Interleukin-2 Receptors in Experimental Chagas’ Disease

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Mammals infected with the protozoan parasite Trypanosoma cruzi develop suppressed cellular and humoral immune responses. This immunosuppression has been correlated with reduced T-cell responses involving deficient interleukin-2 (IL-2) production and is apparently mediated primarily by suppressor macrophages. Various forms of immunosuppression in other systems have been associated with increased levels of soluble IL-2 receptors (sIL-2R), and in the present study levels of sIL-2R in the sera of T. cruzi-infected mice during the course of infection were examined in enzyme-linked immunosorbent assays. It was found that serum levels of sIL-2R were elevated only during the third week of acute infection, a time of intense immunosuppression. In addition, IL-2R on the surface of T cells were examined by flow cytometric analyses to determine whether there is an alteration in the number of IL-2R-positive cells and whether there is a change in expression of these receptors as infection progresses. The results revealed no significant change in the percentage of cells expressing IL-2R, nor did T cells become suppressed in their ability to express IL-2R in response to concanavalin A during the course of infection.

Experimental Chagas’ disease in mice (caused by the protozoan parasite Trypanosoma cruzi) is accompanied by the development of severe immunosuppression of both cellular and humoral responses to heterologous and parasite-specific antigens (14). This suppression is associated with reduced T-helper-cell activity and deficient interleukin-2 (IL-2) production and is mediated principally by suppressor macrophages (IL-2R) are expressed on T-cell surfaces (19, 25). During the third week of infection, however, when suppression is most intense, IL-2 production, mitogen-induced proliferation of T cells, and IL-2R expression are decreased (25). However, a number of studies using T. cruzi-infected mice have shown that T cells can be stimulated to respond in the suppressed host and suggest that T-cell responsiveness can be restored (3–5, 24, 32).

Activated cells (both T and B cells) release from their surface the p55 subunit of the IL-2R which is referred to as the soluble IL-2R (sIL-2R). Although it is not known what instigates the release of IL-2R from cell surfaces, it appears that this release may be controlled by the action of IL-2 (15) and IL-5 (16). sIL-2R can bind IL-2 efficiently (26) but with low affinity (10). When serum sIL-2R levels increase in the microenvironment around cells, the concentration of sIL-2R may be sufficient to effectively compete with high-affinity surface IL-2R for binding IL-2 and thus render the immune system deficient in responsiveness (26).

In some experimental situations and disease states it has been determined that immunosuppression correlates with an increase in serum levels of sIL-2R (1, 11, 18, 22, 27, 28, 34). Sera from patients with visceral leishmaniasis, a disease caused by a protozoan parasite of the genus Leishmania, nonspecifically suppress the responses of normal lymphocytes. Recently sIL-2R were shown to be involved in this serum-mediated suppression (2).

In the present study, we monitored sIL-2R levels in sera of mice during the course of infection with T. cruzi as well as the expression of IL-2R on splenocytes of infected mice in the presence and absence of stimulation with concanavalin A.

To determine serum levels of sIL-2R, 8- to 12-week-old C3HeB/FeJ female mice from Jackson Laboratories, Bar Harbor, Maine, were used. Mice were injected intraperitoneally (i.p.) with 10^3 blood-form trypanomastigotes of the Brazil strain of T. cruzi. Parasitemia levels were monitored at the time of serum collection from these mice. Increasing parasitemia levels with time were observed (data not shown), indicating that the mice were infected with T. cruzi. Sera from these mice were collected at various times after infection and tested for sIL-2R in a sandwich enzyme-linked immunosorbent assay (ELISA) (3), using two monoclonal antibodies, PC 61.5.3 (American Type Culture Collection, Rockville, Md.), which produces a rat immunoglobulin G (IgG) reactive with the mouse IL-2R, and 7D4 (American Type Culture Collection), which produces a rat IgM that binds to the mouse IL-2R at a different epitope, as described before (3). Concanavalin A (ConA)-stimulated murine splenocyte-conditioned medium (CAS) was used as a standard source of sIL-2R for the ELISA (3).

Very low but measurable levels of sIL-2R were detected in normal C3H serum (batches of pooled sera, each batch from three normal mice) in an ELISA using CAS as a positive control. sIL-2R levels in sera obtained from mice 7, 14, 21, and 28 days after infection were measured, and elevated levels of sIL-2R were found between the third and fourth weeks of infection. This set of experiments was repeated twice at shorter time intervals. A positive control (i.e., CAS) and a negative control (i.e., normal sera) were measured in triplicate on each ELISA plate. Whereas normal levels of the receptor were detected at days 7, 10, 13, 16, and 19 after infection, elevated serum levels of sIL-2R were detected on

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Levels of sIL-2R in sera of C3HeB/FeJ mice infected with T. cruzi measured in a sandwich ELISA that uses two monoclonal antibodies that bind to different epitopes on the receptor. ConA-stimulated spleen cell supernatant was used as a standard source of sIL-2R, and a complete titration of CAS was done on each plate. Serum from each mouse, in a group of three, was tested individually in triplicate. Mean values for each mouse in a group were compared with normal values for three uninfected mice (open squares), using a Student’s t test. Data are presented as mean values for a group of three mice, and the error bars indicate ±2 standard deviations. Serum levels of sIL-2R in T. cruzi-infected mice (closed squares) were within the normal range at day 7 (A) and day 36 (D) after infection but were significantly elevated (P < 0.05) at day 22 (B) and day 28 (C) after infection. O.D., optical density.

Day 22 (2.04 times the normal level) and day 28 (2.15 times the normal level) after infection (Fig. 1). These levels were considered significant if the mean experimental values (three mice per time point, each mouse tested individually in triplicate) were more than 2 standard deviations different from the negative control, i.e., normal serum. The data were also analyzed in a Student’s t test. The serum levels of sIL-2R in infected mice returned to within the normal range by day 36 postinfection. Previous work done in this laboratory using the same mouse and parasite strains and the same dose for infection has reproducibly shown suppression of mitogen-induced T-cell proliferation and IL-2 production to be most intense during the third week of infection (32), when increased levels of sIL-2R are observed.

Increased levels of sIL-2R in the microenvironment around cells have been hypothesized to play a role in immunosuppression by effectively competing with cell surface receptors for IL-2 (26). However, spleen cells from T. cruzi-infected mice have been shown not to contain message levels for IL-2 in Northern blot (RNA blot) analysis, indicating that the suppression of IL-2 production is at the level of transcription (21). Because IL-2 levels are decreased due to deficient transcription, it is unlikely that the presence of increased levels of sIL-2R contribute significantly to suppressed responses. Also, the increased levels of sIL-2R observed in this study are not as high as those observed in other parasitic infections (2).

IL-2R may be shed from the surface of cells as part of normal membrane turnover, especially during an intense immune response, as during T. cruzi infection. Elevated levels of sIL-2R have been shown to correlate with increased levels of β2-microglobulin, which is shed from the surface of activated T cells during normal membrane turnover (12). The increased levels of sIL-2R in experimental Chagas’ disease observed in the present study may result from the massive T-cell activation that occurs during the course of infection (19). IL-2R may also be shed as part of an immunomodulatory process regulated either by the parasite or the host to prevent antigen-specific T-cell clonal expansion. The resultant suppression may play a role in the establishment of the parasitic infection. At the present time, the relationship between pathogenesis and increased levels of sIL-2R is unknown.

Fluorescence-activated cell sorter analysis was used on spleen cells from infected mice to determine cell surface expression of the p55 subunit of the IL-2R (at days 7, 10, 13, 16, 19, 22, and 28 after infection) both before and after ConA stimulation. Spleens from infected mice, whose serum sIL-2R levels were monitored, were removed and teased apart in RPMI 1640 (GIBCO Laboratories, Grand Island,
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were cells Spleen (100 U/ml), N.Y.) for standard fluorescence analysis (29). The rest of the nucleated spleen cells were stimulated in vitro with 5 μg of ConA per ml for 18 h and then examined for IL-2R expression.

The cells were suspended in complete RPMI 1640 at 10^6/ml. Aliquots of 100 μl of this cell suspension were incubated with 10 μl of 10-fold-concentrated 7D4 hybridoma culture supernatant fluid at 4°C for 30 min (6). The cells were then washed with RPMI 1640 without phenol red (Sigma Chemical Co., St. Louis, MO.) and incubated with 5 μg of fluorescein isothiocyanate (FITC)-labelled goat anti-rat IgM (Cappel, Organon Teknika Corp., West Chester, Pa.) in 100 μl of RPMI 1640 without phenol red at 4°C for 30 min. The cells were then washed, and the fluorescence was measured on a Facstar Plus instrument (Becton Dickinson, Mountain View, Calif.). The data were analyzed with the Consort 30 system.

Spleen cells of individual mice were examined for IL-2R expression. Spleen cells from three normal mice examined individually were used as negative controls, as their spleen cells do not express the p55 subunit of the IL-2R, and as positive controls, as they express the p55 subunit after ConA stimulation (29, 33). Three controls were run for each sample: an autofluorescence control, background fluorescence after the binding of only the first antibody, and background fluorescence after binding of only the second antibody.

The number of IL-2R-positive cells were the same in samples from uninfected mice and from infected mice, both before (data not shown) and after ConA stimulation, at each of the time points after infection that were examined (i.e., days 7, 10, 13, 16, 19, 22, and 28 after infection). Unstimulated cells from normal and infected mice were essentially IL-2R negative; i.e., <5% of the cells expressed IL-2R. Similar results were also obtained in an immunogold labeling study (data not shown). However, the number of activated T cells in the spleens of T. cruzi-infected mice is higher than the number in normal mice, as is the total number of spleen cells (31), during the time when siIL-2R levels are increased. The higher number of cells might account for the increased siIL-2R levels since these cells are capable of expressing surface IL-2R upon ConA stimulation in vitro (Fig. 2) and may also be capable of shedding the receptor from their surface. T cells may not be the only cells contributing to the increased levels of siIL-2R that occur during T. cruzi infection. B cells (17) and macrophages (9) have been shown to express IL-2R, and B cells have also been shown to shed a soluble form of the receptor (17). The number of B cells is also increased in mice infected with T. cruzi, but there is no evidence to indicate whether they are involved in contributing to increased levels of siIL-2R in T.
cruzi infections. The source of sIL-2R in the sera of mice infected with *T. cruzi* is unknown.

As stated above, the results of the present study show that splenectomized T cells from mice are not suppressed in their ability to express the p55 subunit of the IL-2R upon ConA stimulation at any time during *T. cruzi* infection. In contrast, studies by Rottenberg et al. (25) using BALB/c mice and the Tulaheun strain of *T. cruzi* have shown suppression of the p55 subunit of the IL-2R 21 days after infection. They were, however, unable to duplicate earlier studies on the enhancing effect of exogenously supplied IL-2 (3, 5). IL-2R expression appears to be normal in *T. cruzi*-infected C3HeB/FeJ mice, since these mice can respond to IL-2 administered during the course of infection by increased longevity, decreased parasitemias, and enhanced immune responses (3, 5). Spleen cells from *T. cruzi*-infected mice also contain normal levels of IL-2R mRNA (30). It is possible that strain differences might account for the conflicting results. BALB/c mice infected with the Tulaheun strain of *T. cruzi* may be unable to respond to exogenous IL-2 because of a suppression of the ability of their T cells to express the p55 subunit of the IL-2R, whereas C3HeB/FeJ mice infected with the Brazil strain of the parasite may respond to IL-2 because their T cells are able to express the p55 subunit of the IL-2R.

The mechanisms involved in the suppression of IL-2 and the expression of the p55 subunit of the IL-2R during the course of a *T. cruzi* infection in mice are not clearly understood. It appears that the lymphokine and its receptor are regulated differently during the course of a *T. cruzi* infection in mice, since IL-2 production is suppressed at the transcriptional level in ConA-stimulated infected spleen cells but the IL-2R are expressed. The regulation may involve the different stages of activation and intracellular signaling pathways that result in the expression of the IL-2 and IL-2R genes. Further studies will be necessary in order to elucidate whether intracellular signaling pathways are suppressed during the course of experimental Chagas' disease.

REFERENCES


the serum of normal animals and of animals bearing IL-2 receptor positive tumours with high or low metastatic capacity. Br. J. Cancer 55:583-587.


