INHIBITION OF ANGIOGENESIS IN RATS BY IL-1 RECEPTOR ANTAGONIST AND SELECTED CYTOKINE ANTIBODIES

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Abstract—Daily administration of 50 ng recombinant human interleukin 1-alpha (IL-1α), 25 ng IL-8, 50 ng tumor necrosis factor-alpha (TNF-α), or 100 ng basic fibroblast growth factor (bFGF) caused intense neovascularization in a rat sponge model. These cytokine-induced neovascular responses were inhibited by coadministration of IL-1 receptor antagonist (IL-1ra; 50 μg), IL-8 antiserum (IL-8-AS; 1:1000), TNF-α antibody (TNF-AB; 500 ng), or a monoclonal antibody to bFGF (DG2; 1000 ng), respectively. These data suggest that it is possible to manipulate the angiogenic response elicited by a defined cytokine by its receptor antagonist or neutralizing antibody. In the absence of exogenous cytokines, the sponge-induced angiogenesis was profoundly suppressed by dexamethasone (5 μg/day), but not modified by IL-1ra, IL-8-AS, TNF-AB, and DG2 alone. However, the combination of these four reagents was able to inhibit the sponge-induced neovascular response almost completely. These findings provide direct evidence that IL-1α, IL-8, TNF-α and/or bFGF have an intrinsic role in angiogenesis. Further work is necessary to characterize the profile of these cytokines during angiogenesis and to elucidate the nature of their interactions.

INTRODUCTION

Angiogenesis is an important process in many physiological conditions such as embryonic development and wound healing. However, defects in the controlling mechanism of angiogenesis often result in pathological conditions such as rheumatoid synovial hypertrophy, proliferative retinopathy, and solid tumors. Recent
studies have established that neovascularization can be induced by a variety of angiogenic factors/cytokines (1–3). Some of these soluble factors are produced by macrophages [e.g., interleukin 1-alpha (IL-1α) and tumor necrosis factor-alpha (TNF-α)]. Others are derived from endothelial cells [e.g., basic fibroblast growth factor (bFGF) and endothelial cell-derived IL-8], or platelets [platelet-derived endothelial cell growth factor (PD-ECGF)]. Although it is not certain if all of these factors play an intrinsic role in neovascular growth in vivo, recent data clearly demonstrated the potential interactions between angiogenic factors, cytokines, and other inflammatory mediators. For example, the angiogenic effect of IL-1α in a rat sponge model can be enhanced by bradykinin (4) or substance P (5).

Since more than one cytokine is involved in most chronic inflammatory diseases, it is plausible that inhibition of the synthesis of specific cytokines, or blockade of their activity by receptor antagonists and neutralizing antibodies, would provide potential benefit (6). In a recent study, we have shown that daily administration of recombinant human IL-1α, IL-8, TNF-α or bFGF caused intense neovascularization in a rat sponge model (7, 35). Thus, it was initially decided to test if the angiogenic activity of these cytokines could be manipulated by their respective receptor antagonist or neutralizing antibodies. In subsequent experiments, these reagents were used to further analyze the role of IL-1α, IL-8, TNF-α, and bFGF in angiogenesis. Our results suggest that IL-1α, IL-8, TNF-α, and/or bFGF are the principal mediators of sponge-induced neovascularization in the rat and provide direct evidence that these cytokines have an intrinsic role in angiogenesis.

**MATERIALS AND METHODS**

*Materials.* 133Xe injection and the endothelial cell marker, Bandeiraea simplicifolia lectin I, isolectin B₄ (BSL-B₄) were obtained from Medgenix plc, Fleurus Belgium, and Vector Laboratories, Peterborough, U.K., respectively. Recombinant human endothelial cell-derived IL-8 and TNF-α antibody (TNF-AB) were purchased from Genzyme, Cambridge, Massachusetts. Other cytokine-related reagents were generous gifts: recombinant human IL-1α and TNF-α (Dr. J. Saklatvala, Strangeways Research Laboratory, Cambridge, U.K.); human recombinant bFGF (Farmitalia-Carlo Erba, Milan, Italy); monoclonal antibody to bFGF (DG2, Dr. T. M. Reilly, Du Pont Merck, Wilmington, Delaware); sheep anti-IL-8 antiserum (IL-8-AS; Dr. R. Thorpe, National Institute of Biological Standard and Control, South Mimms, U.K.); IL-1 receptor antagonist (IL-1ra; Dr. R. C. Thompson, Synergen Inc., Boulder, Colorado).

*Sponge Implant Model.* Sterile circular polyether sponge disks with central cannulae were implanted subcutaneously in male Wistar rats. Starting on day 1 after implantation, test substances (50 μl) were injected daily into the sponges through the cannulae. The angiogenic response was assessed as a function of blood flow through the implants over a period of 14 days, using a 133Xe clearance technique (8) and confirmed histologically. Briefly, animals were anesthetized with Hypnorm as before and 10 μl 133Xe in sterile PBS was injected into the sponges through the cannulae.