The leishmaniases are diseases caused by vector-borne pathogens that represent a major public health problem affecting the lives of millions of people worldwide (http://www.who.int/whr/en). Depending on the parasite species and on the immunological response of the human host, leishmaniasis ranges from an asymptomatic infection to a self-limiting cutaneous lesion(s) or a fatal visceral form.

No anti-\textit{Leishmania} vaccine is available at the moment. Different studies showed that development of Th1- and \textit{Leishmania}-specific cytotoxic immune responses correlate with healing of patients with cutaneous leishmaniasis (CL) (3, 12). An intense effort is being made to identify antigens that could induce an immune state similar to that developed by individuals who recover from symptomatic infection and are resistant to a subsequent natural challenge. Such antigens may contribute to the development of an anti-\textit{Leishmania} vaccine.

Diagnostic tools targeting leishmaniasis are available. However, parasite detection is invasive and poorly sensitive. Serological diagnosis using various techniques based on detection of \textit{Leishmania}-specific antibodies that are developed during acute disease are often more sensitive, less time-consuming, and more user friendly (4, 11, 15).

In an attempt to identify new antigens to be used as vaccines or for serodiagnosis, we focused on \textit{Leishmania} virulence factors. Indeed, several studies described these factors as potentially immunogenic in humans, mice, and, more recently, dogs (6, 8, 9, 13). Here, cellular and humoral immune responses of healed CL (hCL) and Mediterranean visceral leishmaniasis (MVL) patients were evaluated against results for \textit{Leishmania major} virulence proteins \textit{L. major} protein disulfide isomerase (LmPDI) and mitogen-activated protein kinase kinase (MAPKK). Only MAPKK induces significant peripheral blood mononuclear cell proliferation with gamma interferon production as well as antibody responses. Thus, MAPKK may be of interest in \textit{Leishmania} vaccination and serodiagnosis.
healthy individuals, respectively) (Fig. 3A and B). Interestingly, the difference in production between the two groups was highly significant only for IFN-\(\gamma\)/\(H9253\) (\(P = H11005\) 0.007) (Fig. 3A). However, no IFN-\(\gamma\) or IL-10 productions were observed after stimulation of PBMC with LmPDI (Fig. 3A and B). A positive correlation was found between IFN-\(\gamma\)/\(H9253\) and IL-10 levels (Spearman rank correlation \(r = H11005\) 0.594) and between PBMC proliferation and IFN-\(\gamma\) or IL-10 production (Spearman rank correlation \(r\) values of 0.6 \([P = H11005\] 0.03\] and 0.583 \([P = H11005\] 0.036\] for IFN-\(\gamma\) and IL-10, respectively) in response to MAPKK. These results show that MAPKK could constitute a potential vaccine candidate. It is well established that IFN-\(\gamma\)/\(H9253\) is a key cytokine in resistance against CL and is implicated in parasite killing by activated macrophages (14). However, IL-10 is the main downregulating cytokine of the Th1 immune response and exhibits macrophage-deactivating properties (5, 7). Interestingly, the MAPKK IL-10-inducing capacity was not sufficient for suppression of significant proliferation and high, significant levels of IFN-\(\gamma\) in hCL patients. The IL-10 production by PBMC from hCL as well as healthy individuals might be due to the high level of conservation of MAPKK proteins in eukaryotic species. Although MAPKK stimulates high IL-10 levels, it could constitute a potential vaccine candidate since it was recently reported that the human immune response to crude and defined \textit{Leishmania} antigens generated during immunization can differ from that induced by natural infection (1). Similarly, MAPKK used as a vaccine might induce a dominant Th1 response compatible with protection.

For the second part of the study, we chose to analyze humoral responses to LmPDI and MAPKK in MVL patients rather than CL patients for the following reasons: (i) human sera from CL patients generally show low-level reactivity against \textit{Leishmania} antigens, compared to sera from MVL patients; (ii) diagnosis of MVL is more problematic than that of CL; and (iii) LmPDI and MAPKK are highly conserved with their \textit{Leishmania infantum} homologue, with 92% and 97% identities, respectively. The reactivities of MVL patients to LmPDI (10 \(\mu\)g/ml), MAPKK (5 \(\mu\)g/ml), and SLA (2 \(\mu\)g/ml) were assayed by ELISAs with two groups. The first group consisted of 12 MVL children (age range, 1 to 5 years; mean age \(\pm\) SD, 35 \(\pm\) 21 months) living in the governorate of Kairouan (a region of MVL endemicty), blood sampled before treatment. Diagnosis of MVL was established on clinical criteria (fever, anemia, splenomegaly, and weight loss) and on demonstration of \textit{Leish}-

![FIG. 1. Expression of recombinant proteins in \textit{Escherichia coli}. Recombinant LmPDI (lane 1) and MAPKK (lane 2) were synthesized in BL21, purified by affinity chromatography over Ni-nitrilotriacetic acid resin, and analyzed on 14% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, followed by Coomassie blue staining. MW, molecular mass markers (kDa).](http://cvi.asm.org/)

![FIG. 2. Lymphoproliferative response induced by MAPKK or LmPDI. Levels of lymphocyte proliferation in response to either SLA (10 \(\mu\)g/ml) or recombinant proteins MAPKK (10 \(\mu\)g/ml) and LmPDI (5 \(\mu\)g/ml) incubated for 5 days at 37°C and 5% CO\(_2\) are expressed as SI. The cutoff value (SI = 2.5) is indicated by a horizontal bar.](http://cvi.asm.org/)

![FIG. 3. Immune response induced by MAPKK or LmPDI: IFN-\(\gamma\) (A) and IL-10 (B) induction. PBMC from hCL or healthy subjects were stimulated with either SLA (10 \(\mu\)g/ml) or recombinant proteins MAPKK (10 \(\mu\)g/ml) and LmPDI (5 \(\mu\)g/ml). Supernatants were harvested at 48 h and 72 h and were used for quantification of IL-10 and IFN-\(\gamma\). The dashes indicate the mean cytokine values obtained for the different groups of individuals. NS, nonstimulated cultures.](http://cvi.asm.org/)
FIG. 4. ELISA reactivity of sera from patients with MVL (P) and healthy controls (C) with SLA (2 μg/ml) and recombinant *Leishmania* proteins MAPKK (5 μg/ml) and LmPDI (10 μg/ml). Bars show the cutoff value for each ELISA, which is defined as the mean OD + 2 SDs obtained with sera from healthy controls. The significance of differences between P and C groups was evaluated by the Mann-Whitney test. P values of <0.05 were considered significant. Arrows indicate mean OD values.

**TABLE 1.** Sensitivities and specificities of ELISAs using crude SLAs and recombinant *Leishmania* proteins MAPKK and LmPDI.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
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<tbody>
<tr>
<td>Crude SLA</td>
<td>100</td>
<td>92.3</td>
<td>92.3</td>
<td>100</td>
</tr>
<tr>
<td>MAPKK</td>
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<td>100</td>
<td>100</td>
<td>92.9</td>
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<tr>
<td>LmPDI</td>
<td>66.7</td>
<td>100</td>
<td>100</td>
<td>76.5</td>
</tr>
</tbody>
</table>

*PPV, positive predictive value; NPV, negative predictive value.*