Alteration of Interleukin-2 (IL-2) and Soluble IL-2 Receptor Secretion in the Sera and Urine of Patients with Rickettsial Boutonneuse Fever

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Sera and urine samples from 115 Sicilian patients with boutonneuse fever (BF), obtained at the time of diagnosis and after clinical recovery, were analyzed for concentrations of interleukin (IL)-2 and soluble IL-2 receptor (sIL-2R). There were significantly high levels of sIL-2R in the urine and sera of patients with acute BF compared with healthy controls, and the values returned to normal following successful chemotherapy. The data indicate that the sIL-2R urine concentrations correlated directly with the sIL-2R sera levels. In contrast, in all tested sera and urine samples, IL-2 levels were normal. Furthermore, a reduction in IL-2 production by peripheral blood mononuclear cells from acute BF patients was also observed. sIL-2R represents an unspecific marker useful to monitor the evolution of BF.

Materials and Methods

Patients and samples. The 188 serum and urine samples were obtained from 115 patients with confirmed BF at various stages of the disease. BF was confirmed when at least two of the most characteristic signs were present (fever, tache noire at the site of the tick bite, and macular rash) and there were significant levels of specific antibody titers against *R. conorii* by ELISA or indirect IFA (or both) [17]. The length of the illness before diagnosis was <2 weeks. Serum and urine samples were collected at different stages of the disease; at the time of diagnosis without any specific treatment, every week after treatment, and after clinical recovery. The day of onset of fever was recognized as the first day of the illness. The acute stage was considered to be from day 2 to 14 after the onset of the symptoms and before specific treatment; the convalescent stage was after day 21. The third week of illness was considered to be borderline between the two stages.

In addition, 25 serum samples from blood donors, preliminarily tested for anti-*R. conorii* antibodies and negative by ELISA, were stored at −80°C in multiple aliquots until examined. For each determination, a new aliquot was used since refreezing and thawing of a once-used sample may partially destroy its biologic activity.

Patients were treated with tetracycline (500 mg four times/day for 7 days). Urine specimens were collected on awakening and were centrifuged (2000 g) for 20 min at 4°C. Supernatants were recovered, filtered (0.22-μm Millex filter; Millipore, Molshiem, France), and tested for IL-2 and sIL-2R concentrations.

Materials. Medium was RPMI 1640 (Flow Laboratories, Irvine, UK). Fetal calf serum was from Hyclone Laboratories (Logan, UT). Ficoll-hypaque (Lagitre, Milan, Italy) was used to isolate peripheral blood mononuclear cells (PBMC).

Lymphokine production. PBMC were adjusted to 10^6 cells/mL and incubated for 24 h with or without 5 μg/mL phytohemagglutinin. Supernatants were recovered, filtered through 0.22-μm filters (Millex; Millipore), and stored at −70°C until tested.

Assay for IL-2 and sIL-2R. Commercially available kits were used to determine the concentrations of IL-2 and sIL-2R (IL-2 and
Figure 1. sIL-2R levels in sera (open bars) and urine specimens (solid bars) from controls and patients with rickettsial BF, according to phase of illness. Data are mean ± SE.

Results

Figure 1 shows the serum and urine levels of sIL-2R in BF patients at the time of diagnosis and at different stages of the disease compared with levels in healthy subjects. As is clearly shown, serum levels of sIL-2R were significantly increased ($P < .01$) in patients with active BF but gradually declined in the third week of infection; levels in patients were not significantly different from those of healthy subjects from the fourth week on. Exactly the same trend was observed in the urine samples. In fact, sIL-2R concentrations were significantly high in weeks 1 and 2 of infection compared with weeks 3 and 4 and 8–9 times higher than levels in healthy controls ($2131 ± 296$ vs. $221 ± 31$ U/mL; figure 1). A direct correlation was observed between the sIL-2R levels in sera and urine ($r = .4694; P < .001$) from BF patients (figure 2). On the contrary, an inverse relation was observed between sIL-2R levels and IL-2 production; in fact in all phases of disease, urine and serum IL-2 levels were below the threshold value, as in controls (data not shown).

To investigate the possibility that reduced production of serum IL-2 could be caused by the inability of PBMC to synthesize it, PBMC from both BF patients and healthy controls were tested for IL-2 production. The data indicate that IL-2 from mitogen-stimulated PBMC was significantly reduced in the acute phase of infection compared with levels in controls and was in the normal range after clinical recovery (figure 3).

Discussion

IL-2R is the main cellular mediator of the actions of IL-2 as a growth and differentiation factor for T, B, and NK cells and as an activator for macrophages [1, 7, 16]. It is shed in soluble form (sIL-2R) into the cell culture supernatant after mononuclear cell activation [3, 4, 18, 19].

As already detected in several clinical conditions [5–8], sIL-2R levels in our study were significantly increased in acute BF and returned to normal when patients recovered from the disease. Serum levels of sIL-2R correlated positively with those detected in urine, indicating that sIL-2R undergoes glomerular filtration. Since the increase in sIL-2R in the urine of BF patients was 8–10 times greater than levels in controls, whereas in serum it was 3–4 times greater, it is possible that sIL-2R is produced in part within the urinary tract. In agreement with these data is the observation that glomerular mesangial cells secrete IL-1, which is involved in inducing the expression of CD25 molecules on the lymphocyte surface [9, 20, 21]. On the other hand, rickettsiae invade and proliferate in the endothelial cells of small vessels [10, 12], and mesangial cells are active producers of IL-1 [22].

Such findings indicate that sIL-2R is a marker of disease activity and represents a sign of immune activation [3, 4]. This is also attested to by the high levels of interferon-γ [15, 16], which could be due to an elevated response of T [23, 24] or NK [25, 26] cells to a continuous high antigen load.

Because of the reduction in CD4+ T cell subsets [16], the active production of sIL-2R could depend on CD8+ T cells [4, 27] or, more likely, on monocytes. Monocytes are not only significantly increased but also strongly activated by interferon-γ [15, 16, 19] and, hence, are able to produce considerable quantities of sIL-2R [28]. However, the tissues could likely be the major source of sIL-2R [5, 27, 29, 30].

Although there were high levels of sIL-2R, the concentrations of IL-2 were never increased. Since sIL-2R is capable of binding to free IL-2 [4], sIL-2R could down-regulate IL-2–driven proliferation and IL-2–dependent cell-mediated responses, acting as a physiologic inhibitor of IL-2 [3–5]. In line with this is the observation of reduced production of IL-2 by PBMC in acute BF that returns to normal after clinical recovery, when sIL-2R levels also return to normal. Yet immunosuppression may be suspected only in the early stage of BF [31, 32]. Thus the increased release of sIL-2R could be the means...
by which activated cells reduce surface receptor density below a critical threshold level, preventing an undesirable response. Alternatively, sIL-2R could serve as a binding protein, prolonging the IL-2 half-life [3, 4]. Furthermore, failure to detect IL-2 in serum could indicate that IL-2 is produced in paracrine concentrations by the lymphocytes and macrophages present in the perivascular area adjacent to infected endothelial cells [33].

In conclusion, serum and urine sIL-2R levels seem to be useful markers in monitoring the evolution of BF. The urine level of sIL-2R appears to be particularly relevant, because it increases in acute BF more than the serum level and decreases more quickly after clinical recovery. In a patient with persistently high levels of urine sIL-2R after the fifth week of infection, BF was associated with alterations in renal function (hypercetyzotemia, hypercreatinemia), which confirms that impairment in renal function causes an active transport of sIL-2R in the urine [9, 21]. This also indicates that there is active production of sIL-2R in the urinary tract.

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References