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Soluble Murine IL-1 Receptor Type I Induces Release of Constitutive IL-1α

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IL-1α and IL-1β are proinflammatory cytokines involved in the pathogenesis of many infectious and noninfectious inflammatory diseases. To reduce IL-1 toxicity, extracellular domains of the soluble (s) IL-1R are shed from cell membranes and prevent triggering of cell-bound receptors. We investigated to what extent murine sIL-1RI can neutralize the IL-1 produced by LPS-stimulated macrophages. When mouse peritoneal macrophages were incubated with LPS, addition of sIL-1RI significantly inhibited the bioactivity of IL-1. Stimulation of cells with sIL-1RI alone induced no bioactive IL-1. When immunoreactive cytokine concentrations were measured with specific radioimmunoassays, sIL-1RI alone appeared to induce a significant release of IL-1α in a concentration-dependent manner. This effect was independent of new protein synthesis. The production of IL-1β or TNF-α was not influenced by sIL-1RI. There was no interference of sIL-1RI with the IL-1α radioimmunoassay. In mice, an i.v. injection of sIL-1RI alone induced a rapid release of IL-1α, but not of TNF-α or IL-1β. Treatment of mice with sIL-1RI improved the survival during a lethal infection with Candida albicans. In conclusion, sIL-1RI induces a rapid release of IL-1α from cells, as well as into the systemic circulation. Although this IL-1α may be inactivated in circulation by the same sIL-1RI, this phenomenon probably has immunostimulatory effects at local levels where the sIL-1RI-induced IL-1α acts in a paracrine or autocrine manner. *The Journal of Immunology, 1999, 162: 4876–4881.

Interleukin-1α and IL-1β are proinflammatory cytokines that play a central role in the regulation of inflammatory reactions occurring during severe infections (1). When infused into animals, IL-1 causes a syndrome resembling sepsis, with fever, increased vascular permeability, hypotension, multiple organ failure, and eventually death (2). In humans, IL-1 infusion is accompanied by fever, chills, hypotension, and production of IL-6 (1, 3). These effects are potentiated by concomitant administration of TNF-α, another main proinflammatory mediator in sepsis (2). 

Inhibition of IL-1 or TNF production with pharmacological agents (4), or blockade of membrane IL-1Rs with IL-1R antagonist (IL-1Ra)3 (5, 6) in experimental models of lethal endotoxemia results in protection of the animals. These experimental data have indicated that IL-1 inhibition might be a potential therapeutic approach in severe infections.

For IL-1Ra to work, high levels are needed to block all receptors on relevant cells. This requires large amounts of IL-1Ra, since only 1% of IL-1Rs need to be occupied to induce a signal (1). In contrast, neutralizing the circulating IL-1 is easier, since IL-1 concentrations during diseases are in the picomolar range, with relatively small amounts of IL-1 being produced during disease. Therefore, use of soluble receptors acting like neutralizing Abs can prove more successful.

Soluble IL-1Rs (sIL-1R) are naturally occurring regulatory proteins that influence the biological activities of IL-1. Type I IL-1R (IL-1RI) is found on most cells and it appears to mediate all the IL-1 actions (1). In contrast, type II IL-1R (IL-1RII) has no signaling function and is considered to act as a “decoy” receptor (7). Soluble recombinant forms of IL-1RI (sIL-1RI) have been developed, and their binding characteristics to IL-1 are similar to those of membrane IL-1RI (8). Administration of sIL-1RI in vivo improves the survival of heart allografts (9), protects from experimental autoimmune diabetes (10), improves the severity of active arthritis (11), and autoimmune encephalomyelitis (12). A recent study by Preas et al. (13) has shown that sIL-1RI administration to human volunteers is able to reduce some of the inflammatory effects of LPS, such as IL-1β induction, while it has stimulatory effects on TNF-α and IL-8 synthesis.

Despite the data indicating that sIL-1RI is a potential modulatory agent of IL-1-mediated pathways, few data are available about the mechanisms responsible for these effects or the possible direct actions of sIL-1RI, as suggested by the study of Preas et al. (13). The aim of the present study was to assess the capacity of recombinant murine sIL-1RI to bind and neutralize bioactive IL-1, and to investigate the possible stimulatory effects of sIL-1RI on cytokine synthesis by murine cells.

Materials and Methods

Materials

sIL-1RI was generously provided by Dr. M. B. Widmer (Immunex, Seattle, WA). Recombinant IL-1α was a gift of Dr. P. Lomedico (Hoffmann-La-Roche, Nutley, NJ) and recombinant IL-1β a gift of Dr. P. Graber (Glaxo, Geneva, Switzerland). LPS (Escherichia coli serotype O55:B5) and cycloheximide were obtained from Sigma (St. Louis, MO). IL-1α and IL-1β were radioiodinated as previously described (14).

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3 Abbreviations used in this paper: IL-1Ra, IL-1R antagonist; sIL-1R, soluble IL-1R; IL-1RI, type I IL-1R; IL-1RII, type II IL-1R; IL-1RAcP, IL-1R accessory protein.
The in vitro binding of IL-1 with the sIL-1RI was investigated using HPLC. The elution profile of \(^{125}\text{I}-\text{IL-1}\) on a Shodex KW-802.5 gel column with a molecular mass range of 100–50,000 Da (8 × 300 mm; Millipore, Milford, MA) was determined before and after incubation with sIL-1RI. \(^{125}\text{I}-\text{IL-1}\) (100 ng/ml) was incubated with sIL-1RI (10 μg/ml) in PBS for 15 min at room temperature. Phosphate buffer (100 mM, pH 6.8) was used as the elution buffer. The flow rate was 0.5 ml/min.

**Animals**

CBA mice were obtained from The Jackson Laboratory (Bar Harbor, ME). For the experiments, 6–8-wk-old mice, weighing 20–25 g, were used. The animals were fed standard laboratory chow (Hope Farms, Woerden, The Netherlands) and housed under specific pathogen-free conditions. The experiments were approved by the ethical committee on animal experiments of the Catholic University Nijmegen.

**In vitro cytokine production**

Resident peritoneal macrophages were harvested by rinsing the peritoneal cavity aseptically with cold PBS containing 0.38% (w/v) sodium citrate. After centrifugation for 10 min at 500 × g at 4°C, cells were resuspended in RPMI 1640 DM (Flow Laboratories, Irvine, CA) containing 5% normal mouse serum, 1 mM pyruvate, 2 mM t-glutamine, and 100 μg/ml gentamicin. A total of 10⁶ cells/well were cultured in 96-well microtiter plates (Costar, Cambridge, MA) in RPMI medium (final volume, 200 μl), with or without LPS (1 ng/ml) and/or sIL-1RI (10 ng/ml), unless indicated otherwise. Stimulants were collected after 24 h of incubation at 37°C and stored at −70°C until assay. To the remaining macrophages, 200 μl RPMI 1640 was added and the cells were disrupted by three freeze-thaw cycles to determine the cell-associated cytokine contents (15). The samples were stored at −70°C until cytokine measurements.

To test whether the detection of cytokines in the presence of sIL-1RI is due to an artifact in the radioimmunoassay or due to the presence of sIL-1RI in the medium, we coated the bottom of the wells with 1 μg/ml sIL-1RI by overnight incubation at 4°C. After blockage of nonspecific binding sites with 2% BSA and three vigorous washes with sterile PBS, 10⁶ peritoneal macrophages were added to each well, and LPS stimulation was performed for 24 h at 37°C. Control wells were precoated with 2% BSA.

In separate experiments, we investigated whether stimulation of cytokines by sIL-1RI is dependent on new protein synthesis. Mouse peritoneal macrophages were collected and stimulated with sIL-1RI as described above in the absence or in the presence of 1 μg/ml cycloheximide (16).

All the in vitro experiments used peritoneal macrophages from 10 animals and were performed at least twice.

**In vivo stimulation of cytokine production**

Groups of CBA mice were injected i.p. with LPS (10 μg/mouse), sIL-1RI (2 μg/mouse), or a combination of both. The kinetics of IL-1α, IL-β, and TNF-α production was studied by collecting the blood in EDTA from the retroorbital plexus of separate subgroups of five mice before and 30 min, 90 min, and 4 h after the challenge with LPS and/or sIL-1RI to determine plasma cytokine concentration. To test whether sIL-1RI could also induce IL-1α in neutropenic mice, the animals were injected s.c. 4 days before treatment with sIL-1RI with 150 mg/kg cyclophosphamide (Bristol-Myers Squibb, Weesp, The Netherlands), followed by injection of 100 mg/kg 1 day before the challenge. This model of neutropenia maintains blood neutrophils at levels lower than 100/mm³ (data not shown) (17). Cytokine concentrations were measured 90 min after the challenge, as described above.

The influence of sIL-1RI on IL-1α distribution and clearance in vivo

Groups of mice were treated with either 2 μg/ml sIL-1RI i.p. or saline, immediately before i.v. infusion of 10 μCi radiiodinated IL-1α or IL-1β in 0.2 ml saline. Five minutes, 15 min, 30 min, 1 h, 6 h, and 24 h later, subgroups of five animals were sacrificed and the distribution and clearance of \(^{125}\text{I}-\text{IL-1}\) was compared in the sIL-1RI-treated and control mice by measuring the percentages of the injected dose in the blood, liver, spleen, kidneys, thyroids, and lungs.

The effect of sIL-1RI treatment on Candida albicans infection

It has been previously shown that infection with *C. albicans* can be beneficially influenced by treatment with IL-1 (17). To investigate whether the induction of IL-1α release by sIL-1RI may have beneficial effects on the course of murine candidiasis, groups of 15 CBA mice were treated with either 0.1 mg/ml sIL-1RI i.p. or saline immediately before the mice were infected i.v. with 5 × 10⁵ CFU *C. albicans* (strain UC 820) (18). From both groups, five animals were sacrificed on day 1 after the infection, and the circulating cytokine concentrations were measured in plasma. In the remaining 10 mice/group, survival was assessed daily for 4 wk. To test whether sIL-1RI could also exert beneficial effects in neutropenic mice, the animals were injected s.c. 4 days before infection with 150 mg/kg cyclophosphamide, followed by injection of 100 mg/kg of cyclophosphamide 1 day before and 1, 3, 5, 7, and 9 days after i.v. infection with 10⁵ CFU *C. albicans*. The repeated cyclophosphamide administrations were performed to assure persistent neutropenia during the prolonged infection with *C. albicans*.

**Cytokine measurements**

IL-1 bioactivity was determined using the murine thymoma cell line EL-4 NOB-1 (ECACC, Porton Down, Salisbury, U.K.) as an IL-1-specific cell-producing IL-2 response, in combination with the IL-2-sensitive CTLL-2 cells (ECACC) (19). TNF-α, IL-1α, and IL-1β immunoreactive concentrations were determined using specific fluid phase RIAs, as described previously (20). Detection limits were 40 pg/ml for TNF-α and 20 pg/ml for IL-1α and IL-1β. Control experiments were performed to determine whether sIL-1RI alters the cytokine RIAs. When added to standard cytokine samples used for the calibration of the standard curves, sIL-1RI in the range tested (0.1 ng/ml to 1 μg/ml) had no influence on the assay (Fig. 1).

**Statistical analysis**

Differences between groups were analyzed using the Mann-Whitney U-test. Differences were considered significant at *p* < 0.05.

**Results**

sIL-1RI binds and neutralizes LPS-induced bioactive IL-1

Upon in vitro incubation with 0.1 mg/ml sIL-1RI, the retention time of \(^{125}\text{I}-\text{IL-1}\) on the Shodex KW-802.5 gel filtration column was reduced from 22 to 16 min, indicating almost complete complexation of the radioiodinated IL-1α with the sIL-1RI. When mouse peritoneal macrophages were incubated with sIL-1RI alone, no bioactive IL-1 could be detected in the supernatant. LPS stimulation led to synthesis of bioactive IL-1 (699 ± 588 pg/ml), and addition of 10 ng/ml sIL-1RI was able to significantly reduce this amount (223 ± 105 pg/ml, *p* < 0.03) (Fig. 2).

sIL-1RI induces the release of immunoreactive IL-1α in vitro

The synthesis and release of immunoreactive IL-1α, IL-1β, and TNF-α in response to LPS or sIL-1RI stimulation was investigated in the same set of experiments. Stimulation of murine macrophages with sIL-1RI resulted in high concentrations of IL-1α in the supernatant. This release was 2-fold higher than that observed...
after LPS stimulation, and the combination of sIL-1RI with LPS did not induce more IL-1α than sIL-1RI challenge did itself (Fig. 3). The effect of sIL-1RI on IL-1α induction was concentration-dependent (Fig. 4). Levels of IL-1β or TNF-α were unaffected (Fig. 3). The concentration of cell-associated cytokines was not influenced by sIL-1RI stimulation (data not shown).

To exclude the possibility that sIL-1RI would cross-react with IL-1α in the RIA (although control experiments demonstrated the contrary; see Fig. 1), we precoated the bottom of the plates with sIL-1RI. After washing the remaining sIL-1RI, macrophages were added and incubated. In these culture supernatants, significant release of IL-1α (152 ± 39 vs 28 ± 8 pg/ml, *p* < 0.01) was found again.

In a separate set of experiments we assessed whether induction of IL-1α release by sIL-1RI is dependent on new protein synthesis. Reducing new protein synthesis with cycloheximide (16) did not affect the IL-1α release induced by sIL-1RI (192 ± 20 pg/ml in the absence, vs 218 ± 21 pg/ml in the presence of cycloheximide, *p* > 0.05). This finding points to release of preformed IL-1α.

**sIL-1RI induces the release of immunoreactive IL-1α in vivo**

The capacity of sIL-1RI to induce IL-1α release in vivo was investigated by challenging mice with 2 μg/mouse sIL-1RI i.p. Challenge of mice with the vehicle alone had no stimulatory activity on cytokine production. sIL-1RI induced a rapid rise of IL-1α concentration in the circulation, with a peak already after 30 min (Fig. 5a). LPS-induced IL-1α had slower kinetics, and LPS induced lower IL-1α concentrations (Fig. 5a). No induction of IL-1β (Fig. 5b) or TNF-α (Fig. 5c) by sIL-1RI challenge was apparent. Similarly, 90 min after challenge of neutropenic mice with sIL-1RI, there was a significant increase in plasma concentrations of IL-1α (88 ± 21 pg/ml) compared with the concentration in the placebo-treated mice (<20 pg/ml, *p* < 0.01).

In two separate experiments we investigated whether sIL-1RI influenced the distribution of radiolabeled IL-1α or IL-1β to the organs or their clearance from blood. Radioiodinated IL-1α or IL-1β was injected into mice pretreated with vehicle only or sIL-1RI. The distribution of radiolabeled cytokines to liver, spleen, kidneys, lungs, and thymus was not influenced by sIL-1RI treatment (data not shown). The clearance of radiolabeled IL-1α (Fig. 6) or IL-1β (data not shown) from the blood was not modified by sIL-1RI treatment of the mice.
The effect of sIL-1RI treatment on a lethal C. albicans infection

While none of the mice infected with $1 \times 10^5$ CFU C. albicans died (data not shown), all control mice infected with $5 \times 10^5$ CFU C. albicans died during the first 10 days of infection. Treatment of mice with sIL-1RI significantly prolonged the survival of the mice infected with the high inoculum of Candida (Fig. 7a). In addition, sIL-1RI also prolonged survival of neutropenic mice infected with C. albicans (Fig. 7b). The IL-1α concentrations one day after infection with C. albicans were higher in mice treated with sIL-1RI than that in mice receiving placebo ($290 \pm 20$ vs $110 \pm 50$ pg/ml, $p < 0.05$). Each experiment involved minimally 10 mice/group and was performed twice.

Discussion

The results presented in this study show that sIL-1RI has a remarkable effect by inducing the release of preformed IL-1α, both in vitro and in vivo. The potential importance of this effect is illustrated by the beneficial effects of sIL-1RI administration on the course of murine disseminated candidiasis, an experimental infection in which IL-1 has protective effects. These protective effects are probably due to local autocrine and paracrine effects of sIL-1RI-induced IL-1α, since several studies in the literature suggest that the bioactive IL-1α released into the circulation is neutralized.

FIGURE 5. Effect of sIL-1RI injection in mice. CBA mice were injected i.p. with LPS (10 μg/mouse), sIL-1RI (2 μg/mouse), or a combination of both. The kinetics of IL-1α, IL-1β, and TNF-α production was studied by collecting the blood from the retroorbital plexus of subgroups of mice. sIL-1RI induced a rapid release of IL-1α (a), but not IL-1β (b) or TNF-α (c). * $p < 0.05$. Each experiment involved 10 mice/time point and was performed twice.

FIGURE 6. Effect of sIL-1RI on IL-1α distribution and clearance. Groups of mice were treated with either 2 μg/ml sIL-1RI i.p. or placebo, immediately before 0.2 μg (specific activity, 10 μCi/μg) of radioiodinated IL-1α was infused i.v. in 0.2 ml saline. No influence of sIL-1RI on the clearance of $^{125}$I-IL-1α was apparent. * $p < 0.05$. Each experiment involved 10 mice/time point and was performed twice.

FIGURE 7. a, Effect of sIL-1RI on lethal C. albicans infection. Groups of CBA mice were infected i.v. with $5 \times 10^5$ CFU C. albicans and treated s.c. with either placebo or one dose of 2 μg/ml sIL-1RI. The sIL-1RI treatment significantly improved the survival. b, Effect of sIL-1RI in mice rendered granulocytopenic with repeated injections of cyclophosphamide and infected i.v. with $10^4$ CFU C. albicans. The treatment of neutropenic mice with sIL-1RI beneficially influenced the survival time, although statistical significance was not reached. The experiments involved minimally 10 mice/group and were performed twice.
by the same sIL-1RI (9–12), which was also confirmed by our observations.

It is well established that recombinant forms of sIL-1RI have similar IL-1 binding characteristics as the membrane-associated form (8). In our experiments, we confirm that the recombinant form of murine sIL-1RI binds to both mouse IL-1 species. When mouse peritoneal macrophages were stimulated with LPS, the IL-1 biological activity of the resulting supernatants was significantly reduced. Our data are in line with the report of Preas et al. (13), who also reported decreased circulating IL-1β concentrations after sIL-1RI infusion during experimental endotoxemia in humans. This inhibition may account for the beneficial effects of sIL-1RI treatment in experimental models of autoimmune diabetes (10), heart transplantation (9), active arthritis (11), and autoimmune encephalitis (12).

In contrast to bioactivity, the immunoreactive concentrations of cytokines measured after sIL-1RI challenge revealed that a significant increase of IL-1α bioactivity occurred before apparent enhancement of IL-1β bioactivity (Fig. 3a). sIL-1RI exerted this effect in a concentration-dependent manner (Fig. 4). An artifact in the immunoassay was excluded by control experiments showing no influence of high sIL-1RI concentrations on the RIA standard curves, indicating that our RIA measures both free and sIL-1RI-complexed IL-1. Moreover, to further exclude artefacts in the assay, wells were precoated with sIL-1RI and stimulation of IL-1α release by fixed sIL-1RI was performed. The finding of high IL-1α concentrations in these supernatants in which no sIL-1RI was present proves a true effect of the soluble receptor on IL-1α release and demonstrates that this effect of sIL-1RI is not an artefact. Incubation of macrophages with sIL-1RI in vitro did not result in elevated IL-1β or TNF-α levels. The stimulatory action of sIL-1RI was also investigated in vivo, and the same phenomenon was observed: sIL-1RI induced a rapid increase in IL-1α concentrations that already reached peak elevation 30 min after the administration, without a comparable increase in IL-1β or TNF. The rapid kinetics suggests release of “preformed” IL-1α from the cells, rather than de novo protein synthesis. Indeed, the blockade of protein synthesis with cycloheximide showed that IL-1α induction by sIL-1RI is independent on de novo protein synthesis. A similar rapid release of preformed IL-1α has been previously reported in rats subjected to thermal injury (21). Interestingly, sIL-1RI was also able to induce a significant release of IL-1α in neutropenic mice, indicating that other cell types than neutrophils, such as for example endothelial cells, are also a source of the cytokine (1). However, the fact that the concentrations of sIL-1RI-induced IL-1α were an order of magnitude higher in nonneutrophic mice compared with those in neutrophic mice, indicates that neutrophils are a major source of preformed IL-1α.

Whereas sIL-1RI could not induce IL-1β, in contrast with the strong stimulation of this cytokine by LPS, the soluble receptor was much more potent than LPS for the induction of IL-1α. This differential induction of the two members of the IL-1 family, together with the higher affinity of sIL-1RI for IL-1α than for IL-1β (1), may explain why sIL-1RI present in the supernatants of the experiments shown in Fig. 2 was able to completely inhibit the IL-1β bioactivity induced by sIL-1RI alone, but not by the combination LPS+sIL-1RI. Indeed, sIL-1RI alone would induce only IL-1α, which would be bound with high affinity by the same sIL-1RI in the supernatant, and thus be neutralized completely. In contrast, the LPS would also stimulate IL-1β synthesis. The lower affinity of sIL-1RI for IL-1β probably results in certain amounts of unbound cytokine, explaining the residual IL-1 bioactivity after stimulation with the combination of LPS and sIL-1RI.

Stimulatory effects of sIL-1RI during experimental endotoxemia in humans have been recently reported by others (13). When administered together with LPS, sIL-1RI induced higher levels of C-reactive protein and higher peak concentrations of TNF-α and IL-8 than in volunteers challenged with LPS alone. It was suggested that these effects are, at least in part, due to binding and inactivation of IL-1Ra by sIL-1RI (13). However, inactivation of IL-1Ra was found 3 h after LPS administration, whereas TNF-α was induced before that, as early as 90 min after the LPS challenge (13). The hypothesis that at least some of these effects are exerted directly by sIL-1RI are supported by our data showing a rapid release of IL-1α, with the peak levels already present 30 min after LPS challenge.

It should be kept in mind that sIL-1RI induced other cytokines in mice than those found in humans by Preas et al. (13), and in this respect the mouse model may not represent what occurs in humans. There are several other differences between our study and that of Preas et al. which may account for some of the differences observed. First, whereas we investigated mainly the direct effects of sIL-1RI, Preas et al. (13) assessed the effect of sIL-1RI on LPS-induced cytokine production. Second, even though the latter study reported increased TNF and IL-8 release in the sIL-1RI treatment groups, it was not investigated whether these cytokines were bioactive or not. Thirdly, the release of IL-1α was not investigated (13), as that might have been also influenced by sIL-1RI. Possible effects of sIL-1RI on distribution or clearance of IL-1 in vivo have been excluded by our experiments in which radioiodinated IL-1α or IL-1β were infused into mice together with sIL-1RI or placebo. An important question is whether the IL-1α induced by sIL-1RI is biologically active. The biological activity of IL-1 could be exerted at distance in an endocrine manner, and in the close vicinity of cells in an autocrine and paracrine manner. The data presented in Fig. 2 show that the cytokine is at least in part bound and neutralized by the circulating sIL-1RI present in the system. It is therefore unlikely that the released IL-1α would exert remote effects. However, the rapid release of IL-1α from the cells upon sIL-1RI stimulation may have important consequences at a local level, where paracrine and/or autocrine actions of high local concentrations of IL-1α may be expected. This hypothesis is underlined by the beneficial effects of sIL-1RI during murine disseminated candidiasis, an experimental model in which IL-1 treatment has proven beneficial (17). Even the relatively low amounts of IL-1 induced by sIL-1RI in the neutrophic mice had beneficial effects, as only few IL-1 molecules are necessary to achieve full biological activity (1). We hypothesize that locally released IL-1α induced by sIL-1RI, which is not neutralized by sIL-1RI in the tissues, accounts for this effect. However, beneficial effects of sIL-1RI which are independent on IL-1α release cannot be excluded. To settle the question whether the effects of sIL-1RI in vivo are IL-1α-dependent, experiments with sIL-1RI may be performed in IL-1α knockout mice (22).

Important biological effects of sIL-1RI are also suggested by studies in which sIL-1RI administration was able to decrease inflammatory reactions to intradermal allergens, even when given at the contralateral site (23). Given the potential usage of soluble receptors for treatment of disseminated candidiasis and other inflammatory diseases, a thorough investigation of the in vivo effects of sIL-1RI is warranted. In view of the evidence that the overall effect of soluble cytokine receptors is dependent on the dose, future studies should thoroughly address this issue.

It is presently unknown which mechanisms are responsible for the effects of sIL-1RI in these models. One hypothesis is that the cleavage of the extracellular IL-1RI domain unmasks a domain with catalytic properties which is able to induce release of IL-1α from the cell. An alternative stimulatory pathway may involve binding of sIL-1RI to IL-1R accessory protein (IL-1RAcP)
through the cell-associated IL-1α, resulting in cell stimulation (24). Although stimulation of cells through formation of a sIL-1RI/IL-1/IL-1RAcP complex has not been reported to date, it is tempting to speculate that this may be a new regulatory function of sIL-1RI in vivo. Examples of other soluble cytokine receptors shown to associate with membrane bound subunits to effect signal transduction are common among members of the IL-6 receptor family and G-CSF receptors (25).

We hypothesized that the cell-associated IL-1α necessary for bridging of sIL-1RI to IL-1RAcP is stimulated by LPS leaking from the gut. However, this did not prove to be the case, because sIL-1RI had the same stimulatory activity in germ-free mice in which no such leakage occurs (M.G.N., B.J.K., C.A.D., and J.W.M.V., unpublished results). Therefore, even in the absence of gut flora shedding LPS, there is constitutive IL-1α production that has access to the blood compartment within a very short period of time. Alternatively, the IL-1α may be bound to other plasma proteins and hence “liberated” by sIL-1RI. Additional experiments are currently performed to elucidate the mechanisms responsible for these effects of sIL-1RI.

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