DIFFERENT REGULATION OF TNFα AND IL-1ra SYNTHESIS IN LPS-TOLERANT HUMAN MONOCYTES

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INTRODUCTION

In vivo administration of Gram-negative bacteria or endotoxin (lipopolysaccharide, LPS) induces a transient refractoriness to a subsequent challenge by LPS. Manifestations such as fever, hypoglycemia, hypotension, shock and death are decreased or avoided for a period of 3-4 days following an initial injection with non-lethal doses of LPS (He et al., 1992). This observation could reflect an endotoxin tolerance. Endotoxin tolerance is mainly a macrophage-mediated phenomenon (Freudenberg and Galanos, 1988). We and others have previously shown that monocytes isolated from patients with sepsis syndrome had a reduced ability to produce IL-1α, IL-1β, IL-6 and TNFα upon in vitro stimulation (Luger et al., 1986; Helminen, 1991; Munoz et al. 1991, Simpson et al., 1991). Van Deuren et al. (1994), studying patients with acute meningococcal infections, have confirmed this observation using a whole blood assays. In addition, they reported that the ex vivo IL-1ra production, after LPS stimulation, was enhanced or maintained but never reduced as it was observed for the inflammatory cytokines. The authors suggested that the down-regulation of inflammatory cytokines production and up-regulation of IL-1ra production during acute infection could serve as a mechanism of protection.

In an in vitro model of LPS-tolerance using human monocytes from healthy donors (Matic and Simon, 1991; Cavaillon et al. 1994), we have investigated whether such different regulation exists between TNFα and IL-1ra.
METHODS AND RESULTS

Human monocytes selected by adherence were incubated for 22 h in culture medium supplemented with 0.2% normal human serum and 1 μg/ml indomethacin, alone or in the presence of 2 μg/ml Neisseria meningitidis (N.m.) LPS. The cells were then washed extensively, and cultured for different period of times in the same culture medium, in the absence or the presence of 2 μg/ml N.m. LPS. TNFα and IL-1ra were assessed at transcriptional level using Northern blot analysis and at protein level using specific ELISAs. As shown in figure 1a, 1 h of restimulation with LPS was unable to induce significant amounts of TNFα mRNA in LPS-tolerized human monocytes while transcription of the TNFα gene occurred in non LPS-pretreated cells. Absence, or weak expression of TNFα mRNA was similarly observed after both 1 h or 4 h of LPS restimulation in the tolerized monocytes. It is worth noting that, while TNFα mRNA expression is maximum after 1h in freshly isolated monocytes (Wollenberg et al. 1993), in untreated pre-culture monocytes expression of TNFα mRNA was greater 4 h after stimulation with LPS than after 1 h of stimulation.

In contrast, 1 h after restimulation with LPS, transcription of IL-1ra in LPS-tolerized human monocytes was enhanced in comparison with untreated precultured mono-

![Graph](image)

**Figure 1:** TNFα mRNA/β-actin mRNA ratio (a) and IL-1ra mRNA/β-actin mRNA ratio (b) in human monocytes cultured in the absence or the presence of N.m. LPS (2 μg/ml) for 22 h, and monocytes were challenged or not in the presence of N.m. LPS (2 μg/ml) for different time. Relative densities were obtained by scanning the northern blots by laser densitometer.
cytes (figure 1b). Even after 18 h of LPS restimulation, IL-1ra mRNA expression remained high in the tolerated monocytes.

We also assessed TNFα and IL-1ra release in LPS-tolerized and untreated precultured monocytes supernatants. One hour after LPS restimulation, TNFα release in LPS-tolerized monocytes was close to that measured in untreated precultured monocytes (TNFα release = 327 ± 77 pg/ml vs 309 ± 92 pg/ml by LPS-tolerized monocytes vs untreated precultured monocytes; n = 5). These results did not reflect those obtained at transcriptional level. A further incubation (4 h) of LPS-tolerized human monocytes with LPS led to a low TNFα release (Table 1) as compared to untreated pre-cultured cells. This last result correlates with those obtained at the transcriptional level.

One hour after LPS restimulation, IL-1ra release in LPS-tolerized monocytes was sometimes enhanced or identical to IL-1ra release measured in untreated precultured monocytes (IL-1ra release = 1361 ± 319 pg/ml vs 1227 ± 314 pg/ml by LPS-tolerized monocytes vs untreated precultured monocytes; n = 5). Moreover, the enhancement observed sometimes at protein release level was not identical to that observed at transcriptional level. IL-1ra released by LPS-tolerized monocytes and restimulated 4 h with LPS was slightly decreased (10%) as compared to untreated precultured monocytes (Table 1). This result did fully reflect what was observed at transcriptional level, since IL-1ra mRNA expression was higher than that obtained with untreated precultured monocytes (figure 1b). Twenty-six hours-culture supernatants of control human monocytes (untreated precultured and unchallenged monocytes) were not able to release TNFα (30 pg/ml) but spontaneous IL-1ra was measured in supernatants (3475 pg/ml).

As previously described (Cavaillon et al., 1994), 18-24 h after restimulation with LPS of LPS-tolerized human monocytes, a more complete inhibition of TNFα release (94%) was observed (figure 2), whereas only a weak decrease of IL-1ra protein release (34%) was noticed (figure 2).

Table 1: TNFα and IL-1ra release upon N.m. LPS (2 μg/ml) activation by untreated precultured and LPS-tolerized human monocytes (22 - 26 h). This experiment was representative of five.

<table>
<thead>
<tr>
<th>pretreatment (T = 0 - 22 h)</th>
<th>Challenge (T = 22 -26h)</th>
<th>Cytokines recovered (T = 26h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>none</td>
<td>TNFα (pg/ml) IL-1ra (pg/ml)</td>
</tr>
<tr>
<td>none</td>
<td>LPS</td>
<td>46,430</td>
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<tr>
<td>LPS</td>
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<td>8,676 (-81%)</td>
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<table>
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<tr>
<th>pretreatment (T = 0 - 22 h)</th>
<th>Challenge (T = 22 -26h)</th>
<th>Cytokines recovered (T = 26h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>none</td>
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</tr>
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<td>LPS</td>
<td>10,494</td>
</tr>
<tr>
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<td>9,479 (-10%)</td>
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DISCUSSION

We have compared the in vitro synthesis of TNFα and IL-1ra in monocytes of healthy donors, using a LPS-tolerance model. It appears that TNFα and IL-1ra were differently regulated in LPS-tolerized monocytes model. These data are in agreement with ex vivo results reported by Van Deuren et al. (1994) during meningococcal infection. We have previously showed that TNFα was completely down-regulated, when LPS-tolerized-monocytes were challenged with LPS (Cavaillon et al., 1994). In the present work, we demonstrate that one hour of restimulation with LPS leads to a significant reduction of TNFα translation, whereas within 1h no down-regulation was observed at protein release level. Four hours after LPS restimulation of LPS-tolerized monocytes, both transcriptional and protein level were down-regulated as compared to untreated precultured monocytes. Interestingly, we observed a shift in the kinetic of TNFα mRNA expression. TNFα mRNA was better expressed 4 h after LPS stimulation than after 1 h in untreated precultured monocytes whereas it is the opposite observation in freshly isolated human monocytes. Another difference between translation and protein release was found with IL-1ra. We showed that 1 and 4 h after LPS restimulation, IL-1ra mRNA was
enhanced in LPS-tolerized monocytes as compared to untreated precultured cells. This enhancement was not associated with an increase of protein release. In LPS-tolerized monocytes restimulated 18 h with LPS, a weak low protein release was observed as compared to untreated precultured monocytes, whereas TNFα protein release was totally inhibited in LPS-tolerized cells. At this time, no TNFα mRNA expression was observed in both LPS-tolerized and untreated precultured monocytes. Another set of experiments to evaluate cell-associated IL-1ra protein is required in an attempt to further analyse the discrepancy between the observed enhanced IL-1ra mRNA expression and the absence of increased release of IL-1ra.

We have previously shown that IL-1, IL-10 and TGFβ could render human monocytes hyporeactive to further activation by LPS, leading to a reduced TNF (Cavaillon et al., 1994). IL-10 and TGFβ are produced by monocytes after LPS stimulation and it is well established that these 2 cytokines can enhance IL-1ra synthesis in LPS-stimulated monocytes (Wahl et al., 1993; de Waal Malefyt et al., 1993; Jenkins et al., 1994). Moreover, IL-1 produced after LPS stimulation could also induce IL-1ra synthesis (Wahl et al., 1993). We could hypothesize that IL-1ra enhancement seen in LPS-tolerized monocytes was the consequence of the synthesis of these cytokines.

In vivo, additional mechanism could be involved. IL-4 might play a role in this process. Wong et al. showed that after IL-4 therapy, monocytes from cancer patients expressed a marked increase in IL-1ra mRNA, which was reflected by significant increase in serum level (Wong et al., 1993). Furthermore, IL-4 down-regulates the LPS-stimulated pro-inflammatory cytokines production by human PBMC and monocytes, whereas IL-4 increases the synthesis of IL-1ra by these cells (Te Velde et al., 1990; Vannier et al., 1992; de Waal et al., 1993). Further studies on the regulatory mechanism of TNFα and IL-1ra synthesis in LPS-tolerant human monocytes could help us for the comprehension of the complex cytokines network involved in tolerance phenomenon.

REFERENCES


