IL-8 Is an Angiogenic Factor in Human Coronary Atherectomy Tissue

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Background—Interleukin-8 (IL-8), a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, is a potent angiogenic factor that may play a role in atherosclerosis. Previously, IL-8 has been reported in atherosclerotic lesions and circulating macrophages from patients with atherosclerosis. Therefore, we sought to determine whether IL-8 plays a role in mediating angiogenic activity in atherosclerosis.

Methods and Results—Homogenates from 16 patients undergoing directional coronary atherectomy (DCA) and control samples from the internal mammary artery (IMA) of 7 patients undergoing bypass graft surgery were assessed for IL-8 content by specific ELISA, immunohistochemistry, and in situ hybridization for IL-8 mRNA. The contribution of IL-8 to net angiogenic activity was assessed using the rat cornea micropocket assay and cultured cells. IL-8 expression was significantly elevated in DCA samples compared with IMA samples (1.71±0.6 versus 0.05±0.03 ng/mg of total protein; P<0.01). Positive immunolocalization of IL-8 was found exclusively in DCA tissue sections, and it correlated with the presence of factor VIII–related antigen. In situ reverse transcriptase polymerase chain reaction revealed the expression of IL-8 mRNA in DCA tissue. Corneal neovascular response, defined by ingrowth of capillary sprouts toward the implant, was markedly positive with DCA pellets, but no constitutive vessel ingrowth was seen with IMA specimens. Neutralizing IL-8 attenuated both the in vivo corneal neovascular response and the in vitro proliferation of cultured cells.

Conclusions—The results suggest that, in human coronary atherosclerosis, IL-8 is an important mediator of angiogenic activity and may contribute to plaque formation via its angiogenic properties. (Circulation. 2000;101:1519-1526.)

Key Words: angiogenesis ■ atherosclerosis ■ cytokines ■ coronary disease

Coronary atherosclerosis continues to be the leading cause of morbidity and mortality in the United States. The pathogenesis of coronary atherothrombotic plaque formation is a complex process that demonstrates features of exaggerated injury and repair, including the recruitment of mononuclear cells, fibroproliferation, deposition of extracellular matrix, and angiogenesis, which lead to progressive fibrosis, calcification, and eventual luminal occlusion. Angiogenesis is an essential process that supports fibroplasia, the deposition of extracellular matrix during wound healing, and chronic inflammation; it contributes to progressive fibrosis in several chronic inflammatory states, such as rheumatoid arthritis, psoriasis, and idiopathic pulmonary fibrosis. Angiogenesis also occurs in atherosclerosis.

Chemotactic cytokines (chemokines) are polypeptide molecules that have proinflammatory activities. Interleukin-8 (IL-8) is an ELR (glutamic acid-leucine-arginine) CXC chemokine that was initially discovered using chemotaxis and the activation of neutrophils. It has endothelial cell chemo-

tactic activity in vitro and induces neovascularization in the cornea of rats and rabbits in vivo. In addition, the angiogenic (IL-8) and angiostatic interferon-γ–inducible protein 10 (IP-10) CXC chemokines were recently reported to regulate angiogenesis associated with non–small cell lung cancer and idiopathic pulmonary fibrosis. Because IL-8 has previously been reported in atherosclerotic lesions, we hypothesized that IL-8 may contribute to the pathogenesis of atherosclerosis via its angiogenic properties. Therefore, we analyzed human coronary atherosclerotic lesions for their IL-8 content, performed immunohistochemical examinations to localize IL-8, and determined the angiogenic activity of IL-8 in patients undergoing directional coronary atherectomy (DCA) for anginal syndromes.

Methods

Tissue Collection

Atherectomy specimens were collected from 16 patients who underwent DCA at the Cardiac Catheterization Laboratories of the
University of Michigan Medical Center after they presented with symptomatic coronary disease (unstable or exertional angina or angina following a recent myocardial infarction or coronary intervention). Samples of the left IMA from 7 patients undergoing elective coronary artery bypass graft surgery were used as controls. After performing each coronary atherectomy, tissue was promptly retrieved from the atherectomy catheter (Devices for Vascular Intervention Inc), rinsed in sterile saline, and either snap-frozen in liquid nitrogen for subsequent homogenization or fixed in 4% formaldehyde and imbedded in paraffin. IMA specimens were obtained in the operating room during the bypass procedure, rinsed in sterile saline, and processed in a manner similar to that used with the DCA specimens. All DCA patients had clinical and angiographic findings consistent with the clinical diagnosis of unstable angina and had coronary luminal stenoses >70%, as measured by quantitative angiographic analysis.

**Tissue Homogenization**

Frozen DCA or IMA tissue was homogenized and sonicated in an “anti-protease” buffer on recovery from the cardiac catheterization laboratory using a method that was previously described.12,13 Specimens were centrifuged at 1500g for 15 minutes, filtered through 1.2-μm sterile Acrodiscs (Gelman Sciences), and kept frozen at −70°C until they were thawed for assay by specific IL-8 ELISA. In addition, a portion of the specimen was lyophilized (SpeedVac, Savant), normalized to an equivalent amount of total protein, and used in the corneal micropocket model of neovascularization for the analysis of angiogenic activity.

**Reagents**

Polyclonal anti-human IL-8 specific anti-sera were produced by the immunization of rabbits with human recombinant IL-8 (R&D Systems) in multiple intradermal sites with complete Freund’s adjuvant.10,11 The specificity of this antisera was assessed by Western blot analysis against a panel of other human recombinant cytokines.15 Antibodies were specific in our sandwich ELISA, without cross-reactivity to a panel of 12 human recombinant interleukins, including interleukin-1 receptor antagonist IL-1ra (IRAP), IL-1, IL-2, IL-4, IL-6, tumor necrosis factor α, interferon-γ, and other members of the CXC and CC chemokine families (eg, CXC: epithelial neutrophil activating protein-78 [ENA-78], growth-related gene [GRO]-α, GRO-β, GRO-γ, neutrophil-activating peptide-2 [NAP-2], platelet factor 4 [PF-4], IP-10, and macrophage inflammatory protein-α and β, and regulated on activation normal T cell expressed and secreted [RANTES]).10,15 The anti-protease buffer for tissue homogenization consisted of 1×PBS with 2 mMol/L phenylmethylsulfonyl fluoride and 1 μg/mL each of antipain, aprotinin, leupeptin, and pepstatin A. Goat anti–factor VIII–related antigen antibodies were purchased from BioGenex. Anti-macrophage antibodies (HAM 56) were purchased from Enzo Diagnostics.

**IL-8 ELISA**

IL-8 was quantitated by specific ELISA using a modification of a double-ligand method.16,17 Briefly, flat-bottomed, 96-well microtiter plates (Nunc) were coated with 50 μL/well of specific IL-8 polyclonal antibodies (1 ng/μL in 0.6 mol/L NaCl, 0.26 mol/L H3BO4, and 0.08 mol/L NaOH; pH 9.6) for 24 hours at 4°C and then washed with PBS and 0.05% Tween-20 (wash buffer). Non-specific binding sites were blocked with 2% BSA. Plates were rinsed, and samples were added (50 μL/well). This was then followed by incubation for 1 hour at 37°C. Plates were then washed, and 50 μL/well of specific anti-IL-8 biotinylated polyclonal antibodies (3.5 μg/μL in wash buffer and 2% FCS) was added for 45 minutes at 37°C. Plates were washed 3 times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories) was added, and the plates were incubated for 30 minutes at 37°C. Chromogen substrate (Dako) was then added, and the plates were incubated at room temperature to the desired extinction. Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Inc). Standards were half of the log dilution of recombinant IL-8 from 100 ng to 1 pg/mL (50 μL/well).

**Immunohistochemistry of IL-8, Factor VIII-Related Antigen, and HAM 56**

Paraffin-embedded tissue from DCA and IMA specimens was processed for immunohistochemical localization of IL-8, factor VIII-related antigen, and HAM 56, as previously described.16,17 Briefly, consecutive tissue sections were dewaxed with xylene and rehydrated with graded concentrations of ethanol. Tissue nonspecific binding sites were blocked using normal goat serum (BioGenex). Tissue sections were then washed and overlaid with a 1:500 dilution of either control (rabbit) or polyclonal rabbit anti-IL-8 antibodies, either control (goat) or goat anti-factor VIII–related antigen antibodies, or either control (mouse) or mouse anti-macrophage (HAM 56) antibodies. Sections were then washed and incubated for 60 minutes with either secondary goat anti-rabbit, rabbit anti-goat, or goat anti-mouse biotinylated antibodies (BioGenex). After washing twice with Tris-buffered saline, slides were overlaid with a 1:35 dilution of peroxidase conjugated with streptavidin (Vector) and incubated for 60 minutes. The tissue sections were then washed twice in Tris-buffered saline and incubated with alkaline phosphatase conjugated with streptavidin (BioGenex) for 60 minutes. Fast Red (BioGenex) reagent was used for chromogenic localization of antigen. After optimal color development, tissue sections were immersed in sterile water, counterstained with Mayer’s hematoxylin, and cover-slipped using an aqueous mounting solution.

**Localization of Tissue IL-8 mRNA by In Situ Reverse Transcriptase Polymerase Chain Reaction**

IL-8 mRNA was localized to coronary arteries using a modification of an in situ reverse transcriptase polymerase chain reaction analysis, as previously described.17 Briefly, 3-μm-thick paraffin-embedded sections on PLUS slides (Fisher) were dewaxed in xylene and rehydrated in graded concentrations of ethanol. Slides were then rinsed in diethylpyrocarbonate H2O2 followed by a protease K digest. Slides were then treated with DNase overnight. Reverse transcriptase polymerase chain reaction was performed using the Promega Access System in the presence of digoxigenin (11-dUTP; Boehringer Mannheim) with sense (sequence: 5’ AAG-CTG-GCC-GTG-GCT-CTC-TTG 3’) or antisense (sequence: 5’ AGC-CCT-CCT-AA-AAT-CTT 3’) primers. Slides were then washed in Tris-buffered solution, blocked for 30 minutes, and then incubated for 30 minutes with anti-digoxigenin antibody conjugate. Slides were then washed and placed for 10 minutes in a buffered solution of 100 mM/L Tris-HCl, 100 mM/L NaCl, and 50 mM/L MgCl2 with a pH of 9.5 at 20°C. Slides were then exposed to a color solution containing nitroblue tetrazolium, X-phosphate (Boehringer Mannheim), and levamisole and incubated in a darkened, humidified chamber until adequate color visualization was determined. The reaction was then terminated by immersing slides in H2O2. Slides were counterstained with Contrast Red (KPL), rinsed, and cover-slipped.

**Corneal Micropocket Assay of Angiogenesis**

The angiogenic activity of DCA and IMA homogenates was assayed in vivo in the avascular cornea of hooded Long-Evans rat eyes, as previously described.15,17 Briefly, equal volumes of lyophilized DCA or IMA tissue specimens were normalized to total protein and combined with a sterile Hydran (Interferon Sciences Inc) casting solution. Aliquots of 5 μL were pipetted onto the flat surface of an inverted sterile polypropylene specimen container and polymerized overnight in a laminar flow hood under UV light. Before implantation, pellets were rehydrated with normal saline. Animals were anesthetized with intraperitoneal ketamine (150 mg/kg) and atropine (250 μg/kg). Rat corneas were anesthetized with 0.5% proparacaine hydrochloride ophthalmic solution, which was followed by implantation of the Hydran pellet into an intracorneal pocket (1 to 2 mm from the limbus). Six days after implantation, animals received 1000 U of heparin and ketamine (150 mg/kg) intraperitoneally, which was
followed by a 10-mL perfusion of colloidal carbon via the left ventricle. Corneas were harvested and photographed. No inflammatory response was observed in any of the corneas treated with the above specimens. Positive neovascularization responses were recorded only if sustained directional ingrowth of capillary sprouts and hairpin loops toward the implant were observed. Negative responses were recorded when either no growth was observed or when only an occasional sprout or hairpin loop displaying no evidence of sustained growth was detected. All animals were handled in accordance with the guidelines of the Unit for Laboratory Animal Medicine.

**Cell Culture and DNA Synthesis**

To further illustrate the role of IL-8 as an angiogenic factor, we measured the effects of anti-IL-8 antibody on coronary artery smooth muscle cell DNA synthesis. Human coronary artery smooth muscle cells were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum, recombinant epidermal growth factor (0.5 ng/mL), recombinant fibroblast growth factor-B (2 ng/mL), insulin (5 μg/mL), and gentamycin (100 μg/mL). All experiments were conducted on cells at passages 3 to 6, and cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 and 95% air. DNA synthesis was determined using the method of [3H]-thymidine (Amersham International) incorporation using 96-well plates. Cells (104 cells per well) were made quiescent by starving them in serum-free media for 48 hours; then, cells were stimulated by being incubated with Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and either varying titers of IL-8 relative to factor VIII–related antigen. The presence of IL-8 protein, as measured by ELISA, was significantly elevated in DCA tissue compared with IMA controls, suggesting that the expression of IL-8 protein was associated with increased mRNA levels in cells comprising atherosclerotic plaque. Given the heterogeneity of our patient population and our sample size, the predominant cellular source of IL-8 mRNA could not be distinguished because endothelial cells, macrophages, and smooth muscle cells were each associated with a localization to IL-8.

**Statistical Analysis**

Comparison of IL-8 levels was made using Student’s t test. Data were considered statistically significant at P≤0.05.

**Results**

DCA samples came from patients with a mean age of 59.5±5 years, whereas the specimens from the internal mammary artery (IMA) that were used for comparison were harvested from patients with a mean age of 63.8±4 years.

**IL-8 Protein is Present in Coronary Atherectomy Specimens**

The presence of IL-8 in DCA and IMA samples was measured by specific ELISA. DCA tissue constitutively expressed more IL-8 than IMA controls, as measured by specific ELISA standardized to total protein (1.71±0.6 versus 0.05±0.03 ng/mL; P<0.01). Because IL-8 protein, as measured by ELISA, was significantly elevated in DCA tissue homogenates when compared with IMA samples, we assessed its presence in paraffin-embedded tissue using immunohistochemistry. Figures 1B and 1C represent the result of immunolocalization using antibodies to IL-8. IL-8 was predominantly expressed in DCA tissue (Figures 1B and 1C).

**IL-8 Relative to Factor VIII–Related Antigen**

Because endothelial cell recruitment and growth is required to support angiogenesis, we determined whether the immunolocalization of IL-8 was associated with vascular endothelial cells in DCA samples. Figures 1E and 1F represent the result of immunolocalization using antibodies to factor VIII–related antigen, an endothelial cell marker. The presence of endothelial cells is evidenced by the immunolocalization of factor VIII–related antigen (Figures 1E and 1F) in regions that were colocalized by antibodies to IL-8. In addition, areas of new vessel formation, lined by endothelial cells positively staining for factor VIII–related antigen, were also evident in DCA specimens.

**Presence of IL-8 mRNA**

Because the IL-8 protein was present in atherosclerotic (DCA) tissue, as determined by ELISA and immunolocalization of IL-8, we used in situ hybridization for IL-8 mRNA to localize the source of IL-8. Figure 2 represents the result of in situ hybridization for IL-8 mRNA in DCA tissue. IL-8 mRNA was present in DCA specimens but was undetected in IMA controls, suggesting that the expression of IL-8 protein was associated with increased mRNA levels in cells comprising atherosclerotic plaque. Given the heterogeneity of our patient population and our sample size, the predominant cellular source of IL-8 mRNA could not be distinguished because endothelial cells, macrophages, and smooth muscle cells were each associated with a localization to IL-8.

**Presence of Macrophages Relative to IL-8**

Mononuclear cell recruitment and activation has been postulated to be an important event in the development of atherosclerotic plaque; thus, we examined whether localization of IL-8 was also associated with that of macrophages in atherosclerotic lesions. Figure 3 represents the result of immunolocalization using HAM 56, an anti-macrophage antibody. The expression of IL-8 associated with neovascularization in coronary plaques was also associated with the presence of macrophages.

**Corneal In Vivo Angiogenesis Assay**

To substantiate that IL-8 may be modulating tissue-derived angiogenic activity in coronary atherosclerotic lesions, we next assessed the in vivo angiogenic activity of random pooled samples of either DCA or IMA tissue in the presence or absence of preimmune (control) or neutralizing IL-8 antibodies using the rat cornea micropocket model of neovascularization. These antibodies did not contain significant quantities of lipopolysaccharide contamination, as assessed by limulus assay, and all samples were normalized to total protein. Corneal neovascular response, as defined by ingrowth of capillary sprouts and hairpin loops toward the implant, was markedly positive with DCA pellets (Figure 4A) in 6 of 6 corneas, without evidence of leukocyte infiltration (assessed by light microscopy). In contrast, in corneas embedded with hydron pellets containing DCA homogenates with neutralizing IL-8 antibodies, the angiogenic response was completely abrogated (Figure 4B). This complete inhibition of angiogenic activity by a neutralizing antibody to IL-8 underscores the importance of IL-8 in stimulating angiogenesis in DCA tissue. No growth was seen with the IMA pellets in either the presence or absence of the neutralizing antibody (Figures 4C and 4D; n=6 for each manipulation; Table).
activity of IL-8; thus, the role of IL-8 on DNA synthesis in cultured coronary artery smooth muscle cells was examined in the presence and absence of a neutralizing antibody to IL-8. Compared with stimulated cells treated with control sera, DNA synthesis, as measured by $[^3H]$-thymidine incorporation, was significantly reduced in cells treated with both 1:300 and 1:1000 titers of neutralizing antibody to IL-8 (33±10% and 55±12%, respectively; Figure 5). These results suggest that the antagonism of IL-8, a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, may at least partly inhibit the proliferation of coronary artery cells.

**Discussion**

These results suggest that CXC chemokines are produced in the coronary atherosclerotic plaques of patients presenting with unstable angina. In addition, IL-8, a CXC chemokine previously shown to be an angiogenic factor in vivo and a chemotactic and mitogenic factor for vascular smooth muscle and endothelial cells in vitro, is present in elevated levels in atherectomy specimens and may be a primary signal for angiogenesis in atherosclerotic tissue. Furthermore, the presence of IL-8 in coronary plaques was associated with that of vascular endothelial cells by virtue of the colocalization with factor VIII–related antigen. These findings strongly support a role for chemokines in atherogenesis and elucidate potential mechanisms by which IL-8, a molecule only recently described in human atherosclerosis, may contribute to the development and progression of coronary atherosclerotic disease in humans.

Clinically significant coronary atherosclerosis is the result of a multifactorial process that includes the accumulation and incorporation of lipids, the recruitment of inflammatory mononuclear cells and T lymphocytes, intimal proliferation of smooth muscle cells, the production and deposition of connective tissue or basement membrane matrix, and angiogenesis. The notion of chronic inflammation and fibroproliferation as mechanisms in the pathogenesis of atherosclerotic lesions was proposed by Virchow in the mid-nineteenth century and evolved over the next century into the response-to-injury hypothesis described by Ross and Glomset. According to this model, injury to the endothelium by multiple
sources, including oxidized low-density lipoproteins, mechanical stress, toxins, and viruses, results in endothelial cell dysfunction and the increased adherence and activation of mononuclear cells, platelets, and T-lymphocytes. In turn, these activated monocytes/macrophages and the other cells involved in the developing lesion elaborate growth factors and cytokines that stimulate the fibroproliferative response; this leads to the formation and progression of atherosclerotic plaques. Indeed, observations of plaque progression in coronary disease supported, at least in part, the claim that these processes featured a continuum of chronic inflammation, an influx of granulation tissue, and fibroplasia, which ultimately led to luminal occlusion.22,23

CXC chemokines are a family of recently identified cytokines that are characteristically basic heparin-binding proteins. These cytokines in their monomer forms are 10 kDa and seem to have proinflammatory and reparative activities. This family displays 4 highly conserved cysteine amino acid residues, with the first 2 cysteines separated by 1 nonconserved amino acid residue (CXC cysteine motif).24 Over the last decade, several human CXC chemokines have been identified; they possess either angiogenic (IL-8; ENA-78; GRO-α, -β, and -γ; granulocyte chemotactic protein [GCP-2]; connective tissue activating protein III [CTAP-III]; NAP-2; and platelet basic protein [PBP]) or angiostatic (IP-10, PF-4, and monokine induced by gamma interferon [MIG]) activity at physiological concentrations. These properties are based on the presence or absence of the Glu-Leu-Arg (ELR) motif, which immediately precedes the first conserved cysteine at the NH2 terminal portion of the primary sequence.25–28

Angiogenesis characterized by neovascularization is a critical event in wound healing and inflammation, and it also occurs in a variety of pathological states, such as granuloma formation, chronic inflammatory disease, and tumorigenesis.5–8,29 During neovascularization, normally quiescent endothelial cells are stimulated, degrade their basement membrane and proximal extracellular matrix, migrate directionally, divide, and organize into new functioning capillaries invested by a basal lamina. This process is at least
partly dependent on a number of specific interactions between endothelial cells, the extracellular matrix, and various families of adhesion molecules in the presence of growth and regulatory factors. These factors include IL-8, a CXC chemokine possessing the ELR motif, which induces in vitro endothelial cell migration and proliferation, 2 essential components of angiogenesis. The angiogenic activity of IL-8 has been shown in vitro to be equipotent to that of basic fibroblast growth factor, vascular endothelial growth factor, and other previously recognized heparin-binding proteins that promote angiogenesis. The results of the present study are consistent with the evolving paradigm of exaggerated wound repair, aberrant angiogenesis, and inflammation as mechanisms in the pathogenesis of atherosclerosis. The immunohistochemical and in situ hybridization data, together with the complete attenuation of angiogenic bioactivity with neutralizing IL-8 antibodies presented in this study, suggest that IL-8 is produced in human coronary atheromas and may function as a mediator of tissue-derived angiogenesis in atherosclerotic lesions. The expression and action of several angiogenic factors have been well described during embryogenesis, wound healing, and tumorigenesis.

Neovascularization seems to be an important process in the pathogenesis of atherosclerotic plaque and the angiogenic molecule; vascular endothelial growth factor has been a recent focus of investigation in vascular pathology for its ability to modulate endothelial repair and smooth muscle growth. Moreover, a recent report in which the attenuation of plaque growth was achieved by the use of angiogenesis inhibitors further illustrates the potential importance of neovascularization in the progression of atherosclerosis. In this study, IL-8 was detected almost exclusively in DCA tissue samples compared with those from the left IMA, and it was associated with a profound angiogenic response that was completely inhibited with neutralizing anti–IL-8 antibodies. In addition, IL-8 antisera also attenuated the in vitro proliferation of stimulated human coronary artery cells. These findings suggest that IL-8 is a mediator of angiogenesis that may contribute to the evolution of clinically significant coronary lesions.

Other monocyte-derived proteins have been investigated for their role in the development of atherosclerosis. MCP-1 was strongly expressed by infiltrating macrophages after acute arterial injury. The expression of IL-8 protein and mRNA has been reported in the plaque macrophages and circulating monocytes of patients presenting with clinically significant atheromas, as well as in their macrophages and foam cells in response to cholesterol loading. In addition, other investigators have separately demonstrated the presence of the IL-8 protein and the evidence of neovascularization in atherosclerotic plaques. Furthermore, depletion of the IL-8 receptor CXCR2 in transgenic hypercholesterolemic mice has been associated with a reduction of macrophage accumulation and plaque size in atherosclerotic lesions.

The findings of the current study agree with the above observations. The colocalization of IL-8 with factor VIII–related antigen supports a role for IL-8 as a contributor to endothelial cell recruitment (and, hence, neovessel formation) in human coronary plaques. This evidence would, therefore, support a mechanism by which IL-8 is, at least partly, a mediator of the angiogenesis associated with atherosclerosis and by which IL-8 may support the fibroproliferation and matrix deposition that lead to plaque formation.

The lack of a longitudinal analysis of coronary plaque formation for the presence and activity of IL-8 is a limitation to our interpretation of the data presented in this study. Such a temporal analysis, however, would be impractical without an appropriate animal model because the tissue specimens analyzed were obtained from symptomatic patients with clinical indications for coronary atherectomy and, therefore, advanced disease. Although we cannot comment on the specific role of IL-8 in each of the clinical subsets represented by the specimens, the data reported do suggest that IL-8 is present in the atherosclerotic tissue of patients presenting with symptomatic coronary disease. In addition, our ability to
comment on the possible regulatory interactions of other chemokines, particularly between those of the CXC family possessing and lacking the ELR motif, was limited by sample size. However, as demonstrated by our results, the content and angiogenic activity of IL-8 in the coronary plaques analyzed were overwhelming when compared with controls. A larger survey of coronary tissue would allow us to examine inherent chemokine interactions in plaque formation and to characterize their expression and activity in lesions from patients who present with the syndromes of unstable angina, chronic exertional angina, and restenosis.

In conclusion, we reported the relative concentrations of a CXC chemokine in human coronary atherosclerotic tissue and demonstrated the significant expression and angiogenic activity of IL-8. This report extends previous observations in atheromas to include a role by which IL-8 may significantly contribute to angiogenesis, which seems to be germane to the pathogenesis of coronary atherosclerosis. Thus, our findings suggest that in human atherosclerosis, IL-8 may be chemo-

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\( n=6 \) experiments for each condition. NRS indicates normal rabbit serum; Ab, antibody.

Figure 4. IL-8 is a significant angiogenic factor in DCA compared with IMA (control) specimens in rat cornea micropocket assay for angiogenesis. A and B are representative photomicrographs of angiogenic response to DCA specimens in presence of control and anti–IL-8 antibodies, respectively. C and D are representative photomicrographs of angiogenic response to IMA (control) specimen in presence of control and anti–IL-8 antibodies, respectively.

3H Thymidine/anti-IL8

Figure 5. Neutralizing antibody to IL-8 inhibits proliferation of human coronary smooth muscle cells. [3H]-thymidine incorporation in cells treated with IL-8 antisera at titers of 1:300 and 1:1000 was significantly reduced (−33±10% and −55±12%, respectively) compared with cells treated with normal serum.
tactic and mitogenic toward endothelial cells and smooth muscle cells as well as a signal for angiogenesis. Future investigations are required to further elucidate the role of CXC chemokines in atherosclerosis as well as to identify a potential role for therapies directed against IL-8 in mitigating the development and complications of disease.

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References
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