Endogenous Neutralizing Antibodies Against Platelet-Derived Growth Factor-AA Inhibit Atherogenesis in the Cholesterol-Fed Rabbit

David J. Lamb, Tony Y. Avades, Gordon A.A. Ferns

Abstract—Previous studies have shown that the B chain of platelet-derived growth factor (PDGF) has an important role in atherogenesis. In this study we have investigated the contribution of PDGF-A chain in cholesterol-induced atherogenesis in the New Zealand White rabbit. High titters of antibodies to PDGF-AA or to platelet cytosolic protein (PCP) were induced in these animals by immunization against recombinant human PDGF-AA or human PCP. Rabbits were then fed a 0.25% to 1% cholesterol–containing diet for 10 weeks to induce atherosclerotic lesions; the rabbits were then humanely killed and perfusion-fixed and their aortas were removed. The extent of atherosclerosis in the thoracic aortas was determined by quantitative morphometry after staining with oil red O. The intimal and medial areas in histological sections taken at the level of the first intercostal branch were quantified by image analysis. Immunization against PDGF-AA and PCP, but not against adjuvant alone, resulted in rising titters of antibodies within 2 weeks, the levels of which reached a plateau by 8 weeks. The antibodies to PDGF-AA were isoform-specific, recognized both human and rabbit PDGF-AA, and neutralized the biological activity of PDGF-AA in vitro. Integrated plasma cholesterol levels were similar in both groups. Compared with nonimmune rabbits (n = 10), animals immunized against PDGF-AA (n = 10) or PCP (n = 10) had significantly smaller areas of the aorta covered by atherosclerotic lesions (24.6 ± 5.1% and 18.7 ± 4.2%, respectively, vs 34.4 ± 4.3%; P < 0.05). This was associated with a reduced aortic intimal-medial area ratio in PDGF-AA-immunized (0.009 ± 0.006) and PCP-immunized (0.025 ± 0.017) rabbits than in nonimmune animals (0.159 ± 0.066; P < 0.05). These data suggest that PDGF-AA is actively involved in cholesterol-induced atherosclerosis in the rabbit. (Arterioscler Thromb Vasc Biol. 2001;21:997-1003.)

Key Words: platelet-derived growth factor-AA ■ endogenous antibody ■ atherosclerosis ■ cholesterol-fed rabbit

The fatty streak is the earliest macroscopically evident lesion of atherosclerosis. This lesion is predominantly composed of macrophage-derived foam cells, which accumulate beneath the endothelial layer. The formation of the more clinically relevant fibrofatty plaque appears to depend on smooth muscle cell migration into the intima and subsequent proliferation (reviewed in Ross1). The factors responsible for these processes are still unknown. Previous studies, however, suggest that smooth muscle cell migration and proliferation may be a consequence of synergistic interplay between growth factors and cytokines released by several arterial or lesional cell types (reviewed in Ross1). Of particular importance to atherogenesis may be platelet-derived growth factor (PDGF).2

PDGF is a cationic protein with a molecular weight of ≈30 kDa whose active molecule consists of 2 homologous polypeptide chains. The dimeric molecule can contain a combination of PDGF-A and -B chains; hence, the 3 isomeric forms found are PDGF-AA, PDGF-AB, and PDGF-BB (reviewed in Ross et al3). The biological effects of PDGF are mediated by plasma membrane receptors, which are also composed of dimers of 2 subunits, the α-subunit and β-subunit. The β-subunit has a high affinity for the PDGF-B chain, whereas the α-subunit binds the PDGF-A and -B chains with equal affinity.4 The biological responses of smooth muscle cells to the 3 isoforms of PDGF are dependent on the relative levels of expression of the PDGF receptor subunits on their surfaces.5

Initially, atherogenic growth factors such as PDGF-AA and PDGF-BB may be derived from dysfunctional endothelium.6,7 The subsequent accumulation of activated macrophages within the lesion provides a rich source of potentially atherogenic growth factors, including transforming growth factor-β (TGF-β), tumor necrosis factor-α, insulin-like growth factor-1 (IGF-1), and interleukin-1β (IL-1β) (reviewed in Ross1). Resting monocytes constitutively express both the PDGF-A and -B chains but express predominantly the PDGF-B chain as they mature into tissue macrophages.8 As smooth muscle accumulates and the lesion becomes fibrous, smooth muscle cells may also secrete mitogenic factors such as PDGF-AA, IGF-1, and TGF-β, which may act in a paracrine or...
autocrine manner. As the lesion becomes severe, the endothelium can be compromised, and thrombogenic elements of the extracellular matrix beneath become exposed. This event may be associated with platelet deposition and degranulation. Platelets contain several mitogenic and potentially proatherogenic growth factors, including PDGF-AA, PDGF-BB, epidermal growth factor, IGF-1, and TGF-β.

Recent studies have placed particular emphasis on the role of PDGF-BB in atherosclerosis. The PDGF-B chain has been found to be expressed in both atherosclerotic lesions induced in primates and in advanced lesions found in humans. The uptake of oxidized LDL by macrophages and the subsequent cholesterol accumulation, thought to be an initially important process in atherogenesis, are also accelerated by PDGF-BB. We have previously reported that endogenous neutralizing antibodies against PDGF-BB elicited in the cholesterol-fed rabbit inhibits atherosclerotic lesion formation in the aortas of these animals. These antibodies were found to inhibit atherosclerosis to a similar extent as did antibodies raised against proteins from rabbit platelets.

The role of PDGF-AA in atherosclerosis is less clear, although PDGF-AA is expressed in human atherosclerotic lesions and is upregulated in areas of neointimal thickening. In addition, smooth muscle cells isolated from spontaneously hypertensive rats express greater numbers of PDGF-α receptors than do cells from control animals. Both lisinopril (an angiotensin-converting enzyme inhibitor) and verapamil (a calcium channel blocker), which inhibit arterial neointimal thickening in vivo, downregulate the expression of the PDGF-A chain mRNA in endothelial cells. This may be a subsidiary mechanism by which these pharmacological agents reduce the incidence of coronary heart disease.

In this study, we have investigated the effect of eliciting endogenous neutralizing antibodies against PDGF-AA and human platelet cytosolic protein (PCP) in the cholesterol-fed rabbit on the formation of atherosclerotic lesions in their aortas. This may help to clarify the importance of PDGF-A chain in atherogenesis in this model.

Methods

Materials

Cholesterol-containing rabbit diets were supplied by Special Diet Services. All reagents were of analytical grade unless stated otherwise.

Rabbit Colonies

Juvenile New Zealand White rabbits (10 weeks old) weighing ~2.0 kg were housed in the Experimental Biology Unit at the University of Surrey, Guildford, in accordance with Home Office regulations. Food and water were allowed ad libitum.

Immunization Groups

Each experimental group consisted of 10 rabbits. Human recombinant PDGF-AA was purchased from NBS Biologicals. Human and rabbit PCPs were prepared from heparinized whole blood. Platelet-rich plasma was obtained by centrifugation at 200 g for 20 minutes at 4°C. Prostacyclin (Sigma) was added to a final concentration of 0.33 μg/mL, and the platelet-rich plasma was centrifuged at 800 g for 15 minutes at 4°C. The platelet pellet was resuspended in phosphate-buffered saline (PBS, pH 7.4), and the platelets were lyzed by 5 cycles of freeze-thawing. Platelet debris was removed by centrifugation at 10 000 g for 1 hour at 4°C, and the protein concentration of the supernatant was determined by a modification of the Lowry protein assay. Immunogens were administered subcutaneously at 4 sites in a volume of 0.25 mL per site in complete nontoxic Freund’s adjuvant (NFA). In brief, primary immunizations of 75 μg PDGF-AA, 75 μg PCP, or saline were prepared in 0.9 mL saline and mixed with 0.1 mL BCG vaccine (John Bell & Croydon) in a 5-mL syringe. Two volumes of incomplete NFA (a kind gift from Mr B.A. Morris, School of Biomedical and Life Sciences, University of Surrey, UK) were taken in a second syringe, and a stable emulsion was formed by mixing the contents of both syringes by several passes through a twin Luer-hubbed syringe connector with a fine channel (Guildhay). Four weeks after the primary immunization, booster injections containing 50 μg PDGF-AA, 50 μg PCP, or saline were prepared without BCG vaccine in incomplete NFA and administered as described above. Antibody titers were measured biweekly.

Blood Sampling

Blood was drawn before the start of the experimental diet and biweekly thereafter from the ear vein into heparinized containers, and plasma was obtained by centrifugation at 4°C. Plasma was stored at −70°C before analysis.

Dietary Manipulation

Rabbits were maintained on a chow diet for the first 8 weeks of the experiment. Animals were then fed a chow diet supplemented with 1% cholesterol for 10 weeks after the first immunization. The plasma cholesterol levels were measured biweekly. This schedule enabled modification of the cholesterol content of the cholesterol-containing diets to maintain the plasma cholesterol level at ~20 mmol/L. This was achieved by mixing the 1% cholesterol–containing diets with a standard chow diet to produce individually tailored diets with different cholesterol contents.

Cholesterol Measurement

Plasma cholesterol levels were measured on a Boehringer Accutrend meter with Accutrend test strips (Boehringer Mannheim) after the plasma samples were diluted in PBS if necessary.

Plasma Antibody Titers

Plasma antibody titers to PDGF-AA, PCP, and mycobacterial tuberculosis (John Bell & Croyden) were measured with a sandwich ELISA. Microtiter plates (Nunc Maxisorp, Merck Ltd) were coated with 10 ng recombinant PDGF-AA or PCP in 0.1 mol/L carbonate buffer, pH 9.6, per well for 18 hours at 4°C under humidified conditions. The wells were washed 3 times in wash buffer (PBS containing 0.05% Tween-20). Nonspecific binding was reduced by blocking each well with 20% (wt/wt) milk in PBT (PBS containing 1% bovine serum albumin and 0.1% Tween-20) for 1 hour at 37°C. Wells were washed 3 times with wash buffer. Plasma was diluted 1:100 with PBT, and 100 μL per well was incubated for 30 minutes at 37°C. After being washed, the wells were incubated with 100 μL per well of biotinylated anti-rabbit IgG antibody (Sigma) diluted 1:20 000 in PBT for 30 minutes at 37°C. After being washed, the wells were incubated with 100 μL premixed avidin D and biotinylated horseradish peroxidase (both from Vector Laboratories) diluted 1:500 in PBT for 30 minutes at 37°C. o-Phenylenediamine (0.04%; Sigma) was dissolved in 0.05 mol/L citrate/0.1 mol/L phosphate buffer, pH 5, containing 10 μL H₂O₂ per 25 mL, Substrate (100 μL per well) was incubated at room temperature for 5 minutes, and the reaction was terminated by adding 50 μL of 3 mol/L HCl. Optical density at 492 nm was measured with a Labsystems iEMS reader MF microtiter plate reader with Genesis 2 software (Life Sciences).

Antibody Partial Purification

Plasma was isolated from whole, heparinized blood by centrifugation at 1000 g for 10 minutes at 4°C. Saturated ammonium sulfate (Fisher) was added dropwise until 25% saturation was achieved. The large proteins and lipoproteins were allowed to precipitate at 4°C overnight. The supernatant containing the smaller–molecular-weight species was retained after centrifugation at 15 000 g at 4°C for 1 hour. The low-molecular-weight proteins were precipitated by adding an equal volume of saturated ammonium sulfate to the supernatant and incubating the resulting mixture at 4°C overnight. The precipitate
was pelleted by centrifugation at 15000g at 4°C for 1 hour and resuspended in 0.1 mol/L PBS. The fractions in the 50000- to 300000-Da molecular-weight range were obtained by filtration through a 300000-Da molecular-weight-cutoff Mini-Ultrasette filtration system (Filtron Technology Corp) according to the manufacturer’s instructions. The filtrate was then filtered through a 50000-Da molecular-weight-cutoff Mini-Ultrasette filtration system (Filtron) and washed with several volumes of 0.1 mol/L PBS. The retentate containing the IgG fraction was sterilized by membrane filtration and assayed for protein by a modified Lowry assay.16

Rabbit Smooth Muscle Cell Lysate Preparation
Rabbit smooth muscle cells were grown from medial explants from rabbit aortas and cultured in Dulbecco’s modified Eagles’ medium containing 10% fetal bovine serum, 10 IU/mL penicillin, and 10 μg/mL streptomycin at 37°C under 5% CO2. Cells were washed with ice-cold PBS and lysed in 10 mmol/L Tris, 158 mmol/L NaCl, 0.1 mmol/L EDTA, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 1 mmol/L PMSF, 1 mmol/L DTT, and 1:100 Sigma protease inhibitor cocktail, pH 7.4, for 30 minutes at 4°C. Lysates were centrifuged at 15000g for 30 minutes at 4°C, and the supernatants were stored at −80°C until required.

Antibody Specificity Assessment
The specificity of the raised antibodies was assessed by Western blotting analysis under denaturing, nonreducing conditions by using Vector Elite ABC anti-rabbit kit and the Vector DAB substrate kit (both from Vector Laboratories) according to the manufacturer’s instructions. Samples were boiled for 5 minutes in sample buffer (0.19 mol/L Tris, 2% SDS, 10% glycerol, and 0.005% bromophenol blue, pH 8.8), and 30 μL (100 ng protein) was then applied to each well. The samples were separated by electrophoresis at 80 mA for 2 hours in a 12.5% mol/L Tris, 0.2 mol/L glycine, and 0.1% SDS, pH 8.3, tank buffer. Samples were transferred to nitrocellulose membranes at 200 mA for 3 hours in 25 mmol/L Tris, 0.1 mol/L glycine, 10% methanol, and 0.05% SDS, pH 8.3, transfer buffer. Nonspecific protein binding was reduced by incubation with Superblock (Pierce & Warriner) at room temperature for 1 hour. The partially purified anti–PDGF-AA or anti-human PCP antibodies were diluted to 100 μg/mL in 0.1 mol/L Tris, 0.1% Tween-20, and 0.1 mol/L NaCl, pH 7.5, and incubated with the membrane for 30 minutes at room temperature. After being washed, the blot was developed by using Vector Elite ABC anti-rabbit kit and the Vector DAB substrate kit (both from Vector Laboratories) according to the manufacturer’s instructions.

Antibody Neutralization Capability
Murine (BALB/c) 3T3 cells were cultured in Dulbecco’s modified Eagles’ medium containing 10% fetal bovine serum, 1x glutamax, 50 IU penicillin per mL, 50 μg streptomycin per mL, and 2.5 μg amphotericin B per mL (all from Life Technologies) and plated into 24-well tissue culture plates (Costar, Fisher Scientific). Cells were made quiescent by incubation in Dulbecco’s modified Eagles’ medium containing 0.4% fetal bovine serum, 1x glutamax, 50 IU penicillin per mL, 50 μg streptomycin per mL, and 2.5 μg amphotericin B per mL for 48 hours. The cells were then restimulated with 30 ng/mL PDGF-AA in the presence or absence of increasing concentrations of anti–PDGF-AA antibody. After 18 hours, 1 μCi of [3H]methyld thymidine (1 μCi/mL, Amershams Pharmacia Biotech) was added to each well and incubated for a further 4 hours. The medium was removed, the wells were washed with 5% ice-cold trichloroacetic acid at 4°C, and the resulting material was dissolved in 1 mol/L NaOH at room temperature. [3H]methyld thymidine uptake was measured in a Wallac 1410 Wizard scintillation counter (EG&G Wallac), and cell protein was measured by a modification of the Lowry assay.16

Animal Sacrifice
Eighteen weeks after the primary immunization and 10 weeks after initiating a cholesterol-containing diet, the animals were anesthetized with xylazine (3.5 mg Rompun per kg IM; Bayer Plc) and ketamine (18 mg Ketaset per kg IM; Willows Francis Veterinary) and subsequently heparinized (300IU/kg IV; Sigma), and the abdominal aorta was cannulated. Rabbits were then killed with an overdose of pentobarbitone (Rhine Merieux), and the jugular veins were transected for perfusion runoff. Rabbits were perfused with isotonic saline at a rate of 100 mL/min and a pressure of 120 mm Hg. When the runoff was clear, the saline was replaced with 4% paraformaldehyde in isotonic saline as described previously.17 After perfusion, the entire thoracic aorta was isolated, cleaned of fascia, and placed in fresh paraformaldehyde for 48 hours. The aortas were split longitudinally into 2 halves: 1 half was placed in fresh paraformaldehyde before being stained with oil red O and the other half was divided into segments and dehydrated in increasing ethanol concentrations before being embedded in paraffin wax for histology.

Statistical Analysis
Analyses were performed with SPSS software. Statistical significance was assumed for a P value <0.05. All analyses were performed with unpaired t tests after the data were found to be not normally distributed. The relationship between antibody titers and aortic area positively staining for oil red O was calculated by linear regression analysis. Statistical significance was assumed for a P value <0.05. All analyses were performed with spss software.

Results
Immunization With PDGF-AA and Human PCP Elicited Endogenous Specific Antibodies of High Titer
Immunization with recombinant PDGF-AA elicited a rapid increase in plasma levels of antibodies that had a high reactivity with PDGF-AA and a lower reactivity with human PCP as measured by ELISA (Figure 1A). The anti–PDGF-AA antibodies isolated from these animals recognized recombinant human PDGF-AA, but not PDGF-BB or other growth factors, in Western blotting experiments. They also exhibited weaker reactivity with recombinant PDGF-AB and fractions of human PCP but did not bind to human serum albumin (Figure 1B). These data suggest that the antibodies raised against PDGF-AA were PDGF-A chain–specific. These antibodies cross-reacted with rabbit PDGF-AA contained within rabbit smooth muscle cells and platelets (Figure 1C).

The antibodies raised to human PCP also attained high titers. They exhibited no detectable cross-reactivity with the human PCP.
recombinant PDGF-AA as measured by ELISA (Figure 1D). The anti–human PCP antibodies recognized components of human PCP in Western blotting experiments, exhibited weak binding to human albumin, and additionally showed no reactivity with either PDGF-AB or -BB isoforms (Figure 1E). All immunizations were performed in NUFA, which elicited a rapid increase in plasma levels of antibodies that had a high reactivity to mycobacterial tuberculin (Figure 1F). The plasma level of these antibodies was not significantly different in any of the immunization groups.

Endogenous Antibodies to PDGF-AA Were Neutralizing In Vitro

In vitro, increasing concentrations of the partially purified rabbit anti–PDGF-AA antibody inhibited the mitogenic effects of PDGF-AA on 3T3 fibroblasts in a dose-dependent manner (Figure 2). However, at high concentrations of the antibody, the mitogenic response to PDGF-AA was enhanced. This may have been due to the presence of small quantities of contaminating mitogenic factors in the antibody preparations. This may also explain why the partially purified antisera did not completely inhibit the mitogenic effects of PDGF-AA.

Cholesterol Feeding Significantly Raises Plasma Cholesterol

The cholesterol content of the diet was manipulated to range between 0.25% and 1% so that plasma cholesterol levels were allowed to peak at ≈20 to 25 mmol/L in all animals. There were no significant differences in plasma cholesterol levels between the experimental groups, either during the experimental period (expressed as integrated cholesterol levels) or at sacrifice (the Table). There was also no significant differ-
Inhibition of 3T3 mitogenic response to PDGF-AA by anti–PDGF-AA antibodies

![Graph showing inhibition of 3T3 mitogenic response to PDGF-AA by anti–PDGF-AA antibodies](image)

**Figure 2.** Effects of increasing concentrations of partially purified anti–PDGF-AA rabbit antibodies on PDGF-AA-induced fibroblast proliferation.

ence in mean body weights of the animals from the 3 experimental groups (the Table).

**Endogenously Elicited Antibodies to PDGF-AA and PCP Inhibit Aortic Lesion Development**

Fatty streaks developed in the thoracic aortas of all animals and were visualized by positive staining to oil red O (Figure 3A). Lesions were most severe in the aortic arch and became less prominent in the distal thoracic aorta, where lesions were mostly confined to intercostal artery branch points. Immunization of cholesterol-fed rabbits with PDGF-AA significantly ($P<0.05$) reduced the area of the aortas staining positively with oil red O compared with those from control animals (Figure 3B). The oil red O–stained area was also significantly ($P<0.01$) reduced in animals immunized with human PCP compared with control animals. There was a highly significant positive relation between integrated plasma cholesterol levels and the area of aorta staining positively to oil red O in the control animals ($R^2=0.792$, $P<0.001$). However, there was a weak correlation ($R^2=0.465$, $P<0.05$) in animals immunized with PDGF-AA and a nonsignificant ($R^2=0.125$, $P>0.05$) relation in animals from the human PCP group (data not shown). The weaker associations between integrated cholesterol levels and lesion severity in the latter 2 groups may be related to the variability in antibody responses of the individual animals within these groups.

A similar relation between lesion development and antigen administered was found when the extent of atherosclerosis was measured by the ratio of thickness of the intima and media at the level of the first intercostal branch (Figure 4). Animals immunized with either PDGF-AA or human PCP exhibited significantly ($P<0.05$) smaller aortic intimal-medial area ratios compared with animals within the control group.

**Discussion**

**The Role of PDGF-AA and Other Platelet Proteins in Atherosclerosis**

Platelets contain several mitogenic and potentially proatherogenic growth factors, including PDGF-AA, -AB, and -BB; epidermal growth factor; IGF-1; and TGF-β. PDGF-AA has been shown to mediate the mitogenic effect of exogenous TGF-β$^1$ and IL-1$^β$ on smooth muscle cells via autocrine pathways. The presence of neutralizing antibodies to PDGF-AA may therefore be expected to modulate the potential atherogenic effects of PDGF-AA, IL-1β, and TGF-β.

Immunization of cholesterol-fed rabbits with PDGF-AA was associated with a significant reduction in the extent of atherosclerotic lesions in the thoracic aorta. This reduction was of similar magnitude to that observed in rabbits immunized with cytosolic protein from human platelets. The antibodies isolated from rabbits immunized with PDGF-AA showed cross-reactivity with human recombiant PDGF-AA, rabbit platelet protein, and rabbit smooth muscle cell lysates, but not with human platelet protein. The antibodies from rabbits immunized with PCP showed no cross-reactivity with PDGF-AA. The presence of high titers of plasma anti–PDGF-AA immunoglobulin may inhibit atherosclerosis by neutralizing the effects of PDGF-AA. However, it is uncertain whether PDGF-AA is the principle growth factor involved or whether its importance is due to its mediation of the effects of other growth factors and cytokines, including IL-1, TGF-β, and PDGF-BB.

The antiatherogenic effect of the antibodies raised against PCP, however, appear to be mediated through mechanisms independent of PDGF-AA activity. Nevertheless, it is likely that the platelet antibody exerted its antiatherogenic effects by inhibiting other platelet-associated factors. The strong association between plasma cholesterol and lesional area in the control animals was attenuated in the animals immunized against PDGF-AA. This finding probably reflects the action of the anti–PDGF-AA antibodies inhibiting lesion formation. The association between integrated plasma cholesterol and lesional area was even weaker in the animals immunized with PDGF-AA. The presence of high titers of plasma anti–PDGF-AA immunoglobulin may inhibit atherosclerosis by neutralizing the effects of PDGF-AA. However, it is uncertain whether PDGF-AA is the principle growth factor involved or whether its importance is due to its mediation of the effects of other growth factors and cytokines, including IL-1, TGF-β, and PDGF-BB.

The antiatherogenic effect of the antibodies raised against PCP, however, appear to be mediated through mechanisms independent of PDGF-AA activity. Nevertheless, it is likely that the platelet antibody exerted its antiatherogenic effects by inhibiting other platelet-associated factors. The strong association between plasma cholesterol and lesional area in the control animals was attenuated in the animals immunized against PDGF-AA. This finding probably reflects the action of the anti–PDGF-AA antibodies inhibiting lesion formation. The association between integrated plasma cholesterol and lesional area was even weaker in the animals immunized against PCP, indicating that human platelets contain other factors that contribute to atherogenesis in this model. Hence, although PDGF-AA appears to play an important role in atherogenesis in the cholesterol-fed rabbit, other agents such

**Mean Body Weights, Plasma Cholesterol Levels, and Aortic Wall Characteristics of Cholesterol-Fed Rabbits Immunized With Various Substances**

<table>
<thead>
<tr>
<th></th>
<th>PDGF-AA</th>
<th>PCP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>3.37±0.11</td>
<td>3.37±0.11</td>
<td>3.15±0.09</td>
</tr>
<tr>
<td><strong>Plasma cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At sacrifice, mmol/L</td>
<td>22.5±2.1</td>
<td>24.8±1.6</td>
<td>27.2±1.2</td>
</tr>
<tr>
<td>Integrated, (mmol/L) · weeks</td>
<td>108.1±10.2</td>
<td>109.9±5.9</td>
<td>124.5±6.0</td>
</tr>
<tr>
<td><strong>Aortic wall</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal-medial area ratio</td>
<td>0.009±0.006$^*$</td>
<td>0.025±0.017$^*$</td>
<td>0.159±0.066</td>
</tr>
<tr>
<td>% Lesional area</td>
<td>24.6±5.1$^*$</td>
<td>18.7±4.2$^†$</td>
<td>34.4±4.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

$^*P<0.05$, $^†P<0.01$ vs control rabbits.
as growth factors, chemokines, or other vasoactive molecules associated with platelets are also likely to be important. Although the atherogenic immunogen(s) were platelet derived, it does not exclude the possibility that these factors may be secreted by other atherogenic cells in this model.

The Importance of Immunomodulation in Atherosclerosis

The inflammatory nature of atherosclerosis was first described by Virchow in 1856. It has since been proposed that modulating the immune response during lesion formation in the arterial wall may influence both the size and morphology of these lesions. It is therefore possible that eliciting antibodies against either PDGF-AA or platelet proteins may influence atherogenesis independently of their ability to neutralize the effects of growth factors. The adjuvant administered to both experimental and control groups, however, contained *Mycobacterium bovis*, which is well known to produce both strong humoral and cellular immune responses. Indeed, we found equivalent levels of anti-*Mycobacterium* antibodies in the plasma of animals from all groups. We consider it unlikely that the immune responses to PDGF-AA, platelet proteins, and saline (other than the specific activity of the antibodies) differentially effected atherogenesis in the 3 groups against the background of the immune response to *Mycobacterium bovis*.

Conversely, exogenous administration of immunoglobulins has been reported to inhibit atherosclerosis in the apoE-knockout mouse. This effect may be due to the endogenous superoxide dismutase activity found in isolated immunoglobulins. However, we found that animals immunized with saline alone did not exhibit significantly different levels of atherosclerosis from animals immunized with adjuvant alone (D.J. Lamb and G.A.A. Ferns, unpublished data, 1998).
Possible Clinical Implications

Our data indicate that PDGF-AA plays an important role in the development of aortic atherosclerosis in the cholesterol-fed rabbit. PDGF-AA may be released from degranulating platelets, and PDGF-A chain is also expressed by macrophages. PDGF-α receptors are found on the plasma membrane of endothelial cells and smooth muscle cells. Although the 3 PDGF isoforms display a marked cross-species homology, there are also interspecies differences in cellular distribution and expression. Hence, the extrapolation of these data to humans should be treated with some caution.

These data suggest that PDGF-AA plays a critical role in the evolution of atherosclerotic plaque formation, either directly or indirectly by mediating the effects of other growth factors such as PDGF-BB and TGF-β. This effect appeared to be independent of the immunomodulatory effect of immunization. In addition, PDGF-AA does not appear to be the only platelet-associated growth factor that contributes to cholesterol-induced atherogenesis.

Acknowledgments

This work was supported by a grant from the British Heart Foundation. The authors would like to thank Brian Morris for his gift of NUFA and Graham Mooray for his technical assistance.

References

Endogenous Neutralizing Antibodies Against Platelet-Derived Growth Factor-AA Inhibit Atherogenesis in the Cholesterol-Fed Rabbit
David J. Lamb, Tony Y. Avades and Gordon A. A. Ferns

doi: 10.1161/01.ATV.21.6.997

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/21/6/997

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/