Adoptive Transfer of CD4+ T Cells Reactive to Modified Low-Density Lipoprotein Aggravates Atherosclerosis

Xinghua Zhou, Anna-Karin L. Robertson, Charlotta Hjerpe, Göran K. Hansson

Objective—Atherosclerosis is associated with immune responses to oxidized low-density lipoprotein (oxLDL). The presence of activated macrophages and T cells in lesions suggests that cell-mediated immune reactions are taking place during the disease process. However, the role of specific immune responses has remained unclear. We have previously shown that transfer of CD4+ T cells from apolipoprotein E knockout mice (apoE−/−) into immunodeficient apoE−/− scid/scid mice accelerates disease.

Methods and Results—To test whether this effect is dependent on specific disease-associated antigens, purified CD4+ T cells from oxLDL-immunized mice were transferred into apoE−/− scid/scid mice. CD4+ T cells from mice immunized with a nonrelevant antigen, keyhole limpet hemocyanin (KLH), and naïve CD4+ T cells were used as controls. After 12 weeks, all mice that received T cells had larger lesions than untouched apoE−/− scid/scid controls. However, mice receiving CD4+ T cells from oxLDL immunized mice had substantially accelerated lesion progression compared with those receiving naïve or KLH-primed T cells. Circulating levels of interferon-γ were increased in proportion to the acceleration of atherosclerosis.

Conclusion—These data show that adoptive transfer of purified CD4+ T cells from oxLDL-immunized mice accelerates atherosclerosis. They support the notion that Th1 cellular immunity is proatherogenic and identify oxLDL as a culprit autoantigen. (Arterioscler Thromb Vasc Biol. 2006;26:864-870.)

Key Words: atherosclerosis ▪ lymphocytes ▪ low-density lipoprotein ▪ immune system ▪ mice, knockout−/−

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Research over the last decades has identified immune mechanisms as pathogenically important in atherosclerosis.1,2 T lymphocytes and macrophages are present in lesions throughout the development of disease, both in man and experimental models.3-6 Most of the T cells in the lesions are CD4+ TCRαβ+ T cells expressing activation markers such as human leukocyte antigen-DR (HLA-DR) and interferon-γ (IFN-γ).3,4,7-9 The expression of major histocompatibility complex (MHC) class II molecules such as HLA-DR on activated macrophages and activated T cells adjacent to these macrophages in the lesions strongly suggests that cell-mediated immune reactions are taking place in atherosclerosis.

A series of investigations have suggested modified low density lipoproteins (LDLs) as autoantigens in atherosclerosis.10 LDL accumulates in lesions, where it is modified by oxidative and enzymatic processes.11-15 Circulating autoantibodies to epitopes of oxidized LDL (oxLDL) have been detected in patients and experimental animals with atherosclerosis.13,16 and antibodies isolated from atherosclerotic lesions recognize oxLDL.17 Macrophages can take up, process, and present neoantigens such as oxLDL to CD4+ T cells, eliciting cellular immune responses.18 Indeed, ∼10% of the CD4+ T cells cloned from human lesions recognize oxLDL in an MHC class II–dependent manner.9

The apolipoprotein E knockout (apoE−/−) mouse is a useful model of human disease because it develops severe hypercholesterolemia and spontaneous atherosclerosis.19,20 In this model, a local immune response dominated by CD4+ T cells occurs in all phases of atherosclerosis.5,21 T cells from immune organs of apoE−/− mice exhibit strong reactivity toward oxLDL.22 In addition, high titers of circulating autoantibodies to oxLDL are detected in this model.23 However, the role of the T-cell immune response to modified LDL in the disease process has remained unclear.

To study the role of adaptive immunity in atherosclerosis, we generated an immunodeficient apoE−/− scid/scid mouse by crossing apoE−/− mice with the scid/scid strain, which lacks T and B cells.24 We reported previously that transfer of CD4+ T cells from aged apoE−/− mice into immunodeficient apoE−/− scid/scid mice accelerates the progression of atherosclerosis.24 To determine whether this effect is dependent on specific disease-associated antigens, CD4+ T cells from mice immunized with oxLDL were transferred into apoE−/− scid/
scid mice. Controls included CD4+ T cells from mice immunized with a nonrelevant antigen, keyhole limpet hemocyanin (KLH), or with incomplete Freund’s adjuvant (IFA) alone, as well as untouched mice. Our results demonstrate an important role for the oxLDL-specific T-cell response as an accelerating factor in atherosclerosis.

Methods

Animals, Diets, and Serum Analysis
C57BL/6J mice obtained from Taconic Europe, Ry Denmark, and apoE−/− scid/scid mice on the C57BL/6J background were generated in our laboratory,24 kept under sterile conditions, and fed standard mouse chow. All experiments were approved by the local ethics committee. The apoE−/− scid/scid mice were injected intravenously with CD4+ T cells at 7 weeks of age and euthanized at 16 weeks. To exclude a potential “leakage” (ie, appearance of immune cells), which has been reported in aged scid/scid animals, we assessed the frequency of immunocompetent cells by flow cytometry. Splenocytes were stained with fluorescein isothiocyanate–conjugated rat anti-CD3, phycoerythrin-conjugated rat anti-CD8, phycoerythrin-conjugated rat anti-CD19, and CyChrome conjugated rat anti-CD4. All antibodies were from PharMingen. Blood was obtained by heart puncture at euthanization and allowed to clot. Sera were centrifuged at 14,000 g for 20 minutes to remove chyomicrons. The serum IFN-γ, interleukin-4 (IL-4), and IL-5 levels were analyzed by sandwich ELISA with OptElla antibody sets (PharMingen) and serum cholesterol by the cholesterol oxidase method (Boehringer Mannheim).

Homologous LDL Isolation and Modification
Blood was obtained by heart puncture from 8- to 10-week-old apoE−/− mice and pooled into vacuum tubes containing K2EDTA. LDL was isolated from plasma by ultracentrifugation through a discontinuous NaCl gradient of 1.020 to 1.063 mg/mL for 20 hours at 4°C in a Beckman L8-80 ultracentrifuge with a 50.3-Ti Beckman fixed-angle rotor.16,25 In plasma from apoE−/− mice, this density cutoff may contain chyomicron remnants in addition to LDL.16,26 The protein content was determined by the Lowry method, and the LDL preparation with added Na2EDTA (1 mg/mL) was sterile filtered, kept at 4°C under N2, and used within 2 weeks. Malondialdehyde (MDA) modification of LDL was performed as described previously.25

Immunization Protocol and Analysis of Antigen Specificity
At 6 weeks of age, male C57BL/6J donor mice were randomly divided into 3 groups (n=40 per group). Two groups were injected peritoneally with either homologous MDA-LDL (100 μg protein per mouse) or KLH (Pierce; 100 μg protein per mouse) and boosted once after 2 weeks. MDA-LDL and KLH were emulsified with IFA before immunization. The third group was used as donors of naïve T cells.

Splenocytes were obtained on day 5 after the booster. The splenocytes from individual mice were cultured in duplicate in serum-free medium including 1:100 ITS+Premix (Collaborative Biomedical), 1 mg/mL BSA, 10 mmol/L HEPES, 1 mmol/L Na pyruvate, 1 mmol/L nonessential amino acids, and 50 μg/mL gentamicin sulfate (Sigma) after osmotic lyses of red blood cells. These cells were incubated in the absence of or in the presence of titrated amounts of homologous MDA-LDL or KLH for 72 hours. The incubation was followed by overnight incorporating with 3H-thymidine for the assay of cell proliferation. To quantify the T-cell cytokines secreted by the splenocytes, supernatants harvested 72 hours after incubation were measured using a Th1/Th2 Cytokine CBA Kit (BD Biosciences) according to manufacturer instruction.

Immunization with:

- KLH in IFA
- MDA-LDL in IFA

Donors: C57BL/6J

Recipient: ApoE−/− x scid/scid

7 weeks of age

Figure 1. Illustration of experimental design. At 6 weeks of age, C57BL/6J mice were injected peritoneally with either homologous MDA-LDL or KLH in IFA and boosted after 2 weeks. An untreated group served as donors of naïve T cells. Purified splenic CD4+ T cells were transferred intravenously into the apoE−/− scid/scid mice.

Negative Selection and Adoptive Transfer of CD4+ T Cells
Splenocytes from immunized mice were pooled and incubated in plastic dishes for 90 minutes at 37°C after red blood cells were lysed. Nonadherent cells were incubated at 4°C for 30 minutes with biotin-conjugated rat-anti-mouse-CD19/CD22, anti-mouse–CD8, anti-mouse–Mac-1 (CD11b), and anti-mouse–c-kit (CD117) to remove potential peripheral stem cells. This was followed by addition of streptavidin-coated microbeads and elimination on MiniMACS columns. The purified cells were >95% viable and contained >99% CD3+CD4+ T cells and <1% CD22+B cells as judged by flow cytometry. At 7 weeks of age, female apoE−/− scid/scid mice were randomly divided into 4 groups (n=5 to 9 per group) as follows: (1) transfer of CD4+ T cells from mice immunized with MDA-LDL (meanLDLCD4+), (2) transfer of CD4+ T cells from KLH-immunized mice (mdsCD4+), (3) transfer of CD4+ T cells from unimmunized mice (laaCD4+), and (4) untouched controls. Every mouse received 12×106 CD4+ T cells via the tail vein. All recipients were euthanized at 16 weeks of age (ie, 9 weeks after cell transfer).

Quantitation of Plaque Size, Cellular Components, and Markers of Immune/Inflammatory Activation
Recipient apoE−/− scid/scid mice were euthanized in a CO2 chamber at 16 weeks of age and perfused transcardially with PBS. The heart was dissected out and the tissue segment from sinus aorticus to the lower tips of the right and left atria was isolated, snap-frozen in liquid nitrogen, and embedded with O.C.T. (Tissue-Tek) compound. The tissue was sectioned at a thickness of 10 μm. For morphometric analysis, 5 sections starting at the level of the aortic valves were chosen at 100-μm intervals. Sections were stained with Oil Red O and counterstained with hematoxylin. The size of the plaque was measured with Leica Q500MC image analysis software. The mean value of plaque cross-section areas from 5 sections was used to estimate the extent of atherosclerosis.24

To evaluate T-cell infiltration and inflammatory molecule expression in the plaques, immunohistochemistry staining was performed by using monoclonal biotin-conjugated rat anti-CD4, anti-I-Aκ (MHC class II) and anti–vascular cell adhesion molecule-1 (VCAM-1) followed by avidin-borseradish peroxidase.24

Statistics
Results are expressed as mean±SEM. Data were analyzed by the Mann–Whitney test. The significance level was set at P<0.05.

Results

Induction of Antigen-Specific T Cells
The adoptive transfer experiment was performed to assess the role of specific antigen in the disease-aggravating action of
CD4⁺ T cells on atherosclerosis. Six-week-old C57BL/6J (denoted B6) rather than apoE⁻/⁻ mice were chosen as donors because their T cells are largely naïve, whereas the autoimmune process starts early in apoE⁻/⁻ mice. The experimental design is outlined in Figure 1. Donor B6 mice were immunized twice with MDA-LDL or the control antigen KLH. To avoid inducing a T-cell response to other antigens (e.g., heat shock proteins), IFA rather than complete Freund’s adjuvant

Figure 2. Induction of antigen-specific T cells in immunized donor mice and effects of CD4⁺ T cell transfer on circulating IFN-γ levels. A and B, Splenocyte proliferation assay. The cells from individual mouse after lyses of red blood cells were cultured in serum-free medium with titrated amounts of homologues MDA-LDL or KLH and the antigen-specific proliferation was measured by ³²P-thymidine incorporation. C through I, Antigen-specific T-cell cytokine secretion. Analysis of the cytokines in cultures of the cells from individual mouse was performed after 72-hour incubation with different amounts of antigens. J, IFN-γ concentration (pg/mL) in peripheral blood of the T-cell receivers at 16 weeks old. Mean±SD; n=6; *P<0.05 vs control.
was used in our immunization protocol. The splenocytes of MDA-LDL–immunized mice showed a dose-dependent proliferation in response to in vitro stimulation with MDA-LDL but not to KLH (Figure 2A and 2B). Strong systemic IgG responses were registered to the respective antigens (Figure I, available online at http://atvb.ahajournals.org). T-cell proliferation was accompanied by increased expression of the T-cell cytokines IFN-γ and IL-2 (Figure 2C and 2D). Splenocytes collected from KLH–primed mice exhibited significant proliferation when exposed to KLH but not to MDA-LDL in vitro and was accompanied by increased expression of the T-cell cytokines IFN-γ, IL-2, and IL-5 (Figure 2B, 2F through 2H). The results of the proliferation assay and the expression pattern of the T-cell cytokines therefore indicated the induction of antigen-specific T cells to the respective antigens used for immunization. No IL-4 production was detected in any of the groups, as is usually the case with B6 antigens used for immunization. No IL-4 production was detected in any of the groups (data not shown). Robust IL-5 secretion was registered in the group transferred with MDA-LDL–immunized T cells, but not in the group transferred with KLH–immunized T cells (data not shown). Phenotype of Recipient ApoE<sup>−/−</sup> scid/scid Mice At 16 weeks of age, no differences were registered in body weight or serum cholesterol levels between treatment groups (Table). No T cells, B cells, or immunoglobulins could be detected in the untouched apoE<sup>−/−</sup> scid/scid mice. As expected, CD4<sup>+</sup> T cells but not CD8<sup>+</sup> T cells or B cells were found in mice that had received CD4<sup>+</sup> T cell transfer. There was no significant difference in the number of CD4<sup>+</sup> T cells in spleens between the groups that received T cells from different donor groups (Table). Interestingly, serum concentrations of the Th1 cytokine IFN-γ were very low both in untreated apoE<sup>−/−</sup> scid/scid mice and in mice receiving either naïve CD4<sup>+</sup> or KLH CD4<sup>+</sup> T cells but increased significantly in the group transferred with MDA-LDL–immunized CD4<sup>+</sup> T cells (Figure 2J). This may reflect an antigen-specific activation of MDA-LDL–specific CD4<sup>+</sup> cells in vivo. Serum levels of the Th2 cytokines IL-4 and IL-5 were below the detection limit for all groups (data not shown).

Lesion Development in ApoE<sup>−/−</sup> scid/scid Mice After Adoptive Transfer

Compared with untreated apoE<sup>−/−</sup> scid/scid mice, fatty streak lesions in the aortic root were on average 227% larger in apoE<sup>−/−</sup> scid/scid mice that received MDA-LDL–immunized CD4<sup>+</sup> T cells, 112% larger in the KLH–immunized CD4<sup>+</sup> T cell transfer group, and 127% larger in the group receiving naïve CD4<sup>+</sup> T cells from untouched B6 mice (Figure 3). Lesion development was significantly increased in apoE<sup>−/−</sup> scid/scid mice that received MDA-LDL–immunized CD4<sup>+</sup> T cells compared with those receiving either KLH–immunized or naïve CD4<sup>+</sup> T cells (P<0.008 and 0.019; Figure 3). The latter 2 treatment groups did not differ significantly from each other with regard to lesion size but differed from lesion size in untreated mice (Figure 3).

T-Cell Homing and Inflammatory Molecule Expression in the Lesions After Specific T-Cell Transfer

Immunohistochemical analysis showed a strikingly increased infiltration of CD4<sup>+</sup> cells into the lesions after MDA-LDL–immunized T cell transfer (Figure 4A and 4B) compared with other treatments. Furthermore, expression of the MHC class II molecule I-A<sup>+</sup> was increased several-fold in mice receiving MDA-LDL–immunized CD4<sup>+</sup> T cells (Figure 4A and 4C). VCAM-1 expression was significantly increased in the lesions of all apoE<sup>−/−</sup> scid/scid mice that had received CD4<sup>+</sup> T cells, irrespective of the specificity of the transferred cells (Figure 4A and 4D).

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<td>Spleen CD4&lt;sup&gt;+&lt;/sup&gt;CD3&lt;sup&gt;+&lt;/sup&gt;, % of splenocytes</td>
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Serum cholesterol was determined enzymatically and the cell type analyzed by flow cytometry. n=4 to 6 mice per group. Mean±SEM.
Discussion

The results of this study show, for the first time, that adoptive transfer of cell-mediated immunity to oxLDL accelerates atherosclerosis. Thus, transfer of CD4+ T cells from MDA-LDL–immunized mice caused a 3.4-fold increase in lesion size in apoE−/− scid/scid animals. This was nearly a doubling of lesion size when compared with the situation in mice receiving CD4+ T cells from mice immunized with another antigen: KLH. This increase was accompanied by systemic elevation of the Th1 cytokine IFN-γ, by increased penetration of CD4+ T cells into lesions, and by augmented expression of (the IFN-γ–inducible) I-Ab protein in the lesions, supporting the notion that Th1 cells producing IFN-γ play an important role in the disease process. The fact that transfer of CD4 cells into immunodeficient mice aggravates the disease is in line with our previous finding that absence of CD4 cells in apoE−/− scid/scid mice leads to reduced atherosclerosis.26 A proposed mechanism by which the transferred MDA-LDL–specific CD4+ T cells promote atherosclerosis was illustrated (Figure 5). It should be noted that the cholesterol levels were not different among the groups, therefore the acceleration of the disease progression was unlikely because of effects on lipid metabolism. In parallel with augmented lesion development, the mice that received MDA-LDL CD4+ T cells showed an increase in the number of CD4+ T cells in lesions. However, the proportion of CD4+ T cells in the spleen did not differ between the recipients of MDA-LDL CD4+ T cells and of KLH CD4+ T cells. These findings suggest that the MDA-LDL–specific CD4+ T cells homed to and expanded in the atherosclerotic lesions, where the MDA-LDL antigen accumulates.

A Th1 response to MDA-LDL was registered when splenocytes harvested from the mice immunized with MDA-LDL were challenged with MDA-LDL in vitro. In contrast, Binder et al reported recently that immunization with MDA-LDL induces Th2-biased responses,27 although complete Freund’s adjuvant-containing mycobacteria, which was used in their study, is well known to be more prone to induce Th1 responses than IFA, which we used.28 The discrepancy between 2 studies may be explained by the different types of adjuvant, the dose of antigen used for the immunization, the number of boost injections, the composition of the culture medium, or the concentration of antigen applied in vitro. Serum-free medium was used in our study because sera used in cell culture contain high levels of LDL, which may obscure any T-cell response to exogenous LDL particles. Importantly, the peak response of the T cells in vitro to MDA-LDL was detected when a low concentration of oxLDL was added to the cultures, whereas a concentration of oxLDL >20 μg/mL was found to induce cytotoxicity. The precise T-cell epitopes in the oxLDL preparations remain to be identified.

Interestingly, transfer of naïve CD4+ or KLH CD4+ T cells resulted in modestly increased lesions with signs of increased...
inflammatory activity when compared with untouched control apoE \(^{-/-}\) scid/scid mice. This may reflect the presence in the naive CD4\(^+\) and the K/Lb CD4\(^+\) T cell preparations of a T-cell population with the capacity to recognize modified LDL or other proatherogenic antigens. Such cells would be activated in situ during the course of the disease, respond immunologically, and aggravate disease development. Alternatively, the homeostatic proliferation of cells transferred in an immunodeficient host could accelerate atherosclerosis because of cytokine secretion or other immune interactions.

VCAM-1 is abundantly expressed in atherosclerotic lesions.\(^ {29-31} \) It can be induced via the nuclear factor \( \kappa B \) pathway, which is activated by proinflammatory cytokines, endotoxins, and several other stimuli.\(^ {32} \) In the current study, VCAM-1 was induced to approx the same extent, irrespective of the antigenic specificity of transferred T cells. Our findings suggest that VCAM-1 expression reflects vascular inflammation but is less dependent on antigenic specificity than I-A.

Several gene products of activated T cells are likely to be important in atherosclerosis. Numerous studies point to a proatherogenic role of Th1 products such as IFN-\( \gamma \) and TNF-\( \alpha \).\(^ {1,4,33,34} \) Not only does IFN-\( \gamma \) promote antigen presentation by upregulating MHC genes, it also controls scavenger receptor expression as well as cholesterol efflux in macrophages, proliferation, differentiation and collagen production in smooth muscle cells, and hemostatic properties of the endothelium.\(^ {1,35,36} \) TNF-\( \alpha \) released by activated Th1 cells has profound effects on adhesion molecule expression, lipoytic enzymes, and several other phenomena. The cell surface molecule CD40 ligand (CD154) can activate macrophages and vascular cells, which can express CD40; this leads to secretion of proteolytic enzymes, tissue factor, and other important factors in the atherogenic process.\(^ {37} \) Other costimulatory proteins expressed by immune cells include OX40L, a genotype of which was recently found to associate with coronary artery disease in man.\(^ {38} \) All these effector molecules may be involved in the proatherogenic activity of CD4\(^+\) T cells, but it remains to be determined whether the properties of the activating antigens affect the effector repertoire. At any rate, our results confirm the notion that CD4\(^+\) T cells are proatherogenic, support the notion that their disease-aggravating activity may be mediated via Th1 effector molecules, and show that antigenic specificity for the disease-associated antigen oxLDL is associated with dramatic proatherogenic activity. This may be because oxLDL accumulates in the lesions, where it provides a strong local stimulus for T-cell activation.

It has been shown that systemic immunization with oxLDL decreases lesion development with significant elevation of IgG antibodies against oxLDL.\(^ {25,39-41} \) The finding that immunization with MDA-LDL protects from atherosclerosis in immunocompetent apoE\(^{-/-}\) mice\(^ {25,26,39-41} \) whereas transfer of CD4\(^+\) T cells from mice immunized with MDA-LDL into immunodeficient apoE\(^{-/-}\) mice aggravates disease in this study may appear puzzling. However, the apoE\(^{-/-}\) scid/scid mice lack both B and T cells. Therefore, any effects of B cells, including antibody-producing plasma cells derived from them as well as effects of CD8\(^+\) T cells, were absent in the present transfer study. In addition, the antigen-presenting cells in apoE\(^{-/-}\) scid/scid are never directly exposed to the antigens in the preparation used for immunization, including adjuvant, precluding a possible development of tolerogenic antigen-presenting cells. Furthermore, any early protective mechanisms induced by the immunization may not be transferred if the transferred cells are isolated as late as 20 days after the immunization. The fact that the immunization-induced atheroprotection remains in the mice lacking CD4 suggests that other cells than CD4\(^+\) T cells may play an atheroprotective role after immunization.\(^ {26} \) Recent reports also suggest that humoral immune responses may have protective effects on disease development.\(^ {9,42} \) Because immunization with oxLDL enhances both cellular and humoral immune responses to the atherosclerosis-related antigen, it is possible that some of the mechanisms activated by immunization are deleterious, whereas others are protective. The timing for the induction of such reaction may also relevant. Further studies are necessary to explore how the balance between such protection and aggravation induced by immunization can be controlled.

To summarize, the present data identify by adoptive transfer methodology that oxidatively modified LDL is an autoantigen that promotes atherosclerosis in hypercholesterolemic mice.

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References

2. Zhou X, Hansson GK. Immunomodulation and vaccination for athero-
12. Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, Currist LK, Corr M, Witztum JL. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from athero-
13. Billiau A, Mathys P. Modes of action of Freund’s adjuvants in experi-
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Figure 1

A

IgG anti-KLH

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IgG anti-MDA-LDL

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Figure I. Induction of T cell dependent IgG antibody responses in immunized donor mice. ELISA of sera from donor B6 mice immunized with KLH (A) and MDA-LDL (B) show induction of high-titer IgG antibodies to the specific immunogen but not to another antigen. n=6.