Differential Gene Expression for IL-1 Receptor Antagonist, IL-1, and TNF Receptors and IL-1 and TNF Synthesis May Explain IL-1-Induced Resistance to Infection

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IL-1 pretreatment prolongs survival in lethal infection in normal and in neutropenic mice. We investigated whether this protection occurs by interference with deleterious cytokine effects. The effect of IL-1 pretreatment on concentrations of IL-1α, IL-1β, IL-6, and TNF-α circulating in vivo and the ex vivo cytokine production capacity of macrophages was assessed in uninfected, non-neutropenic and neutropenic Swiss mice, in Swiss mice infected with Klebsiella pneumoniae (non-neutropenic mice) or Pseudomonas aeruginosa (neutropenic mice), and in neutropenic C3H/HeN and C3H/HeJ mice infected with P. aeruginosa. In Swiss and C3H/HeN mice, IL-1 pretreatment enhanced survival and reduced circulating TNF-α and IL-6 as well as LPS-stimulated production of IL-1α and TNF-α. In C3H/HeJ mice, a lack of IL-1-induced protection was associated with low cytokine concentrations and production. In contrast, up-regulation of mRNA for the IL-1 receptor antagonist (IL-1 Ra) was observed in several organs of IL-1-pretreated mice, suggesting that IL-1 Ra could attenuate deleterious IL-1 effects. In addition, IL-1 pretreatment down-regulated steady state mRNA for the type I IL-1 R and the type I TNFR in several organs at the time of infection, suggesting desensitization of target cells as an additional mechanism of IL-1-induced protection. We conclude that the IL-1-induced protection is at least partially mediated by down-regulating cytokine production, and that the induction of IL-1 Ra and the desensitization of target cells by receptor down-modulation may also contribute to this phenomenon. The Journal of Immunology, 1994, 153: 5772.

Two 17-kDa IL-1 proteins (IL-1α and IL-1β) produced by a variety of cell types, possess a wide spectrum of biologic effects (1). In animals, administration of IL-1 has been shown to enhance nonspecific resistance to bacteria, fungi, and plasmodia (2, 3). For example, pretreatment with a low dose (3 to 30 μg/kg) of recombinant human IL-1β (rhIL-1β) 24 h before a lethal Gram-negative infectious challenge enhances survival of normal and neutropenic mice (4, 5). The mechanism for this protection has only partially been clarified. A direct antimicrobial effect of IL-1 has been excluded in vitro (4), and whether IL-1 induces enhanced clearance of microorganisms in vivo is controversial (4, 6). The protective effect of IL-1 in granulocytopenic mice argues against a major role of neutrophils (2, 4, 7, 8). Glucocorticosteroids, eicosanoids, or cytokines such as IL-6, IL-8, and granulocyte macrophage-CSF do not mediate the IL-1-induced resistance to infection (9–12). However, IL-1-induced TNF-α and IL-1-induced acute phase proteins play some role as mediators of the IL-1 effect (11, 12).

In severe Gram-negative infection, excessive production of several cytokines including TNF-α, IL-1, IL-8, and IFN-γ, sometimes referred to as “lethal cytokinemia,” has been implicated in morbidity and lethality (13–15). To limit these deleterious effects, several counterregulatory mechanisms seem to exist: cytokine production may be inhibited (16), and host sensitivity to proinflammatory cytokines may be reduced by production of endogenous inhibitors (17, 18) or tolerance of target cells, for example...
by down-regulation of receptors (19, 20). IL-1 might enhance host resistance to lethal infection by one or more of these mechanisms. IL-1 has been shown to induce its specific inhibitor, the competitive receptor antagonist (IL-1Ra) in vivo in humans and in vitro (21–23). IL-1 is also capable of desensitizing to a lethal dose of LPS, TNF-α, and IL-1 (19) in vivo, and of reducing mRNA and protein levels for the type I IL-1R (IL-1RI) and the TNFR in several types of cells in vitro (24, 25). In the present study, we have investigated whether these mechanisms play a role in the IL-1-induced protection to infection by assessing the effect of IL-1 pretreatment on in vivo and in vitro cytokine production and on organ mRNA levels of IL-1RI, TNFRI, and IL-1Ra in infected animals.

Materials and Methods

Mice
Female outbred Swiss mice (weight, 20 to 25 g; TNO, Rijswijk, The Netherlands), female inbred C3H/HeN and C3H/HeJ mice (weight, 18 to 25 g; Bomholt Gard, Ry, Denmark), and New Zealand white rabbits (from a local colony) were kept under specific pathogen-free conditions.

Materials

Recombinant murine and human IL-1β (rmIL-1β and rhIL-1β) and polyclonal rabbit anti-murine IL-1α and IL-1β antisera were generously provided by Dr. P. Graber (Glaxo, Geneva, Switzerland); rmIL-1α and rhIL-1α were a kind gift of P. Lomedico, Hoffmann-La Roche (Nutley, NJ), and mTNF-α and rhTNF-α were kindly donated by Dr. G. Adolf, Ernst-Boehringer Institut (Vienna, Austria). Inactivated IL-1β was prepared by heating rhIL-1β at 95°C for 30 min. For in vivo administration, rhIL-1β or inactivated rhIL-1β (control treatment) was dissolved in pyrogen-free PBS, pH 7.4 (PBS), with 2% (v/v) mouse serum. Kind donations were received of cDNA for mIL-1RI (Dr. Ueli Gubler, Hoffmann-La Roche Inc.), mTNFRI (Dr. D. Wallach, Weizmann Institute, Rehovot, Israel), mIL-1Ra (Prof. A. S. Whitehead, University of Dublin, Dublin, Ireland) and chicken β-actin (Dr. B. Huber, Tufts University, Boston, MA).

Radioimmunoassays for IL-1α, IL-1β, and TNF-α

RIAs for the murine cytokines were performed essentially as described previously (26). To optimize this RIA for murine plasma and cell culture samples, several modifications were necessary. Because standard curves in assay buffer and sample medium did not superimpose, the medium of the standards was chosen to be identical to that of the samples. To improve sensitivity, the RIA was performed at 4°C, and the assay buffer in which the radiolabeled cytokines and second Ab were dissolved was changed to 0.25 M sodium phosphate (pH 7.4), 12.75 mM EDTA, 0.25% BSA (ORHD21/22 grade; Sigma Chemical Co., St. Louis, MO), 0.02% sodium azide with addition of 0.1% (v/v) Triton-X, and 1,000 kallikrein inactivating U/ml of aprotinin, with omission of normal rabbit serum.

The mean of triplicate standards was calculated, the value for non-specific binding (without rabbit antisera) was subtracted, and standard curves were fitted by weighted (27) nonlinear regression using a four-parameter logistic model (28).

IL-6 bioassay

IL-6 levels in serum were quantitated as hybridoma growth factor activity by using the IL-6-dependent murine hybridoma 7TD1 (a kind gift from J. van Snick, Ludwig Institute for Cancer Research, Brussels, Belgium) as described previously (29). Hybridoma growth factor activity resulting in half-maximal stimulation was arbitrarily defined as 1 U. The detection limit of the assay was 50 U/ml.

Experimental protocols

1) Survival experiments. To ascertain the effectiveness of rhIL-1β pretreatments, enhancement of survival of P. aeruginosa infection was assessed simultaneously with the preparation of samples for RIA and mRNA determinations. Because P. aeruginosa (ATCC 27853) does not kill normal mice, mice were rendered neutropenic (<0.5 × 10⁶ granulocytes per liter) by two s.c. injections of cyclophosphamide (ASTA Pharma, Frankfurt, Germany) on day -4 and on day -1 (150 and 100 mg/kg, respectively). On day -1, 800 ng of rhIL-1β or control treatment was injected i.p. (0.1 ml), and 24 h later, at time zero, a lethal challenge with 0.5 to 1 × 10⁷ CFU of P. aeruginosa was given intramuscularly (i.m.) in the left thigh muscle. Six hours after the infection, gentamicin (120 mg/kg; Schering, Kenilworth, NJ) was administered s.c. to postpone the time of death, and thus accentuate the differences between treatment groups. Mortality was recorded for 48 h in the P. aeruginosa infection, because thereafter bone-marrow recovery starts to play a role, and also cyclophosphamide effects on cell types other than phagocytic cells may be involved.

2) Preparation of samples for cytokine protein measurements. Mice were pretreated i.p. with 800 ng of rhIL-1β or control treatment 24 h before an i.m. infectious challenge with P. aeruginosa (neutropenic mice) or with 1 to 5 × 10⁷ CFU K. pneumoniae ATCC 43816 (non-neutropenic mice). All bacterial challenges (0.1 ml per mouse) were prepared by appropriate dilution of overnight cultures washed three times in saline. For assessments of circulating cytokines, blood was collected individually in EDTA-coated tubes on ice at several time points after infection. Plasma was prepared within 1 h by 10 min centrifugation at 400 × g at 4°C and stored at −70°C. For assessment of ex vivo cytokine production, resident peritoneal macrophages were harvested by rinsing the peritoneal cavity aseptically with cold PBS with 0.38% NaCl and 0.01% sodium citrate. After centrifugation at 400 × g at 4°C, cells were resuspended in RPMI 1640 (Dutch modification; Flow Laboratories, Irvine, UK), containing 1 mM pyruvate, 2 mM l-glutamine, and 100 μg/ml gentamicin. After perfusion of peritoneal macrophages (10⁶ cells/ml for 24 h) in 96-well microtiter plates in RPMI medium with or without LPS (from Escherichia coli serotype O55:B5; Sigma Chemical Co.) at a final concentration of 100 ng/ml, the culture supernatants were harvested, replaced by RPMI medium, and cells and supernatants were frozen at −70°C. Two more freeze-thaw cycles were performed before assay of cell-associated cytokines to accomplish lysis.

3) Preparation of tissues for mRNA assessments. Because surface protein expression of murine IL-1 and TNF receptors are below detection limit for proper statistical analysis (30, 31), and the murine IL-1Ra protein and specific Abs were not available, in vivo receptor expression and IL-1Ra regulation were studied by using gene expression. At 4, 12, or 24 h after i.p. administration of 800 ng of rhIL-1β or control injection i.p., Swiss mice were bled, killed by cervical dislocation, and the liver, spleen, kidneys, lungs, thymus, and brain were frozen immediately in liquid nitrogen, and stored at −70°C. Total RNA was extracted by using the guanidine-isothiocyanate-cesium chloride method (32). For Northern hybridization, performed to verify the integrity of RNA isolates, 20 μg of total RNA was subjected to electrophoresis in a 1.2% agarose-formaldehyde gel and transferred by capillary action to Hybond N+ membrane (Amersham, Buckinghamshire, UK). RNA was fixed to the membrane with UV light suggested by the manufacturer. Because all cDNA probes to be used were specific for the respective mRNA (24, 33–35), and analysis of both Northern and dot blots revealed a direct quantitative relationship (24, 35), gene expression was analyzed by using dot blots. Dot blots (50 μg and four twofold dilutions) also employed nylon membranes, and were sequentially probed with cDNA for mIL-1RI (2.2 kb), mIL-1Ra (1.7 kb), mTNFRI (1.5 kb), and β-actin. cDNAs were labeled with 32P by using a random-primed DNA labeling kit (Pharmacia, Piscataway, NJ). Membranes were exposed at −70°C to Kodak XAR5 films with Dupont Cronex intensifying screens. Autoradiograms were scanned by laser densitometry (Ultrascan XL, Pharmacia LKB, Bromma, Sweden). Ratios of densities of mIL-1Ra, mIL-1RI, and mTNFRI mRNA’s after IL-1 vs control pretreatment (inactivated IL-1β) were calculated and related to β-actin by dividing by the ratios of β-actin expression after IL-1 vs control pretreatment.

Statistical analysis

Survival data were analyzed by use of the log rank test (36). Comparisons of cytokine concentrations between treatment groups were made by the
FIGURE 1. Survival of neutropenic Swiss, C3H/HeN, and C3H/HeJ mice of a lethal i.m. challenge with *P. aeruginosa* at \( t = 0 \) after i.p. pretreatment at \(-24\) h with \( 800 \) ng of IL-1\( \beta \) or control pretreatment (inactivated IL-1\( \beta \)). Survival is significantly enhanced by IL-1 pretreatment in Swiss and in C3H/HeN mice \( (\chi^2 = 4.22 \text{ and } 8.03, p < 0.05, \text{ and } p < 0.005, \text{ respectively}) \), but not in C3H/HeJ mice (23 to 28 mice/group).

Results

**Specificity and validation of RIAs for murine cytokines**

Specificity of the three antisera was confirmed by assessing the lack of displacement of tracer from the respective Ab by mrlL-1\( \alpha \), mrlL-1\( \beta \), mrTNF-\( \alpha \), hrIL-1\( \alpha \), hrIL-1\( \beta \), and hrTNF-\( \alpha \), all tested in concentrations up to 5 \( \mu \)g/ml. Sensitivities of the RIAs for mIL-1\( \alpha \), mIL-1\( \beta \), and mTNF-\( \alpha \) with freshly prepared tracers ranged from 50 to 80, 20 to 70, and 80 to 140 pg/ml, respectively. The intra-assay coefficient of variation was less than 15% from the detection limit to typically 1800 pg/ml (IL-1\( \alpha \) and IL-1\( \beta \)) and 6000 pg/ml (TNF-\( \alpha \)). Interassay precision, expressed as coefficient of variation, was estimated by measuring two different plasma concentrations of IL-1\( \alpha \), IL-1\( \beta \), and TNF-\( \alpha \) (320 and 625 pg/ml) in duplicate on 10 different occasions, and was 15.0% and 12.9% for IL-1\( \alpha \), 3.8% and 7.1% for IL-1\( \beta \), and 15.4% and 11.0% for TNF-\( \alpha \). Recovery in these occasions was 91% and 98% for IL-1\( \alpha \), 87% and 94% for IL-1\( \beta \), and 91% and 112% for TNF-\( \alpha \), with SEM always <6.3%. When supernatant from unstimulated peritoneal macrophages was spiked with 160 or 320 pg/ml mrlL-1\( \alpha \), mrlL-1\( \beta \), and mrTNF-\( \alpha \), and measured on 10 occasions, recovery was 111% and 94% for IL-1\( \alpha \), 110% and 89% for IL-1\( \beta \), and 120% and 112% for TNF-\( \alpha \), with SEM always <16%.

Effect of rhIL-1\( \beta \) pretreatment on survival in different strains of mice

The LPS-hyporesponsive C3H/HeJ mice (38) were compared with the closely related LPS-sensitive C3H/HeN mice and with Swiss mice. The responses of the mice were verified by the lack of rise in plasma TNF-\( \alpha \) levels 2 h after i.p. LPS (50 \( \mu \)g) in C3H/HeJ mice, and a rise in C3H/HeN mice (39) (not shown). We examined survival and cytokine production in C3H/HeJ and C3H/HeN mice infected with *P. aeruginosa*.

As shown in Figure 1, pretreatment with 800 ng rhIL-1\( \beta \) 24 h before lethal *P. aeruginosa* infection enhanced survival significantly in neutropenic C3H/HeN and Swiss mice \( (\chi^2 = 4.33 \text{ and } 8.03, p < 0.05, \text{ and } p < 0.005, \text{ respectively}) \), but only slightly in neutropenic C3H/HeJ mice \( (\chi^2 = 1.67, \text{ NS}) \). Survival of infected mice not treated with IL-1 was somewhat better in C3H/HeJ mice compared with untreated C3H/HeN mice \( (\chi^2 = 2.78, p = 0.09) \). Survival of IL-1-pretreated C3H/HeN mice did not differ significantly from that in C3H/HeJ mice not treated with IL-1.

Measurement of in vivo cytokine production

Plasma concentrations of IL-1\( \alpha \) and IL-1\( \beta \) were at or below the detection limit at each time point after an i.m. challenge with *K. pneumoniae* (non-neutropenic mice) or *P. aeruginosa* (neutropenic mice).

As shown in Figure 2, plasma TNF-\( \alpha \) concentrations rose in Swiss mice at different time points in both types of infection. TNF-\( \alpha \) concentrations peaked at 1 h after *K. pneumoniae* inoculation, and then declined to the detection limit at 15 to 20 h. After *P. aeruginosa* injection, TNF-\( \alpha \) levels were lower than after the *K. pneumoniae* challenge and peaked later (1900 pg/ml at 5 h). These differences seem to reflect the more indolent course of the *P. aeruginosa* infection, because we and others have observed that if the same stimulus for TNF production is used in neutropenic mice and normal mice, the former have higher TNF concentrations in their blood (Ref. 40 and M. G. Netea, J. W. M. van der Meer, W. L. Blok, G. Francot, and W. A. Buurman, manuscript in preparation).

The effect of rhIL-1\( \beta \) pretreatment on plasma TNF-\( \alpha \) levels in Swiss mice after infection is shown in Figure 3. After a *K. pneumoniae* challenge, TNF-\( \alpha \) plasma levels in
p = 0.009 and TNF-α, the effect of IL-1 pretreatment on cytokine production ex vivo. Because macrophages are principal sources of IL-1 and TNF-α, the effect of IL-1 pretreatment on cytokine production capacity was studied with peritoneal macrophages from uninfected or infected non-neutropenic or neutro-

IL-1-pretreated normal mice were lower, albeit not statistically significant. In neutropenic P. aeruginosa-infected Swiss mice, TNF-α levels in the IL-1-pretreated group were reduced at each time point (significant at 5 and 8 h, p = 0.009 and p = 0.0043, respectively; 6 to 10 mice/group).

In neutropenic C3H/HeJ mice pretreated with rhIL-1β or control injections, plasma TNF-α 5 h after a P. aeruginosa challenge was undetectable. In C3H/HeN mice, IL-1β pretreatment caused a significant reduction of TNF-α levels at this time point (p = 0.04); also, in Swiss mice examined in the same experiment this difference was highly significant (p = 0.0015) (Fig. 4). In each strain of mice and in both pretreatment groups, TNF-α levels were undetectable at 24 h.

IL-6 serum levels, measured 3 and 8 h after P. aeruginosa infection in neutropenic Swiss mice, were reduced by IL-1 pretreatment (t = 3 h: from 6826 to 1736 U/ml, p = 0.004; t = 8 h: from 2746 to 1463 U/ml, NS; 6 to 7 mice/group).

**Measurement of cytokine production ex vivo**

FIGURE 3. Plasma TNF-α concentrations in Swiss mice pretreated at −24 h with 800 ng of IL-1β (hatched bar) or control pretreatment (inactivated IL-1β) (solid bar), and infected at t = 0 with K. pneumoniae (normal mice; panel A) or P. aeruginosa (neutropenic mice; panel B). Differences with control mice are significant at 5 and 8 h after P. aeruginosa infection (p = 0.009 and p = 0.0043, respectively; 6 to 10 mice/group).

FIGURE 4. Effect of i.p. pretreatment at −24 h with 800 ng of IL-1β (hatched bar) or control pretreatment (inactivated IL-1β) (solid bar) on plasma TNF-α concentrations at 5 h after i.m. P. aeruginosa infection administered at time zero in neutropenic Swiss, C3H/HeN, and C3H/HeJ mice. The reduction of TNF-α is significant in C3H/HeN mice (p = 0.04) and in Swiss mice (p = 0.0015), but not in C3H/HeJ mice (8 to 10 mice/group).
FIGURE 5. Ex vivo measurements of cytokine concentrations in cultured LPS-stimulated supernatants (s) and cells (c) isolated from mice pretreated in vivo with IL-1β (hatched bar) or control pretreatment (inactivated IL-1β) (solid bar) without infection, or after infection with K. pneumoniae (normal mice) or P. aeruginosa (neutropenic mice) (4 to 8 mice/group). A. Macrophages isolated from non-neutropenic uninfected Swiss mice 24 h after in vivo pretreatments. Secreted TNF-α is significantly reduced after IL-1 pretreatment (p = 0.01). B. Macrophages isolated from neutropenic uninfected Swiss mice 24 h after in vivo pretreatments. Secreted TNF-α is significantly reduced after IL-1 pretreatment (p = 0.009). C. Macrophages isolated from non-neutropenic IL-1 or control pretreated Swiss mice 5 h after infection with K. pneumoniae. Cell-associated IL-1α and secreted TNF-α from LPS-stimulated cells are significantly reduced after IL-1 pretreatment (p = 0.01 and p = 0.017, respectively). D. Macrophages isolated from neutropenic IL-1 or control pretreated C3H/HeJ mice 5 h after infection with P. aeruginosa. E. Macrophages isolated from neutropenic IL-1 or control pretreated C3H/HeN mice 5 h after infection with P. aeruginosa. Cell-associated IL-1α and secreted TNF-α from LPS-stimulated cells are significantly reduced after IL-1 pretreatment (p = 0.014 and p = 0.019, respectively). F. Macrophages isolated from neutropenic IL-1 or control pretreated C3H/HeJ mice 5 h after infection with P. aeruginosa. Cell-associated IL-1α and secreted IL-1β and TNF-α from LPS-stimulated cells are significantly reduced after IL-1 pretreatment (p = 0.0048, p = 0.0076, and p = 0.024, respectively). G. Macrophages isolated from neutropenic IL-1 or control pretreated C3H/HeN mice 24 h after infection with P. aeruginosa. H. Macrophages isolated from neutropenic IL-1 or control pretreated C3H/HeN mice 24 h after infection with P. aeruginosa. I. Macrophages isolated from neutropenic IL-1 or control pretreated Swiss mice 24 h after infection with P. aeruginosa. Secreted TNF-α from LPS-stimulated cells is significantly reduced after IL-1 pretreatment (p = 0.04).

present; however, these were still significantly reduced in IL-1-pretreated cells (190 vs 140 pg/ml, p = 0.0059; data not shown).

Twenty-four hours after P. aeruginosa infection in control or IL-1-pretreated C3H/HeJ mice, cytokine concentrations in cellular fractions or supernatants of unstimulated or stimulated macrophage cultures were negligible (panel G). Macrophages from neutropenic C3H/HeN mice obtained 24 h after P. aeruginosa infection and cultured with LPS contained cell-associated IL-1α concentrations of 1570 and 850
Table I. Median expression of steady state mRNA of IL-1Ra, IL-1RI, and TNFRI 12 and 24 h after IL-1 treatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Time (h)</th>
<th>IL-1Ra Ratio* (n)**</th>
<th>Range</th>
<th>IL-1RI Ratio</th>
<th>Range</th>
<th>TNFRI Ratio</th>
<th>Range</th>
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<td>Spleen</td>
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<td>1.03 (2)</td>
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<td>0.64 (3)</td>
<td>0.55-0.76</td>
<td>0.81 (3)</td>
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<td>24</td>
<td>1.83 (2)</td>
<td>1.34-2.31</td>
<td>0.65 (8)</td>
<td>0.40-0.84</td>
<td>0.69 (9)</td>
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<td>0.83 (3)</td>
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<tr>
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<td>0.73 (3)</td>
<td>0.63-0.9</td>
<td>0.87 (1)</td>
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<td>1.46 (1)</td>
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<td>0.87 (1)</td>
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<tr>
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<td>24</td>
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* Ratios of densities of IL-1Ra, IL-1RI, or TNFRI mRNAs after IL-1 vs control pretreatment (inactivated IL-1β) are divided by the ratios of densities of β-actin mRNA after IL-1 vs control pretreatment in the same blot.
** (n) denotes the number of observations.

Effect of IL-1 pretreatment on IL-1Ra gene expression in organs

The steady state mRNA expression of the IL-1Ra was not influenced by IL-1 pretreatment in any organ at early time points, but at 24 h a 1.8- and 9-fold up-regulation was present in spleen and liver, respectively (Table I and Fig. 6).

Effect of IL-1 pretreatment on IL-1RI and TNFRI gene expression

As shown in the dot blot (Fig. 6), a 60 and 54% down-regulation of steady state levels of mRNA for the IL-1RI and TNFRI, respectively, was found in the spleen 24 h after IL-1 pretreatment in comparison with control treatment. In Table I, the results of steady state mRNA levels 12 and 24 h after IL-1 pretreatment are shown for the various organs. A down-regulation (±30%) of steady state levels of mRNA for the IL-1RI and for the TNFRI were present in the spleen, thymus, and lung at 12 and 24 h after IL-1 pretreatment in comparison with control treatment, and no effect occurred in the brain. A clear up-regulation of IL-1RI and TNFRI mRNA was present in the liver at 24 h after IL-1 pretreatment, following a down-regulation at 12 h. In the kidney, after a 12-h pretreatment there was no IL-1 effect; at 24 h an up-regulation was observed for the TNFRI but not for the IL-1RI.

Discussion

In the present study, we have shown that IL-1-induced enhancement of survival of lethal Gram-negative infec-

FIGURE 6. Effect of IL-1 treatment on steady state levels of IL-1Ra, IL-1RI, and TNFRI mRNA isolated from the spleen at 24 h. Equivalent loading of the lanes was monitored by the constitutive expression of the β-actin housekeeping gene.
cytokine concentrations after a Gram-negative bacterial challenge are expected to be low. Therefore, if reduction of cytokine production would be the principal mechanism of IL-1-induced protection to infection, IL-1-induced enhanced survival should not occur in these mice. Our results are in accordance with this assumption. We observed a stepwise increase in survival to P. aeruginosa infection by IL-1 pretreatment in C3H/HeJ mice (nonsignificant), C3H/HeN mice (significant), and Swiss mice (highly significant). In the plasma of neutropenic C3H/HeJ mice, 5 h after the P. aeruginosa challenge cytokines were not detectable; in C3H/HeN mice, TNF-α plasma concentrations were elevated, and significantly reduced by IL-1 pretreatment; in Swiss mice, these effects of IL-1 pretreatment were even more pronounced. The increasing protective effect of IL-1 in C3H/HeJ, C3H/HeN, and Swiss mice, respectively, is also paralleled by an increasing down-regulation of cytokine production ex vivo.

The down-regulation of proinflammatory cytokines by pretreatment with IL-1 seems to be associated with up-regulation of IL-1Ra mRNA at the time of infection. IL-1Ra inhibits many effects of IL-1 by blocking the IL-1R (45). Attenuation of IL-1 effects by IL-1Ra during severe infections has been demonstrated (42, 46), and two recent studies demonstrate that administering neutralizing Abs to IL-1Ra enhances the severity of disease (47, 48). Induction of IL-1Ra synthesis in vitro by IL-1 has recently been reported (21–23, 49). Our finding of enhanced gene expression of IL-1Ra in spleen and liver after IL-1 pretreatment could imply that IL-1Ra induction plays a role in IL-1-induced protection. Interestingly, a similar pattern, i.e., down-regulation of proinflammatory cytokines with up-regulation of IL-1Ra occurs once infections such as meningococcal infection and typhoid fever have been established (50, 51). It is likely that in natural overwhelming infections (such as meningococcal sepsis in humans) the protective down-regulation of cytokine production up-regulation of anti-inflammatory cytokines occurs too late.

In addition to the modulation of pro- and anti-inflammatory cytokine production, we also investigated whether a desensitization of target cells by down-regulation of receptors plays a role in the IL-1-induced enhanced survival of infection. This possibility has been suggested by several in vitro studies (24, 25), although this does not apply to all types of cells (52). Because of the low numbers of cytokine surface receptors, we assessed steady state gene expression of the IL-1RI and the TNFRI in organs of Swiss mice 24 h after IL-1 or control injection, which is the time of the infectious challenge.

IL-1 pretreatment induced a down-regulation in spleen, thymus, and lung levels of both IL-1RI and TNFRI mRNA. These results suggest that IL-1-induced protection to infection is partially mediated by down-regulation of these receptors for IL-1 and TNF.

We have not been able to evaluate the effect of IL-1 on the type II IL-1R (IL-1RII). However, it is likely that this receptor is of lesser biologic relevance, because it has been recently reported that IL-1 signaling occurs exclusively via the type I receptor (53). Also, the TNFRI seems to be of greater biologic relevance than the TNFRII, because TNFRI-dependent signaling cascades have been proposed to be important in the effects of TNF-α as a proinflammatory factor (54), and because mice deficient for the TNFRI are resistant to septic shock (55).

In the liver, IL-1RI and TNFRI mRNA steady state levels were up-regulated at 24 h. The role of this up-regulation is unclear for the IL-1-induced protection. On the other hand, it could be argued that this up-regulation at the level of the hepatocyte is beneficial by augmenting acute phase proteins (12). In addition, because IL-1 pretreatment ameliorates the biochemical and histologic signs of liver damage, increased IL-1R on hepatocytes may augment this effect. Receptor up-regulation may also enhance clearance of IL-1 and TNF-α and thus add to the reduction of plasma cytokine levels (56, 57). The observed TNFR up-regulation in the kidney could also contribute to clearance of TNF-α (58).

Wallach et al. (19) also reported a protective effect of IL-1 pretreatment in galactosamine-sensitized mice to lethal effects of IL-1, TNF, and LPS. Similar to our observations, these investigators suggested that IL-1-induced protection is mediated by decreased cytokine production after LPS, and demonstrated decreased cytokine sensitivity of target cells. In contrast, Libert et al. (59), who studied the desensitization by IL-1 pretreatment to TNF and LPS in galactosamine-treated mice, did not observe reduced TNF-α plasma concentrations or modulated surface TNF-α receptors in the liver 12 h after IL-1 pretreatment. Because of the differences in the models used (especially the galactosamine sensitization), it is difficult to compare these results with ours.

Sheppard and Norton (60) reported protection by IL-1 pretreatment to a lethal TNF-α challenge and also suggested desensitization of target cells as a mechanism. Contel, Parant, and Parant (61) observed decreased TNF-α levels after LPS administration in IL-1-pretreated mice. In ex vivo studies with macrophages, desensitization for LPS was observed for only 2 h; these authors suggest a role of in vivo-induced corticosterone in the TNF-α down-regulation. In our models, however, IL-1-induced glucocorticosteroids do not play a role (10).

Although numerous studies report a deleterious role of overproduced cytokines during infection, physiologic quantities of TNF, IL-1, and IL-6 during infection may be protective (46, 62–64). Our recent studies with an antimurine TNF-α mAb argue for a deleterious role of TNF-α in the rapidly lethal experimental infections studied (11). Further studies performed with the use of specific Abs
could confirm a causal role in reduction of these cytokines in our model of IL-1-induced protection.

In addition to modulation of inflammatory and anti-inflammatory cytokines and cytokine receptors, other mechanisms of IL-1-induced protection could be operative. Induction of soluble TNFRs by IL-1, which neutralized TNF-α, has been reported in vivo (22). We have previously reported the role of IL-1-induced protective acute phase proteins (12). These proteins may be responsible for the slight protection induced by IL-1, which we observed in C3HHeJ mice. The mechanisms put forward in our studies might well be responsible for other IL-1-induced beneficial effects induced by IL-1 such as radiation protection, prevention of IL-2 toxicity, prevention of cytosine arabinoside-induced alopecia, and prevention of acetaminophen-induced liver damage (65–68).

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References


