Induction of IG9 Monocyte Adhesion Molecule Expression in Smooth Muscle and Endothelial Cells After Balloon Arterial Injury in Cholesterol-Fed Rabbits

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Abstract—The expression of monocyte-specific adhesion molecules and chemokines by cell types within the vessel wall plays an important role in foam cell accumulation during atherosclerotic plaque development. We previously identified IG9, a novel monocyte adhesion protein that is expressed on endothelial cells (ECs) overlying human and rabbit advanced atherosclerotic plaques. The present study was designed to determine the temporal and spatial expression of IG9 and the chemokine, monocyte chemoattractant protein-1 (MCP-1), after balloon injury with (double injury) or without (single injury) prior air desiccation EC injury in the femoral arteries of rabbits fed a high-cholesterol diet. By immunohistochemical analyses, intense reactivity with monoclonal antibodies to IG9 and MCP-1 was detected 24 hours after single injury in medial smooth muscle cells (SMCs) and in SMCs of adventitial microvessels. However, monocyte infiltration of the tunica media was minimal or not detected in these sections. IG9 and MCP-1 antibody reactivity in vessel sections 28 days after single injury and 24 hours, 7 days, and 28 days after double injury was localized to medial and neointimal SMCs, foam cells, and luminal ECs overlying the plaques. Uninjured rabbit (cholesterol or normal diet) vessel sections exhibited minimal IG9 and MCP-1 immunostaining. In vitro studies using human aortic SMCs demonstrated IG9 protein induction after 24 hours of treatment with platelet-derived growth factor-BB and interferon-γ or epidermal growth factor. IG9 expression was further increased by pretreatment of SMCs with the proatherogenic lipid, minimally oxidized low density lipoprotein. After balloon injury (24 hours), IG9 is induced in vascular SMCs before the detectable accumulation of monocytes within the vessel wall. Thus, the expression of IG9 by SMCs as well as by ECs may be an important factor in the accumulation of foam cells in atherosclerotic plaque development after arterial injury. (Arterioscler Thromb Vasc Biol. 2000;20:1293-1300.)

Key Words: arterial injury ■ atherosclerosis ■ smooth muscle ■ adhesion molecules ■ LDL, minimally oxidized or modified

The adherence of circulating monocytes to endothelial cells (ECs) is one of the earliest events in the development of atherosclerotic plaques. Injury to the vascular endothelium induces EC dysfunction and promotes an inflammatory response characterized by the adherence of monocytes and T lymphocytes to the vessel wall. On entering the subendothelial space, monocytes take up lipids, become activated, and release inflammatory and chemotactic molecules, including the cytokine, tumor necrosis factor-α (TNF-α), and the chemokine, monocyte chemoattractant protein-1 (MCP-1). Chemokine expression contributes to the recruitment of additional monocytes (and T lymphocytes) into the developing lesion, and inflammatory mediators produced by the infiltrating leukocytes stimulate resident cells within the vessel wall to secrete additional proatherogenic molecules, including growth-regulatory factors, cytokines, and other chemokines. These molecules promote medial smooth muscle cell (SMC) migration and proliferation and the continued influx of monocytes and T lymphocytes, thereby amplifying this inflammatory process and promoting neointimal hyperplasia. An understanding of the mechanisms involved in the initial recruitment and adhesion of monocytes to the vessel wall and their subsequent retention within the developing atherosclerotic lesion might facilitate the design of interventional strategies to attenuate this process.

Monocyte binding is mediated, in part, by the expression of adhesion proteins that are induced on the surface of ECs by a
variety of stimuli, including cytokines, P-selectin, intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1 (VCAM-1) have been detected in human atherosclerotic vessels.

All of these adhesion proteins were found to be expressed by ECs, with VCAM-1 localized mainly to vessels within neovascularized areas of plaques and the other adhesion proteins expressed by luminal ECs overlying the plaques. ICAM-1 and VCAM-1 were also shown to be focally expressed by SMCs within plaques.

We identified previously a novel monocyte adhesion molecule that is recognized by monoclonal antibody (mAb) IG9 and is expressed on the surface of activated ECs. The 105-kDa adhesion molecule, referred to as the IG9 protein, is not expressed in vitro on untreated human umbilical vein endothelial cells but is induced after treatment with TNF-α, interleukin-1β, lipopolysaccharide, or phorbol myristate acetate. IG9 expression is first detected 3 hours after treatment of human umbilical vein endothelial cells with these agonists, and maximal levels are induced after 4 to 9 hours, returning to baseline after 48 hours. Minimally oxidized or modified LDL (MM-LDL) also induces IG9 expression in human umbilical vein endothelial cells and in human aortic ECs. E-selectin, VCAM-1, and ICAM-1 are not induced or upregulated in MM-LDL–treated ECs. Monocyte adhesion to TNF-α– and MM-LDL–treated ECs is significantly inhibited by mAb IG9, whereas lymphocyte and granulocyte binding to TNF-α–treated cultures is unaffected.

In human and rabbit advanced atherosclerotic plaques, IG9 protein expression in vivo has been localized to arterial luminal endothelium, suggesting a possible functional role for this protein in monocyte adhesion to ECs overlying the plaques during lesion progression. The present study examines the cell type–specific expression of the IG9 adhesion molecule for monocytes and the monocyte chemoattractant, MCP-1, after balloon-induced arterial injury in the cholesterol-fed rabbit models of single and double injury. We found that IG9 and MCP-1 are prominently expressed by medial SMCs very early after balloon injury (BI) and before detectable monocyte infiltration of the tunica media. In cell culture studies, SMCs were induced to express IG9 protein after treatment with MM-LDL, platelet-derived growth factor (PDGF)-BB, and either TNF-α or epidermal growth factor (EGF) for 24 hours. Thus, vascular SMC expression of IG9, in addition to MCP-1, may be a significant contributor to the extensive accumulation of macrophages within the vessel wall after arterial injury.

**Methods**

**Animals and Induction of Experimental Lesions**

Thirty (n = 30) male New Zealand White rabbits (4.0 ± 0.2 kg) were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). After a high-cholesterol diet (2% cholesterol and 6% peanut oil for 28 days), atherosclerosis was induced by BI in femoral artery segments with or without prior EC injury by air desiccation with the use of nitrogen gas as described previously.

Rabbits were assigned to 3 intervention groups: (1) non–cholesterol-fed (normal diet) rabbits (n = 4) subjected to air desiccation EC injury only and analyzed 28 days after EC injury, (2) rabbits subjected to high-cholesterol diet without prior EC injury followed by BI (single-injury model) and analyzed 24 hours after EC injury, and (3) rabbits subjected to BI after EC injury (double-injury model) and a high-cholesterol diet and analyzed 24 hours after BI (n = 7), 7 days (n = 4), or 28 days (n = 6) after BI. Control animals included uninjured rabbits fed a noncholesterol diet (normal diet, n = 3) and uninjured rabbits fed a high-cholesterol diet (n = 1). The control and intervention groups are shown in the Table.

**Balloons Injury**

Details of the BI protocol have been published previously. Briefly, BI was performed through a right common carotid artery incision with a 2.5-mm-diameter balloon dilation catheter (Advanced Cardiovascular Systems Inc) after bolus heparin administration (150 U/kg, Solopak Laboratories). Three 1-minute 10-atm inflations were performed 1 minute apart by use of a hand inflator. After the procedure, the catheter was removed, the carotid artery was ligated, and the wound was sutured. Rabbits were then fed standard rabbit chow until the time of death. Animals were administered an overdose of sodium pentobarbital, and the femoral arteries were perfused at 100 mm Hg with 4% paraformaldehyde in PBS through a catheter inserted in the carotid artery and positioned above the aortoliac bifurcation. Segments of femoral arteries at the site of previous BI and/or EC injury (2 to 3 cm in length) were excised, postfixed in 4% paraformaldehyde in PBS, cross-sectioned at 2-mm intervals, dehydrated in ethanol and xylene, and embedded in paraffin.

**Histomorphometry**

Percent luminal cross-sectional area narrowing by plaque (%CSAN-P) was determined by computerized planimetry with use of a CUE-2 Image Analyzer (Galai Production Ltd) in association with an Olympus BH-2 microscope system as described previously. %CSAN-P was calculated as the area bound by the internal elastic lamina minus luminal area times 100 divided by the area bound by the internal elastic lamina.

**Immunohistochemistry**

Serial sections (5 μm) were cut onto poly-L-lysine–coated slides (Sigma Chemical Co), deparaffinized through ethanol and xylene, and rehydrated. Sections were blocked with 2% horse serum in Tris-buffered saline for 1 hour at 37°C, washed with Tris-buffered saline, and incubated for 2 hours at room temperature with primary antibody. mAb IG9 ascites (IgG3, 1:1000 dilution) was used to detect IG9 protein; RAM-11 mAb (mouse anti-rabbit monocye/macrophage antibody, IgG1, 1:50 dilution, Dako Corp) was used for identification of monocytes; and mAb 1A4 (mouse anti-human...
α-smooth muscle actin, IgG2A, 1:200 dilution, Dako Corp) was used for identification of SMCs. MCP-1 expression was detected with the mouse anti-human mAb, 3F11 (IgG1, 1:200 dilution; a generous gift of Dr Charles Mackay, formerly of Leukosite Inc, Cambridge, MA). After washes with Tris-buffered saline, sections were incubated with biotinylated horse anti-mouse IgG (1:800 dilution) for 1 hour, followed by a 45-minute incubation with avidin-biotin alkaline phosphatase (ABC Vectastain, Vector Laboratories). The alkaline phosphatase substrate consisted of nitro blue tetrazolium chloride and 5-bromo-4-chloro-3 indolyl phosphate p-toluidine salt (GIBCO-BRL) and 1 mmol/L levamisole (Sigma), an inhibitor of endogenous alkaline phosphatase activity. Positive reactivity is indicated by a blue-purple precipitate. Serial sections from all intervention groups were also incubated with negative control, isotype-matched, mouse myeloma proteins (IgG1, IgG2A, and IgG3, Cappel-Organon Teknika Corp). Nonspecific antibody reactivity was not detected in any of the sections.

**Cell Culture**

Human aortic SMCs were purchased from Clonetics or isolated from human thoracic aortas harvested from explanted hearts at the time of cardiac transplantation. Primary cultures were established by removing adventitial and connective tissue, dissolving the remaining arterial intima and media into 1-cm² segments, and incubating the tissue fragments in tissue culture dishes with 2.5 mg/mL collagenase and 15% FBS. Adherent cells were washed free of debris, and cells were grown in DMEM with 10% FBS, 50 U/mL penicillin, and 50 μg/mL streptomycin (Pen-Strep). Cells were identified as SMCs by their typical microscopic appearance and by immunostaining with antibody to α-smooth muscle actin. The absence of endothelial cell contamination was confirmed by immunostaining with antibody F8/80 to human von Willebrand factor (1:100 dilution, Dako Corp).

**Growth Factors and Other Reagents**

Recombinant human PDGF-BB homodimer, EGF, and interferon-γ (IFN-γ, 200 IU/mL; endotoxin <0.2 μg/μg) were purchased from Genzyme. Human TNF-α (100 IU/mL, endotoxin <0.1 ng/μg) was purchased from R&D Research Systems. DMEM, 100X Pen-Strep, and FBS were purchased from GIBCO-BRL, and collagenase type II was purchased from Worthington Biochemical.

**Cell-Based ELISA**

Human aortic SMCs were grown to confluence in 96-well culture plates, and before treatment, SMCs (passages 7 to 12) were rendered “quiescent” by incubation in low-serum medium consisting of DMEM+1% FBS for 24 to 36 hours. Cells were treated (4 or 8 wells per treatment) with TNF-α, IFN-γ, EGF, or PDGF-BB for 4, 16, or 24 hours at 37°C and 5% CO₂ in low-serum medium. In addition, cells were treated with PDGF-BB in combination with TNF-α, IFN-γ, or EGF. SMCs were also pretreated with MM-LDL (250 μg/mL) for 24 hours in low-serum medium before cytokine and/or growth factor treatment. LDL was isolated by density-gradient centrifugation of serum and stored in PBS containing 0.01% EDTA. MM-LDL was obtained by storage of LDLs at 4°C for 3 to 6 months, as previously described, or by enzymatic oxidation. Three to 5 separate experiments were performed for each treatment group except for MM-LDL (n=2). Treated SMCs were fixed in 2% formaldehyde in PBS, blocked with 1% BSA in PBS, and incubated with mAb IG9 ascites (1:1000 dilution) for 2 hours at 37°C. After PBS washes, alkaline phosphatase–coupled goat anti-mouse IgG3 secondary antibody (1:1000 dilution, Southern Biotechnologies) was added for 1 hour at 37°C, followed by the addition of the alkaline phosphatase substrate p-nitrophenyl phosphate disodium (Sigma). Absorbance was read at 405 nm. Results are expressed as mean±SEM. Statistical significance was determined by 2-tailed Student t test. A value of P<0.05 was considered significant.

**Results**

**IG9 and MCP-1 Expression After EC Injury**

Control femoral artery sections from non–cholesterol-fed (normal diet) uninjured rabbits lacked neointimal formation (%CSAN-P=0±0 and exhibited minimal to undetectable MCP-1, RAM-11(monocyte), or IG9 antibody reactivity (not shown). After cholesterol feeding for 28 days, femoral artery sections from an uninjured rabbit exhibited minimal to no neointimal formation; only small focal areas of luminal monocyte accumulation were detected. IG9 and MCP-1 expression was marginally elevated within the medial SMC layer of this vessel (not shown).

Femoral arteries subjected to adescicatation EC injury without subsequent high-cholesterol feeding exhibited mild to moderate neointimal formation (%CSAN-P=26±6) consisting almost entirely of SMCs, with monocyte infiltration localized to focal areas in the adventitia at 28 days after EC injury (not shown). All sections from this intervention group exhibited minimal to undetectable levels of IG9 and MCP-1 expression in the vessel wall (not shown).

**IG9 and MCP-1 Expression After BI in High-Cholesterol–Fed Rabbits (Single Injury)**

Analysis of sections of arteries of high-cholesterol–fed animals excised 24 hours after BI (single injury) showed undetectable neointimal thickening (%CSAN-P=0±0) with monocytes localized only to small focal areas of the adventitia (Figure 1A). Intense IG9 antibody reactivity was detected in medial SMCs in all analyzed vessel sections (Figure 1B). Adventitial microvessel SMCs also expressed high levels of IG9 immunostaining. MCP-1 antibody reactivity closely paralleled that of mAb IG9 in intensity and localization (Figure 1C). IG9 and MCP-1 expression preceded detectable monocyte infiltration of the tunica media and neointimal formation in injured vessels. There were numerous IG9 (not shown) and MCP-1–positive cells (Figure 1D) adherent to or just below the internal elastic lamina, which were unreactive with the RAM-11 (monocyte) and 1A4 (SMC) antibodies. The identity of these cells is not certain, but they could possibly be T lymphocytes, neutrophils, and/or SMCs that have lost α-smooth muscle actin expression.

Twenty-eight days after BI (single injury), significant neointimal formation (%CSAN-P=50±4) consisting of SMCs (Figure 2A) and monocytes (Figure 2B) was present. Substantial numbers of monocytes and monocyte-derived foam cells (RAM-11 positive) were seen within the media just below the internal elastic lamina and in the adventitia as reported previously. Prominent IG9 and MCP-1 immunoactivity was present in medial SMCs and to a lesser extent in foam cells (Figure 2C and 2D). Adventitial microvessel SMCs also showed marked IG9 and MCP-1 antibody reactivity. Neointimal SMCs and monocytes were focally IG9 and MCP-1 positive (Figure 2C and 2D). A continuous layer of luminal cells, probably ECs, were seen overlying plaques at this time point. These cells expressed moderate to high levels of IG9 and MCP-1 immunoreactivity (Figure 2E and 2F) and were unreactive with the RAM-11 (monocyte) and 1A4 (SMC) antibodies (not shown).

**IG9 and MCP-1 Expression After EC Injury and BI in High-Cholesterol–Fed Rabbits (Double Injury)**

Substantial neointimal formation, consisting of SMCs and monocytes, was detected in 6 of 7 animals analyzed 24 hours after double injury (%CSAN-P=52±9, with numerous
monocytes and foam cells localized to the adventitia, neointima, and the media below the internal elastic lamina (Figure 3A) as reported previously. In the 6 vessels with plaque development, IG9 (Figure 3B) and MCP-1 (Figure 3C) expression was localized to neointimal, medial, and adventitial microvessel SMCs and to monocytes and foam cells within the vessel wall and adventitia. IG9 immunoreactivity appeared less prominent than that of MCP-1 in all of the analyzed vessel sections.

The cell type–specific expression of IG9 did not change at 7 days (%CSAN-P=29±3, not shown) and 28 days (%CSAN-P=61±6, Figure 3E) after double injury, although IG9 immunoreactivity was greater than at 24 hours. In addition, luminal ECs overlying the plaques were also moderately to highly reactive with mAb IG9 (28 days after BI). MCP-1 expression at 7 days (not shown) and 28 days (Figure 3F) after BI (double injury) was also very similar to that seen at 24 hours, with the addition of luminal EC expression of MCP-1 28 days after BI.

Growth Factor, Cytokine, and MM-LDL Regulation of IG9 Expression in Human SMCs

The novel finding of SMC expression of IG9 after cholesterol feeding and BI initiated a study to characterize further the expression of the IG9 protein in this cell type. Primary human aortic SMC cultures were treated with TNF-α, IFN-γ, EGF, or PDGF-BB. IG9 expression was analyzed by ELISA after treatment for 4, 16, or 24 hours. Untreated SMCs expressed minimal amounts of cell surface IG9 protein. Treatment with TNF-α (1 to 500 U/mL), IFN-γ (1 to 1000 U/mL), EGF (1 to 30 ng/mL), or PDGF-BB (1 to 50 ng/mL) for 4, 16, or 24 hours did not induce significant IG9 expression (data not shown). However, treatment with PDGF-BB (10 ng/mL) in combination with IFN-γ (100 U/mL) or EGF (3 ng/mL) for 24 hours induced significant IG9 expression compared with no treatment (P<0.05, Figure 4).

We have previously shown that the proatherogenic oxidized lipid, MM-LDL, at 250 μg/mL induces IG9 protein expression in human and rabbit ECs after 24 hours and that this expression mediates a significant portion of MM-LDL–induced monocyte adhesion. To determine whether MM-LDL also induces IG9 expression in SMCs, cultures were treated with this concentration of MM-LDL for 24 hours. Unlike ECs, IG9 expression was not significantly induced in human aortic SMCs (Figure 5). However, MM-LDL pretreatment of SMCs for 24 hours increased the level of IG9 protein induced by TNF-α, IFN-γ, EGF, or PDGF-BB, with signif-

Figure 1. Immunohistochemical analysis of serial sections of a femoral artery from a cholesterol-fed rabbit 24 hours after BI (single injury). A, Section incubated with RAM-11 antibody illustrating the absence of monocyte infiltration of the vessel wall at this time with minimal numbers of monocytes localized to focal areas of the adventitia. B and C, Adjacent sections showing intense medial SMC expression of IG9 (B) and MCP-1 (C). Magnification ×17 (A to C), bar=1 mm. D, Higher magnification of an adjacent section showing MCP-1 expression (mAb 3F11 reactivity) within the tunica media. Magnification ×66 (D), bar=0.1 mm.
significant synergistic increases seen after treatment with EGF or PDGF-BB in combination with TNF-α or EGF (P<0.02 versus no treatment, Figure 5; IFN-γ was not included in these studies).

**Discussion**

In the present study, we demonstrate that the IG9 monocyte adhesion protein is prominently expressed by SMCs within large areas of the tunica media as well as in adventitial microvessels 24 hours after a single BI of cholesterol-fed rabbit femoral arteries. This expression precedes the detectable influx of monocytes into the injured arterial vessel wall. The pronounced IG9 immunoreactivity, as well as that of MCP-1, detected in adventitial microvessels at this early time point is suggestive of adventitial contributions to lesion formation after arterial vessel injury. From previous immu-
nohistochemical studies, it has been postulated that a large number of the foam cells in the neointima and media in this model of injury-induced atherogenesis are derived from cells of the media and adventitia rather than from the lumen. Support for this was derived from the finding of contiguity between neointimal and medial foam cells and those of the adventitia, with convincing evidence of association with neovasculature invasion from the adventitia. Thus, adventitial microvessel expression of a monocyte chemoattractant and a monocyte adhesion protein, as found in the present study, may establish an important route for monocyte trafficking from the adventitia into the injured vessel wall.

In a study by Scott et al., increased cell proliferation and growth factor synthesis in the adventitia were detected at early times after balloon overstretch injury of pig coronary arteries. Migration of adventitial myofibroblasts across the external elastic lamina to the developing neointima was demonstrated, and these results support the hypothesis that the adventitia may play a role in vascular lesion formation after vessel injury. In a recent report, enhanced leukocyte-endothelial cell interactions and P-selectin, ICAM-1, and VCAM-1 expression were found to occur in vivo in the rabbit mesenteric microcirculation during the first 2 weeks of hypercholesterolemia. The authors suggest that the microvasculature is an early site of vascular dysfunction, enabling circulating leukocytes to traffic from microvessels to large

Figure 3. Immunohistochemical analysis of serial sections of femoral arteries from cholesterol-fed rabbits 24 hours and 28 days after BI with prior EC injury (double injury). At 24 hours after BI, RAM-11 reactivity is evident throughout the neointima, media, and adventitia (A). In adjacent sections, low levels of IG9 (B) and moderate to high levels of MCP-1 (C) antibody reactivity are shown on SMCs and monocytes. By contrast, at 28 days after BI, abundant monocytes are localized within the neointima and media (D). Adjacent sections show high IG9 (E) and moderate MCP-1 (F) antibody reactivity on medial and neointimal SMCs, monocytes, foam cells, and luminal ECs. Magnification ×17 (A to F), bar=1 mm.

Figure 4. Induction of IG9 protein expression in human aortic SMCs as analyzed by ELISA. SMCs were treated with TNF-α (100 U/mL), IFN-γ (100 U/mL), or EGF (3 ng/mL) alone or in combination with PDGF-BB (10 ng/mL) for 24 hours. Values represent absorbance (OD) at 405 nm. Data are mean±SEM for 3 to 5 separate experiments. *P<0.05 compared with untreated cells.

Figure 5. MM-LDL–mediated modulation of IG9 induction in human aortic SMCs as analyzed by ELISA. SMCs were pretreated with medium only or MM-LDL (250 μg/mL) for 24 hours, followed by treatment with TNF-α (100 U/mL), EGF (3 ng/mL), or PDGF-BB (10 ng/mL) alone or in combination for 24 hours. Values represent absorbance (OD) at 405 nm. Data are mean±SEM for 3 to 5 separate experiments without MM-LDL pretreatment and 2 separate experiments with MM-LDL pretreatment. *P<0.05 and **P<0.02 compared with untreated cells.
arterial vessels during plaque development. Thus, monocytes within the developing neointima and media of atherosclerotic vessels may not originate entirely from the lumen of the arterial vessel.

Neointimal formation 28 days after BI is characterized by significant monocyte accumulation throughout the vessel wall and is accompanied by prominent IG9 positivity on neointimal SMCs, monocytes, foam cells, and luminal ECs overlying the plaque. This is in contrast to the negligible monocyte accumulation that occurs 28 days after EC injury of normolipemic rabbit arteries and the minimal levels of IG9 expression in the fibrocellular lesions elicited by this type of vessel injury. The expression of IG9 on multiple cell types within the vessel wall at later time points after single BI is associated with marked monocyte infiltration into the neointima and media during injury-induced plaque progression and may result from the establishment of a lipid–, cytokine–, and growth factor–enriched microenvironment within the developing plaque.5,9 We demonstrate that cytokines and growth factors expressed in vivo during atherogenesis32–35 are inducers of IG9 expression in cultured SMCs and ECs. Thus, the expression of IG9 by multiple cell types within the vessel wall during injury-induced plaque progression may contribute to monocyte accumulation within the developing neointima, in addition to the media.

Our finding that IG9 is expressed in vivo by SMCs in the early response to vessel injury initiated the study of the regulation of IG9 expression in this cell type. We found that TNF-α did not induce IG9 expression in cultured human aortic SMCs, even though this cytokine is a positive regulator of IG9 expression in ECs.8,9 PDGF-BB or EGF, potent modulators of SMC function,36–38 also did not induce significant IG9 expression in cultured SMCs. However, PDGF-BB in combination with EGF or the activated T-lymphocyte product, IFN-γ, induced significant IG9 protein expression. The regulation of IG9 protein expression in SMCs may be dependent on activation of signaling pathways by multiple growth factor and cytokine receptors to provide a threshold level of protein expression that can be detected by mAb IG9.

Cytokine– and growth factor–induced IG9 expression in SMCs was upregulated by MM-LDL pretreatment. Oxidized lipoproteins are postulated to play a role in the development of atherosclerosis,40–42 and active oxidized phospholipids derived from MM-LDL were found to be increased in rabbit atherosclerotic lesions.43 Enhanced levels of MM-LDL within vessel walls may function as activators of SMCs and ECs because MM-LDL is mitogenic to cultured SMCs44 and induces MCP-1 in SMCs and ECs45 and macrophage colony–stimulating factor46 and tissue factor44 in ECs. In addition, MM-LDL also selectively stimulates ECs to bind monocytes,22 and this adhesion is dependent on MM-LDL–mediated increases in intracellular cAMP.27 MM-LDL–mediated signaling mechanisms in SMCs have not been extensively characterized to date, but the present study suggests that additive signal transduction pathways activated by cytokines, growth factors, and MM-LDL may significantly contribute to the induction of IG9 expression in SMCs. Another possibility is that MM-LDL may be modifying the plasma membrane, resulting in enhanced cytokine– and growth factor receptor–mediated induction of IG9 or in increased recognition of IG9 surface protein by mAb IG9 in treated SMCs.

IG9 expression in vivo at early time points after balloon arterial injury in cholesterol-fed rabbits is localized to vascular SMCs, and cytokines, growth factors, and oxidized lipids associated with atherogenesis, including PDGF-BB, IFN-γ, and MM-LDL, are potential inducers of IG9 expression in this cell type. Therefore, our results suggest that IG9 is a cellular adhesion molecule that contributes to the accumulation of monocytes within the media of arterial vessels during the initiating phase of injury-induced atherogenesis in cholesterol-fed rabbits.

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