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Atheroprotective Effect of CD31 Receptor Globulin Through Enrichment of Circulating Regulatory T-Cells

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Objectives This study was designed to evaluate whether replacing CD31 (PECAM-1) signaling can restore the regulation of lymphocyte activation and improve experimental atherosclerosis.

Background Atherosclerosis, the principal cause of myocardial infarction and stroke, is due to the development of a pathogenic immune response within the vascular wall and is aggravated by the reduction of regulatory T-cells. CD31, a transmembrane adhesion molecule with inhibitory signaling functions, is physiologically expressed on blood and vascular resting cells but is lost in pathologic conditions associated with atherosclerosis.

Methods Replacement therapy with a CD31 receptor globulin (Rg) was delivered by in vivo gene transfer in 6-week-old apolipoprotein E knockout mice ($n = 14$ per group) every 5 weeks for 6 months. Control groups were treated with a truncated CD31Rg or with vehicle alone. At the end of the study, plaque size and morphology and blood T-cell compartment were analyzed in all mice.

Results Atherosclerotic lesions of CD31Rg-treated mice were smaller ($p < 0.01$) and showed less neovascularization and intraplaque hemorrhage ($p < 0.05$) compared with control subjects. Furthermore, circulating regulatory T-cells were increased in vivo ($p < 0.01$) and showed normal suppressive function on proliferation of conventional T-cells in vitro. Indeed, CD31Rg treatment led to blunted blood T-cell activation ($p < 0.05$) and reduced T-cell infiltration within plaques ($p < 0.01$).

Conclusions Our data suggest that CD31 plays a key role in the regulation of the immune response linked to atherosclerosis. CD31-targeting therapeutic approaches may therefore be envisaged for preventing and treating atherosclerotic diseases. (J Am Coll Cardiol 2007;50:344–50) © 2007 by the American College of Cardiology Foundation

Atherosclerosis results from the infiltration of lipoproteins in the intimal space and the development of a pathogenic immune response to modified lipoproteins and other inflammatory stimuli within the arterial wall (1). Such response develops at the blood–vessel interface between cells lining the vascular wall (endothelial cells) and cells flowing within the bloodstream such as platelets, lymphocytes, granulocytes, and monocytes. Under physiologic conditions, activated blood cells can circulate within the arteries without attacking each other or the cells of the arterial wall, indicating the presence of a constitutive physiologic control mechanism able to prevent inappropriate cell activation at the blood-vessel interface. Such a regulation might be efficiently coordinated by leukocytes-platelets-endothelial cell-cell communication through a common receptor: CD31. This hypothesis is supported by the following considerations: 1) all (and exclusively) cells of the blood-vessel interface express CD31 (2); 2) CD31 functions as both a homophilic and heterophilic adhesion receptor (3), which facilitates dialog between cells of different origin such as leukocytes, platelets, and endothelial cells; 3) the CD31 promoter contains 2 consensus sites for nuclear factor-kappa B binding (4), which suggests a function for CD31 in inflammatory contexts; 4) the phosphorylation of the 2 immunoreceptor tyrosine-based inhibitory motifs in the intracellular domain of CD31 allows the activation of the bound SHP2 tyrosine phosphatase, which leads to the inhibition of cell functions mediated by protein tyrosine kinases (5); and 5) homophilic ligation of CD31 mediates mutual repulsion of viable cells by transmitting "detachment" signals (6). Interestingly, up to 50% of circulating T

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lymphocytes in adult human blood lack detectable CD31 at their surface (7), and such loss may be a consequence of their activation (8–10).

Furthermore, activated endothelial cells and dendritic cells upon maturation lose CD31 in response to inflammatory stimuli (11,12). We therefore hypothesized that the pathologic interaction between activated leukocytes and endothelial cells in atherosclerosis occurs because the physiological inhibition driven by CD31-CD31 and/or CD31-heterophylic (13) signaling is lost. Indeed, we have recently shown (14) that loss of CD31 at the surface of circulating T-cells positively correlates with the occurrence of atherothrombosis in mice and of (atherothrombotic) abdominal aortic aneurysm in patients (15). The presence of CD31 on T lymphocytes showed immunoregulatory properties on the effector function of leukocytes over cells of the vascular wall in vitro (15).

In the present study, we sought to evaluate whether a substitute CD31 signaling could restore the regulation of the immune response at the blood-vessel interface in an in vivo experimental model of atherosclerosis.

Abbreviations and Acronyms

CD31Rg = CD31 receptor globulin (domain 1-6)

CD31Rg Δ1-2 = truncated CD31 receptor globulin lacking domain 1-2 (domain 3-6)

HRP = horseradish peroxidase

Ig = immunoglobulin

MT = Masson's trichrome staining

PBS = phosphate-buffered saline

Treg(s) = regulatory T-cell(s)

Materials and Methods

Study protocol. Female apolipoprotein E knockout mice from our breeding facility were maintained on a regular chow diet and kept under standard conditions. All experiments conformed to the "Position of the American Heart Association on Research Animal Use," adopted on November 11, 1984. We expressed our deoxyribonucleic acid plasmids encoding CD31 receptor globulin (Rg), composed of the entire extracellular portion of CD31 fused to constant parts of human immunoglobulin (Ig) G1 (13,16) in vivo in mice (n = 14) by intramuscular gene electrotransfer as previously described (17). Control mice received either CD31Rg Δ1-2 that lacked the two N-terminal Ig domains of CD31 (n = 14) or phosphate-buffered saline (PBS) (n = 14). Briefly, 15 μg of plasmid deoxyribonucleic acid was injected in each tibial cranial muscle, and 8 transcutaneous electric pulses (20 ms, 200 V/cm, 2 Hz, ECM 800 electroporator, BTX, San Diego, California) were applied through 2 plate electrodes on both sides of the limb. These were the optimal conditions for skeletal muscle gene transfer, as determined by Mir et al. (18). Plasmid products were detected up to 4 weeks by plasma protein electrophoresis and immunoblotting for the human IgG1-Fc portion of CD31 fusion proteins (data not shown). Over 6 months, we repeated the gene transfer every fifth week starting from age 6 weeks. At the end of the study, the mice were euthanized under anesthesia.

Plasma and circulating cell analysis. Blood was drawn from the right heart ventricle and collected in heparinized tubes. Plasma was analyzed for protein (Lowry method) and cholesterol (France-méthode "CHOD-PAP," Boehringer Mannheim, Mannheim, Germany) content and for IgG antibodies directed against the human IgG1-Fc of the constructs by enzyme-linked immunosorbent assay (coating = polyclonal human IgG, detection = horseradish peroxidase [HRP]-conjugated rabbit antimouse IgG antibodies and reading at 450 to 570 nm). The cell pellet was subjected to density gradient centrifugation over 1-ml Ficoll in a 5-ml round-bottom tube. The peripheral blood mononuclear cells were processed for flow cytometry analysis. Fluorescent monoclonal antibodies (CD3-APC, CD4-PerCP, CD8-PE, CD69-PE-Cy7, CD45-FITC; all from BD Biosciences, San Jose, California) were used simultaneously for characterization of lymphocyte population and activation status. Circulating regulatory T cells (Treg) were quantified using a staining protocol from eBioscience, San Diego, California; (CD4-FITC, CD25-APC, FoxP3-PE antibodies) and a LSRII flow cytometer with the DIVA analysis software (BD Biosciences).

Atherosclerotic plaque analysis. We measured atherosclerotic plaque extent on 4 oil-red O-stained 10-μm-thick aortic root cryosections at fixed distance points from the beginning of the valve as previously described (19). We also analyzed brachiocephalic artery lesion size on 5 cross-sections cut at 50-μm intervals starting from its origin from the aortic arch. Plaque composition was analyzed by specific polyacid histologic stains using a customized orcein/Masson's trichrome (MT) staining protocol.

Immunohistochemistry. The presence of CD31Rg gene transfer products was evaluated by immunohistochemistry on aortic root cryosections using a biotinylated monoclonal antibody directed against the Fc portion of human IgG1 (13). Staining was revealed by either Vectastain ABC-HRP and DAB substrate (Vector, Burlingame, California) and light microscopy or by streptavidin-FITC and fluorescence microscopy using a Zeiss Axiovert 200M microscope and AxioVision image capture software. Endothelial cells were stained with FITC-conjugated lectin BSI (Bandeiraea Simplicifolia-I). CD31-positive cells were visualized with a biotinylated monoclonal antibody directed against mouse CD31 (clone MEC 13.3, BD Biosciences) and AlexaFluor 555-conjugated streptavidin. T lymphocytes and macrophages in aortic root plaques were assessed by using monoclonal antibodies directed against Thy1.2 (Serotec, Oxford, United Kingdom) and Mac3 (BD Biosciences), respectively, and an AlexaFluor 555-conjugated secondary antibody. Immunostained slides were cover-mounted with Vectashield HardSet with DAPI (Vector). Plaque-infiltrating Thy1.2- and Mac3-positive cells (red) were counted by computer-assisted image analysis and reported as percentage of total cells (blue).

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Table 1 Antibody Response to Treatment and Rate of Plaque Inflammatory Cells

	PBS	CD31Rg Δ1-2	CD31Rg
Antihuman Fc IgG (a.u.)*	0.309 ± 0.035	0.417 ± 0.055†	0.516 ± 0.051†
Mac3+ (% cells)‡	29.0 ± 6.9	29.9 ± 6.5	36.8 ± 7.4
Thy1.2+ (% cells)‡	8.3 ± 3.5	1.6 ± 0.8†	0.4 ± 0.2†

Data are expressed as mean ± SEM, n = 14 mice/group. *a.u. = arbitrary units. Plasma levels of antihuman Fc immunoglobulin G (IgG) antibodies were measured by enzyme-linked immunosorbent assay on individual mouse sera (diluted 1:50) and detected by HRP/TMB substrate (450−570 nm optical density). †$p < 0.05$ versus PBS. ‡Percentage of macrophages (Mac3+, red) and T lymphocytes (Thy1.2+, red) among all cells (DAPI, blue nuclear staining) was evaluated by immunohistochemistry in consecutive aortic root sections. Five sections at 100-μm intervals were analyzed for each staining in all mice. Nine random fields delimited by the internal elastic lamina (green autofluorescence) were used for each section at 200x magnification.

CD31Rg = CD31 receptor globulin; CD31Rg Δ1-2 = truncated CD31 receptor globulin lacking domain 1-2; PBS = phosphate-buffered saline.

T-cell suppression assay. Treg function was assessed as previously described (20). Briefly, $CD4^+$ T-cells from cell suspensions of spleen and peripheral lymph nodes were enriched by magnetic cell sorting and sequentially incubated with a biotin-conjugated CD25 monoclonal antibody (BD Biosciences) and streptavidin microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Two consecutive magnetic cell separations using LS columns (Miltenyi Biotec) yielded ≥90% $CD4^+CD25^+$ cells. The $CD25^-$ cells were harvested from the flow-through and contained ≤0.3% of $CD4^+CD25^+$ T-cells. The $CD4^+$ $CD25^-$ T-cells were plated alone or in co-culture with Tregs ($CD4^+CD25^+$) at a ratio of 1:1, 2:1, 4:1, or 8:1 in flat-bottomed 96-well microplates (0.5×10^5 cells/well; total volume 200 μl/well in RPMI 1640 supplemented with Glutamax, 10% FCS, 0.02 mM 2 beta-mercaptoethanol, and antibiotics). Cells were cultured at 37°C for 72 h and pulsed with 1 μCi of ^{3}[H]thymidine (Amersham, Piscataway, New Jersey) for the last 18 h of culture. Thymidine incorporation was assessed using a TopCount NXT scintillation counter (Perkin Elmer, Waltham, Massachusetts).

Statistical analysis. Data are expressed as mean ± SEM unless otherwise indicated in the text. Differences between groups were analyzed by 1-factor ANOVA and Bonferroni post hoc test. For the analysis of atherosclerotic lesion size and T-cell suppression, we used 1-factor ANOVA with repeated measures and Bonferroni post-hoc test. Differences were considered statistically significant when the p value was <0.05.

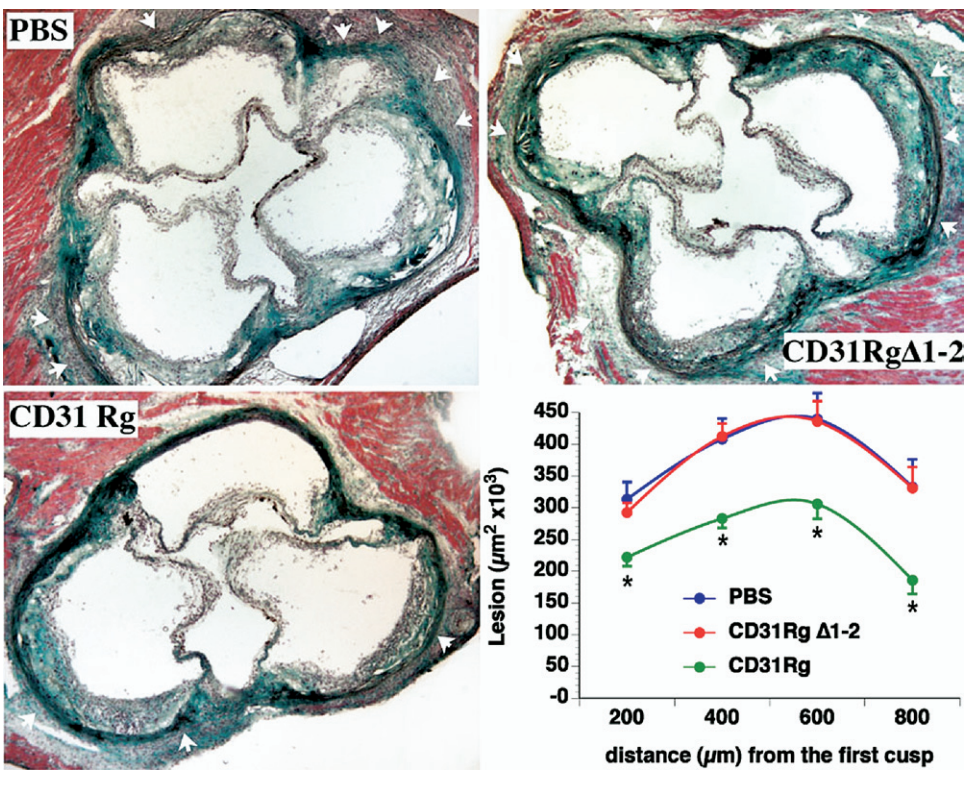

Figure 1 Atherosclerotic Lesions Are Reduced by CD31Rg Treatment

(Micrographs) Representative micrographs of MT-stained sections (original magnification 40x). Myocardiocytes are stained **red,** elastin fibers and nuclei appear **black,** extracellular matrix appears **green.** Fibrofatty atherosclerotic lesions are present in the 3 aortic cups in PBS and CD31Rg Δ1-2 groups, whereas they are limited to 2 of the 3 cusps by CD31Rg treatment. Adventitial inflammation was reduced in the CD31Rg group **(white arrows)**. **(Bottom right panel)** Morphometric analysis was performed at 200, 400, 600, and 800 μm from the appearance of the first aortic cusp, starting from the ventricular side of the aortic root, by oil-red O staining and computer-assisted image analysis. The CD31Rg-treated group showed significantly smaller lesions as compared to the CD31Rg Δ1-2 and PBS control groups. *$p < 0.01$ versus CD31Rg Δ1-2 and PBS, n = 14 mice/group. CD31Rg = CD31 receptor globulin; CD31Rg Δ1-2= truncated CD31 receptor globulin lacking domain 1-2; MT = Masson's trichrome staining; PBS = phosphate-buffered saline.

Results

Mice groups did not differ for body weight, total cholesterol, plasma protein levels, and blood cell count (data not shown). Plasma IgG directed against human IgG1-Fc was similarly increased in CD31Rg and CD31Rg Δ1-2–treated mice as compared with PBS control mice (Table 1), suggesting that the in vivo responses to CD31Rg treatment were not due to secondary immune effects.

Reduced atherosclerosis plaque extent and vulnerability upon CD31Rg treatment. Atherosclerotic lesions at the level of the aortic root were significantly smaller in CD31Rg-treated mice as compared the PBS and CD31Rg Δ1-2 control mice (Fig. 1). Lesion size in the brachiocephalic artery was not different in the 3 groups, but a very large intragroup variability was observed because the site of lesion development is not constant at this location. Median (min-max) plaque size was 172 (138 to 350) $\times 10^3$ μm^2 in PBS, 213 (113 to 407) $\times 10^3$ μm^2 in CD31Rg Δ1-2, and 194 (146 to 262) $\times 10^3 \mu m^2$ in CD31Rg (p = NS). Plaque composition as evaluated on MT staining did not differ among the 3 groups at the aortic root level (Fig. 1). At variance, analysis of MT-stained brachiocephalic atherosclerotic plaques of comparable size among the 3 groups showed that CD31Rg treatment was associated with blunted neovascularization and intraplaque hemorrhage as compared CD31Rg Δ1-2 and PBS control treatment (Fig. 2). We found that MT staining documented consistent intraplaque fibrin (red) deposition in 9 of 14 (PBS) and 6 of 14 (CD31RgΔ1-2) control mice (p = NS), whereas tiny fibrin deposits could be detected in only 3 of 14 CD31Rg-treated mice ($p < 0.05$ vs. PBS, chi-square test). Fibrin deposits were topologically distributed in areas of rich neovascularization, as detected by FITC-lectin BSI staining of endothelial cells within the plaques (Fig. 2). Of note, most of the intraplaque neovessel endothelial cells were negative for CD31, as shown by the merged red and green fluorescent channels (Fig. 2).

The whole cell density (number of nuclei/μm^2 tissue) was not different in the 3 treatment groups. Specific immunofluorescence analysis showed that most of the infiltrating cells were macrophages ($Mac3^+$ cells) and that the rate of macrophage infiltration was not affected by treatment. Interestingly, although considerably lower in all groups, the percentage of infiltrating T-cells was reduced by 20-fold in lesions of CD31Rg and by 5-fold in the CD31Rg Δ1-2–treated mice as compared to PBS control mice ($p < 0.01$ vs. PBS) (Table 1).

CD31Rg binds to plaque infiltrating cells. Immunohistochemistry demonstrated that CD31Rg, but not CD31RgΔ1-2, bound a portion of the plaque infiltrating cells (Fig. 3), suggesting that stable binding is dependent on the presence of the extracellular CD31 domains 1-2 that are lacking in

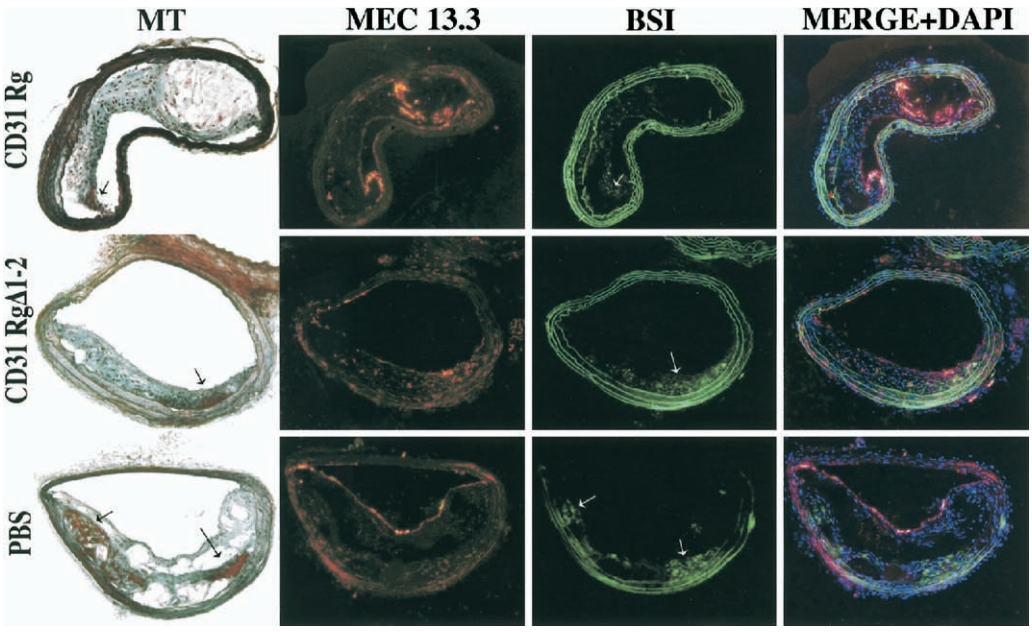

Figure 2 Reduced Neovascularization and Plaque Hemorrhage Upon In Vivo Expression of CD31Rg

Masson's trichrome staining and double immunohistochemistry for CD31 (MEC 13.3) and endothelia (lectin BSI) on comparable brachiocephalic plaques. Representative micrographs showing blunted neovascularization and intraplaque hemorrhage in CD31Rg-treated mice (3 of 14 mice, 21%, $p < 0.05$, chi-square test) as compared to CD31Rg Δ1-2 (6 of 14 mice 42%) and PBS (9 of 14 mice, 64%) control mice. Intraplaque fibrin deposition (**red,** MT column, **black arrows**) localizes at areas of rich neovascularization as detected by FITC-lectin BSI staining of endothelial cells within the plaques (**green,** BSI column, **white arrows**). Of note, most of the intraplaque neovessels were negative for CD31 (**red,** MEC13.3 column) as showed by the merge of fluorescent images (MERGE + DAPI column). Original magnification 100×. Abbreviations as in Figure 1.

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the CD31Rg Δ1-2 plasmid product. Interestingly, CD31Rg binding could be detected not only on cells highly expressing CD31 ($CD31^{bright}$ cells), but also on cells that had partially lost their surface CD31 expression ($CD31^{low}$ cells) (Fig. 3). This binding is probably due to binding of CD31 to heterophilic ligands, including cell surface glycosaminoglycan, alpha-V-beta-3 integrin, CD38, or TCD31L (13,16).

Enhanced Treg content in the blood of CD31Rg-treated animals. Flow cytometry analysis of peripheral blood mononuclear cells showed that the percentages of $CD3^+$ (total), $CD4^+$ (helper), and $CD8^+$ (cytotoxic) T-cells within the lymphocyte population (light scatter x CD45 double gate) were similar in the 3 groups. In contrast, the percentage of $CD4^+/CD69^+$ double-positive (activated T-helper) cells among total T lymphocytes was significantly reduced in the blood of CD31Rg-treated mice (Fig. 4). In vitro proliferation studies showed that T-cell activation was hampered in the CD31Rg group as compared with the control group, because splenocyte growth was significantly reduced in response to the mitogen concanavalin A (Fig. 5).

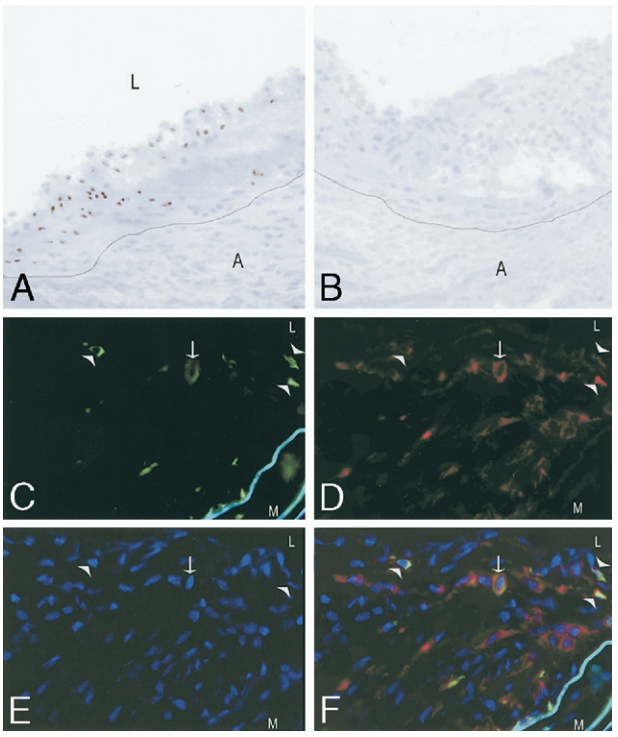

Figure 3 **CD31Rg Binds to Plaque-Infiltrating Cells**

Immunohistochemistry of CD31Rg in aortic root cryosections. CD31Rg **(A),** but not CD31Rg Δ1-2 **(B),** binds a consistent number of plaque-infiltrating cells. CD31Rg binding was detected on $CD31^{bright}$ cells **(white arrows)** as well as on $CD31^{low}$ cells **(white arrow heads)**. CD31Rg staining was revealed by HRP/DAB **(brown, A to B; green, C and F)**, nuclei were counterstained by hematoxylin **(light blue, A to B)** or DAPI **(blue, E to F)**. $CD31^+$ cells were visualized by a monoclonal rat antimouse CD31 antibody (MEC13.3) and an Alexafluor 555-conjugated secondary antibody **(red, D and F)**. Original magnification 200×. A = adventitia; L = lumen; M = media; other abbreviations as in Figure 1.

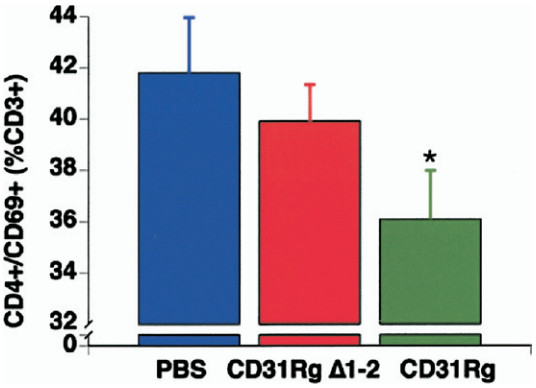

Figure 4 **Circulating Activated T Lymphocytes Are Reduced Upon In Vivo Expression of CD31Rg**

The percentage of circulating activated ($CD69^+$) helper ($CD4^+$) cells within T lymphocytes (double gate FSC/SSC x $CD3^+$) was analyzed by flow cytometry. At the end of the study, CD31Rg-treated mice showed significantly reduced numbers of circulating activated T-helper lymphocytes as compared to PBS and CD31Rg Δ1-2 controls. Similar data were obtained 1 week after the first electroporation round. *$p < 0.05$ versus PBS and CD31Rg Δ1-2, n = 14 mice/group. Abbreviations as in Figure 1.

In agreement with this finding, the percentage of Tregs was significantly increased in the blood of CD31Rg-treated mice (9 ± 0.7%) as compared to CD31Rg Δ1-2 (5 ± 0.8%, $p < 0.01$) or PBS (5 ± 1%, $p < 0.01$) control groups (Fig. 6). Of note, the suppressive function of Treg was not altered by the CD31Rg treatment; Tregs derived from

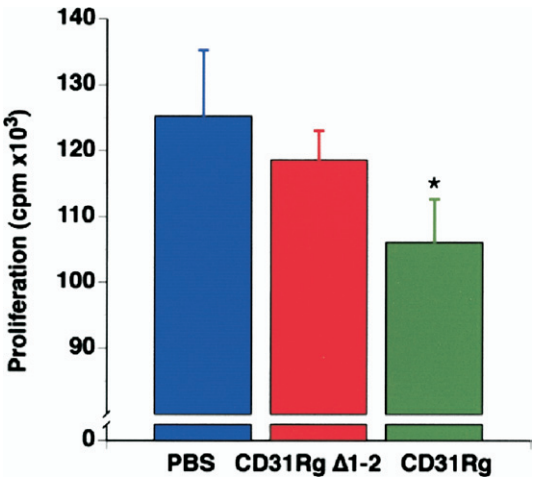

Figure 5 **Reduced T Lymphocyte Response Upon In Vivo Expression of CD31Rg**

Spleen cells were stimulated with concanavalin A for 3 days. ^{3}[H]thymidine was added to the cultures for the last 18 h. Cell proliferation was measured in a beta counter and is expressed in counts per minute (cpm). T-cell proliferation of CD31Rg-treated mice was significantly reduced as compared control mice, even in response to a powerful stimulus such as the polyclonal mitogen concanavalin A. *$p < 0.05$ versus CD31Rg Δ1-2 and PBS, n = 14 mice/group. Abbreviations as in Figure 1.

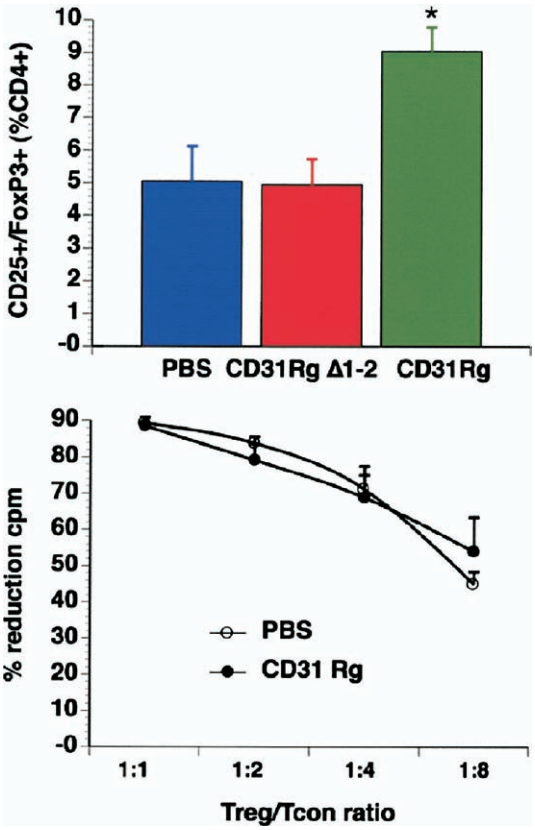

Figure 6 **In Vivo CD31Rg Expression Increases Circulating Tregs**

(Top) The percentage of $CD25^+/FoxP3^+$ regulatory T cells (Tregs) within total $CD4^+$ T lymphocytes was significantly increased in the peripheral blood of CD31Rg-treated mice as compared with the controls. *$p < 0.01$, $n = 14$ mice/group. **(Bottom)** The suppressive capacity of Tregs was not altered by CD31Rg treatment ($n = 14$ mice/group, NS). $CD4^+CD25$ negative T-cells were incubated with Tregs at the indicated ratios, and proliferation was measured as before. Other abbreviations as in Figure 1.

CD31Rg- or PBS-treated animals reduced proliferation of $CD4^+$ CD25-negative cells to similar extent (Fig. 6).

Discussion

Recent studies suggest that CD31 (PECAM-1) could function as a regulatory molecule in vascular biology and immunity because it is expressed exclusively by endothelial and blood cells. Homophilic as well as heterophilic CD31 binding was shown to drive inhibitory signals between apposing cells (13,21) as well as detachment signals, preventing, for instance, phagocyte ingestion of viable cells (6). In this perspective, the presence of CD31 could serve as a "leave me alone" signal for flowing cells and the cells lining the vascular wall (21). Interestingly, atherosclerosis and its acute clinical manifestations are characterized by the activation of circulating lymphocytes (22,23), and T-cell activation is associated with the loss of CD31 expression (24).

We have recently reported (14) that the loss of CD31 on circulating T-cells is associated with enhanced activation of the immune response toward the vascular wall and the occurrence of plaque complications in atherosclerotic mice, as well as with the incidence of atherosclerotic abdominal aortic aneurysm in patients (15). In this study, we sought to evaluate the hypothesis that replacing CD31 signaling could restore the regulation of lymphocyte activation, leading to reduction of disease extent in experimental atherosclerosis. Therefore, we expressed a soluble form of CD31, CD31Rg, which had previously demonstrated regulatory capacities in vitro (13), in atherosclerosis-prone apoE KO mice by intramuscular gene transfer technology.

Repeated CD31Rg treatment over 6 months resulted in a significant reduction of atherosclerotic lesion extent. We found that CD31Rg, but not a truncated form lacking the first 2 extracellular domains of CD31, bound to plaque-infiltrating cells. In parallel, the truncated construct was not able to affect disease extent, suggesting that the first 2 domains of the CD31 ectodomain are important for the beneficial effect. In this respect, previous studies had suggested that individually distinct CD31 extracellular domains might mediate distinct function in CD31 signaling (3).

Independently of lesion extent, CD31Rg treatment also reduced the occurrence of fibrin deposits in areas of plaque neovascularization. The plaque neovessels have been recently indicated as a major mechanism of plaque complication and growth both in patients (25,26) and atherosclerosis-prone mice (27) because of their immature state and consequent fragility (25). Interestingly, the endothelial cells of plaque neovessels were frequently negative for CD31 staining, as reported in a previous study in atherosclerotic patients (28). Our data suggest that replacement of CD31 signaling could improve neovessel stability (29) and protect from intraplaque hemorrhage.

The beneficial effects of CD31Rg on atherogenesis were accompanied by prevention of T-cells from entering the developing plaque: though in the CD31Rg Δ1-2–treated animals the number of T-cells was reduced (5-fold), virtually no plaque-infiltrating T-cells could be detected in mice treated with CD31Rg (20-fold reduction). Furthermore, CD31Rg blunted the T-cell activation, as detected by the reduced expression of activation markers on circulating T-cells, as well as by a lower proliferative response to polyclonal stimulation. This work is in agreement with our previous findings that CD31Rg induces in vitro hyporesponsiveness of T-helper lymphocytes (13,16). Combining the latter work with the new finding, we reasoned that CD31Rg might produce T-cell hyporesponsiveness by converting some of its target T-cells into Tregs. Indeed, our data show that CD31Rg treatment in vivo results in increased numbers of circulating Tregs. We can also show that the suppressive function of circulating Tregs is not altered by CD31Rg treatment, implying that their increased number might explain the concomitant observed reduction in circulating activated T-cells in our study.

In accord with this finding, it was shown that the aberrant T-cell activation observed in patients with acute coronary syndromes is linked to a lack of T-cell regulation (30) and reduced numbers and function of circulating Tregs in the acute phases of coronary artery diseases (31). Furthermore, experimental studies have demonstrated that Tregs play a crucial role in the control of atherogenesis in mice (20). Although the molecular mechanisms involved in the CD31-mediated induction of circulating Treg remain to be elucidated, the present study indicates that CD31-targeting therapeutic approaches may be envisaged for preventing and treating atherosclerotic diseases.

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