Transfer of CD4⁺ T Cells Aggravates Atherosclerosis in Immunodeficient Apolipoprotein E Knockout Mice

Xinghua Zhou, MD, PhD; Antonino Nicoletti, PhD; Rima Elhage, PhD; Göran K. Hansson, MD, PhD

Background—Atherosclerosis is associated with immune responses to oxidized lipoproteins and certain microorganisms, but the role of specific immunity has remained unclear.

Methods and Results—To study the role of immunity in atherosclerosis, we crossed atherosclerosis-prone apoE⁻/⁻ mice with immunodeficient scid/scid mice. The offspring showed a 73% reduction in aortic fatty streak lesions when compared with immunocompetent apoE⁻/⁻ mice. Transfer of CD4⁺ T cells from apoE⁻/⁻ to immunodeficient apoE⁻/⁻/scid/scid mice increased lesions by 164%. This was associated with the infiltration of transferred T cells into lesions, increased circulating interferon-γ levels, and increased I-A expression in lesions.

Conclusions—CD4⁺ T cells carry disease-promoting immunity in atherosclerosis. (Circulation. 2000;102:2919-2922.)

Key Words: atherosclerosis ■ lymphocytes ■ immune system ■ mice, knockout

Atherosclerosis is characterized by focal arterial lesions containing cholesterol, fibrosis, and inflammatory infiltrates.¹ The latter consist mainly of macrophages and T lymphocytes, many of which are immunoreactive against oxidized LDL.²⁻³ Patients with atherosclerosis exhibit systemic immune responses against oxidized LDL,⁴⁻⁵ heat shock proteins,⁶ Chlamydia pneumoniae,⁷ and other antigens, but the role of adaptive immunity in the disease process has remained unclear. New knockout models such as the apoE⁻/⁻ mouse, which develops spontaneous hypercholesterolemia and atherosclerosis, have permitted detailed studies of atherogenesis.⁸⁻⁹

The extent of disease is reduced when selective or generalized immune defects are induced in such mice by crossbreeding with other knockout strains¹⁰⁻¹² or by treatment with blocking antibodies.¹³⁻¹⁴ All these data point to a proatherogenic role for adaptive immunity, but the finding that immunization with oxidized LDL reduces lesions¹⁵ suggests that protective immunity may also occur. The role of immunocompetent cells in the disease process has, therefore, remained unclear.

To study the role of adaptive immunity in atherosclerosis, we generated immunodeficient, atherosclerosis-prone mice by crossing apoE⁻/⁻ mice with the scid/scid strain, which lacks T and B cells. Our data indicate that CD4⁺ T cells play an important role in atherosclerosis.

Methods

Animal Breeding and Immunological Analyses
ApoE⁻/⁻ mice (M&B, Bonholtgaard, Denmark) and scid/scid mice (Jackson Laboratory, Maine), both backcrossed for 10 generations to C57BL/6 background, were mated to generate apoE⁻/⁻/scid/+ offspring. The homozygous apoE⁻/⁻/scid/scid offspring were bred by brother-sister mating in a specific pathogen-free environment. Genetic screening was performed by polymerase chain reaction using apoE primers (Jackson Laboratory) and the scid gene primers 5'-GTCAGTCTCATTTGGCAATTG-3' and 5'-AGTATAACAGCTGGGTGTCG-3'.¹⁶ To minimize background differences, immunocompetent apoE⁺/⁻ mice were from the same founders as the apoE⁻/⁻/scid/scid mice. All experiments were approved by the local ethics committee. Female mice (n=4 to 6 per group) were fed standard mouse chow and killed at 18 weeks of age. Splenocytes were stained with FITC-conjugated anti-CD3, phyroerythrin anti-CD8, and CyChrome anti-CD4 and analyzed by flow cytometry. Interferon-γ, interleukin-4, interleukin-10, IgM, and IgG were analyzed in sera by sandwich ELISA (PharMingen), and serum cholesterol was analyzed by a cholesterol oxidase method (Boehringer Mannheim).

Cell Transfer
Splenocytes from 5-month-old female apoE⁺/⁻ mice were incubated in dishes for 90 minutes. Nonadherent cells were incubated with biotin-rat-anti-mouse-CD19 followed by the addition of streptavidin-coated microbeads and elimination on MiniMACS columns. Harvested cells were depleted of CD8 cells by a similar protocol. The purified cells were >95% viable and contained >99% CD3⁺CD4⁺ T cells and <1% CD22⁺ B cells, as judged by flow cytometry. A total of 18 to 20×10⁶ cells/mouse were injected into the tail veins of 6-week-old female apoE⁻/⁻/scid/scid mice.

Analysis of Atherosclerosis
Frozen sections were collected every 100 μm over a 500-μm interval from the aortic root, stained with oil red O–hematoxylin, and analyzed for lesion size.¹⁷ Immunohistochemical staining was performed using monoclonal anti-CD4 and anti-I-Aγ followed by biotin-avidin-horseradish peroxidase.¹⁷ Data were analyzed by ANOVA, the Mann-Whitney test, and Spearman’s rank correlation.
Results

The apoE–/–/scid/scid mice lacked T and B cells in lymphoid organs, and no immunoglobulins could be detected in sera. No significant differences were observed in body weight (data not shown) or serum cholesterol levels (12.1 ± 1.9 mmol/L in apoE–/–/scid/scid mice and 10.2 ± 0.9 mmol/L in apoE–/– mice).

Fatty streak lesions were significantly reduced in apoE–/–/scid/scid mice compared with apoE–/– animals (Figures 1 and 2). There was a 73% reduction in lesion size, with fewer macrophages, very few I-Aβ–expressing cells, and a complete absence of CD4+ cells in apoE–/–/scid/scid mice when compared with apoE–/– mice (Figures 1D, 1E, 1G, and 1H).

To explore the role of CD4+ T cells, such cells were isolated from the spleens of 5-month-old female apoE–/– mice, which have significant atherosclerosis,18 and injected into 6-week-old female apoE–/–/scid/scid recipients. Cell transfer did not affect body weight or serum cholesterol (data not shown). Serum levels of the Th1 cytokine interferon-γ were very low in apoE–/–/scid/scid mice, but they increased substantially (∼8×) after cell transfer, to a level not significantly different from that in immunocompetent apoE–/– mice (Figure 2B). The correlation between interferon-γ concentration and lesion size was r = 0.75 in the apoE–/–/scid/scid mice with and without CD4+ cell transfer and r = 0.56 in the entire material (P < 0.05). The Th2 cytokines interleukin-4 and interleukin-10 were not detected in sera from any of the groups. CD4+ but not CD8+ T cells were increased in the spleens of apoE–/–/scid/scid mice after cell transfer. Of the total spleen cells, CD4+ cells were 1.2 ± 0.5% of the total in apoE–/–/scid/scid mice, 7.7 ± 1.5% in apoE–/–/scid/scid mice transferred with CD4+ cells, and 13.3 ± 1.6% in apoE–/– mice; CD8+ cells were 0.2 ± 0.1%, 0.7 ± 0.3%, and 3.7 ± 0.8%, respectively.

Fatty streak lesions in the aorta were 164% larger in apoE–/–/scid/scid mice that received CD4+ T cells compared with untreated apoE–/–/scid/scid mice and not significantly smaller than those in fully immunocompetent apoE–/– mice (Figures 1 and 2). Substantial numbers of CD4+ cells infiltrated the lesions (Figure 1F), and abundant I-Aβ expression was also observed (Figure 1I).

Discussion

The present study shows that (1) mice lacking adaptive immunity exhibit reduced development of fatty streak lesions, (2) transfer of CD4+ T cells from atherosclerotic donors into immunodeficient recipients aggravates the atherosclerotic process, (3) CD4+ T cells home to lesions, and (4) T-cell transfer is accompanied by elevated systemic interferon-γ levels and expression of the interferon-γ–induced I-Aβ gene in the lesions. These findings are compatible with a proatherogenic role for CD4+ T cells and suggest that it is exerted, at least in part, by local action of the (interferon-γ–producing) Th1 subset in the artery wall.

The finding that mice lacking T and B cells have reduced atherosclerosis is in line with previous studies of mice lacking the recombinase-activating gene.10 That adaptive immunity plays an important accelerating role in...
LDL. Further experiments will be needed to test whether secretion suggests a Th1 dominance among effector cells. The spleen T cells of apoE–/– mice exhibit strong reactivity toward oxidized LDL. It is noteworthy that the spleen T cells of apoE–/– mice exhibit strong reactivity toward oxidized LDL. The spleen T cells of apoE–/– mice exhibit strong reactivity toward oxidized LDL. Furthermore, interferon-γ increases expression of I-Aβ in mice receiving CD4+ T cells suggests that transferred T cells induce gene expression in lesions through cytokine secretion. The present findings suggest that interferon-γ has an important aggravating effect on lesions.

Summary, our results demonstrate an important role for adaptive immunity in early atherosclerosis. It will now be important to identify antigens and effector molecules that activate proatherogenic immunity.

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References


Figure 2. Effects of immunodeficiency and CD4+ T cell transfer on lesion size and interferon-γ levels. A, Morphometric quantitation of lesion size (μm²) in aortic root. B, Interferon-γ concentration (pg/mL) in peripheral blood. Median is shown by line. *P<0.05 versus apoE–/– mice and apoE–/–/scid/scid animals. Although the low interferon-γ levels in the latter mice were probably caused by natural killer cell secretion, the high levels after the transfer of CD4+ T cells suggest that the Th1 activity of transferred cells generated large amounts of interferon-γ. Interestingly, interferon-γ receptor–deficient apoE–/– mice exhibit a substantial reduction in atherosclerosis and a changed lipid metabolism. Furthermore, interferon-γ accelerates transplant vascular lesions in arteries xenografted into scid/scid mice. Because the I-Aβ gene is induced by interferon-γ, increased expression of I-Aβ in mice receiving CD4+ T cells suggests that transferred T cells induce gene expression in lesions through cytokine secretion.

12.13 The CD4+ subset of T cells dominates in the lesions of patients and apoE–/– mice, and the pattern of local cytokine secretion suggests a Th1 dominance among effector cells. The present adoptive transfer experiments provide evidence for a proatherogenic role of these T cells. Injecting CD4+ T cells into apoE–/–/scid/scid recipients accelerated atherosclerosis to a level almost as high as that in immunocompetent apoE–/– mice. Because the transferred CD4+ T cells were obtained from atherosclerotic apoE–/– mice, it is likely that specific immunity to disease-related antigens was instrumental in producing this effect. It is noteworthy that the spleen T cells of apoE–/– mice exhibit strong reactivity toward oxidized LDL. Further experiments will be needed to test whether responses to this antigen account for the proatherogenic effect. The fact that transferred CD4+ T cells homed to atherosclerotic lesions suggests the presence of powerful recruitment mechanisms. It is also possible that some of the transferred cells may proliferate locally after encountering specific antigens in the lesions.

14.15 Interferon-γ levels were elevated in CD4+ T cell–recipient apoE–/–/scid/scid mice but low in untouched apoE–/–/scid/scid animals. Although the low interferon-γ levels in the latter mice were probably caused by natural killer cell secretion, the high levels after the transfer of CD4+ T cells suggest that the Th1 activity of transferred cells generated large amounts of interferon-γ. Interestingly, interferon-γ receptor–deficient apoE–/– mice exhibit a substantial reduction in atherosclerosis and a changed lipid metabolism. Furthermore, interferon-γ accelerates transplant vascular lesions in arteries xenografted into scid/scid mice. Because the I-Aβ gene is induced by interferon-γ, increased expression of I-Aβ in mice receiving CD4+ T cells suggests that transferred T cells induce gene expression in lesions through cytokine secretion. The present findings suggest that interferon-γ has an important aggravating effect on lesions.

In summary, our results demonstrate an important role for adaptive immunity in early atherosclerosis. It will now be important to identify antigens and effector molecules that activate proatherogenic immunity.
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