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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-

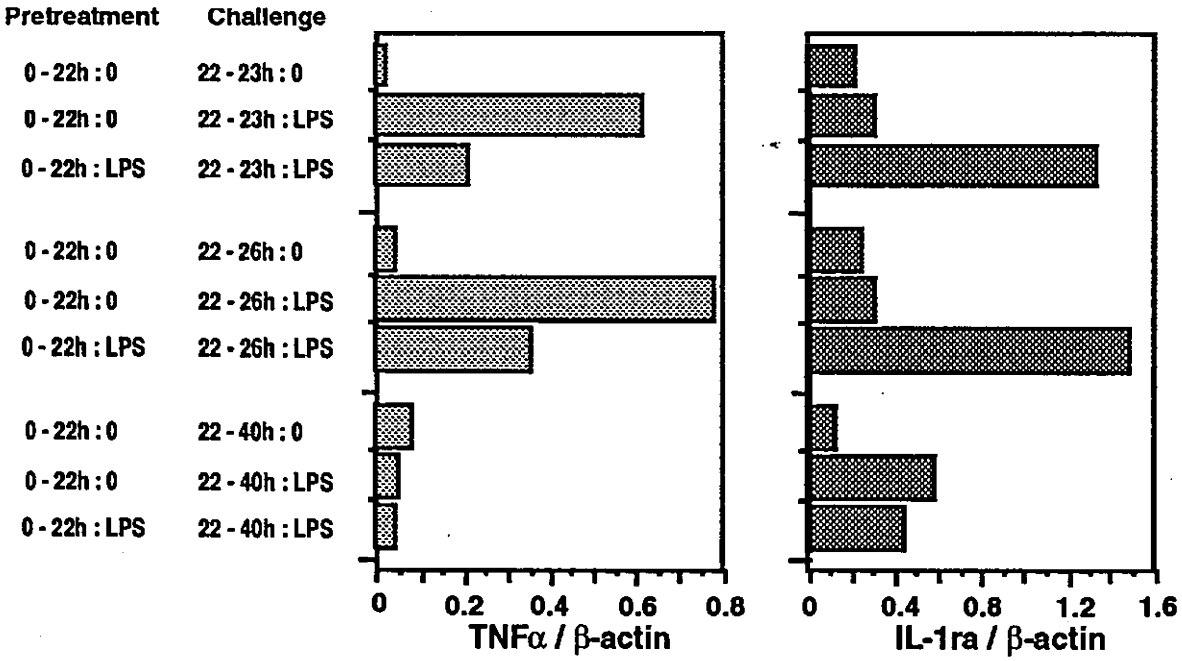

Figure 1: TNFα mRNA/β-actin mRNA ratio (a) and IL-1ra mRNA/β-actin mRNA ratio (b) in human monocytes cultured in the absence or the presence of *N.m.* LPS (2 µg/ml) for 22 h, and monocytes were challenged or not in the presence of *N.m.* LPS (2 µg/ml) for different time. Relative densities were obtained 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 tolerized 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), 18-24h 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.

pretreatment (T = 0 - 22 h)	Challenge (T = 22 -26h)	Cytokines recovered (T = 26h) TNFα (pg/ml)	IL-1ra (pg/ml)
none	none	30	3475
none	LPS	46,430	10,494
LPS	LPS	8,676 *(-81%)*	9,479 *(-10%)*

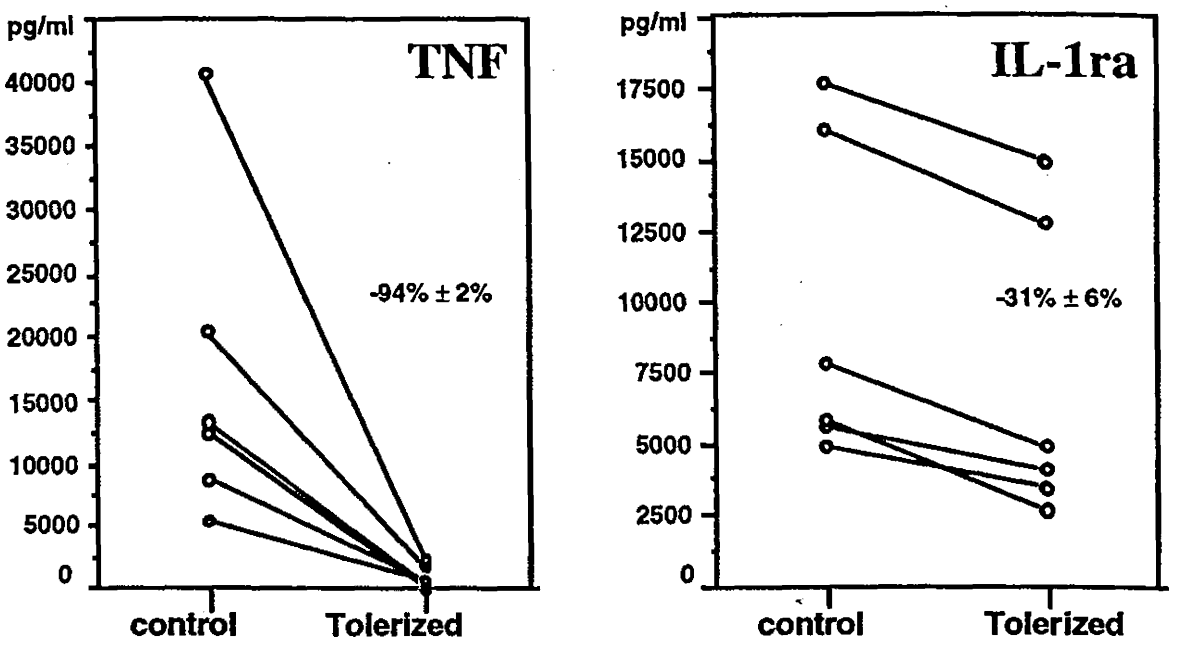

Figure 2: TNFα and IL-1ra release upon *N.m.* LPS (2 µg/ml) activation by untreated precultured and LPS-tolerized human monocytes (22 - 40 h). Each line represent an individual donor (n = 6)

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 whithin 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). Furthemore, 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.

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