Brucella abortus Lipopolysaccharide in Murine Peritoneal Macrophages Acts as a Down-Regulator of T Cell Activation

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Macrophages play a central role in host immune responses against pathogens by acting as both professional phagocytic cells and as fully competent APCs. We report here that the LPS from the facultative intracellular Gram-negative bacteria *Brucella abortus* interferes with the MHC class II Ag presentation pathway. LPS inhibits the capacity of macrophages to present hen egg lysozyme (HEL) antigenic peptides to specific CD4+ T cells but not those of OVA to specific CD8+ T cells. This defect was neither related to a decrease of MHC class II surface expression nor to a deficient uptake or processing of HEL. In addition, *B. abortus* LPS did not prevent the formation of SDS-resistant MHC class II complexes induced by HEL peptides. At the cell surface of macrophages, we observed the presence of LPS macrodomains highly enriched in MHC class II molecules, which may be responsible for the significant down-regulation of CD4+ T cell activation. This phenomenon may account for the avoidance of the immune system by certain bacterial pathogens and may explain the immunosuppression observed in individuals with chronic brucellosis. The Journal of Immunology, 2000, 165: 5202–5210.
induction of SDS-resistant MHC class II molecules by HEL is not impaired by the presence of LPS. Interestingly, the LPS macrodomains we previously described at the macrophage plasma membrane (18) are highly enriched in MHC class II molecules. We propose that the LPS-MHC class II macrodomains may impair the appropriate recognition of HEL peptide-MHC class II complexes by CD4+ T cell hybridomas.

Materials and Methods

Macrophage preparation

Eight-week-old female C3H/HeNe (H-2k) and C57BL/6 (H-2d) mice were purchased from Jackson ImmunoResearch (West Grove, PA). Peritoneal fluids were harvested as described in (18), washed at 1200 rpm for 10 min at 4°C, and the pellets were resuspended in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS, 10 mM HEPES (Life Technologies), 10 mM sodium pyruvate (Life Technologies), 10 mM nonessential amino acids (Life Technologies), 2 mM glutamine (Life Technologies), 2 x 10^{-5} M 2-ME (Sigma, St. Louis, MO), and 1 mM penicillin/streptomycin (Life Technologies). Peritoneal cells were plated and incubated for 2 h at 37°C in a 5% CO2 atmosphere. Nonadherent cells were removed from the wells by aspiration, and the adherent macrophages were rinsed and incubated in fresh medium.

Hybridomas and Ags

HEL and OVA were purchased from Sigma. The HEL, 34–45 and HEL, 46–61 peptides were synthesized using an Applied Biosystems peptide synthesizer (Perkin-Elmer Biosystems, Courtaboeuf, France) and the OVA, 237–264 peptides were provided by Dr. Patrick Machy (Centre d’Immunologie de Marseille Luminy, Marseille, France). OVA was passively absorbed to latex beads (Polysciences, Warrington, PA) in accordance with the manufacturer’s protocol, followed by extensive washing in medium. The HEL-specific CD4+ T cell hybridomas 3A9 and 3B11 are specific for HEL, 46–61, Kk and HEL, 44–45, Kk, respectively (19). The OVA-specific CD8+ T cell hybridoma 2B10 is specific for OVA, 237–264, Kk (20). The IL-2-dependent CTLL cell line was used for detecting the presence of secreted IL-2 in the culture medium. All of these cell lines were cultured in the supplemented RPMI medium described above.

Lipopolysaccharide

Brucella abortus 2308 and Shigella flexneri 5a LPSs were provided by Dr. I. Moriyon (University of Navarra, Pamplona, Spain) and Dr. P. Sansonetti (Pasteur Institute, Paris, France), respectively. The purity and the characteristics of these preparations have been published elsewhere (21, 22). Macrophages activated in vivo by LPS or activated in vitro and in vivo by 10 ng/ml of IFN-γ or 10 ng/ml of rLPS for 8 h at 37°C. After washing, cells were further incubated with HEL, synthetic HEL peptides, OVA, synthetic OVA peptides, and the corresponding specific T cell hybridomas (105 cells/well). After a 24-h incubation, culture supernatants were assayed for the presence of the T cell growth factor IL-2 by measuring the incorporation of [3H]thymidine in the IL-2-dependent CTLL cell line (105 cells/well).

Flow cytometric analysis of Ia molecules

Macrophages activated in vivo by LPS or activated in vitro and in vivo by IFN-γ were plated at 106 cells/well (Dish, Nunc, Naperville, IL) and incubated for 30 min at 4°C in PBS/10% FCS for analysis by FACSscan flow cytometry (Becton Dickinson, Mountain View, CA).

Ag uptake and degradation

HEL was labeled with 35S by the iodogen method (Pierce, Rockford, IL) to a specific activity of ~0.5 mCi/mg (106 cpm/μg). Macrophages (105 cells/well) were cultured in 24-well culture plates with the radiolabeled HEL (2 μg/well) in cell culture medium for 1 h at 37°C. Cells were extensively washed in PBS containing 0.5% BSA until no radioactivity could be detected in the washing medium and then reincubated in radiolabeled HEL-free medium for various times at 37°C. At each time point, macrophages were solubilized in PBS/1% Triton X-100 (Sigma) and centrifuged for 20 min at 14,000 rpm at 4°C. Lysate supernatants were incubated for 30 min at 4°C with 20% TCA to separate soluble from insoluble fractions, and the cell-associated radioactivity was counted in each fraction using a Cobra 5005 counter (Packard, Meriden, CT).

35S metabolic labeling and immunoprecipitations

106 macrophages from LPS-injected mice were cultured in cytostatin/me-thionine-free RPMI 1640 medium containing 5% dialyzed FCS supplemented or not with 2 ng of HEL. After 1 h at 37°C, cells were pulsed in medium containing 0.25 μCi/ml of [35S]methionine and 20% [35S]cysteine mix (DuPont-NEN, Harrisburg, PA). After 2 h at 37°C, samples were washed and lysed in solubilization buffer (1% Nonidet P-40 (NP40), 10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 2 mM EDTA; and I mM PMSF) for 30 min at 4°C. Samples were precleared by an incubation with protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) before incubation with the 10.2.16 anti-class II Ab preadsorbed on protein A-Sepharose beads. Immunoprecipitates were collected by centrifugation, washed three times with 1% NP40, 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 0.1% sodium deoxycholate, and 0.5% SDS, then twice with the same buffer without SDS and sodium deoxycholate, twice with 0.5% NP40, 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 2 mM EDTA, then twice with 10 mM Tris-HCl, pH 7.5. The washed beads were resuspended in 40 μl SDS-PAGE sample buffer containing 5 mM DTT. In the presence of HEL (Sigma), half of each sample was heated at 55°C for 5 min and the other half was left at room temperature for 1 h. The eluted proteins were then analyzed by SDS-PAGE on 12% acrylamide gels. Gels were treated with Enhance (DuPont-NEN, Hanover, MA), dried, and submitted to autoradiography. Quantification of the band signal intensities were performed using a MacBAS v2.2 program.

Confocal microscopy

Immunofluorescence experiments were performed on macrophages from LPS-injected mice without cell permeabilization to detect cell surface-associated molecules, as previously described (18). Macrophages (5 x 105 cells/well) were plated on 12-mm glass coverslips in 24-well tissue culture
plates (Costar) and were fixed at room temperature with 3.7% paraformaldehyde in PBS, pH 7.4, for 20 min followed by a 10-min incubation with 0.1 M glycine. Then, cells were incubated with a PBS solution containing 5% mouse serum/5% horse serum for 20 min to block unspecific binding. Primary Abs diluted in the same buffer were added to the cells for 30 min. After extensive washings with PBS, macrophages were incubated for 30 min with fluorescein secondary Abs. LPS was revealed using an antiserum from *B. abortus*-infected cows followed by anti-cow IgG Ab conjugated to Texas Red. MHC class II molecules were revealed by the FITC-conjugated 10.2.16 anti-I-Ak mouse IgGs. For MHC class I detection, cells were fixed with paraformaldehyde, incubated with the rat anti-mouse Fc receptor Ab (24G2), and incubated with the mouse H1005/28 anti-H-2K Ab, which was revealed by goat anti-mouse IgG Abs conjugated to fluorescein. Coverslips with adherent macrophages were washed, mounted in Mowiol ( Hoechst, Frankfurt, Germany), and viewed under a Leica TCS 4D confocal microscope (Leica Lasertechnik, Heidelberg, Germany).

**Results**

**Brucella abortus LPS affects the Ag presentation of two distinct HEL epitopes**

In APCs, association of antigenic peptides with MHC class II molecules can occur in different compartments of the endocytic pathway (26). For instance, the HEL46–61 epitope requires newly synthesized MHC class II molecules and the invariant chain expression to be presented to 3A9 hybridoma cells and has been shown to associate with class II molecules inside late endocytic compartments. In contrast, the HEL34–45 epitope, which is more peripheral, can associate with mature or immature class II molecules inside early endocytic compartments and can be presented to 3B11 hybridoma cells independently of protein synthesis and of invariant chain (27). We have analyzed the effect of *B. abortus* LPS on these different Ag presentation pathways. We observed that LPS inhibits the presentation by macrophages of both HEL46–61 (Fig. 1A) and HEL34–45 epitopes (Fig. 1D) generated from exogenously added HEL. In parallel, we analyzed the availability of surface MHC class II molecules by testing the capacity of macrophages to present exogenously added purified peptides to T cell hybridomas (Fig. 1, B and C). The presentation of class II-restricted epitopes such as the HEL46–61 (Fig. 1B) and the HEL34–45 (Fig. 1D) peptides was also reduced in the presence of LPS. The inhibitory effect of LPS was Ag dose-dependent (Fig. 1, A and B), and increasing Ag concentrations could only partially restore the ability of macrophages to present HEL. Altogether, these results show that *B. abortus* LPS inhibits the presentation of different HEL epitopes generated in distinct endocytic compartments, affecting in this way the presentation by neosynthesized MHC class II, recycled MHC class II, and vacant surface MHC class II molecules.

**LPS does not affect the MHC class I-mediated Ag presentation**

Because MHC class II presentation pathway is inhibited by the presence of LPS in macrophages, we tested whether *B. abortus* LPS could affect the MHC class I presentation pathway by using the OVA Ag and its specific CD8 T cell hybridoma. Fig. 2 shows that LPS does not significantly impair the presentation by MHC class I molecules of both OVA (Fig. 2A) and OVA257–264 Peptide (Fig. 2B) to CD8 T cells. These results indicate that the inhibitory effect of LPS is specific to the MHC class II pathway. This can be correlated with our previous results showing a specific association of *B. abortus* LPS with MHC class II molecules in B lymphocytes (17).

**B. abortus LPS inhibits MHC class II-mediated Ag presentation in a dose-dependent manner**

The effect of Brucella LPS on Ag presentation by macrophages was compared with that of a classical endotoxin, the *Shigella flexneri* LPS, which displays distinct structural and functional properties from *B. abortus* LPS (28). At low doses (0.1 μg/ml), *Brucella* LPS already inhibited 48% of HEL presentation, whereas *Shigella* LPS had a negligible effect (Fig. 3). The inhibition rate increased with the LPS dose, reaching 90% for *B. abortus* LPS and 70% for *S. flexneri* at 20 μg/ml. Similar results were observed when we used purified HEL46–61 and HEL34–45 peptides (data not shown), which do not require either internalization or processing in APCs. These results indicate that both LPSs were able to prevent HEL presentation to T cells in a dose-dependent manner; however, *B. abortus* LPS appears as the most active LPS. One parameter that could be responsible for the decreased ability of macrophages to present Ags is the cell toxicity displayed by LPSs. In agreement with previous reports, both LPSs exhibit very similar Limulus lysate gelation activity (1 ng for *Shigella* and 2 ng for *Brucella*). However, the classical *S. flexneri* endotoxin displayed very high toxicity when compared with that of *B. abortus* LPS (Fig. 4). For instance, at 12 nM of KDO, *Shigella* LPS was already 7 times more toxic than *Brucella* LPS. At 27 nM of KDO, *Shigella* was 18 times more toxic as compared with *Brucella* LPS. Moreover, at higher concentrations (200 nM of KDO), *Brucella* LPS did not display a significant toxic effect on cells, in agreement with previous reports (15, 29). Because LPS toxicity is dependent on time, it is very difficult to dissociate the toxic effect induced by *Shigella* LPS from the inhibition of HEL presentation. In contrast, the Ag presentation impairment observed in *B. abortus* LPS-treated macrophages was not related to a toxic effect on APCs, but rather to an inhibitory phenomenon. We then investigated further the mechanisms by which *B. abortus* LPS down-regulates T cell activation.

**B. abortus LPS does not affect the capacity of macrophages to internalize and catabolize native HEL**

We previously showed that *Brucella* LPS concentrates in lysosomes between 6 and 24 h after its internalization (18). This retention could alter the intracellular trafficking of internalized proteins and the ability of these APCs to process Ags. To examine this possibility, HEL was radioiodinated, incubated with macrophages, and the cell-associated radioactivity of both normal and LPS-pulsed macrophages was determined after various times of chase. The efficiency of uptake was determined by incubating macrophages with 125I-HEL and by measuring the radioactivity present in Triton X-100 lysates just after the pulse. Table I shows that the same amounts of native Ag (TCA-insoluble fraction) were associated with macrophages loaded or not with LPS, indicating that uptake of HEL was not affected by the presence of LPS. To analyze the catabolism of native HEL, the radioactivity was measured after different times of chase. Cell-associated TCA-precipitable radioactivity progressively diminished with incubation time, reflecting the degradation of native HEL by macrophages. No increase of radioactivity was detected in the cell-associated TCA-insoluble fraction due to the release into the cell culture supernatants of peptidic fragments resulting from HEL processing. The time course of degradation and the amount of HEL degraded appeared very similar in both cell preparations, demonstrating that macrophages conserved their capacity to process native HEL even in the presence of LPS. So, the inhibition of presentation of HEL by LPS-loaded macrophages is not due to either a decrease in HEL uptake or in its intracellular processing.

**The level of surface class II molecule expression is not impaired by the presence of LPS in vitro**

The responsiveness of CD4 T lymphocyte hybridomas to protein Ags depends on the expression of MHC class II molecules bound to a given Ag. Because LPS may modify the level of MHC class II molecule expression, we analyzed by flow cytometry the total
amount of surface class II molecules present on IFN-γ-activated macrophages loaded or not with LPS. Fig. 5 clearly indicates that IFN-γ, in contrast to LPS, up-regulates MHC class II molecule expression in vitro, and that subsequent addition of *B. abortus* LPS on IFN-γ-activated macrophages does not affect the surface expression of MHC class II molecules. Therefore, the inhibition of Ag presentation by macrophages in the presence of LPS cannot be attributed to a reduced amount of MHC class II molecules at the macrophage surface.

**B. abortus LPS induces the expression of MHC class II molecules in vivo**

We also measured the level of MHC class II expression at the cell surface of macrophages obtained after injection of LPS into the...
peritoneal cavity of mice. The injection of LPS resulted in a strong up-regulation of MHC class II molecules on the macrophage cell surface (Fig. 6), probably due to an indirect mechanism, the level of which was similar to that of macrophages from IFN-γ-injected mice (data not shown).

MHC class II presentation pathway is inhibited in vivo by LPS

We then compared the antigenic presentation abilities between macrophages from LPS- and IFN-γ-injected mice. Fig. 7 clearly shows that LPS-treated macrophages are less efficient in presenting HEL peptides than macrophages treated with IFN-γ. These results corroborate those presented in Fig. 1 showing an inhibitory effect of LPS on processed HEL presentation in macrophages treated in vitro. In addition, as in vitro, the MHC class I presentation pathway was not affected (data not shown). We further investigated what could be the defect explaining the interference with the Ag presentation process using the in vivo model.

LPS forms macrodomains, which are enriched in class II molecules

We recently demonstrated that after internalization in mouse peritoneal macrophages, Brucella LPS is recycled to the cell surface where it forms macrodomains (18). These membrane domains were observed in macrophages treated either in vitro or in vivo with LPS or after infection with an attenuated strain of B. abortus (18). In the absence of LPS, MHC class I and II molecules analyzed by confocal microscopy on macrophages from IFN-γ-treated mice displayed an homogenous random distribution at their cell surface (Fig. 8, A and B). In contrast, the surface distribution of MHC class II molecules, but not that of class I, was modified after LPS treatment (Fig. 8, E and F). MHC class II molecules were concentrated in cell surface macrodomains, most of which colocalized with LPS (Fig. 8C). In contrast, MHC class I (Fig. 8F) did not display any specific localization with LPS.
not colocalize with LPS (Fig. 8D). These results led us to hypothesize that the LPS-MHC class II molecule macrodomains may correspond to specialized plasma membrane units, which could be involved in the modulation of Ag presentation by macrophages.

**LPS does not modulate the formation of SDS-resistant MHC class II dimers induced by processed HEL**

Because MHC class II molecules and LPS were clustered at the cell surface of macrophages, we hypothesized that the intrinsic capacity of Ia molecules to bind antigenic peptides could be modified in the presence of LPS. Macrophages were first treated with IFN-γ to allow MHC class II expression followed by an incubation or not with LPS in the presence or the absence of HEL. We analyzed the influence of LPS on the formation of SDS-resistant MHC class II dimers (compact forms) induced upon HEL internalization, a hallmark of HEL peptide binding to MHC class II molecules. In the absence of HEL and LPS, macrophages from IFN-γ-primed mice expressed compact forms (Fig. 9, −HEL, −LPS), the addition of LPS did not significantly change their amount (Fig. 9, −HEL, +LPS). Indeed, the value of the compact forms/free α and β forms ratio was 0.65 in the absence and 0.5 in the presence of LPS. As expected, in the presence of HEL, a strong induction of compact forms was observed (Fig. 9, +HEL, −LPS), the amount of which remained constant in the presence of LPS (Fig. 9, +HEL, +LPS). The compact forms/free α and β forms ratio reached 0.9 and 0.87 after HEL addition in the absence or the presence of LPS, respectively. These results show that the generation of HEL-induced compact forms correctly occurred even in the presence of LPS and suggest that LPS does not prevent the access of processed peptides to the MHC class II binding groove.

**Discussion**

Brucella LPS, like those from other intracellular pathogens such as Legionella, Campylobacter, Coxiella, and Chlamydia, presents major differences when compared with enterobacterial LPSs (15–18, 24, 29–32). For instance, the lipid A moieties and the core oligosaccharides of these LPSs are structurally different from the classical enterobacterial LPSs, being, in general, more hydrophobic and less substituted, and possessing longer aliphatic chains. The LPSs from these intracellular bacteria display attenuated biological activities and exert very low endotoxicity for animals. In this report, we provide new insights into the mechanisms of cellular immune response modulation by the low endotoxic LPS, purified from *B. abortus*, a facultative intracellular parasite capable of infecting both animals and humans.

Here we demonstrate that the low endotoxic *B. abortus* LPS dramatically reduces the protein Ag-dependent stimulation of T lymphocytes. This event can be related to the intracellular trafficking of *Brucella* LPS and its capacity to form macrodomains with MHC class II molecules. The inhibitory effect on Ag presentation cannot be due to a direct suppressive action of LPS on T cells without any macrophage requirement, because LPS was never found free in the culture medium. Indeed, we have previously shown that intracellular LPS is not exocytosed or recycled by macrophages in the external milieu (18). It has been generally established that endotoxins enhance the accessory function of APCs by inducing the expression of molecules implied in T cell activation. For instance, *Escherichia coli* and *Salmonella* LPSs have been shown to up-regulate B7 costimulatory molecules in B lymphocytes (11) and in human monocytes (33). Endotoxins also trigger the maturation of both human and murine dendritic cells

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**Table I. Uptake and catabolism of 125I-HEL in the presence or absence of LPS**

<table>
<thead>
<tr>
<th>Chase Time (min)</th>
<th>TCA-Precipitable (cpm) ~LPS</th>
<th>TCA-Soluble (cpm) ~LPS</th>
<th>TCA-Precipitable (cpm) +LPS</th>
<th>TCA-Soluble (cpm) +LPS</th>
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</thead>
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<td>0</td>
<td>3143 ± 528</td>
<td>291 ± 13</td>
<td>2912 ± 497</td>
<td>234 ± 27</td>
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<td>30</td>
<td>1742 ± 341</td>
<td>160 ± 41</td>
<td>1479 ± 187</td>
<td>152 ± 18</td>
</tr>
<tr>
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<td>1184 ± 156</td>
<td>213 ± 30</td>
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<tr>
<td>120</td>
<td>897 ± 65</td>
<td>89 ± 1</td>
<td>618 ± 20</td>
<td>65 ± 3</td>
</tr>
</tbody>
</table>

*Macrophages were incubated with IFN-γ for 48 h and treated (+LPS) or not (~LPS) with LPS for 8 h. Then, 125I-HEL (2 μg) was added to the cells for 1 h at 37°C and, after extensive washing, the radioactivity was chased for various periods of time. Cell-associated TCA-soluble and -insoluble radioactivity was measured after each time period.*

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**FIGURE 6.** Macrophages for LPS-injected mice express MHC class II molecules. Macrophages were harvested from mice injected (or not) with LPS and directly submitted to flow cytometry analysis to detect the amount of MHC class II expression on macrophages cell surfaces (as described in Fig. 4), control macrophages that were not labeled with the fluorescent AB

**FIGURE 7.** LPS inhibits HEL Ag presentation in vivo. Macrophages were recovered from LPS (open columns)- or IFN-γ (filled columns)-injected mice and were tested for their Ag presentation ability (see Materials and Methods) using HEL or HEL_{46–61} and HEL_{34–45} peptides recognized in the context of MHC class II molecules by their specific T cell hybridomas.
In stimulatory molecules in peritoneal macrophages (data not shown). We found that almost completely abrogated, in part, by a reduced expression of Brucella LPS. Loading of peptides on MHC class II were not directly affected by such an effect because the uptake of HEL, its processing, and the class II molecules were not directly affected by this effect because the uptake of HEL, its processing, and the class II molecules within MHC class II-enriched compartments. This differentiation event is characterized by an up-regulation of B7 costimulatory molecules with immunogenic peptides (13). We were able to exclude compartments and to a defect in the loading of MHC class II molecules at the cell surface (12, 34, 35). However, in sinusoidal endothelial cells pretreated with E. coli LPS-treated liver endothelial cells, the major cause of the defect in Ag presentation was due to the accumulation of LPS inside the APC, leading to a modification of the pH of endocytic compartments and to a defect in the loading of MHC class II molecules with immunogenic peptides (13). We were able to exclude such an effect because the uptake of HEL, its processing, and the loading of peptides on MHC class II molecules were not directly affected by Brucella LPS.

To be presented to T cells, protein Ags have to be internalized and processed by APCs into peptides, which associate with MHC class II molecules within MHC class II-enriched compartments. Then, the peptide-MHC class II complexes recycle to the cell surface (36). Several studies have revealed that phagocytosis of some microbial particulate materials, like soluble polysaccharides or polyanions, affected the presentation of proteins by macrophages to specific T cells (7). Although the underlying mechanisms have not been clearly identified, it was suggested that the encounter between Ia molecules and processed peptides could be impaired after ingestion of these poorly degradable molecules. Similarly, we have observed that B. abortus LPS is retained in macrophages without undergoing degradation, it accumulates for several hours within the lysosomes before recycling to the macrophage plasma membrane where it concentrates in some cell surface areas (18). In this study, we report that plasma membrane LPS macrodomains were specifically enriched in MHC class II molecules, confirming the tight association between these two molecules found by coclipitation experiments in murine macrophages (data not shown) and human and murine B lymphocytes (17). Despite this fact, the presence of MHC class II-LPS macrodomains seemed to not prevent the binding of HEL peptides into the groove of MHC class II molecules because the formation of HEL-induced compact forms was similar in both LPS-treated and -untreated macrophages. Therefore, LPS-induced interference on MHC class II Ag presentation is likely to occur distal to intracellular events leading to the meeting of antigenic peptides and MHC class II molecules. Altogether, these results suggest that the functional failure of LPS-loaded macrophages to present exogenous Ags could be related to events involving the meeting of correctly formed peptide-MHC class II complexes with their specific TCR. One hypothesis to explain this inhibition would be that LPS embedded in the membrane of MHC class II-positive compartments would interact with already formed HEL peptide-MHC class II complexes, thus forming a ternary complex, which then recycles to the plasma membrane. In the presence of LPS, an important proportion of class II-peptide complexes seems to be sequestered in the macrodomains present at the cell surface, decreasing the number of functional MHC class II-peptide complexes able to activate T cells. We suggest that the LPS O-chain would prevent the correct interaction between the MHC class II-peptide complex and the TCR, leading to a nonactivation of the T cells.

Another interesting model is related to a superantigen (SAG)-like function in T cell activation. It is known that SAGs simultaneously bind the Vβ domain of the TCR and MHC class II molecules on APC and consequently modify the geometry of TCR-peptide/MHC complexes, which may be less critical for T cell activation than certain other factors, in particular, those involved in the stability of the resulting complex (37, 38). In addition, the serial triggering (39–41) and kinetics proofreading models (42) of
T cell activation suggest that the short half-lives of TCR-peptide/MHC complexes are required for efficient T cell stimulation. It has been proposed that the ternary complex TCR-SAG-MHC has a short half-life similar to that of the TCR-peptide/MHC complex, which renders the complexes unable to serially engage a large number of TCRs (38). In this study, we detected the presence of LPS-MHC class II macrodomains in macrophages even after 60 days post-LPS injection, thus highlighting the remarkable stability of these surface LPS macrodomains. In this model, LPS could down-regulate T cell responses by stabilizing the MHC-peptide complexes at the cell surface of APC. Consequently, in contrast to SAG, LPS would be less efficient at triggering T cells because they form TCR-LPS-MHC complexes with a very long half-life. Because it remains to be established whether ligands that form complexes with very long half-lives are actually less efficient at triggering T cells, further molecular characterization of the surface MHC class II-LPS complexes will certainly be of great interest to better understand the mechanisms by which this molecule alters the activation of T lymphocytes and, thus, inhibits the cellular immune response.

Interestingly, the inhibition of immune response described here in vitro correlates with that observed in vivo upon infection by Brucella. Indeed, it is worth noting that chronic brucellosis is accompanied by a profound general immunosuppression, which can be revealed by using an IL-2 detection system (43, 44). Such mechanisms seem to be related to the location of Brucella within macrophages, the major effectors of microorganism cell-mediated killing. For instance, infected macrophages may exert a negative feedback control, which diminishes lymphocyte proliferation in vitro and in vivo upon infection. In conclusion, this study affords evidence that Brucella LPS plays a central role in the immunosuppression observed upon brucellosis infection and may account for the presence of anergic T cells. For instance, infected macrophages may exert a negative influence leading to the conclusion that macrophages containing chronically infected macrophages may fail to act as a target of T cells. Indeed, it is worth noting that chronic brucellosis is accompanied by a profound general immunosuppression, which can be revealed by using an IL-2 detection system (43, 44). Such mechanisms seem to be related to the location of Brucella within macrophages, the major effectors of microorganism cell-mediated killing. For instance, infected macrophages may exert a negative feedback control, which diminishes lymphocyte proliferation in vitro and in vivo upon infection. In conclusion, this study affords evidence that Brucella LPS plays a central role in the immunosuppression observed upon brucellosis infection and may account for the presence of anergic T cells. For instance, infected macrophages may exert a negative influence leading to the conclusion that macrophages containing chronically infected macrophages may fail to act as a target of T cells.
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