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Interleukin-1 β and Signaling of Interleukin-1 in Vascular Wall and Circulating Cells Modulates the Extent of Neointima Formation in Mice

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Interleukin (IL)-1 is an important mediator of inflammation and cardiovascular disease. Here, we examined the role of IL-1 in arterial neointima formation. Carotid artery neointima was induced by ligation, and arteries were harvested 4 weeks after injury. The neointima/media of mice deficient in the IL-1 signaling receptor (IL-1R1^{-/-}) was significantly reduced compared to IL-1R1^{+/+} controls ($P < 0.01$). IL-1R1^{+/+} mice receiving subcutaneous IL-1ra also had significantly reduced neointima/media compared with placebo ($P < 0.05$). IL-1 β ^{-/-} mice had reduced neointima/media compared to wild-type ($P < 0.05$), whereas IL-1 α ^{-/-} mice were no different from controls. Mice deficient in the P2X₇ receptor (involved in IL-1 release) or caspase-1 (involved in IL-1 activation) did not differ in their response to carotid ligation compared to controls. To examine the site of IL-1 signaling, we generated chimeric mice. IL-1R1^{+/+} mice receiving IL-1R1^{-/-} marrow and IL-1R1^{-/-} mice receiving IL-1R1^{+/+} marrow both had significantly reduced neointima/media compared with IL-1R1^{+/+} to IL-1R1^{+/+} ($P < 0.05$) but had significantly greater neointima/media than IL-1R1^{-/-} to IL-1R1^{-/-} controls ($P < 0.05$). These data confirm the importance of IL-1 β signaling in mediating arterial neointima formation and suggest the involvement of IL-1 signaling in both circulating and arterial wall cells. Furthermore, receptor antagonism may be a better therapeutic target than interruption of IL-1 β processing or release. (*Am J Pathol* 2006; 168:1396–1403; DOI: 10.2353/ajpath.2006.051054)

response to balloon and stent injury. A fundamental mechanism of these processes is inflammation. There is considerable evidence for the involvement of interleukin (IL)-1 in the vessel wall response to injury. In porcine coronary arteries after balloon injury, regenerated endothelium displays increased expression of IL-1 β .¹ IL-1 is also a smooth muscle mitogen and is dependent on autocrine platelet-derived growth factor and/or basic fibroblast growth factor production by vascular smooth muscle cells.^{2–4} Administration of IL-1 β to the coronary artery causes neointima formation in the pig,⁵ whereas the absence of IL-1 receptor antagonist (IL-1ra) promotes, and the absence of IL-1R1 reduces, neointimal formation in mice after injury.^{6,7} Administration of IL-1ra to pigs inhibits neointima formation after both balloon and stent injury.⁸

Intracellular IL-1 β requires specific processing and release to be biologically active by signaling via the type I IL-1 receptor (IL-1R1). Pro-IL-1 β is cleaved to its active form by caspase-1, and release from cells is mediated via the purinergic receptor P2X₇. P2X₇ receptor-deficient macrophages do not process or release IL-1 β in response to ATP.⁹ *In vivo*, wild-type and P2X₇ receptor-deficient mice release the same amount of IL-6 into the peritoneal cavity, but there is no release of mature IL-1 β from P2X₇ receptor-deficient mice.⁶ In an analogous manner, caspase-1^{-/-} mice do not release mature IL-1 β and are resistant to endotoxin.¹⁰ Once processed and released, IL-1 β induces a wide range of inflammatory signals that include the synthesis of downstream cytokines such as IL-6 and IL-8. This system is very tightly regulated by the endogenous receptor inhibitor IL-1ra.¹¹

Intimal hyperplasia is a central part of the many aspects of cardiovascular diseases. (Neo)intima forms in

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Arterial damage and its repair are integral parts of the pathogenesis of both coronary artery disease and the

response to balloon or stent injury as well as in the development of atherosclerosis. The neointima is comprised of vascular smooth muscle cells and extracellular matrix in large part, with variable numbers of inflammatory cells. The origin of intimal cells is a controversial issue. Some studies propose a vascular wall phenomenon with expansion of pre-existing intimal cells, phenotypic modulation of medial smooth muscle cells and their migration into the media, and migration and differentiation of adventitial fibroblasts. Other studies highlight the importance of recruitment and differentiation of mesenchymal stromal cells of the bone marrow.^{12–18} It is likely, therefore, that smooth muscle cells in vascular lesions are composed of cells of diverse origin.

This study aims to delineate further the role of IL-1 signaling in arterial neointima formation and to investigate the relative contribution of IL-1R1 ligation on resident vascular wall cells and circulating cells to neointimal formation after ligation. Data are presented on the effect of genetic deletion of individual agonists and receptors controlling specific stages in the processing and release pathways. Chimeric mice created using adoptive bone marrow transfer were used to investigate the relative importance of IL-1 signaling in circulating and vascular wall cells in neointimal formation after ligation.

Materials and Methods

Mouse Strains Used

IL1R1^{-/-} mice (stock no. 003245)¹⁹ and their genetically matched wild-type strain, IL-1R1^{+/+} (stock no. 000664) were obtained from the Jackson Laboratories (Bar Harbor, ME). Caspase-1^{-/-} mice²⁰ and their genetically matched wild-type strain (C57/129Sv) were a gift from Professor M.K.B. Whyte (University of Sheffield, Sheffield, UK). P2X₇-receptor^{-/-} mice and their control strain (C57BL/6XDBA)²¹ were obtained from Professor R.A. North (University of Manchester, Manchester, UK). IL-1β^{-/-} and IL-1α^{-/-} mice were a gift from Professor Nancy Rothwell (University of Manchester, Manchester, UK)²²; their control strain C57BL/6 wild-types were supplied by Charles River (Kent, UK). All animals were housed in a controlled environment with a 12-hour light/dark cycle at 22°C. All experiments were performed in accordance with UK legislation under the 1986 Animals (Scientific Procedures) Act.

In the carotid ligation experiments, eight animals in each respective group underwent ligation. All mice were male and 8 to 12 weeks of age (equating to ~25 g in weight). For the bone marrow transplantation studies, 9 to 15 animals, 4 to 6 weeks of age, underwent transplantation for each group. However, excess death in IL-1R1^{-/-} mice, presumably because of enhanced radiation sensitivity,^{23,24} reduced the final number of animals available for carotid ligation analysis to between five and nine.

Bone Marrow Transplantation

Female donor mice were killed, and their femurs and tibias removed under aseptic conditions. Marrow cavities were flushed, and single-cell suspensions prepared. Cells were washed and resuspended in Hanks' balanced salt solution before transplantation. Male mice, 4 to 6 weeks old, received a lethal dose of whole-body irradiation (1100 rads, split into two doses, 4 hours apart). Irradiated recipients then received 1 to 2 × 10⁶ cells in Hanks' balanced salt solution, by tail-vein injection. Carotid ligation was performed 5 weeks after bone marrow transfer. The efficiency of bone marrow transplantation was monitored by X- and Y-chromosome painting of metaphase spreads of chimeric mice. Two types of chimeric mice were created: IL-1R1^{+/+} mice with IL-1R1^{-/-} bone marrow and IL-1R1^{-/-} mice with IL-1R1^{+/+} bone marrow. Control groups of IL-1R1^{-/-} to IL-1R1^{-/-} and IL-1R1^{+/+} to IL-1R1^{+/+} transfers were also performed.

Carotid Ligation

Mice were anesthetized by intraperitoneal injection of midazolam (1.25 mg/ml) and hypnorm (2.5 mg/ml) and the right common carotid artery exposed. Arterial neointima formation was induced by ligation of the common carotid artery immediately below the bifurcation, with 6/0 silk, and the wound closed. Mice receiving IL-1ra, or placebo, were then given a subcutaneous dose of analgesia (carprofen, 5 mg/ml) prior to a small incision being made on the dorsal side of the neck, and a subcutaneous pocket formed using blunt dissection. An Alzet pump containing 25 mg/kg/day IL-1ra (Amgen, Thousand Oaks, CA) or placebo was placed into this pocket, the neck sutured, and the mouse allowed to recover. Infusion of drug or placebo was continuous for the duration of the study. Sham-operated animals, in which the carotid artery was slung with silk, but not tied, were performed as surgical controls.

Tissue Preparation, Histology, and Morphometry

Four weeks after surgery, the mice were killed by an overdose of pentobarbital and blood was taken for analysis of cytokine serum levels. The vasculature was then flushed with phosphate-buffered saline and perfusion-fixed by ventricular injection of formalin before the ligated or sham-operated carotid artery was harvested, processed, and embedded into paraffin wax. Five-μm-thick sections were taken along the entire length of each artery at intervals of 100 μm. Typically, this yielded 10 sections per artery. These sections were histologically stained with Alcian blue/elastic van Gieson and the neointima, media, lumen, and total vessel area (all layers of the artery wall) determined by image analysis (Lucia G, Nikon, UK). Neointima, media, and total vessel area were all measured directly. Lumen area was calculated from perimeter measurements to correct for fixation and sectioning errors. The section with the largest neointimal area per artery was used for the statistical analysis reported. In addition, the data were analyzed following

the approach of sampling and analyzing sections at a constant distance from the ligature (500 μ m).

Enzyme-Linked Immunosorbent Assay

Serum levels of IL-1 α and IL-1 β were determined by sandwich enzyme-linked immunosorbent assay (R&D Systems, UK).

Immunohistochemistry

Paraffin-embedded sections of harvested carotid arteries were stained immunohistochemically for α -smooth muscle actin (DAKO, Glostrup, Denmark) to visualize smooth muscle cells, F4/80 (Abcam, Cambridge, UK) to visualize macrophages, and proliferating cell nuclear antigen (DAKO) to visualize proliferating cells. Standard immunohistochemical techniques were applied.

Statistical Analysis

The results are shown as mean $\pm$ SEM. Data groups were analyzed by one-way analysis of variance followed by a Bonferroni multiple comparison posttest or by a Student's *t*-test as appropriate. *P* < 0.05 was regarded as a significant difference.

Results

The two different methods of morphological analysis used in this study yielded the same trends and significant differences. Consequently, only the data from analysis of largest neointimal sections is reported in detail. A comparison of data obtained from the two analytical methods is presented in the online Supplementary Figures 1 to 5 at <http://ajp.amjpathol.org>.

IL-1R1^{-/-} and IL-1 α -Treated Mice Response to Carotid Ligation

At 4 weeks, the neointima/media area of IL-1R1^{-/-} mice was reduced by 19-fold compared to the genetically matched IL-1R1^{+/+} control mice (Figures 1 and 2A). Absolute neointimal area was also significantly reduced (Table 1). Sham-operated mice had no measurable neointima. The lumen area of IL-1R1^{-/-} mice was increased 3.4-fold, and total vessel area was significantly decreased compared to controls (Table 1). Medial area did not differ between groups (data not shown).

The results observed in IL-1R1^{-/-} mice were similar in IL-1R1^{+/+} mice that received subcutaneous IL-1 α (Figures 1 and 2A, Table 1); mice receiving IL-1 α had a sevenfold smaller neointima/media area compared to IL-1R1^{+/+} mice receiving placebo. The lumen of mice receiving subcutaneous IL-1 α was increased 2.9-fold, with total vessel area significantly decreased compared to control (Table 1). Wild-type mice receiving a placebo drug instead of IL-1 α did not differ significantly from mice undergoing carotid occlusion alone. The serum

Figure 1. Alcian blue/elastic van Gieson-stained arterial sections of mice after carotid artery ligation. IL-1R1^{-/-} and IL-1R1^{+/+} mice given an infusion of IL-1 α have little or no neointima compared to IL-1R1^{+/+} and IL-1R1^{+/+} plus placebo. Similarly, IL-1 β ^{-/-} mice have a smaller neointima compared to wild-type control and IL-1 α ^{-/-} mice. Caspase-1^{-/-} and P₂X₇^{-/-} mice do not differ in neointimal formation, compared to wild-type controls. Original magnifications, $\times$ 100.

concentration of IL-1 α in mice receiving subcutaneous IL-1 α was significantly higher than in mice receiving placebo drug (1334.0 $\pm$ 129.2 pg/ml versus 0.222 $\pm$ 0.1343 pg/ml, *P* < 0.001). Serum levels of IL-1 β , however, were not affected by IL-1 α infusion (6.998 $\pm$ 0.4889 pg/ml versus 5.605 $\pm$ 0.6291, respectively; *P* = ns).

IL-1 β ^{-/-} and IL-1 α ^{-/-} Mice Response to Carotid Ligation

The neointima, neointima/media, lumen, and total vessel area of IL-1 α ^{-/-} mice did not differ compared to C57/BL6

Figure 2. Graphical representation of intima/media development in mice after carotid artery ligation. **A:** IL-1R1^{-/-} and IL-1R1^{+/+} mice given an infusion of IL-1ra have a significantly reduced intima/media compared to IL-1R1^{+/+} and IL-1R1^{+/+} plus placebo. **B:** Similarly, IL-1β^{-/-} mice have a smaller intima/media compared to wild-type control. IL-1α^{-/-} mice, however, do not differ from wild-type (B). **C and D:** P2X7^{-/-} and caspase-1^{-/-} mice do not differ in neointimal formation compared to their respective wild-type controls.

wild-type control mice (Figures 1 and 2b, Table 1). IL-1β^{-/-} mice, however, had a fourfold reduction in neointima/media ($P < 0.05$), a threefold reduction in absolute neointima ($P < 0.05$), and a fourfold increase in lumen area ($P < 0.05$) compared to wild-type mice, although there was no difference in total vessel area (Figures 1 and 2B, Table 1).

P2X₇ Receptor^{-/-} Mice Response to Carotid Ligation

Mice deficient in P2X₇ receptor expression were used to determine the role of this receptor in IL-1β release after carotid occlusion. P2X₇^{-/-} mice displayed a trend toward an increased neointima/media ratio compared to control, although this was not significant ($P = 0.12$). Similarly, no difference in absolute neointima, lumen, or total vessel area was seen between P2X₇^{-/-} mice and wild-type controls (Figures 1 and 2C, Table 1).

Caspase-1^{-/-} Mice Response to Carotid Ligation

Mice deficient in caspase-1 expression were used to determine the role of IL-1β processing after carotid occlusion. Although there was a trend toward a reduction in neointima/media, neointima, and total vessel area in

caspase-1^{-/-} mice compared to their wild-type control, this was not significant ($P = 0.36$, $P = 0.34$, and $P = 0.49$, respectively) (Figures 1 and 2D, Table 1). Similarly, lumen area did not differ between the two mouse strains (Table 1).

IL-1R1 Chimeric Mice Response to Carotid Ligation

IL-1R1^{+/+} and IL-1R1^{-/-} chimeric mice were used to determine the relative contribution of circulating cells and cells of the artery wall to the development of neointima after carotid occlusion. As expected, IL-1R1^{+/+} mice receiving IL-1R1^{+/+} bone marrow (control mice) had a large neointima after carotid occlusion, with arteries often being fully occluded, similar to nonirradiated and transplanted IL-1R1^{+/+} animals. Also IL-1R1^{-/-} mice receiving IL-1R1^{-/-} marrow developed little or no lesion, analogous to nonirradiated and transplanted IL-1R1^{-/-} mice.

Chimeric mice, IL-1R1^{+/+} animals receiving IL-1R1^{-/-} bone marrow, developed a 2.8-fold smaller neointima/media compared to the control of IL-1R1^{+/+} mice receiving IL-1R1^{+/+} marrow ($P = ns$) and a 17.6-fold larger neointima/media compared to IL-1R1^{-/-} to IL-1R1^{-/-} controls ($P < 0.05$) (Figures 3 and 4). IL-1R1^{-/-} mice receiving IL-1R1^{+/+} bone marrow also developed a 3.7-fold smaller neointima/media than the IL-1R1^{+/+} to IL-

Table 1. Lumen and Total Vessel Area of Carotid Arteries 4 Weeks after Ligation

	Neointimal area (mm ²)	Lumen area (mm ²)	Total vessel area (mm ²)
IL-1R1 ^{-/-}	0.0033 ± 0.0020*	0.0091 ± 0.0016 ^{§¶}	0.0235 ± 0.0048
IL-1R1 ^{-/-} sham	0.0 ± 0.0	0.0191 ± 0.0048	0.0301 ± 0.0052
IL-1R1 ^{+/+}	0.0546 ± 0.0109	0.0027 ± 0.0013	0.0381 ± 0.0042**
IL-1R1 ^{+/+} sham	0.0 ± 0.0	0.0305 ± 0.0049	0.0384 ± 0.0074
IL-1R1 ^{+/+} + IL-1ra	0.0047 ± 0.0024 [†]	0.0077 ± 0.0017 [†]	0.0384 ± 0.0064
IL-1R1 ^{+/+} + placebo	0.0336 ± 0.0127	0.0005 ± 0.0004	0.0674 ± 0.0156
P ₂ X ₇ receptor ^{-/-}	0.0253 ± 0.0037	0.0042 ± 0.0027	0.0574 ± 0.0057
P ₂ X ₇ receptor ^{+/+}	0.0151 ± 0.0018	0.0023 ± 0.0009	0.0417 ± 0.0032
Caspase-1 ^{-/-}	0.0447 ± 0.0041	0.0032 ± 0.0009	0.0357 ± 0.0023
Caspase-1 ^{+/+}	0.0670 ± 0.0208	0.0031 ± 0.0015	0.0429 ± 0.0094
IL-1β ^{-/-}	0.0103 ± 0.0033 [‡]	0.0073 ± 0.0015 [‡]	0.0278 ± 0.0076
IL-1α ^{-/-}	0.0350 ± 0.0099	0.0022 ± 0.0010	0.0315 ± 0.0035
C57/BL6 ^{+/+}	0.0337 ± 0.0074	0.0019 ± 0.0009	0.0257 ± 0.0026

Data represents mean ± SEM.

P* < 0.001 versus IL-1R1^{+/+}; [†]*P* < 0.001 versus IL-1R1^{+/+} + placebo; [‡]*P* < 0.05 versus C57/BL6^{+/+}; [§]*P* < 0.05 versus IL-1R1^{+/+}, IL-1R1^{+/+} + placebo; ^{||}*P* < 0.01 versus IL-1R1^{-/-} sham; [¶]*P* < 0.001 versus IL-1R1^{+/+} sham; ^{}*P* < 0.01 versus IL-1R1^{-/-}, IL-1R1^{+/+} + IL-1ra.

Figure 3. Alcian blue/elastic van Gieson-stained arterial sections of mice after bone marrow transplantation and carotid artery ligation. IL-1R1^{-/-} and IL-1R1^{+/+} mice given IL-1R1^{+/+} and IL-1R1^{-/-} marrow, respectively, have larger lesions than IL-1R1^{-/-} given IL-1R1^{-/-} marrow and smaller lesions than IL-1R1^{+/+} mice given IL-1R1^{+/+} marrow. The two chimeric bone marrow-transplanted groups do not differ in neointimal formation compared to each other. Original magnifications, ×100.

Figure 4. Graphical representation of intima/media development in mice after bone marrow transplantation and carotid artery ligation. IL-1R1^{-/-} given IL-1R1^{+/+} marrow (WT→KO) have a significantly reduced intima/media compared to IL-1R1^{+/+} mice given IL-1R1^{+/+} (WT→WT, control). There was a trend toward a smaller intima/media in IL-1R1^{+/+} mice given IL-1R1^{-/-} marrow (KO→WT), although this did not reach significance. Both chimeric groups have a larger intima/media compared to the IL-1R1^{-/-} mice given IL-1R1^{-/-} marrow (KO→KO) control. The intima/media between chimeric groups do not differ.

IL-1R1^{+/+} control ($P < 0.05$) and a 13.1-fold larger neointima/media than IL-1R1^{-/-} to IL-1R1^{-/-} mice ($P = \text{ns}$). There was no significant difference in neointima/media ratio between IL-1R1^{-/-} to IL-1R1^{+/+} and IL-1R1^{+/+} to IL-1R1^{-/-} mice (Figure 4). Comparable results were seen with absolute neointima (Table 1).

There was no significant difference in total vessel area between any of the bone marrow-transplanted mice (Table 2), although the IL-1R1^{-/-} to IL-1R1^{-/-} group tended toward a smaller artery size, and these mice were 3 to 5 g smaller in weight at the point of carotid surgery, compared to the other groups (data not shown). The lumen of the IL-1R1^{-/-} to IL-1R1^{+/+}, IL-1R1^{+/+} to IL-1R1^{-/-}, and IL-1R1^{-/-} to IL-1R1^{-/-} groups were all larger than the IL-1R1^{+/+} to IL-1R1^{+/+} group, although only the IL-1R1^{+/+} to IL-1R1^{-/-} group reached significance (Table 2).

The Efficiency of Bone Marrow Conversion in IL-1R1 Chimeric Mice

X- and Y-chromosome analysis of female-male bone marrow-transplanted mice revealed a $94.94 \pm 2.552\%$ conversion of chromosomes, indicating that all host bone

marrow is removed before transplantation and the chimeric mice are true chimeras.

Immunohistochemical Analysis of Neointimal Lesions

The neointimal lesions of each strain of mice did not differ in composition. All were positive for α -smooth muscle actin content. Similarly, each mouse strain displayed positive staining for macrophages 4 weeks after ligation, with little or no proliferation evident.

Discussion

These data indicate that IL-1 β signaling via the IL-1 receptor type I influences arterial neointima formation in mice. IL-1 α does not appear to be involved. Mice deficient in IL-1 β , the signaling receptor (IL-1R1), and wild-type mice infused with IL-1ra develop little or no neointima compared to the genetically matched wild-type controls or wild-type mice receiving placebo. In contrast, mice lacking caspase-1, and those lacking the ability to release IL-1 β by a P2X₇-mediated event, were statistically no different from controls.

It has previously been reported that mice deficient in the IL-1 receptor type I have reduced neointimal lesions after carotid ligation.⁶ Our data agree with this original study and expands on these findings by demonstrating that it is IL-1 β , specifically, but not IL-1 α , signaling via the IL-1 receptor that is involved in neointima formation, suggesting that IL-1 β is the important agonistic ligand in this family of cytokines in the arterial wall response to ligation.

The data arising from studies of mice in which processing and release mechanisms for IL-1 are deficient give additional insights both into the importance of these processes as well as their therapeutic potential. The role of caspase-1 in the cleavage of the 31-kd precursor form of IL-1 β into its active 17-kd form has been extensively demonstrated *in vitro*.^{25–27} *In vivo*, caspase-1-deficient mice do not release mature IL-1 β and are resistant to lipopolysaccharide-induced endotoxic shock.¹⁰ Although caspase-1 is primarily responsible for cleavage of the precursor intracellularly, other proteases such as proteinase-3, elastase, chymotrypsin, a mast cell chymase, granzyme A, and a variety of proteases commonly found in inflammatory fluids can all extracellularly process the IL-1 β precursor into an active cytokine.^{28–34} It may be that these alternative processing mechanisms are operative in this model. This theory is supported by the finding

Table 2. Lumen and Total Vessel Area of Carotid Arteries after Bone Marrow Transplantation and Ligation

	Neointimal area (mm ²)	Lumen area (mm ²)	Total vessel area (mm ²)
IL-1R1 ^{-/-} → IL-1R1 ^{-/-}	0.0012 ± 0.0008*†	0.0068 ± 0.0014	0.0169 ± 0.0019
IL-1R1 ^{+/+} → IL-1R1 ^{+/+}	0.0230 ± 0.0058	0.0032 ± 0.0014†	0.0275 ± 0.0043
IL-1R1 ^{-/-} → IL-1R1 ^{+/+}	0.0070 ± 0.0023	0.0095 ± 0.0024	0.0369 ± 0.0095
IL-1R1 ^{+/+} → IL-1R1 ^{-/-}	0.0067 ± 0.0020†	0.0125 ± 0.0022	0.0277 ± 0.0041

Data represents mean ± SEM.

* $P < 0.05$ versus IL-1R1^{-/-} → IL-1R1^{+/+}; † $P < 0.01$ versus IL-1R1^{+/+} → IL-1R1^{+/+}; ‡ $P < 0.05$ versus IL-1R1^{+/+} → IL-1R1^{-/-}.

that caspase-1-deficient mice have low, but comparable, amounts of circulating active IL-1 β compared to wild-type controls after carotid ligation (data not shown). It is of note that there was a (nonsignificant) trend to reduction in neointima in the caspase-1-deficient mice. If correct, this indicates that these other proteases may be physiologically important in IL-1 β processing. In support of this, caspase-1-deficient mice are known to exhibit a full inflammatory response to subcutaneous turpentine,³⁵ another IL-1 β -dependent process. It would, therefore, seem unlikely that caspase-1 inhibition will be of therapeutic benefit in the prevention of neointima formation.

The results from the P2X₇-deficient mice are also of considerable interest. P2X₇ is a purinergic receptor found in particular on monocytes and macrophages that, in the presence of lipopolysaccharide, activates ATP in such a way as to form a transient pore and induce membrane blebbing associated with the release of mature IL-1 β .³⁶ When activated by ATP, P2X₇ receptor-deficient macrophages do not process or release IL-1 β .⁹ These data would predict that the P2X₇-deficient mice should have reduced neointima formation. The results reported here indicate no significant difference between the groups, and if anything there is a trend toward an increase in neointima formation in this knockout strain. We have recently shown that the P2X₇ receptor is also involved in the release of intracellular IL-1ra from macrophages and endothelial cells.³⁷ It is well known that the IL-1 system is kept in check by members of the IL-1ra family. The IL-1ra gene has a number of products arising from alternative splicing. The secreted form (sIL-1ra) has a leader sequence and follows traditional secretory paradigms. However, the other intracellular isoforms lack this sequence. Our data indicating that the P2X₇ receptor can mediate the release of intracellular IL-1ra raise the possibility that in the mouse carotid tie model, as analyzed here throughout a 4-week period, the inhibition of IL-1 β consequent on P2X₇ deficiency is partly balanced by deficiency of IL-1ra. This is supported by other data from our laboratory that the intracellular IL-1ra isoform is the only isoform synthesized by endothelial cells.³⁸ Indeed, we were unable to detect intracellular IL-1ra release from P2X₇-deficient endothelial cells that had been infected with an intracellular IL-1ra expression construct (data not shown). Re-endothelialization after injury is known to inhibit neointima formation. Thus the suggestion that these mice are devoid of endothelial intracellular IL-1ra may explain the apparently neutral response seen after carotid ligation in the P2X₇-deficient mouse. Additionally, there may be other mechanisms that allow IL-1 β release when P2X₇ receptors are either absent or blocked. As with our results examining the effects of caspase-1 deficiency, the data from the P2X₇ mice indicate that this receptor is unlikely to be of therapeutic potential for the prevention of neointima.

The site of IL-1 signaling in arterial neointima formation was investigated by bone marrow transfer experiments. The arterial response to ligation involves local vessel wall cells as well as recruited cells that arise from the bone marrow (inflammatory cells and progenitor cells).^{12–18} Our data from the bone marrow transplant procedures

show that both types of chimeric mice with deficient IL-1 signaling exhibit a reduced neointima compared to mice with normal IL-1 signaling and an increased neointima compared to mice deficient in signaling in all cells. The size of neointimal lesion, however, is identical between the two types of chimeric mice. Thus, this study suggests that IL-1 signaling in both vascular wall cells and circulating cells plays an equal role in neointimal lesion formation and are additive in the overall response.

These experiments were made difficult by the exquisite sensitivity of IL-1R1-deficient animals to irradiation.^{23,24} Previous work has shown that IL-1 has direct radioprotective effects on the production of bone marrow cells through its ability to induce IL-6 and colony-stimulating factors. Thus, deletion of IL-1 signaling in the animals used in this study rendered them prone to death after irradiation and reconstitution.

In conclusion, our data show that IL-1 β signaling via IL-1R1 plays a key role in the formation of a neointimal lesion after arterial ligation in mice, and that both circulating and arterial wall cells are involved. Therapeutic modulation appears to be best achieved by specific inhibition of the IL-1 signaling receptor.

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