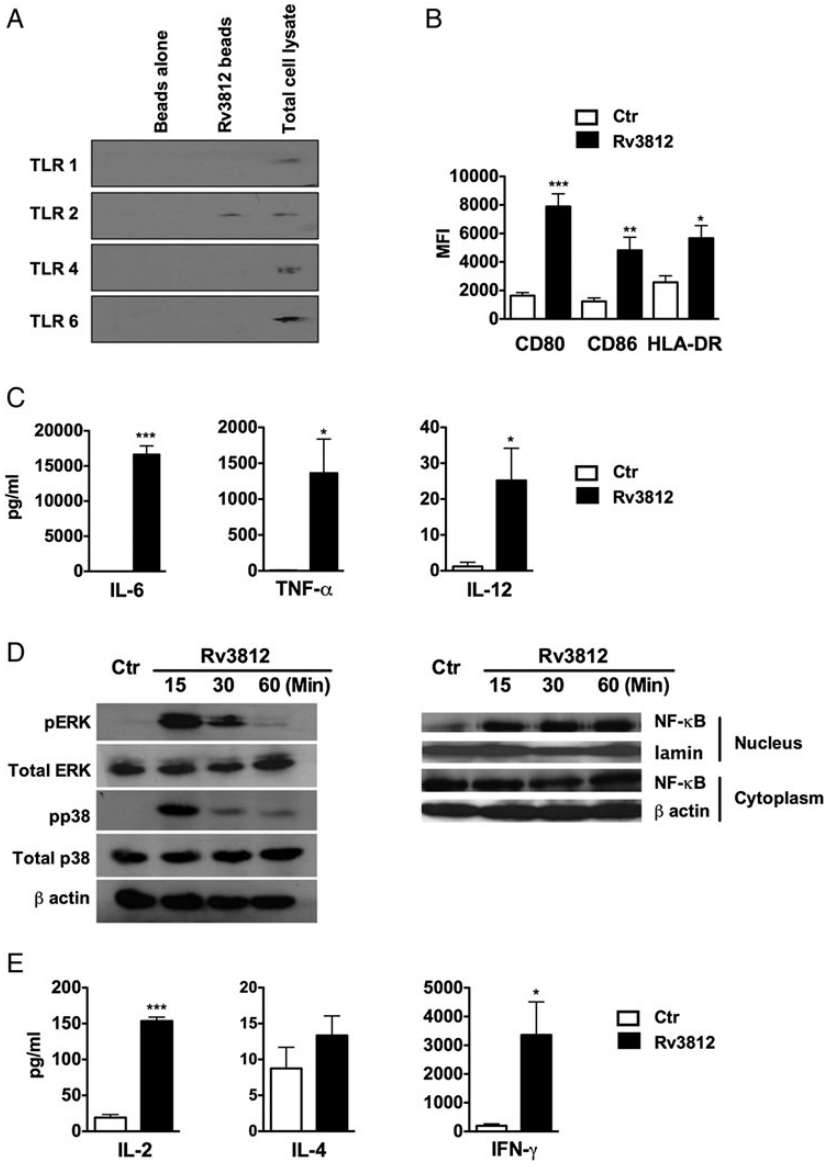

Figure 1. Rv3812 binds to Toll-like receptor 2 (TLR2) and induces interferon γ (IFN-γ) responses in $CD4^+$ T cells by inducing activation of human dendritic cells (DCs). DCs were obtained by culturing peripheral blood monocytes (isolated by using CD14 microbeads; Miltenyi Biotec, France) from healthy blood donors, after receipt of ethical committee permission, in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF; 1000 IU/10^6 cells) and interleukin 4 (IL-4; 500 IU/10^6 cells). *A*, Rv3812 recognizes TLR2. The cell lysates from DCs were incubated with Rv3812 immobilized on Ni-NTA beads, and bead-bound proteins were analyzed for TLR1, TLR2, TLR4, and TLR6 by immunoblotting. *B* and *C*, Rv3812 induces maturation of DCs and secretion of immunoregulatory cytokines. Six-day-old immature DCs (0.5×10^6 cells/mL) were cultured with GM-CSF and IL-4 alone (Ctr) or cytokines and 5 μg Rv3812 for 48 hours. Expression of surface markers (measured in terms of mean fluorescence intensities [MFIs]; *B*) and secretion of cytokines from DCs (*C*) are presented. Data are mean values ± standard error of the mean (SEM) for 4–5 independent donors. *D*, Maturation of DCs stimulated by Rv3812 involves activation of ERK1/2, p38 MAPK, and NF-κB pathways. Data are representative of 3 independent experiments. *E*, Rv3812 triggers strong T-helper 1 cell responses. Rv3812-treated DCs were cocultured with $CD4^+$ T cells at ratios of 1:20 for 96 hours, and T-cell cytokines were analyzed in the cell-free culture supernatants. Data are mean values ± SEM for 4 independent donors. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$, by a 2-way Student *t* test.

or in combination with other PE/PPE proteins with similar properties, such as PE_PGRS17 or PE_PGRS11 [8, 9]. Of note, 2 cell wall antigens, Rv1196 and Rv0125, as fusion proteins are in phase 1 or 2a clinical trials [10].

Notes

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