CD137 Is Expressed in Human Atherosclerosis and Promotes Development of Plaque Inflammation in Hypercholesterolemic Mice

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Background—Atherosclerosis is a multifactorial disease in which inflammatory processes play an important role. Inflammation underlies lesion evolution at all stages, from establishment to plaque rupture and thrombosis. Costimulatory molecules of the tumor necrosis factor superfamily such as CD40/CD40L and OX40/OX40L have been implicated in atherosclerosis.

Methods and Results—This study shows that the tumor necrosis factor superfamily members CD137 and CD137 ligand (CD137L), which play a major role in several autoimmune diseases, may constitute a pathogenic pair in atherogenesis. We detected CD137 protein in human atherosclerotic lesions not only on T cells but also on endothelial cells and showed that CD137 in cultured endothelial cells and smooth muscle cells was induced by proinflammatory cytokines implicated in atherosclerosis. Activation of CD137 by CD137L induced adhesion molecule expression on endothelial cells and reduced smooth muscle cell proliferation. In addition, treatment of atherosclerosis-prone apolipoprotein E–deficient mice with a CD137 agonist caused increased inflammation. T-cell infiltration, mainly of CD8\(^+\) cells, and expression of the murine major histocompatibility complex class II molecule I-A\(^b\) increased significantly in atherosclerotic lesions, as did the aortic expression of proinflammatory cytokines.

Conclusions—Taken together, these observations suggest that CD137-CD137L interactions in the vasculature may contribute to the progression of atherosclerosis via augmented leukocyte recruitment, increased inflammation, and development of a more disease-prone phenotype. (Circulation. 2008;117:1292-1301.)

Key Words: atherosclerosis • cardiovascular diseases • immunology • inflammation • plaque

The development of atherosclerosis is associated with vascular inflammation. Immunocompetent cells, including T cells and macrophages, are recruited to the lesions through an activated endothelium. Activated immune cells produce proinflammatory cytokines and chemokines, which promote local inflammation and cellular influx to the vessel wall. These events in the inflammatory process also are largely responsible for plaque rupture and thrombus formation, which clinically present as ischemia, myocardial infarction, or stroke.\(^1-3\)

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Activation of T cells, the central orchestrators of the adaptive immune response, is critically dependent on costimulatory signals, which are received through, among others, molecules of the tumor necrosis factor (TNF) superfamily.\(^4\) Several members of this family have been implicated in atherosclerosis, eg, CD40/CD40 ligand,\(^6\) LIGHT,\(^6\) and OX40/OX40 ligand.\(^7,8\) In view of this, the signaling interplay between immunocompetent cells and vascular cells is of central interest in pathophysiology.

CD137 (4-1BB), a receptor in the TNF superfamily, is recognized as an independent costimulatory molecule of T cells\(^9\) and activator of monocytes.\(^10\) Studies in several experimental models have shown that CD137 activation in vivo has important immunopathogenetic implications. For example, treatment with agonistic antibodies to CD137 fundamentally affects the development of experimental autoimmune encephalomyelitis,\(^11\) systemic lupus erythematosus–like autoimmune inflammation.
mune disease,\textsuperscript{12,13} and collagen II–induced arthritis\textsuperscript{14} in mice. Moreover, CD137-deficient mice are resistant to endotoxin-induced shock.\textsuperscript{15} In T cells, CD137 expression is triggered by activation\textsuperscript{16} and T-cell receptor (TCR) stimulation,\textsuperscript{17} and CD137/CD137 ligand (CD137L) interaction initiates signaling through TNF receptor–associated factor-2 and activation of nuclear factor-κB and activator protein-1.\textsuperscript{18} Both of these pathways have been shown to induce proatherogenic changes in vitro and in vivo.\textsuperscript{19} Because a number of autoimmune disorders share common pathogenetic mechanisms with atherosclerosis and increase the risk for atherosclerotic cardiovascular disease in humans,\textsuperscript{20} we hypothesized that CD137 activation is pathogenetically important for the progression of atherosclerosis. In the present study, we investigated CD137 in human atherosclerotic lesions and evaluated its implications on disease development in a mouse model of human disease.

Methods

The studies were approved by the regional ethics committees for human and animal studies.

Human Specimen Collection

Eight patients scheduled for nephrectomy and 18 patients scheduled for carotid endarterectomy due to symptomatic disease were included after providing informed consent. Biopsies from renal arteries and atherosclerotic lesions were retrieved peroperatively. Small pieces from all renal and 11 carotid arteries were immediately frozen for RNA and protein extraction or embedded in TissueTEK optical coherence tomography compound (Sakura Finetek Europe BV, Zoeterwoude, the Netherlands) and frozen for immunohistochemistry. Five renal artery biopsies and 5 biopsies from atherosclerotic lesions were incubated with or without bacterial lipopolysaccharide from Escherichia coli (O55:B5, Sigma-Aldrich, St Louis, Mo) at 100 ng/mL for 6 hours. RNA from 144 human carotid endarterectomies and 8 control subjects from the Biobank of Karolinska Endarterectomies (BiKE) were included.\textsuperscript{21} Data from Affymetrix Gene Array U133 Plus 2.0 analysis of RNA from carotid lesions were obtained from 37 patients and 7 control subjects within BiKE.\textsuperscript{21}

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were maintained in endothelial basal medium-2, and human aortic smooth muscle cells (AoSMCs) were maintained in smooth muscle basal medium (all from Cambrex Bio Science, Walkersville, Mass). The following incubations were performed: medium only, TNF-α, interleukin (IL)-1β, or interferon (IFN)-γ (PeproTech EC Ltd, London, UK) at 10 ng/mL or a mix of TNF-α, IL-1β, and IFN-γ (each 10 ng/mL; referred to as cytokine mix), lipopolysaccharide 100 ng/mL, and recombinant CD137 ligand coupled to Dynabeads M280 streptavidin (Dynal Biotech, Oslo, Norway) prepared as previously described\textsuperscript{22} (crosslinked rCD137L) or Dynabeads only. Experiments were repeated at least 3 times. Human pulmonary artery ECs were grown as described above and incubated with or without 10 ng/mL TNF-α for 8 hours.

Immunofluorescence

Acetone-fixed sections were incubated with 10% goat serum in 50 mmol/L NH₄Cl followed by a mouse monoclonal anti-human CD137 antibody (Abcam, Cambridge, UK) or an isotype control (Dakopatts, Glostrup, Denmark) and a polyclonal rabbit anti–human von Willebrand factor (Dakopatts) or a polyclonal rabbit anti-human CD3 (Dakopatts) antibody. Binding was detected with a Texas Red–conjugated goat anti-mouse (Invitrogen-Molecular Probes, Eugene, Ore) or a biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, Calif) detected by an Oregon green-
streptavidin conjugate (Invitrogen, Carlsbad, Calif). Lipid autofluorescence was blocked with 0.003% Sudan black B (BDH Laboratory Supplies, Poole, UK) in 70% alcohol. Formaldehyde-fixed sections, preincubated with Image-iT FX signal enhancer (Invitrogen) and then with 10% goat serum, were incubated with a mouse monoclonal anti-human CD137L antibody (BD PharMingen, San Diego, Calif) or an isotype control (Dakopatts). Binding was detected by a Texas Red–conjugated goat anti-mouse antibody (Invitrogen). Selected sections were also incubated with an FITC-conjugated monoclonal mouse anti-human CD68 antibody (Dakopatts) or an isotype control (Dakopatts), and specific binding was recognized by a biotinylated mouse anti-FITC antibody (Jackson Laboratories, Suffolk, UK), detected by an Alexa Fluor 488-streptavidin conjugate (Invitrogen). An avidin-biotin blocking kit (Vector Laboratories) was used according to the manufacturer’s instructions. Nuclei were visualized with DAPI (Sigma Aldrich), and images were viewed in a Leica fluorescence microscope with subsequent computer-assisted image analysis, including adjustment of brightness and contrast.

Immunohistochemistry

Eight 10-μm sections were collected at 100-μm intervals starting 100 μm from the appearance of the aortic valves. Formaldehyde-fixed sections were stained with hematoxylin and Oil Red O.23 Acetone-fixed frozen sections from murine aortic roots were stained with rat anti-mouse CD68 (Serotec Ltd, Oxford, UK), rat anti-mouse vascular cell adhesion molecule (VCAM)-1, biotinylated mouse anti-mouse I-A^, rat anti-mouse CD4, rat anti-mouse CD8 (all from BD PharMingen), rat anti-mouse CD3 (Southern Biotechnology Associates Inc, Birmingham, Ala), anti–smooth muscle α-actin (Sigma-Aldrich), and rabbit anti–Ki-67 (Novus Biologicals, Littleton, Colo) antibodies or Sirius red (Sigma-Aldrich) as previously described.23 Samples were blinded, and staining and lesion size were analyzed with Leica Q500MC image analysis software. Cells were counted manually.

Enzyme-Linked Immunosorbent Assay

CD137 and CD137L levels in tissue and cell lysates and culture media were measured with an ELISA kit (R&D Systems, Minneapolis, Minn) according to the manufacturer’s instructions.

Flow Cytometry

Resuspended adherent cells were stained with a phycoerythrin-conjugated monoclonal mouse anti-human CD137, mouse anti-human CD106, an FITC-conjugated mouse anti-human CD54 antibody, or a relevant isotype control (all from BD PharMingen); incubated on ice for 30 minutes; washed; and analyzed with a FACSCalibur flow cytometer (BD Biosciences, San Jose, Calif). Cells obtained from meshed murine spleens were stained with the following antibodies (all from BD PharMingen): FITC hamster anti-mouse TCR-β, FITC rat anti-mouse CD4, phycoerythrin rat anti-mouse CD8a, and phycoerythrin rat anti-mouse CD19. Samples were analyzed as above.

Figure 2. Immunostaining of frozen sections of human arterial biopsies. CD137^− and CD137L^− cells are stained red; cell-type markers are stained green. Colocalization of red and green results in yellow. CD137 was expressed on endothelial and T cells in lesions (A through C, E through G, respectively) but not in normal arteries (D). A, anti-CD137; B, anti-von Willebrand factor; C, A and B overlaid; D, anti-CD137 in a normal artery; E, anti-CD137 and anti-CD3; F, anti-CD137 and anti-CD8; G, anti-CD137 and anti-CD4; and H, anti-CD137L and anti-CD68. Nuclei are stained blue with DAPI. White bars are 50 μm.
Semiquantitative Real-Time Reverse-Transcription Polymerase Chain Reaction

Tissue samples were cryohomogenized with a Mikro Dismembrator S (B Braun Biotech International GmbH, Melsungen, Germany), and cells were lysed with RLT buffer (Qiagen, Valencia, Calif). Total RNA was isolated with the RNeasy extraction kit (Qiagen) and reverse transcribed to cDNA using random hexamers and SuperScript II reverse transcriptase (Life Technologies, Rockville, Md). mRNA levels were assessed by real-time reverse-transcription polymerase chain reaction in a TaqMan universal polymerase chain reaction master mix (Applied Biosystems, Foster City, Calif). Primers and probes were purchased as assay on demand (Applied Biosystems). Samples were analyzed in duplicates with an ABI Prism 7700 Sequence Detector (Applied Biosystems). Results were normalized to values for human cyclophilin A or murine hypoxantine-guanine phosphoribosyl transferase (Applied Biosystems).

Proliferation Assay

AoSMCs were maintained in growth medium (Cambrex). Cells were starved for 48 hours in DMEM/F12 (Invitrogen, Carlsbad, Calif) supplemented with 0.5% FBS, 1 mmol/L L-glutamine, 50 μg/mL streptomycin, and 50 U/mL penicillin. Quiescent cells were then stimulated in complete medium with crosslinked rCD137L (at 0.001, 0.01, 0.1, and 1 μg/mL) for 24, 48, or 72 hours or with 0.5 μg/mL crosslinked rCD137L and 3.75 μg/mL soluble rCD137L for 72 hours. Twenty-four hours before harvest, 3H-thymidine (1 μCi/well) was added to the medium. Cultures were harvested and analyzed with a scintillation counter.

Animals and Quantification of Atherosclerosis

Apolipoprotein E–deficient (Apoe−/−) and wild-type C57BL/6 mice were obtained from Taconic Europe A/S (Lille Skensved, Denmark) and fed standard chow and water ad libitum. Eight Apoe−/− mice and 8 C57BL/6 mice were euthanized at 25 weeks of age, and the aortas were freed from connective tissue under microscope and snap-frozen. Five Apoe−/− mice were injected with 200 μg agonistic rat anti-mouse CD137 antibodies (2A13) intraperitoneally at 8, 11, and 14 weeks of age. Five control Apoe−/− mice were injected with rat-IgG2a (BioSite, Täby, Sweden). At 16 weeks of age, the mice were euthanized using CO2 anesthesia, and the hearts and aortas were freed from connective tissue under a dissection microscope. The aortas were snap-frozen, and the hearts were embedded in TissueTek optical coherence tomography compound (Sakura Finetek Europe BV). Serum was collected using a standard protocol, and plasma lipoprotein cholesterol profiles were determined in all individuals by size chromatography using 2 μL plasma.

Statistical Analysis

The Mann–Whitney U test was used for comparisons between 2 groups. For multiple comparisons, 1-way or factorial ANOVA, followed by Fisher’s least-significant-difference test, was used. The Pearson product-moment correlation was used to calculate the correlation coefficient. The Bonferroni correction was used to adjust for multiple comparisons of correlations. Skewed CD137 values were square root transformed before analysis. Values are expressed as mean±SE. Values of P<0.05 were considered significant.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

CD137 Expression in Atherosclerosis

We investigated CD137 expression in peroperatively obtained samples of human arteries. Atherosclerotic arteries were obtained from patients undergoing carotid endarterectomy and from normal renal arteries during nephrectomy. Atherosclerotic arteries expressed significantly more CD137 mRNA than normal arteries (Figure 1A). These results were confirmed in a larger group of 144 patients and 8 control subjects. Mean CD137 levels were 17 times higher in patients...
CD137 Induced by Proinflammatory Stimuli

Lipopolysaccharide stimulation induced CD137 mRNA expression in explanted human normal and atherosclerotic arteries in culture (Figure 1A). Interestingly, although the lipopolysaccharide-induced CD137 response was more prominent in atherosclerotic cultures, CD137 also was induced in normal arteries. Incubation alone also increased CD137 mRNA in normal arterial tissue. T cells are known to express CD137 but are rare in normal vessels and unlikely to account for CD137 expression in normal arteries. Together with the immunohistochemical data, these results suggest that vascular cells can express CD137. Therefore, we investigated CD137 protein levels in cultured HUVECs and AoSMCs. ELISA showed a basal expression of CD137 in vascular cells (35.5±16.5 pg/mg in HUVECs and 61.5±17.1 pg/mg in AoSMCs). Both cell types responded to proinflammatory cytokines with the induction of CD137 mRNA and protein. In HUVECs, TNF-α alone resulted in the strongest CD137 mRNA induction (Figure 1B), whereas TNF-α, IL-1β, and IFN-γ together caused the most powerful mRNA induction in AoSMCs (Figure 1C). This induction also was reflected in the cell-surface protein levels for both cell types (Figure 3A and 3B). TNF-α stimulation caused a >10-fold increase in CD137 protein in HUVEC lysates (from 35.5±16.2 to 410±16 pg/mg). CD137 mRNA induction by TNF-α also was confirmed in pulmonary artery ECs (0.0076±0.004 [unstimulated] versus 3.2±0.8 [stimulated] arbitrary units; P<0.001).

CD137 Was Functional in ECs and SMCs In Vitro

After characterizing the expression in vascular cells, we wanted to evaluate the functional importance of CD137 in these cells. First, we established that the ligand/counterreceptor CD137L is present in vascular tissue. In BiKE, CD137L mRNA levels were significantly higher in patients than in control subjects (0.46±0.02 arbitrary units [range, 0.04 to 1.4 arbitrary units] versus 0.040±0.02 arbitrary units; P<0.001). ELISA of tissue extracts showed that CD137L protein levels were higher in human atherosclerotic compared with normal arteries (310±91 versus 82±9.6 pg/mg; P<0.05). CD137L was localized to CD68+ macrophages in atherosclerotic lesions by use of immunofluorescence (Figure 2H). To investigate the effects of the putative ligand-receptor interaction, cultured cells were exposed to functional rCD137L, ie, biotin-tagged trimeric rCD137L crosslinked by coating on streptavidin beads.22 Fluorescence-activated cell sorter analysis of HUVECs exposed to this crosslinked rCD137L at 5 µg/mL showed increased surface expression of VCAM-1 and intracellular adhesion molecule (ICAM)-1 (Figure 3C and 3D). No significant change was seen in cells treated with streptavidin beads only. In AoSMCs, proliferation, measured as 3H-thymidine incorporation, decreased significantly in response to the crosslinked rCD137L (Figure 4A). Incorporation was significantly reduced when cultures were preincubated with a 7-fold excess of the soluble CD137L (Figure 4B), which is known to block functional ligand binding to CD137.22
Activation of CD137 in Apoe−/− Mice Promoted a Proinflammatory Lesion Phenotype

To study the in vivo effects of CD137 activation in atherosclerosis, we turned to the atherosclerosis-prone Apoe−/− mice. Animals were injected intraperitoneally with the CD137 agonist 2A13 or an IgG2a isotype control every third week from 8 weeks of age and were euthanized at 16 weeks of age. The agonist treatment resulted in a prominent increase in CD3+ T cells in atherosclerotic lesions with no change in CD68+ macrophages as evaluated using immunostaining (Figure 5A and data not shown). Among T cells, substantial numbers of CD8+ cells were detected in lesions of 2A-treated mice (42±14 cells in treated versus 3.4±1.2 cells in control mice) with no change in the number of CD4+ cells (data not shown). The augmented T-cell recruitment to lesions in 2A-treated mice was associated with an increased expression of the major histocompatibility complex class II molecule I-Aβ (Figure 5B and data not shown). T-cell infiltration (A) and I-Aβ expression (B) were significantly augmented on in vivo antibody 2A ligation of CD137 vs control rat IgG2a (n=5). Immunostaining of I-Aβ (C) and CD68 (D) in the aortic root from a control mouse. Immunostaining of I-Aβ (E) and CD68 (F) in a 2A-treated mouse. Black bars indicate 0.2 mm. *P<0.05; **P<0.01.

In line with this, IFN-γ mRNA was significantly increased in the aortas of 2A-treated mice (Figure 6). Similarly, the proinflammatory cytokines TNF-α and IL-1β were significantly upregulated on the mRNA level, as was the adhesion molecule ICAM-1, whereas VCAM-1 showed a nonsignificant trend toward increase after 2A treatment (Figure 6). Atherosclerotic lesions were larger in treated mice than in controls (Figure 7). Smooth muscle content per vessel area did not differ between groups (0.17±0.04 versus 0.15±0.03 arbitrary units in control and treated mice, respectively; P=0.46), but at this early stage of atherosclerosis, no α-actin+ cells were detectable inside the lesions. The content of tightly packed collagen was also similar in both groups (0.14±0.03 versus 0.13±0.03 arbitrary units in control versus treated mice), as was the staining with the proliferation marker Ki-67 (data not shown). There were no differences in the relative amounts of TCR+, CD4+, CD8+, or CD19+ cells in the spleen between the 2 groups (data not shown), and plasma lipoprotein cholesterol profiles did not differ significantly between groups (data not shown).
CD137 Levels Correlated to Factors Associated With a Proinflammatory Phenotype in Human Arteries

To investigate whether the level of CD137 was associated with known markers of vascular inflammation, we examined the correlations between the mRNA levels of CD137 and several markers of inflammation in the human arteries from BiKE. As shown in the Table, CD137 mRNA correlated significantly to several metalloproteinases, HLA class II molecules, members of the T-cell receptor complex, costimulatory factors, and leukocyte adhesion molecules.

Discussion

CD137 is known as a costimulator of T cells, where it activates nuclear factor-κB and promotes proliferation and cytokine production. Recent studies have indicated an important role for CD137 signaling in autoimmune disease.

Table. Correlation Between mRNA Levels of CD137 and Markers of Inflammation Analyzed by Gene Expression Arrays of Human Arteries From the Biobank of Karolinska Endarterectomies

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Pearson’s r</th>
<th>Bonferroni-Adjusted P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP9</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HLA-DP α1</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HLA-DQ α1</td>
<td>0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD68</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMP12</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>0.58</td>
<td>0.003</td>
</tr>
<tr>
<td>CD8α</td>
<td>0.52</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.52</td>
<td>0.02</td>
</tr>
<tr>
<td>CD3ε</td>
<td>0.31</td>
<td>NS</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant.
In our study, 2A treatment did not significantly change the CD8-to-CD4 ratio with previous studies showing expansion of the CD8-to-CD4 ratio is higher than in immune response in human lesions, in which the CD8-to-CD4 ratio is higher than in human plaques are known to inhibit SMC proliferation, which may contribute to destabilization of lesions and may increase the risk for clinical events. In this study, CD137 ligation is likely to occur in atherosclerosis, may enhance activation of CD137+ T cells and ECs in the atherosclerotic lesion, and ultimately may aggravate the ongoing local immune response (Figure 8).

The inflammatory conditions in the lesion influence its composition and stability. The SMC population is an important component for plaque integrity that is believed to improve lesion strength significantly through formation of a fibrous cap and through matrix production and proliferation. However, proinflammatory cytokines commonly found in human plaques are known to inhibit SMC proliferation, which may contribute to destabilization of lesions and may increase the risk for clinical events. In this study, CD137 was inducible in AoSMCs by proinflammatory cytokines associated with atherosclerotic lesions, and stimulation of these SMCs with crosslinked rCD137L reduced proliferation. Consequently, it is conceivable that CD137L synergizes with proinflammatory cytokines to reduce the content of SMCs in advanced lesions. This may contribute to a reduction in lesion stability, ultimately increasing the risk for ischemic events. Analysis of gene expression patterns in human atherosclerotic lesions further supports a role for CD137 in atherosclerosis. CD137 mRNA levels in lesions correlated positively to processes. Our present study demonstrates that CD137 is involved in atherosclerosis.

We detected the CD137 protein in atherosclerotic lesions not only on T cells but also on ECs and showed that CD137 expression in cultured ECs and SMCs is functional. CD137 was induced on ECs and SMCs by proinflammatory cytokines present in atherosclerotic lesions. Activation of CD137 by its specific ligand induced VCAM-1 and ICAM-1 expression on ECs. These adhesion molecules are crucial for leukocyte emigration from the bloodstream into the vessel wall and for formation of atherosclerotic lesions. Through this mechanism, CD137 may contribute to leukocyte diapedesis into the vessel wall and to atheroma formation (Figure 8).

In support of this, atherosclerotic lesions in Apoe−/− mice responded to injection of the CD137 agonist 2A with a remarkably increased infiltration of T cells into atherosclerotic lesions and a dramatic increase in I-Aβ expression, an important sign of immune activation. In agreement with the data from human primary cell cultures, 2A treatment also resulted in increased expression of adhesion molecule mRNA in aortas and augmented levels of a several proinflammatory cytokines. These data suggest that CD137 ligation generates a proinflammatory milieu, which aggravates atherosclerosis. CD137 ligation increased not only inflammatory cells and molecules but also lesion size in the ascending aorta. We speculate that the pattern of lesion development could reflect a heterogeneous distribution of CD137 in the atherosclerotic lesion, similar to that seen in humans and possibly influenced by flow-dependent local factors.

Treatment with the CD137 agonist 2A led to a particularly striking influx of CD8+ T cells into lesions. This is in line with previous studies showing expansion of the CD8+ population in response to treatment with a CD137 agonist. In our study, 2A treatment did not significantly change the splenic CD8+ population but increased the number of CD8+ cells in the lesions. There was no difference in cell proliferation activity between lesions of treated and control mice, suggesting that the increase in the CD8+ population was due to greater infiltration. In atherosclerosis, vascular activation of CD8+ T cells has been shown to augment lesion development dramatically; therefore, it is conceivable that CD137 ligation may accelerate the disease process. Such a mechanism could potentially contribute more to the local cellular immune response in human lesions, in which the CD8-to-CD4 ratio is higher than in Apoe−/− mice. The expression of CD137 on the vascular endothelium also suggests a novel pathway of interaction between blood-borne cells and the atherosclerotic artery wall (Figure 8). A number of immune cells, including monocytes, B cells, and dendritic cells, express CD137L constitutively. Interestingly, binding of CD137L to CD137 can activate B cells and monocytes through bidirectional signaling. We speculate that endothelial CD137 expression could activate adherent immune cells in a similar way and increase their capacity for diapedesis and cytokine production, ultimately adding to the inflammatory milieu in the vascular wall. In line with our data, Drenkard et al recently detected endothelial CD137 expression in inflammatory conditions and showed that CD137 can induce monocyte migration in vivo. Furthermore, infiltrating CD137L+ cells from the circulation could potentially activate CD137 on ECs. Thus, CD137 activation may form part of a positive-feedback, inflammation-promoting circuit in atherosclerosis (Figure 8).

CD137L is known to be expressed not only on leukocytes but also on other cell types at sites of inflammation. We observed that CD137L levels were much higher in human lesions than in normal human arteries and that macrophages in atherosclerotic lesions express CD137L. Therefore, CD137 ligation is likely to occur in atherosclerosis, may enhance activation of CD137+ T cells and ECs in the atherosclerotic lesion, and ultimately may aggravate the ongoing local immune response (Figure 8).

The inflammatory conditions in the lesion influence its composition and stability. The SMC population is an important component for plaque integrity that is believed to improve lesion strength significantly through formation of a fibrous cap and through matrix production and proliferation. However, proinflammatory cytokines commonly found in human plaques are known to inhibit SMC proliferation, which may contribute to destabilization of lesions and may increase the risk for clinical events. In this study, CD137 was inducible in AoSMCs by proinflammatory cytokines associated with atherosclerotic lesions, and stimulation of these SMCs with crosslinked rCD137L reduced proliferation. Consequently, it is conceivable that CD137L synergizes with proinflammatory cytokines to reduce the content of SMCs in advanced lesions. This may contribute to a reduction in lesion stability, ultimately increasing the risk for ischemic events.

Analysis of gene expression patterns in human atherosclerotic lesions further supports a role for CD137 in atherosclerosis. CD137 mRNA levels in lesions correlated positively to...
factors believed to influence lesion stability and the risk of clinical manifestations: levels of matrix metalloproteinases, adhesion molecules, and costimulatory factors; markers of T cell and macrophage infiltration; and major histocompatibility complex class II expression.

Taken together, these observations suggest that CD137-CD137L interactions in the vasculature may contribute to the progression and increased vulnerability of atherosclerotic lesions via augmented leukocyte recruitment, increased inflammation, and development of a more disease-prone phenotype.

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Disclosures
None.

References
Recruitment and activation of inflammatory cells in the vascular wall are key events in the development of atherosclerosis. Intensified inflammation is associated with clinical events such as myocardial infarction. We identified CD137, a T-cell costimulatory molecule, in human atherosclerotic lesions and found that its expression correlated with the local levels of several agents associated with plaque destabilization such as adhesion molecules, proinflammatory cytokines, and matrix metalloproteinases. Activation of CD137 reduced proliferation of cultured smooth muscle cells and increased surface levels of adhesion molecules in endothelial cells. In a murine model of atherosclerosis, treatment with agonistic CD137 antibodies caused significantly increased CD8$^+$ T-cell infiltration and increased levels of murine major histocompatibility complex class II proteins in atherosclerotic lesions, as well as increased vascular levels of proinflammatory cytokines and intercellular adhesion molecule-1. Taken together, these observations suggest that CD137 activation in the vasculature may contribute to the progression and increased vulnerability of atherosclerotic lesions via augmented leukocyte recruitment, increased inflammation, and development of a more disease-prone phenotype.
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