Prevention of endotoxin-induced lethality in neonatal mice by interleukin-13

Interleukin (IL)-13, a cytokine produced by T helper 2 (Th2) cells, is a powerful inhibitor of macrophage functions, including surface expression of CD14 and production of IL-1 and tumor necrosis factor (TNF-α). We tested the effects of recombinant mouse (m)IL-13 in a neonatal mouse model of endotoxin shock; this is a macrophase-dependent condition, which is a model of neonatal sepsis in humans. mIL-13 (0.5 μg/mouse) dramatically reduced the lethal effects of lipopolysaccharide (LPS) if administered either 24 or 4 h prior to or concomitantly with LPS challenge. This action might be mediated by multiple modulatory activities of IL-13 on LPS-induced cytokine secretion since, relative to control animals, the mice treated with mIL-13 had eight times lower peak blood levels of TNF. The IL-1β levels were also decreased, whereas increased levels of IL-6 and IL-10 were observed at several time points after LPS challenge.

1 Introduction

Endotoxin shock is a life-threatening condition characterized by metabolic derangements in the functions of several organs resulting from the activation of the inflammatory system by the lipopolysaccharide (LPS) of Gram-negative bacteria (see [1, 2] for reviews). Central to the pathogenic property of LPS is the binding to CD14, a glycosylprotein expressed on monocytes and neutrophils as a glycosylphosphatidylinositol-anchored molecule [3]. This binding leads macrophages to release inflammatory cytokines such as TNF-α and IL-1α and β [4-5]. Accordingly, LPS lethality cannot be induced in CD14-deficient mice [6] and it is prevented by pretreatment with anti-TNF-α antibody [7] or IL-1 receptor antagonist (IL-1ra) which blocks the effects of IL-1α and IL-1 [8].

IL-13 is a cytokine produced by T helper 2 (Th2) cells and mast cells. The human IL-13 gene has a close chromosomal linkage to the IL-4 gene on chromosome 5 in humans and belongs to the IL-4 gene family (see [9] for review). Although IL-13, unlike human IL-4, does not affect T cell function, both cytokines share several biological properties, including B cell activation with production of IgG4 and IgE, and multiple anti-inflammatory properties primarily exerted through an inhibition of macrophage functions [9]. IL-13 suppresses IL-1β, IL-8, and TNF-α production from both human peripheral blood mononuclear cells (PBMC) and macrophages in vitro [10, 11]. The effect on macrophages seems to be mediated by an inhibitory action of IL-13 on CD14 expression [11] and upregulation of IL-1ra production from PBMC [11, 12].

Mouse(m)IL-13 exhibits about 20% homology with mIL-4 [13]. mIL-13 shares several properties with its human analog, but with a more limited range of activities. It does not affect B cell function and has a less marked inhibitory effect on macrophage production of IL-1β and TNF-α, yet it strongly suppresses the release of IL-12 and nitric oxide [14].

The anti-inflammatory properties make IL-13 a candidate for the treatment of macrophage-mediated inflammatory conditions and therefore prompted us to test its effects in a neonatal mouse model of endotoxin shock. The results demonstrate that prophylactically administered IL-13 dramatically reduces the lethal effect of LPS in these mice. This action may be mediated by multiple modulatory activities of IL-13 on LPS-induced cytokine secretion.

2 Materials and methods

2.1 Reagents

Escherichia coli-derived purified mIL-13 was purchased from R&D Systems (Minneapolis, MN). mTNF-α was obtained from Genzyme (San Francisco, CA). LPS from Salmonella enteritidis was provided by Sigma Chemicals (St. Louis, MO). RPMI 1640, phosphate-buffered saline (PBS) and fetal calf serum (FCS) were from Gibco (Grand Island, NY).

2.2 Animals

Neonatal (<24 h old) BALB/c mice of both sexes were used. Parental mice were obtained from Harlan-Nossan (Milan, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked and kept with the mother. LPS was weighed and diluted in PBS before each experiment. The neonatal mice were weighed and injected subcutaneously with 25 mg LPS per kilogram
body weight. Lethality was assessed at 24-h intervals for 3 consecutive days.

2.3 Cytokine measurement

The mice were killed by decapitation under ether anesthesia prior to and at several time points after challenge with LPS, as described in the legend to Figs. 1 and 2a–d. Mixed arterial and venous blood was collected in heparinized containers and centrifuged. Plasma samples from two animals were pooled for cytokine measurement. Spleens were also collected at the same time points for studies ex vivo. Thereafter, splenic lymphoid cells (SLC) obtained as described [15], were cultured at the final concentration of 2 × 10^6 cells well in flat-bottom microtiter plates at 37°C, 5% CO2, 95% air in complete medium (RPMI 1640 + 10% FCS). After 48 h the cells were centrifuged and the supernatants were collected for cytokine measurements.

mIL-1β, mIL-4, mIL-6, and mIL-10 were measured by solid-phase ELISA kits purchased from Genzyme (IL-1β), Biosource (Camarillo, CA) (IL-10), and Endogen (Boston, MA) (IL-4 and IL-6). The limits of sensitivity of the assays were 10 pg/ml (IL-1β), 5 pg/ml (IL-4), and 15 pg/ml (IL-6, IL-10, and IL-10). To calculate mean values, samples with cytokine values below the level of detection were assigned the limit of sensitivity of the assay as a theoretical value. TNF was measured by a bioassay previously described [16]. TNF activity is expressed in U/ml, and 1 U is defined as the amount of TNF causing 50% lysis of the WEHI 164 cell clone 13. To rule out interference with the bioassay of exogenously administered mIL-13, initial experiments showed that mIL-13 failed to influence WEHI 164 cell lysis induced by mTNF-α. Seven serial twofold dilutions, starting with a 1:20 dilution, were tested in duplicate. The assay was calibrated using mTNF-α as a standard. Since sera were diluted 20 times before assay, the actual lower limit of detection was 20 U/ml TNF activity.

To calculate mean values, results below the detection levels were assigned a theoretical value of 10 U/ml.

3 Results and discussion

3.1 Prophylactic treatment with mIL-13 counteracts lethality in BALB/c mice

To assess whether exogenously administered mIL-13 exerted beneficial effects in a neonatal model of endotoxin shock, several groups of neonatal BALB/c mice were injected with LPS and received either PBS or mIL-13 according to the dose and administration schedule shown in the legend to Fig. 1 and Table 3. Doses of mIL-13 were chosen on the basis of preliminary experiments which showed the lack of toxicity (as judged from suckling and general appearance) in neonatal BALB/c mice not challenged with LPS and treated with 0.1, 0.5, and 1 μg mIL-13/mouse. TNF blood levels were not affected under these experimental conditions (data not shown).

As expected, most control PBS-treated mice died within 96 h after LPS challenge. In contrast, when the animals were treated with mIL-13 4 h prior to LPS challenge, the cytokine counteracted the effects of LPS in a clear dose-dependent fashion (Fig. 1). Thus, while the lowest dose of mIL-13 (0.1 μg/mouse) only offered a slight and insignificant protection from the lethal effects of LPS, pretreatment with 0.5 μg mIL-13/mouse dramatically reduced the rate of mortality (Fig. 1).

Experiments were then carried out in which the effect of mIL-13 administration at different time points and dosages was analyzed in relation to the LPS challenge. While a trend toward a higher degree of protection was noticed when pretreatment with mIL-13 was initiated 24 h prior to LPS challenge, comparable results were obtained when mIL-13 was applied either 4 h prior to or concomitantly with the LPS challenge. Delayed mIL-13 administration either 4 or 24 h after LPS was no longer effective (Table 1 and data not shown). Increasing the dose of mIL-13 to 1 μg/mouse either 4 or 4 h prior to LPS challenge failed to potentiate further the protective effects obtained with 0.5 μg (data not shown).

3.2 mIL-13 profoundly modulates the cytokine secretory response to LPS in BALB/c mice

The lethal effects of LPS in sepsis are known to be mediated by the release from activated macrophages of TNF-α and IL-1α and β [1–5]. Our previous work in a model of neonatal sepsis induced by lethal group B streptococcal infection has also showed that the effects of TNF-α are counteracted by cytokines such as IL-6 and IL-10, both of which are secreted in a tightly coordinated manner in response to LPS [17–19]. Therefore, the beneficial action of mIL-13 might have been mediated by a modification of the cytokine secretory profile of the cells challenged with LPS. To investigate this, several groups of neonatal mice were treated 4 h prior to LPS challenge either with PBS or mIL-13 (0.5 μg/mouse).
Table 1. Effects of time of administration of mIL-13 on lethality induced by S. enteritidis LPS in neonatal mice

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a) Mouse pups were challenged with 25 mg S. enteritidis LPS/kg.
b) Relative to time of challenge with LPS.
c) p < 0.05 by Fisher’s exact test, compared with the respective vehicle controls.

Control neonatal mice challenged with LPS and pretreated with PBS showed increased blood levels of TNF, IL-1β, IL-6, and IL-10, all of which were released with different kinetics in response to LPS (Fig. 2a–d). This cytokine pattern was profoundly modified by pretreatment with mIL-13 which, relative to LPS-challenged control mice, suppressed the peaks of TNF (p < 0.0001) and IL-1β (p < 0.01) which occurred 2 and 4 h after LPS challenge, respectively (Fig. 2a, b) increased the release of IL-6 both at 2 (p < 0.05) and 4 (p < 0.01) h after LPS administration (Fig. 2c) and augmented the peak levels of IL-10 8 h after LPS challenge (Fig. 2d). This latter effect approached, but did not reach, statistical significance (p = 0.06), most likely because of the limited number of mice tested. None of the samples from the control or experimental groups of mice contained detectable amounts of IL-4 at any of the time points examined. This analysis of cytokine production in LPS-challenged neonatal mice thus reveals a profound modification of cytokine secretion in response to mIL-13.

The inhibitory action of mIL-13 on IL-1β and TNF secretion confirms, complements, and extends previous studies in vitro both human and mouse cells [10, 11, 14]. In contrast, the stimulatory effects of mIL-13 on IL-6 production are at variance with one study on mouse macrophages in vitro, which showed a slight IL-13-induced suppression of IL-6 production [14]. Our results, however, are in accord with another study ex vivo where mIL-13 augmented LPS-induced IL-6 production [20]. Deroq et al. [21] have also found that IL-13 stimulates IL-6 release from human keratinocytes. The different experimental conditions employed are likely to account for these differences.

The cytokine secretory capacity of SLC was suppressed ex vivo after LPS challenge perhaps because of functional exhaustion. This finding closely resembles studies on septic shock in humans indicating the dissociation between plasma cytokine levels and cytokine production by PBMC [22]. Thus, the content of IL-4 in culture supernatants...
remained below the limit of sensitivity of our assay both before and after LPS challenge. IL-10 was released in comparable fashion prior to LPS challenge from SLC of both PBS- and mIL-13-treated mice, but declined to below the threshold of detection after LPS challenge (Fig. 2d). In contrast the secretion of both IL-1β and IL-6 ex vivo was less influenced by challenge with LPS in vivo (Fig. 2b, c). However, while SLC from either mIL-13-treated or PBS-treated mice secreted comparable amounts of IL-β (Fig. 2b), the supernatants of SLC mice receiving mIL-13 showed a nonsignificant trend toward production of larger amounts of IL-6 than PBS-treated controls both prior to and 2 h after LPS challenge (Fig. 2c).

Our results show for the first time the beneficial effects of mIL-13 prophylaxis in a model of neonatal endotoxin shock. This action is probably due to the profound modifications induced by mIL-13 of the cytokine secretory profile of LPS-challenged macrophages, with inhibition of IL-1β, and in particular, TNF, along with augmented release of IL-6 and, although to a lesser extent, IL-10. This fits in with the pathophysiology of several mouse models of both adult and neonatal sepsis in which we and others have previously shown the pathogenic role of TNF-α [7, 16] and the protective effect of exogenously administered IL-6 [18, 23] and IL-10 [19, 24-25]. Although evidence for a pathogenic role of IL-1 in neonatal sepsis has not yet been provided, the multiple inflammatory properties of this cytokine, along with its well-known contribution to endotoxin shock in adult models [6], strongly suggest that the observed capacity of mIL-13 to suppress IL-1β release might also be implicated in the prevention of LPS lethality in the neonatal model. Moreover, if, in a manner similar to human IL-13 [11, 12], mIL-13 up-regulates IL-1ra production in response to LPS, this would further reduce the biological effects of IL-1.

As mentioned previously, human IL-13 down-regulates “in vitro” the expression of CD4+ on macrophages [10] and the LPS/CD4 interaction is known to play a pivotal role in triggering the pathogenic events of sepsis [6]. Hence, if CD14 expression on mouse macrophages is also inhibited by mIL-13, this could be envisaged as another mechanism by which IL-13 prevents the toxic effects of LPS. Notice, however, that 72 h are required for human IL-13 to induce 50% inhibition of macrophages CD14 expression [11]. This is much longer than the 4-h pretreatment within which exogenously administered mIL-13 successfully counteracted LPS-induced TNF secretion and lethality in the present study. Thus, unless mIL-13 down-regulates CD14 expression on macrophages “in vitro” more rapidly than human IL-13 does “in vitro”, this phenomenon is unlikely to be a major factor in the preventive action of mIL-13 in neonatal endotoxin shock.

The action of mIL-13 in neonatal endotoxin shock acquires particular biological relevance because mouse neonatal sepsis is more refractory than the adult form to the treatment with several TNF antagonists such as glucocorticoids [26] and sodium fusidate [27], possibly resulting from LPS-induced disturbance in carbohydrate metabolism [28]. Our present results provide evidence for the powerful immunomodulatory and macrophage deactivating properties of IL-13 “in vivo”, which anticipate the use of this cytokine in the treatment of cell-mediated inflammatory conditions. In particular, because neonatal septic shock gives rise to greater lethality and disability than the adult form [29], the use of IL-13 either alone or in combination with other anti-inflammatory cytokines (IL-10) or cytokine inhibitors (anti-TNF-α antibody, IL-1ra) deserves further consideration in the treatment of the syndrome in the clinical setting.

5 References