PRE-CLINICAL RESEARCH

Adipokine Resistin Is a Key Player to Modulate Monocytes, Endothelial Cells, and Smooth Muscle Cells, Leading to Progression of Atherosclerosis in Rabbit Carotid Artery

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Objectives	We investigated the effects of human resistin on atherosclerotic progression and clarified its underlying mechanisms.
Background	Resistin is an adipokine first identified as a mediator of insulin resistance in murine obesity models. But, its role in human pathology is under debate. Although a few recent studies suggested the relationship between resistin and atherosclerosis in humans, the causal relationship and underlying mechanism have not been clarified.
Methods	We cloned rabbit resistin, which showed 78% identity to human resistin at the complementary deoxyribonucleic acid level, and its expression was examined in 3 different atherosclerotic rabbit models. To evaluate direct role of resistin on atherosclerosis, collared rabbit carotid arteries were used. Histological and cell biologic analyses were performed.
Results	Rabbit resistin was expressed by macrophages of the plaque in the 3 different atherosclerotic models. Periadventitial resistin gene transfer induced macrophage infiltration and expression of various inflammatory cytokines, resulting in the acceleration of plaque growth and destabilization. In vitro experiments elucidated that resistin increased monocyte-endothelial cell adhesion by upregulating very late antigen-4 on monocytes and their counterpart vascular cell adhesion molecule-1 on endothelial cells. Resistin augmented monocyte infiltration in collagen by direct chemoattractive effect as well as by enhancing migration toward monocyte chemotactic protein-1. Administration of connecting segment-1 peptide, which blocks very late antigen-4 × vascular cell adhesion molecule-1 interaction, ameliorated neointimal growth induced by resistin in vivo.
Conclusions	Our results indicate that resistin aggravates atherosclerosis by stimulating monocytes, endothelial cells, and vascular smooth muscle cells to induce vascular inflammation. These findings provide the first insight on the causal relationship between resistin and atherosclerosis. (J Am Coll Cardiol 2011;57:99–109) © 2011 by the American College of Cardiology Foundation

Obesity is a well-known principal risk factor for metabolic disorders and cardiovascular diseases, and recent studies have shown that numbers of bioactive molecules secreted from adipose tissue contribute this connection (1–3). Resistin is 1 of these adipocyte-derived molecules, which was first identified as a pivotal hormone linking obesity and insulin resistance in murine models (4). Yet, there are still controversies on its role in human pathology, showing variable associations with insulin resistance (5,6). Interestingly, resistin in humans is known to be expressed in atherosclerotic plaque (7), and its plasma levels are associated with coronary heart disease (8) and future cardiovascular death (9). Several

Abbreviations and Acronyms

Ad.GFP = adenovirus expressing green-fluorescence protein

Ad.Resistin/GFP = adenovirus expressing human resistin and green-fluorescence protein

CS1 = connecting segment-1

EC = endothelial cell

IL = interleukin

MCP = monocyte chemotactic protein

TNF = tumor necrosis factor

VCAM = vascular cell adhesion molecule

VLA = very late antigen

VSMC = vascular smooth muscle cell

in vitro studies demonstrated that resistin upregulates expression of inflammatory cytokines and adhesion molecules in human endothelial cells (10), increases smooth muscle cell proliferation (11) and migration (7), and accelerates foam cell formation (12). Despite these early studies, however, its role in atherosclerosis is now only emerging, and it has not been identified whether resistin expression is just a simple feature of atherosclerotic progression or directly participates in the process. Therefore we investigated the pathologic role of human resistin in atherosclerotic plaque progression and performed mechanistic analysis of the causal relationship between them.

Methods

Cloning of rabbit resistin. Another animal model was required for this study, because of low homology and different expression patterns between murine and human resistin (13–15). We looked for resistin in rabbit and cloned it for the first time from messenger ribonucleic acid of rabbit bone marrow cells, with reverse-transcriptase polymerase chain reaction. The primers were designed from highly conserved regions of human (GeneBank accession number AF205952), pig (AY488504), and cattle resistin (AB117718) complementary deoxyribonucleic acid sequences (forward primer, 5′-CACCTGCAGGATGAAGGCTCT-3′; reverse primer, 5′-GCTCAGGCGTTAGTCCTGATGCG-3′). Further information is described in the Online Appendix. The obtained open reading frame of rabbit resistin was 339 base pair (bp) long (translated into 112 amino-acids), and the nucleotide sequence was submitted to the GeneBank database with accession number FJ424872. Rabbit resistin was mainly expressed in mononuclear cells from peripheral blood and bone marrow, just like human resistin (Online Fig. 1).

Collar placement and gene delivery. Bilateral rabbit carotid arteries were exposed surgically under anesthesia, and 2 biologically inert silicone collars (total length 20 mm, bore diameter 1.8 mm; generous gifts from Dr. Martin, British Heart Foundation Laboratories at University College London) were implanted (1 around the left and 1 around the right carotid artery). After 1 week, the collared arteries were re-exposed. Adenovirus expressing human resistin and green-fluorescence protein (Ad.Resistin/GFP) (100 μl, 1.5×10^9 pfu) was injected within the collar around the left or right carotid artery, at random, whereas adenovirus expressing green-fluorescence protein (Ad.GFP) (100 μl,

1.5×10^9 pfu) was injected within the contra-lateral collar. Then, rabbits were killed at Day 2 (n = 2), Day 7 (n = 3), and Day 28 (n = 7) from gene delivery.

Statistical analysis. All data are presented as mean ± SEM. Comparisons between groups were performed by Student t test. Because experimental and control value were obtained as a pair from each rabbit (Ad.Resistin/GFP- vs. Ad.GFP-transfected carotid artery or monocyte- vs. control phosphate-buffered saline–delivered carotid artery), paired t test was used to compare results of in vivo experiments. The SPSS version 11.0 (SPSS, Chicago, Illinois) was used for analysis, and p < 0.05 was considered statistically significant.

Other methods are described in detail in the Online Appendix.

Results

Resistin expression in rabbit atherosclerosis models. After cloning of rabbit resistin as described previously, expression of resistin was explored in 3 established models of atherosclerosis in aorta or carotid artery of cholesterol-fed rabbit: 1) without any vascular injury (Figs. 1A to 1C); 2) with balloon injury (Figs. 1D to 1F); and 3) with peri-vascular collar application (Figs. 1G to 1I). Resistin was expressed in atherosclerotic plaque of all these 3 models but not in normal vessels (data not shown). Serial section immunohistochemistry and immunofluorescence double-staining for resistin and RAM-11 (a rabbit macrophage marker) showed that resistin was expressed by macrophages in atherosclerotic plaque (Fig. 1), which was consistent with the previous report on human resistin (7).

Atherosclerotic plaque growth by resistin in vivo. Given the high homology and similar tissue expression pattern between rabbit and human resistin, we regarded rabbits as adequate animals for our research. Thus, we delivered Ad.Resistin/GFP or Ad.GFP solution to the peri-adventitial space of the collared rabbit carotid arteries to demonstrate the effects of resistin on atherosclerotic progression. The GFP expression and immunofluorescence staining for resistin examined on Day 2 ascertained stable transduction of adenoviral vector to the arteries (Online Figs. 2A to 2D). Reverse-transcriptase polymerase chain reaction result showing human resistin expression only in the Ad.Resistin/GFP group also supported stable expression of the genes (Online Fig. 2E). Then we measured intima/media area ratio 4 weeks after gene delivery and found that atherosclerotic plaque growth was significantly promoted by resistin (Fig. 2).

Macrophage infiltration and plaque instability by resistin in vivo. Further evaluation of plaque characteristics revealed more complicated effects of resistin on atherosclerotic progression. Immunohistochemistry for RAM-11 revealed that resistin significantly increased macrophage infiltration both in intima and adventitia and was co-localized with resistin expression (Online Fig. 3). Even after adjustment for the area of neointima or adventitia, the number of
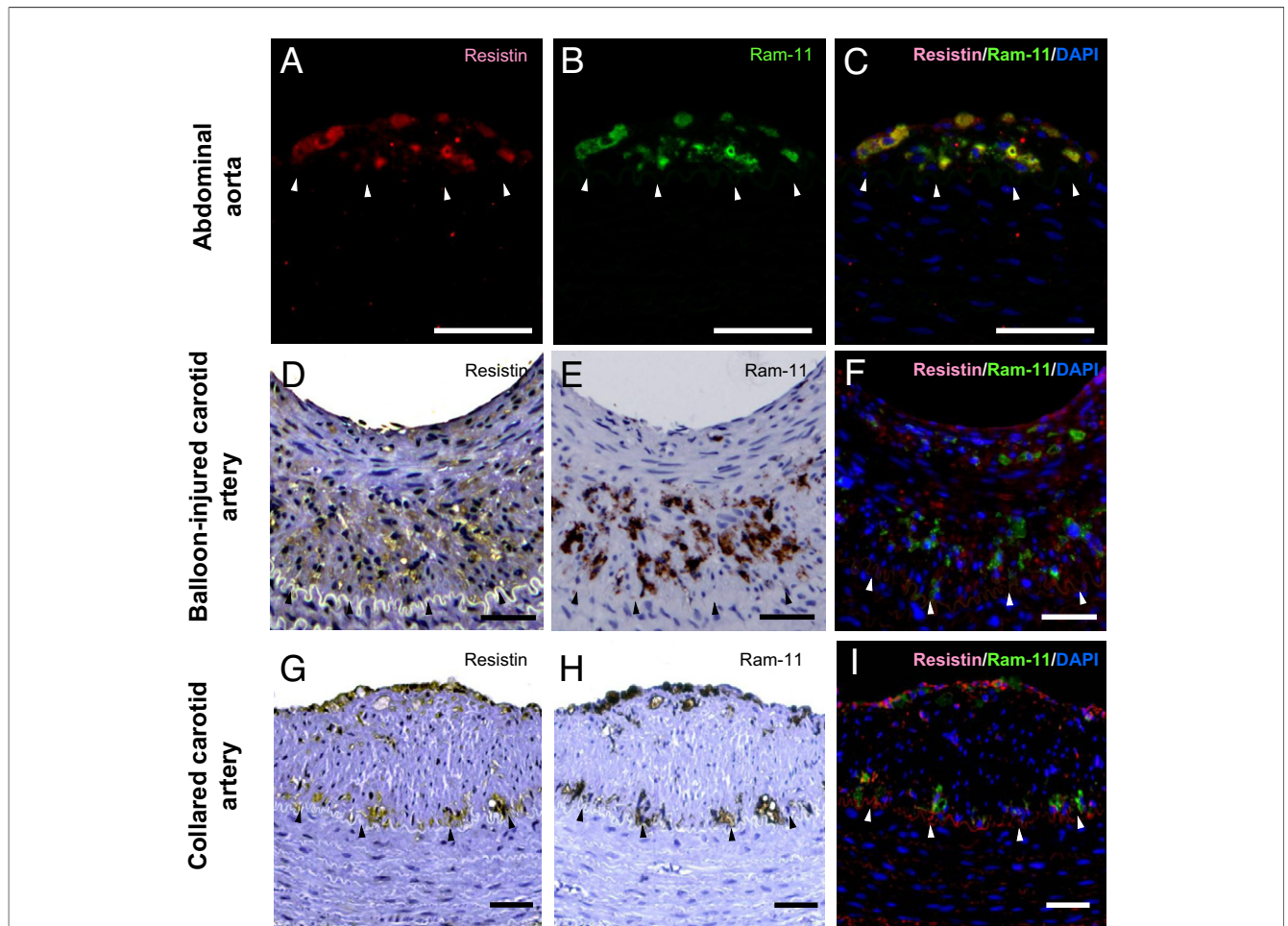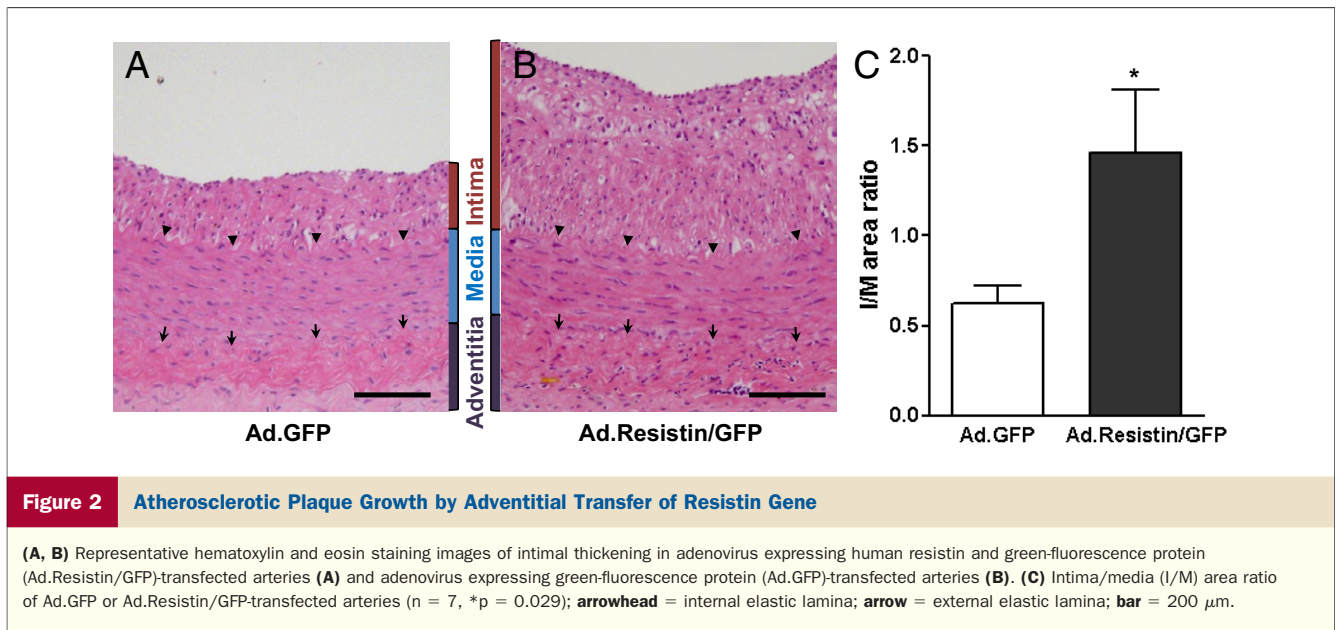
Figure 1   **Expression of Resistin in the Plaques of 3 Different Rabbit Models for Atherosclerosis**

(A to C) Plaque in aorta of cholesterol-fed rabbit without any vascular injury. Immunofluorescence double-staining for resistin **(A, red)** and RAM-11 **(B, green)** showed their colocalization **(C, merged)**. **(D to F)** Plaque in balloon-injured carotid artery of cholesterol-fed rabbit. **(G to I)** Plaque in carotid artery of cholesterol-fed rabbit with peri-vascular collar application. Serial section immunohistochemistry **(D, E, G, H)** and immunofluorescence double-staining **(F, I)** for resistin and RAM-11 demonstrated that resistin was expressed by macrophages in atherosclerotic plaque. The **upper region** indicates luminal side. **Arrowhead** = internal elastic lamina; **Bar** = 100 μm.

infiltrated macrophages was still higher in the Ad.Resistin/ GFP group than in the control group (Fig. 3A) (Table 1). In addition, the proportion of vascular smooth muscle cell (VSMC) area—identified by α-smooth muscle actin immunohistochemistry staining—was significantly reduced, and lipid accumulation tended to be greater in the Ad.Resistin/ GFP group (Figs. 3B and 3C). When calculating plaque stability score by the formula: plaque stabilization score = (α-smooth muscle actin area % + collagen area %)/ (macrophage area % + lipid vacuole area %) (16), it was decreased profoundly by resistin (Fig. 3C). These data suggest that human resistin stimulates atherosclerotic progression in the aspects of plaque stability as well as its size. **Cellular mechanisms of atherosclerosis aggravation by resistin.** Among the pivotal molecules in pathogenesis of atherosclerosis (17), resistin increased expression of inflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]-α) and vascular cell adhesion molecule

(VCAM-1) in carotid arteries but not monocyte chemotactic protein (MCP)-1 (Online Fig. 4). This result suggested that resistin might promote atherosclerotic progression by stimulating monocyte infiltration and vascular inflammation. Therefore, further in vitro experiments were performed to clarify the potential underlying mechanisms; and the effects of resistin on 3 important cellular components of vessel wall—monocytes/macrophages, endothelial cells (ECs), and VSMCs—were explored.

First, resistin stimulated monocyte-EC adhesion by augmenting VLA-4 (α4- and β1-integrin) expression on monocytes (Fig. 4A, Online Fig. 5) and VCAM-1 (counterpart of VLA-4) expression on ECs (Fig. 4B, Online Fig. 6). This was supported by the blocking experiments with VLA-4 and VCAM-1 neutralizing antibodies (Figs. 4C and 4D). Second, resistin incited monocyte infiltration and survival. Resistin increased monocyte infiltration in collagen gel not only by direct

Figure 2   Atherosclerotic Plaque Growth by Adventitial Transfer of Resistin Gene

(A, B) Representative hematoxylin and eosin staining images of intimal thickening in adenovirus expressing human resistin and green-fluorescence protein (Ad.Resistin/GFP)-transfected arteries **(A)** and adenovirus expressing green-fluorescence protein (Ad.GFP)-transfected arteries **(B)**. **(C)** Intima/media (I/M) area ratio of Ad.GFP or Ad.Resistin/GFP-transfected arteries (n = 7, *p = 0.029); **arrowhead** = internal elastic lamina; **arrow** = external elastic lamina; **bar** = 200 μm.

chemoattractive effects on monocytes (Fig. 5A) but also by increasing their mobility toward MCP-1 (Fig. 5B). The WST-1 (Water-Soluble Tetrazolium Salt-1) test revealed that resistin also increased the number of viable monocytes in vitro (Fig. 5C). Third, resistin activated VSMCs to express VCAM-1 (Online Fig. 7).

Finally, to confirm these cellular mechanisms of resistin-induced atherosclerosis, soluble connecting segment (CS)-1 peptides—a VLA-4 binding motif of fibronectin—were administered subcutaneously to block VLA-4 $\times$ VCAM-1 in vivo interaction (18,19). And in CS1 peptide-treated rabbits, resistin neither aggravated atherosclerotic plaque progression nor recruited more macrophages into collared carotid arteries (Fig. 6).

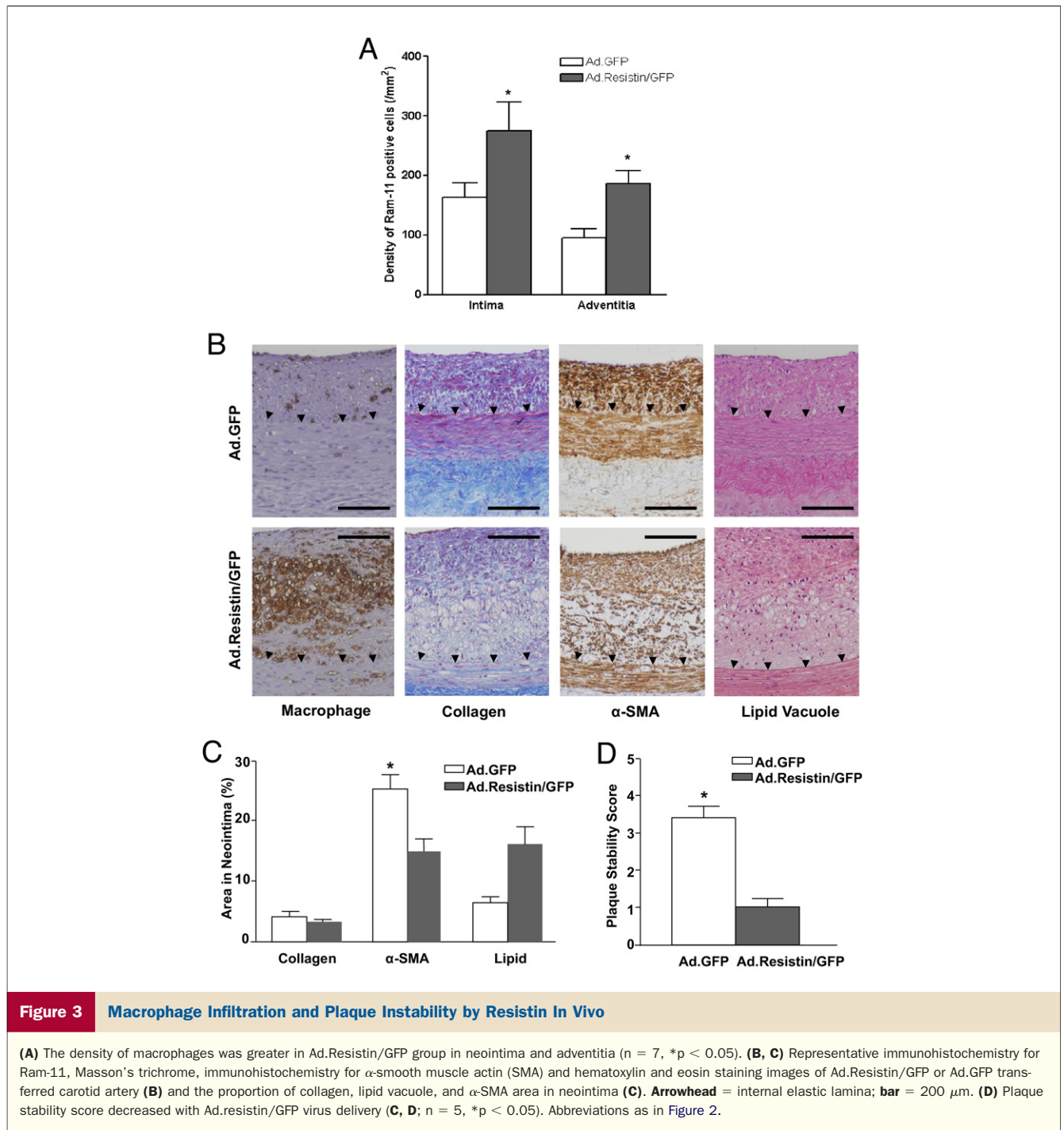**Role of adventitial macrophages in neointimal growth.** From the result that adventitial resistin gene delivery increased macrophage infiltration not only in adventitia but also in intima (Fig. 3A, Online Fig. 3), we designed additional in vivo experiments. First, to determine the effect of peri-adventitial macrophages on plaque progression, we directly delivered rabbit monocytes to adventitia of collared arteries. Second, to elucidate the routes of macrophages infiltrated to neointima, we introduced 2 differently colored monocytes through 2 different routes; red-seminaphthorhodafluor stained monocytes were delivered directly to adventitia of collared arteries, whereas green-carbocyfluorescein succinimidyl ester (CFSE) stained monocytes were introduced intravenously. After 14 days, monocytes delivered to adventitia evidently accelerated neointimal growth (Figs. 7A and 7B), but only green-CFSE monocytes administrated intravenously were detected in neointima (Fig. 7C). These findings suggest that adventitial macrophages might aggravate plaque progression by recruiting circulating monocytes

into neointima remotely but not by infiltrating to the plaque by themselves.

Discussion

The convergence of insulin resistance and inflammation in the pathogenesis of atherosclerotic cardiovascular disease had been recognized over the past decade (20–22). Resistin has emerged as a new molecule to stand at the nodal point of signaling pathways to link metabolic disorders and inflammation (23). Thus we hypothesized that human resistin, as an inflammatory mediator, might be a causal factor of atherosclerosis, and here we report that human resistin can directly aggravate atherosclerotic plaque progression in both size and stability. Resistin increased macrophage infiltration in intima and adventitia of the collared carotid arteries and amplified various inflammatory cytokine expressions in arterial walls. The in vitro studies revealed that resistin affects not only monocytes themselves but also their vascular counter parts, ECs and VSMCs, thereby increasing infiltration and retention of monocytes in the vessel walls. The subsequent in vivo experiment, in which soluble CS1 peptide reversed atherosclerotic plaque progression induced by resistin, also supported the results from the in vitro experiments. Furthermore, we demonstrated for the first time that the peri-adventitial macrophages can recruit circulating monocytes into neointima and aggravate atherosclerosis.

Appropriateness of rabbit model for studying human resistin. A few previous studies tested the role of resistin in various diseases with murine models. Murine resistin, however, is different from that of human in amino-acid sequence (13), expression patterns (14), and functions,

Figure 3   **Macrophage Infiltration and Plaque Instability by Resistin In Vivo**

(A) The density of macrophages was greater in Ad.Resistin/GFP group in neointima and adventitia (n = 7, *p < 0.05). (B, C) Representative immunohistochemistry for Ram-11, Masson's trichrome, immunohistochemistry for α-smooth muscle actin (SMA) and hematoxylin and eosin staining images of Ad.Resistin/GFP or Ad.GFP transferred carotid artery (B) and the proportion of collagen, lipid vacuole, and α-SMA area in neointima (C). **Arrowhead** = internal elastic lamina; **bar** = 200 μm. (D) Plaque stability score decreased with Ad.resistin/GFP virus delivery (C, D; n = 5, *p < 0.05). Abbreviations as in Figure 2.

raising controversies on the implication of resistin in human diseases (15). This discrepancy led us to suspect that the mouse atherosclerosis model, such as apolipoprotein-E knockout mouse, is not suitable for investigation of human resistin in atherosclerosis and to seek for more suitable animal models. Rabbit was 1 of the best candidates for our study, because it has established experimental models mimicking human atherosclerosis (24) and is easy to manipulate. Thus, we cloned rabbit resistin and revealed that it has high homology to that of human (78% and 69% identity at

complementary deoxyribonucleic acid and amino-acid level, respectively), especially at the globular head region (85% amino acid sequence homology) known as the functional domain (25). Rabbit resistin was indeed more similar than mouse resistin to resistin of bigger animals such as pigs or cattle (Table 2). Furthermore, rabbit resistin showed tissue expression pattern comparable to that of human and was expressed by infiltrating macrophages in the atherosclerotic plaque, as shown in the previous report about human resistin (7). Finally, the results showing that rabbit mono-

Table 1	Effect of Resistin Transgene on Lesion Formation in Collared Rabbit Carotid Arteries			
	Ad.GFP (n = 7)	Ad.Resistin (n = 7)	RI of Ad.Resistin*	p Value
Atheroma (mm^2)	1.16 ± 0.21	2.85 ± 0.69	2.51 ± 0.31	0.025
Intima/media area ratio	0.62 ± 0.10	1.46 ± 0.35	2.43 ± 0.36	0.029
Macrophage/mm^2†				
Neointima	163 ± 24	274 ± 49	1.72 ± 0.17	0.010
Adventitia	95 ± 15	187 ± 21	2.24 ± 0.38	0.001
Resistin-positive cells/mm^2				
Neointima	173 ± 33	313 ± 36	1.98 ± 0.20	<0.001
Adventitia	72 ± 14	150 ± 25	2.18 ± 0.13	0.001

Data are expressed as mean ± SEM. *Relative increment (RI) in adenovirus expressing human resistin and green-fluorescence protein (Ad.Resistin/GFP)-infected compared with adenovirus expressing green-fluorescence protein (Ad.GFP)-infected artery. †Number of RAM-11 positive cells/square millimeter in immunohistochemistry staining.
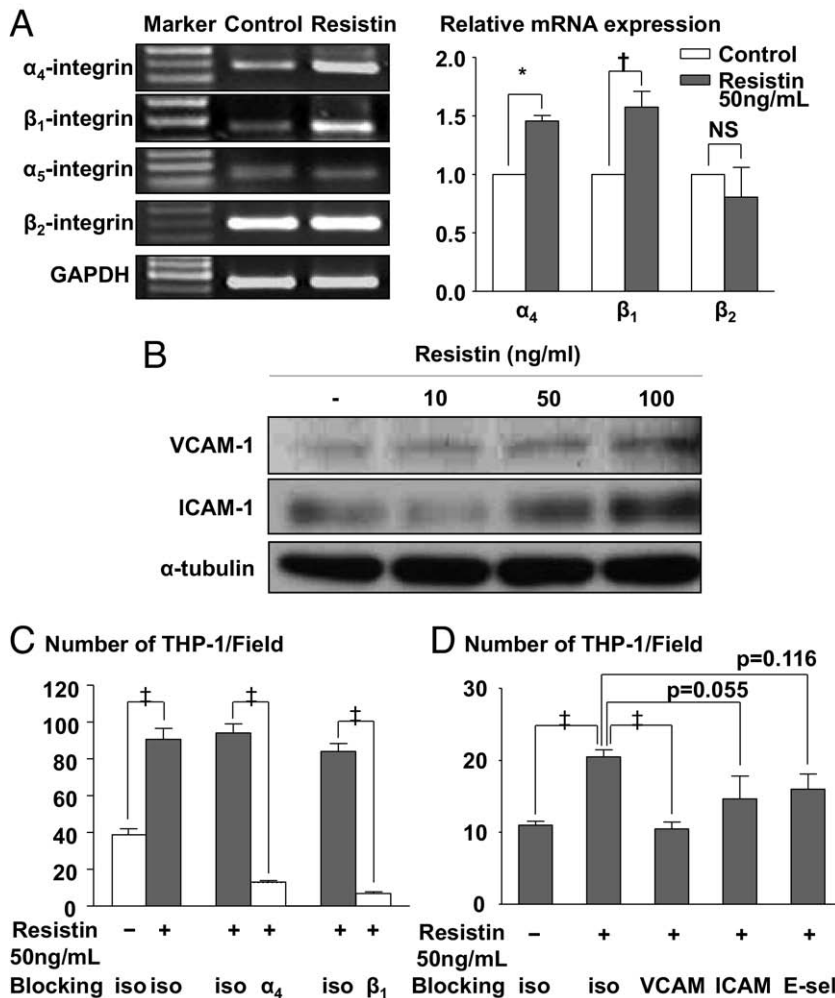
Figure 4   VLA-4 and VCAM-1 in Monocyte-EC Adhesion Mediated by Resistin

(A) Representative figures and densitometry of reverse-transcriptase polymerase chain reaction for integrins show that very late antigen-4 (VLA-4) (α4-, β1-integrin) expression in human acute monocytic leukemia cell line cells (THP-1) was increased by resistin (n = 3, *p = 0.011, †p = 0.051). **(B)** Representative figures of Western blot analysis demonstrate that intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) expression in human umbilical vein endothelial cell (HUVEC) was stimulated by resistin in a dose-dependent manner. **(C)** The THP-1 cells treated with resistin showed enhanced adhesion activity toward HUVEC monolayer, and it was reversed by blocking antibodies against α4 or β1 integrin (n = 9, ‡p < 0.001). **(D)** Incubation of HUVECs with resistin enhanced its adhesion to THP-1s, which was reversed by blocking VCAM-1 with its neutralizing antibodies (n = 6, ‡p < 0.001). mRNA = messenger ribonucleic acid.

Figure 5   Effects of Resistin on Infiltration and Survival of Monocytes

(A) Vertical collagen gel invasion assay showed that human acute monocytic leukemia cell line cells (THP-1) cells significantly infiltrated toward resistin more than control by the virtue of direct chemotactic effect (n = 3, *p = 0.006). **(B)** Resistin treatment on THP-1s also enhanced invasion toward monocyte chemotactic protein-1 (MCP-1) (n = 3, †p = 0.003). **Bar** = 500 μm. **(C)** The THP-1 cell number measured by the WST-1 (Water-Soluble Tetrazolium Salt-1) test was increased by resistin (n = 5, ‡p < 0.001).

cytes responded to human resistin to express inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Online Fig. 8), which was also consistent with the previous report on human cells (26), implied that we could directly use human resistin in rabbit models to identify its role in atherosclerosis.

Proatherogenic action of resistin. As reviewed in the introduction, despite reports that resistin activates ECs and VSMCs in vitro (7,10–11), there has been no direct evidence to support an active proatherogenic action of resistin in vivo. In our study, we observed tangible and significant effects of resistin on atherosclerosis. It increased atherosclerotic lesion area approximately 2.5-fold and intima/media area ratio approximately 2.4-fold. Resistin also raised macrophage accumulation in intima and adventitia by 1.7- and 2.2-fold, respectively, and augmented expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-α as well as adhesion molecule VCAM-1. Furthermore, the proportion of VSMC area in the plaque was decreased by resistin, and plaque stability was also reduced. However, resistin made no significant difference in the number of vasa vasorum in adventitia of our model (data not shown), although there have been a few reports suggesting that resistin can increase angiogenesis in vitro (27). These results propose that resistin, by stimulating vascular inflammation and macrophage accumulation in vessel wall, promotes atherosclerotic plaque formation and destabilization.

Mechanism of resistin inducing vascular inflammation and atherosclerosis. Our in vitro studies revealed additional evidence on how resistin increased macrophage infiltration that resulted in atherosclerotic progression. First, resistin augmented monocyte-EC adhesion both by raising expression of VLA-4 on monocytes and their counterpart VCAM-1 on ECs. Expression of another important adhesion molecule, intercellular adhesion molecule-1, was also increased on ECs by resistin (Fig. 5B), but expression of its counterpart, β2-integrin, was not changed by resistin (Fig. 5A). This would be the reason why intercellular adhesion molecule-1 neutralizing antibodies were less potent than VCAM-1 neutralizing antibodies at blocking resistin-induced adhesion of monocytes on ECs. Second, resistin showed direct chemoattractive effect on human acute monocytic leukemia cell lines and their enhanced migration toward MCP-1 (Fig. 5). This result, with the observations that resistin did not upregulate the expression of MCP-1 in vivo (Online Fig. 4) or in vitro in VSMCs or fibroblasts (data not shown), explains the mechanism of increased macrophage infiltration by overexpression of resistin. Moreover, the WST-1 study suggested that resistin can increase macrophage accumulation in vessel walls by enhancing monocyte survival. Lastly, we have shown that resistin activates VCAM-1 expression on VSMCs, which is known to stimulate monocyte infiltration and retention in the plaque (28). Together these results imply that resistin consistently affects monocytes, ECs, and VSMCs to stimulate monocyte infiltration and retention in vessel walls, thereby augmenting vascular inflammation and plaque development. And considering the results from the previous studies indicating that resistin activates monocytes to express inflammatory cytokines such as
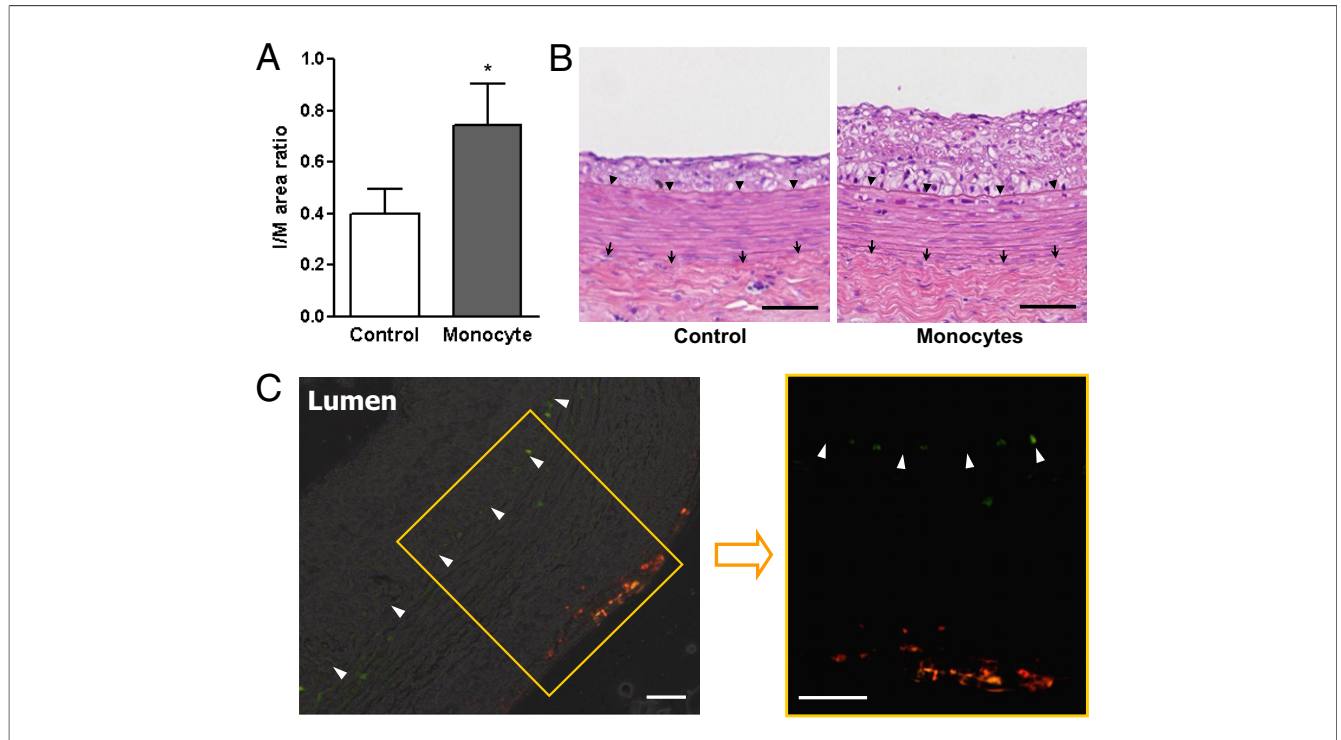
Figure 6   CS1 Peptide Blocked the Effects of Resistin on Plaque Progression

(A) I/M area ratio of Ad.GFP or Ad.Resistin/GFP-transfected arteries in connecting segment-1 (CS1) or its scrambled peptide administered rabbits. The difference of I/M area ratio between Ad.GFP and Ad.Resistin/GFP-transfected arteries shown in scramble CS1 peptide group disappeared with CS1 peptide administration. **(B)** Representative hematoxylin and eosin staining images of intimal thickening in scramble peptides versus CS1 peptides group with Ad.Resistin/GFP or Ad.GFP-transfected arteries. **Arrowhead** = internal elastic lamina; **arrow** = external elastic lamina; **bar** = 200 μm. **(C)** When treated with CS1 peptides, the density of infiltrated macrophages did not differ between Ad.Resistin/GFP- and Ad.GFP-transfected arteries; *p < 0.05. Abbreviations as in Figure 2.

IL-6 and TNF-α (26) and transforms macrophages to foam cells (12), it is possible that monocytes infiltrated to atherosclerotic plaque can be further stimulated by resistin to express inflammatory cytokines and to become foam cells, which results in further progression of atherosclerosis.

Soluble CS1 as a new therapeutic treatment for atherosclerosis. Our in vitro results showed that induction of atherosclerotic plaque by resistin was largely ascribed to interactions between VLA-4 of monocytes and VCAM-1 of ECs/VSMCs. To verify these in vitro findings in vivo, we employed a competitive inhibitor of VLA-4–

VCAM-1 binding, soluble CS1 peptide (19). As a result, administration of CS1 peptides almost completely suppressed aggravating effects of resistin on atherosclerotic plaque—both in size and number of infiltrated macrophages (Fig. 6)—emphasizing the importance of VLA-4–VCAM-1 interaction in resistin-induced atherosclerosis. By contrast, the VLA-4–VCAM-1 pathway is not specific to resistin, and administration of CS1 peptides reduced the size of atherosclerotic plaque even in control GFP-transfected arteries. However, the result that resistin, compared with control, made no difference in the size of atherosclerotic plaque when treated with CS1 peptides

Figure 7   **Acceleration of Plaque Formation by Adventitial Monocytes**

(A) Neointimal thickening was significantly augmented by monocyte delivery to adventitia of collared carotid arteries (n = 4, *p = 0.047). **(B, C)** Representative images of hematoxylin and eosin staining of the arteries **(B)** and those examined under fluorescent microscopy 2 weeks after monocytes delivery **(C)**. **Green** indicates carboxy-fluorescein succinimidyl ester-stained, intravenously administered monocytes, and **red** indicates seminaphthorhodafluor-stained, adventitially delivered monocytes. **Arrowheads** = internal elastic lamina; **arrows** = external elastic lamina; **bar** = 200 μm.

still supports that VLA-4–VCAM-1 interaction was involved in a critical path of atherosclerotic progression by resistin and that the effect of resistin on atherosclerosis progression can be halted by CS1 peptide.

Role of adventitial macrophages in atherosclerosis. In the current study, resistin significantly increased macrophage accumulation in both intima and adventitia. Because the resistin gene was delivered to adventitia, it was shown that resistin recruited macrophages in adventitia through the aforementioned mechanisms demonstrated by in vitro experiments. These adventitial macrophages, in turn, might accelerate intimal plaque growth—as shown in the experiment where monocytes were directly delivered to adventitia of collared arteries (Figs. 7A and 7B). However, the mechanism by which macrophages in adventitia promoted plaque growth was rather unclear. The

subsequent in vivo experiment using monocytes tagged with 2 different colors showed that adventitially infiltrated macrophages (red colored) themselves did not migrate into intima and only circulating monocytes (green colored) were detected in the plaque lesion. Therefore, adventitially infiltrated macrophages might affect the plaque growth remotely, and various cytokines secreted by them were probably involved in the process. Although the actual mechanism remains unclear, and the presence of resident—thus untagged—macrophages of adventitia might limit the interpretation of the results, this is the first report on the role of adventitial monocytes/macrophages in atherosclerotic progression. There have been only a few prior reports on the role of adventitial T-lymphocytes or fibroblasts in atherosclerosis (29,30).

Table 2   **Homology of Mammalian Resistins**

	Human	Pig	Cattle	Rabbit	Mouse
Human	—	75	70	69	56
Pig	75	—	78	71	58
Cattle	70	78	—	69	50
Rabbit	69	71	69	—	57
Mouse	56	58	50	57	—

Data are expressed as percentage of identity at the level of amino-acid sequence.

Conclusions

In summary, our results provide the first evidence that resistin, a kind of adipokine or inflammatory cytokine, can accelerate atherosclerotic plaque progression by aggravating inflammatory conditions in the vessel wall through stimulating monocyte infiltration and activating ECs and VSMCs (Fig. 8). These results might provide new insight on how obesity or inflammation can increase
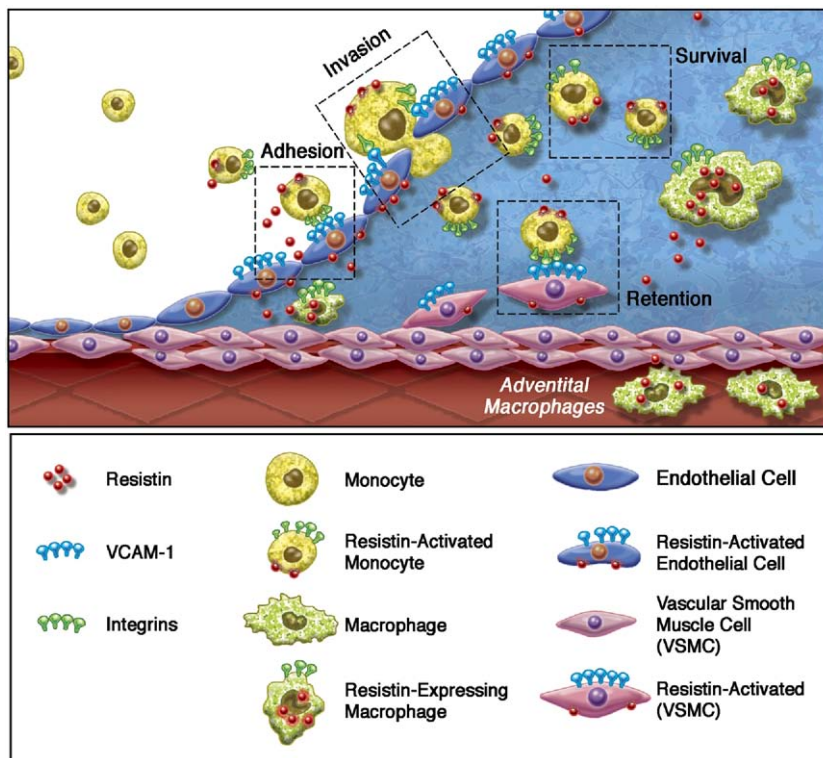
Figure 8   Schematic Illustration of Mechanism by Which Resistin Causes Monocyte-EC–VSMC Interaction to Promote Atherosclerotic Plaque Progression

Resistin expressed by macrophages in atherosclerotic plaque increases: 1) monocyte–endothelial cell (EC) adhesion through upregulating α4- and β1-integrin on mono-cytes and their counterpart vascular cell adhesion molecule (VCAM)-1 on ECs; 2) monocyte infiltration to the plaque by direct chemotactic effect and by increasing motil-ity toward monocyte chemotactic protein-1; 3) monocyte retention in the plaque by exacerbating VCAM-1 expression on VSMCs; and 4) monocyte survival in the plaque. Through these mechanisms resistin might accelerate atherosclerotic plaque progression by aggravating inflammatory condition in the vessel walls.

cardiovascular morbidity and mortality promoting ath-erosclerosis.

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Key Words: atherosclerosis ■ inflammation ■ resistin.

▶ **APPENDIX**

For supplementary methods, figure, and tables, please see the online version of this article.