Interleukin 2 Induced Leukocyte Adhesion to the Normal and Tumor Microvascular Endothelium in Vivo and Its Inhibition by Dextran Sulfate: Implications for Vascular Leak Syndrome

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Abstract

The maximum dose of interleukin 2 (IL-2) alone or with adoptively transferred lymphocytes is limited by the vascular leak syndrome, resulting from an increase in vascular permeability. Using intravital microscopy to quantify cell interaction in vivo and the Miles assay to assess changes in vascular permeability, we have shown that IL-2 increases both leukocyte-endothelial cell adhesion and vascular permeability in the cutaneous tissue of rabbits. Both of these processes can be reversed to a large extent using dextran sulfate (\(M_r \approx 500,000\); 10 mg/kg body weight), a nonspecific blocker of leukocyte-endothelial adhesion. These results suggest that increased leukocyte (probably lymphocyte) adhesion to the postcapillary microvasculature contributes significantly to the IL-2-induced increase in permeability. Therefore, as more specific inhibitors of leukocyte-endothelial adhesion become available, improved strategies could be developed to control or prevent IL-2 toxicity.

Introduction

A major side effect limiting the treatment of solid tumors with IL-2\(^4\) alone or with adoptively transferred cells is a VLS, resulting from an increase in vascular permeability. Although several investigators have measured the increases in vascular permeability in mice, rats, and rabbits following injections of recombinant human IL-2, the mechanisms of this increase are not well understood (1–3). In vitro studies suggest that IL-2 activation increases the adhesion of lymphocytes to cultured endothelial cells (4). These adhered lymphocytes may increase vascular permeability directly (5) or indirectly (6, 7) through the production of interferon, tumor necrosis factor, colony-stimulating factor, or other cytokines. On the basis of these in vitro data, we proposed the following hypothesis. The increase in vascular permeability in vivo is also mediated by the adhesion of endogenous lymphocytes activated by IL-2, and this increase in permeability could be inhibited by preventing this adhesion. We tested this hypothesis by measuring the leukocyte adhesion to the microvasculature using intravital microscopy in normal cutaneous (mature granulation) and neoplastic (VX2 carcinoma) tissues grown in the rabbit ear chamber (8, 9) following IL-2 injection. Since in vivo microscopy does not permit the differentiation between lymphocytes and neutrophils, we measured these two subpopulations and the total in systemic blood at various times post-IL-2 injection to estimate which subpopulation may be adhering to the vasculature. Furthermore, since DS has been used to inhibit nonspecifically the adhesion between granulocytes and endothelial cell monolayer in vitro (10) and in vivo (11, 12), we also used DS to prevent IL-2-induced leukocyte-endothelial adhesion. The effect of IL-2 alone or with DS on vascular permeability was evaluated using the Miles assay (13).

Materials and Methods

 Rabbit Ear Chamber Preparation. Sandison-type transparent chambers were implanted bilaterally in the left and right ears of anesthetized (40 mg/kg body weight i.p. sodium pentobarbital, as Nembutal; Abbott Laboratories, North Chicago, IL) male New Zealand White rabbits, 2.5 kg body weight (Green Meadows Rabbitry, Murrysville, PA) (8). Approximately 6 weeks after the chamber implantation when the granulation tissue became mature (8), VX2 carcinoma was implanted in one chamber following the procedure described in Ref. 9. The other chamber was used for normal tissue studies. The neoplastic tissue was used for intravital microscopy approximately 7 days postimplant.

 Leukocyte Adhesion Analysis. The in vivo interactions between leukocytes and vessel walls were quantified based on the method of Atherton and Born (14). In brief, the number of leukocytes rolling (N\(_r\)) sufficiently slowly to be individually visible was counted for every minute as they rolled past a selected 100-μm section of one side of the venular wall. The rolling velocity (V\(_r\)) of these leukocytes was determined by analyzing videotapes of the microcirculation using the intravital microscope and accessories described elsewhere (8). The number of leukocytes adhering (N\(_a\)) to a selected point of vessel wall for longer than 30 s was also measured. Throughout the observation period the animals remained anesthetized (40 mg/kg i.p., Nembutal). Peripheral blood was sampled from femoral artery every 20 min for 3 h post-IL-2 injection to obtain total leukocyte, lymphocyte, and neutrophil counts.

 Skin Permeability Evaluation. Permeability changes in the preshaved skin of anesthetized rabbits were evaluated using the Miles assay (13). Evan’s blue solution (10 mg/kg) was injected i.v. 10 min before i.c. injection of 0.1 ml of IL-2, EX, or DW. Twenty min after i.c. injection the animals were sacrificed, and the skin lesions were removed. The blue intensity of the skin, measured with an image analysis system under transillumination, was used as a permeability index.

 Protocol. Recombinant human IL-2 and EX (gift of the Cetus Corporation, Emeryville, CA) were diluted in DW at concentrations of 600,000 Cetus units/0.2 ml/kg body weight for i.v. injection and 300,000 Cetus units/0.1 ml for i.c. injection. DW was used as a blank control. The endotoxin level of the IL-2 preparation was 0.0006 ng/ml. Dextran sulfate (\(M_r \approx 500,000\); gift of Dr. Karl E. Arfors, Pharmacia Experimental Medicine, LaJolla, CA) was injected i.v. at doses of 0, 3, 10, and 30 mg/kg body weight in animals undergoing permeability test. Since a 10-mg/kg dose inhibited the permeability increase significantly, only this dose of DS was used in intravital microscopy studies 1 h after IL-2 injection. All values are reported as mean ± SE and \(n = 6\) unless indicated.

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The abbreviations used are: IL-2, interleukin 2; VLS, vascular leak syndrome; EX, excipient; DW, distilled water; DS, dextran sulfate; i.e., intracutaneous.
Results and Discussion

The Miles assay showed that the permeability index was highest with IL-2 (4.33 ± 0.29 arbitrary units), intermediate with EX (3.66 ± 0.62), and lowest with DW (3.16 ± 0.52); however, there was statistical difference only between IL-2 and DW ($P < 0.05$). A similar increase in permeability due to EX has been demonstrated previously (3, 15). Although the Miles assay does not give precise values of the vascular permeability (16, 17), these results show that the IL-2 at this dose is able to induce extravasation of plasma proteins in the skin. Furthermore, this extravasation should be reduced with DS in a dose-dependent manner (Fig. 1), with statistically significant reductions at 10 and 30 mg/kg. This suggests that if leukocyte adhesion is involved in the VLS, DS should be able to reverse it.

Indeed, as shown in Fig. 2, within 1 h of injection IL-2 promotes leukocyte adhesion to the endothelium in the postcapillary venules of normal tissues, and within 20 min DS is able to reverse it. Note that without DS the effect of IL-2 on leukocyte-endothelial cell interaction lasts for at least 1.5–2 h after IL-2 injection. The in vivo adhesion was quantified in terms of three parameters: rolling velocity, $V_r$ (inversely related to adhesion), rolling counts, $N_r$ (a measure of short-term adhesion), and sticking counts, $N_s$ (a measure of long-term adhesion). As shown in Table 1, increase of leukocyte-adhesion induced by IL-2 leads to a decrease in $V_r$, and increases in $N_r$ and $N_s$ in both normal and neoplastic vessels. Values of these three parameters were different in different venules in the same rabbit ear chamber, presumably due to cellular heterogeneity. Compared with DW and EX these changes were statistically significant, except for $V_r$ in normal tissue. Interestingly, EX also induced an increase of leukocyte adhesion, however, to a lesser extent than IL-2. The only parameter that increased significantly was $V_r$ in normal tissue.

As expected, the peripheral blood exhibited decreases in the total leukocyte, neutrophil, and lymphocyte counts within 20 min of IL-2 injection. The neutrophil counts recovered within 1–2 h; however, lymphocyte counts did not recover during the observation period of 3 h (data not shown). These results suggest that activated lymphocytes may be involved in VLS. However, direct identification of cell type adherent to vessel walls in the ear chamber and/or quantification of the leukocyte population and composition in the spleen and lymph nodes along with the peripheral blood are needed to confirm this hypothesis.

Finally, i.v. injections of DS (10 mg/kg) remarkably reduced...
leukocyte adhesion in normal tissue within 30 min (Fig. 3). In addition to decreasing NR, and increasing VR, DS blocked leukocyte rolling almost completely. DS alone increased total leukocyte, neutrophil, and lymphocyte counts in the peripheral blood 3-5 times more than control within 60 min (data not shown). Pretreatment with DS 1 h prior to IL-2 injection prevented decrease in leukocyte counts (data not shown). While these results are in accord with our hypothesis on the relationship between leukocyte adhesion and VLS, they lead to several unanswered questions regarding the mechanism of this increased adhesion. Is this mediated by increased rigidity of lymphocytes due to IL-2 activation (18) or by increased expression of adhesion molecules on leukocytes or endothelial cells (19)? As the molecular nature of this interaction becomes better understood, improved strategies could be developed to reduce or prevent IL-2 toxicity.

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References

* P < 0.01.  
\[ P < 0.05.\]

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Fig. 3. Effect of IL-2 and IL-2 + DS on leukocyte adhesion to postcapillary venules of the cutaneous tissue in the rabbit ear chamber. Note the increase in rolling velocity, VR (um/s), and decreases in the number of rolling, VR (min^-1) and adhered, VR (min^-1) (P < 0.01). Numbers above columns indicate the total number of cells monitored in 4 animals in each type of experiment. Bars, SE.
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