Lesion Development and Response to Immunization Reveal a Complex Role for CD4 in Atherosclerosis

Xinghua Zhou, Anna-Karin L. Robertson, Mats Rudling, Paolo Parini, Göran K. Hansson

Abstract—Atherosclerosis is a complex disease, bearing many of the characteristics of a chronic inflammatory process. Both cellular and humoral immune responses may be involved in the disease development. Oxidized low-density lipoprotein (oxLDL) is suggested to be an autoantigen in atherosclerosis. A protective effect against atherosclerosis has been demonstrated in animals immunized with oxLDL. Such a protection is associated with elevation of T cell–dependent IgG antibodies against oxLDL. In addition, it has been shown that immunization with Freund adjuvant alone also confers protection against development of atherosclerosis. We therefore hypothesized that CD4+ T cells are critical in the development of atherosclerosis and that they are involved in protective immune reactions after immunization. The development of atherosclerosis was studied in apolipoprotein E knockout (apoE KO) mice and CD4/apoE double knockout (dKO) mice that were immunized with either oxLDL in Freund adjuvant or adjuvant alone, or left untreated. Our results show that (1) the absence of CD4+ cells in apoE KO mice leads to reduced atherosclerosis, indicating that CD4+ cells constitute a major proatherogenic cell population, and (2) the atheroprotective effect of LDL immunization does not depend on CD4+ cells, whereas (3) the atheroprotective effect of adjuvant injection is CD4-dependent. These findings demonstrate complex roles of immune cell–cell interactions in the regulation of the atherosclerotic process and point to several possible targets in the treatment and prevention of atherosclerosis. (Circ Res. 2005;96:427-434.)

Key Words: atherosclerosis ■ vaccine ■ low-density lipoprotein ■ CD4+ T lymphocytes ■ antibodies

Atherosclerosis is a complex disease, bearing many of the characteristics of a chronic inflammatory process.1,2 T lymphocytes, occurring concomitantly with macrophages, are found in human lesions with substantial number both in very early stages and in advanced plaques.3,4 Most of the T cells in the lesions are CD4 TCRα/β T cells expressing IFN-γ, and the expression of MHC class II molecules on activated macrophages and the activated T cells adjacent to these macrophages in the lesions, along with evidence of clonal expansion of T cells within the plaque,5 strongly suggests that cell-mediated immune reactions are taking place within the atherosclerotic lesion.3,6–8 Transfer of CD4+ T cells into immunodeficient atherosclerotic mice accelerates the disease.9 In addition, a few TCRγ/δ T cells as well as varying numbers of CD8+ T cells are also present in the plaque.4,10–12 All these findings point to an important role for T cells in atherosclerosis.

A series of investigations have suggested oxidized low-density lipoprotein (oxLDL) to be an autoantigen in atherosclerosis.13,14 LDL consists of the protein apoB100 together with triglycerides, cholesterol, and phospholipids. LDL oxidation has been recognized to play a critical role in the development of atherosclerosis.13,14 Highly reactive products of lipid peroxidation, such as malondialdehyde (MDA), bind to free amino groups in apoB as well as to phospholipids.13 OxLDL is present in atherosclerotic lesions and can stimulate the recruitment of immune cells.15–17 High titers of IgG and IgM autoantibodies specific for oxLDL have been found in the circulation and in lesions of humans and animal models.18,19 Macrophages can present neoantigens such as oxLDL to elicit a T-cell immune response,20 which is supported by the finding that approximately 10% of the T cells cloned from human lesions recognized oxLDL in an MHC class II–dependent manner.8

A protective effect against atherosclerosis has been demonstrated in animals immunized with oxLDL or plaque homogenate.21–24 Such a protection is associated with elevation of T cell–dependent IgG antibodies against MDA-LDL, suggesting a role for T helper cells in antigen-induced disease protection.24 In addition, MDA-LDL–specific CD4+ T cells have been found in draining lymph nodes after immunization.24 Recently, several groups have shown that immunization with Freund adjuvant alone, which stimulates antigen-presenting cells involved in T-cell activation, also confers protection against development of atherosclerosis.25,26 We hypothesized that CD4+ T cells are critical in the development of atherosclerosis and that they are involved in protective immune reactions after immunization.24 We therefore...
studied the effect of CD4 deficiency on atherosclerosis formation and on immunization-induced disease-related responses by using a newly generated CD4/apoE double knockout mouse (CD4/apoE dKO).

**Materials and Methods**

**Mice**

CD4/apoE dKO mice were generated by crossing CD4 KO mice with apoE KO mice and generously provided by Drs Francis Bayard and Rima Elhage in INSERM U397, Institut L. Bugnard, Toulouse, France. Both strains had been backcrossed into a C57Bl/6 background for 10 generations. The apoE single knockout mice were on the same background as the CD4/apoE dKO and were housed identically in the animal department of Microbiology and Tumor Biology Center at Karolinska Institutet. To confirm the absence of the CD4 molecule in CD4/apoE dKO and amount of monocytes in both strains, spleen cells were stained with antibodies against CD4, CD8, and CD19 as well as CD11b (Pharmingen) and analyzed by flow cytometry (Becton Dickinson). All mice were fed with normal chow, and all experiments were approved by the local ethics committee.

**Antigen Preparation and Immunization**

LDL isolation and modification were performed as described before. At 6 weeks of age, both female apoE KO and CD4/apoE dKO mice were randomly grouped (n = 7 to 8). Untreated mice were used as controls. Two groups of mice were immunized subcutaneously either with homologous MDA-LDL (100 μg protein/mouse) or PBS, and boosted five times at 2-week intervals. The antigen or PBS used in the first injection was emulsified with complete Freund adjuvant and incomplete Freund adjuvant was used for booster injections. The mice were euthanized at 18 weeks of age.

**Quantitation of Plaque Size and Evaluation of Lesion Inflammation**

To estimate disease progression, we measured lesions in the aortic root. For morphometric analysis, five sections starting at the level of the aortic valve were collected 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 five sections was used to estimate the extent of atherosclerosis. To evaluate the extent of inflammation in the plaques, immunohistochemical staining was performed by using antibodies anti-CD68, I-A^d^ (murine MHC class II), and VCAM-1 (all from Pharmingen), followed by biotin-avidin-horseradish peroxidase detection.

**Determination of Antibody Titers**

To determine antibody titers and specificity, blood was obtained by heart puncture in conjunction with euthanasia. For the ELISA assay, mouse sera were centrifuged at 14,000 g for 30 minutes to remove chylomicrons. ELISA was used to quantity IgG1, IgG2a, IgG2b, and IgM antibodies against MDA-LDL. Alkaline phosphatase-labeled goat antibodies to mouse IgG1, IgG2a, IgG2b, and IgM were from Southern Biotechnology.

**Real-Time Polymerase Chain Reaction for Quantification of mRNA Expression**

Total RNA from mouse spleens (n = 7/group) was extracted reverse-transcribed to cDNA and analyzed by real-time polymerase chain reaction (PCR) as previously described. The following primers and probes were applied: Mouse IFN-γ: FW, 5'-AGCGAATGACGGCCAAA-3'; RW, 5'-CTGGAGCTTGTTGTTGTTGTA-3'; and TM, 5'-GCCCAACTGACCCTCAGATTTATATTC-3'; Mouse IL-4: FW, 5'-CAAGACAGGAAACGCGCATCA-3'; RW, 5'-GAAGCCCCATAACCCGAGCTCA-3'; and TM, 5'-TCTTCACAGCAAGACAAGA-

AGAACACACACA-3. Mouse CD68: FW, 5'-CAAGGTTCCAGG-GAGGTTGTG-3'; RW, 5'-CCAAGGTAAAGCTGCTCATAAGG-3'; and TM, 5'-GGTACCCATCCACACCATCTC-3'. Mouse SR-A: FW, 5'-AGAGTCGAAAGACATTTCGAACTC-3'; RW, 5'-TTTCG-CCAATGGCTAAATTTCCG-3'; and TM, 5'-AACCGAATGATGTTGTTGTGTCGCTCTG-3'. Mouse CD4: FW, 5'-GCACAGCTTAACTTCATTTCAATAG-3'; RW, 5'-AGATGCTTCTCCGATGTTGTGTC-3'. Mouse HPRT (Hypoxanthine phosphoribosyl transferase): FW, 5'-TAAAACAATGCAAACTTTGCTTTCC-3'; RW, 5'-TCCTTCTCCACCAAGCTTG-3'; and TM, 5'-TCTTTACCCAGCAAGCTTG-3'; and TM, 5'-TCTTTACCCAGCAAGCTTG-3'. Mouse Mac-1: FW, 5'-ACGAGAAGAAGGGACGCCAT-3'; RW, 5'-GAAGCCCTTCAGTCTTCGATGTTGTC-3'. Mouse CD68: FW, 5'-ACAGGAGAAGGGACGCCAT-3'; RW, 5'-GAAGCCCTTCAGTCTTCGATGTTGTC-3'. Mouse CD68: FW, 5'-ACAGGAGAAGGGACGCCAT-3'; RW, 5'-GAAGCCCTTCAGTCTTCGATGTTGTC-3'.

**Statistical Analysis**

Data were analyzed by Wilcoxon nonparametric test. The significance level was set at P < 0.05.

**Results**

**Immunologic and Metabolic Phenotype of CD4/apoE dKO Mice**

ApoE KO mice that also carried targeted deletions in the CD4 gene exhibited a complete absence of CD4+ cells in the spleen, with a compensatory increase in the CD8+ population (P = 0.008; Figure 1a and 1b). However, no difference in percentage of B cells was found between two strains (Figure 1c and 1d). CD4/apoE dKO mice displayed significantly higher serum triglyceride levels (1.82 ± 0.22 versus 1.15 ± 0.04 mmol/L; P = 0.02) and lower body weights (20.9 ± 0.4 versus 23.3 ± 0.7 g; P = 0.008) than apoE KO mice. There was no significant difference in total serum cholesterol levels between the groups. Immunization with PBS or MDA-LDL did not affect triglyceride and cholesterol levels nor weight (data not shown). Gel filtration chromatography of lipoproteins analysis showed significantly increased triglycerides within chylomicron/VLDL fraction (0.62 ± 0.11 versus 0.30 ± 0.03 mmol/L; P = 0.003) and 0.12 ± 0.01 versus 0.05 ± 0.01 mmol/L; P = 0.004) in CD4/apoE dKO mice compared with apoE single-KO animals, whereas cholesterol in chylomicron/VLDL is equally high in both strains (Figure 1f and 1e). In parallel, HDL cholesterol was significantly increased in the CD4/apoE dKO mice (0.63 ± 0.1 versus 0.41 ± 0.09 mmol/L; P = 0.004) (Figure 1e).

**Reduced Atherosclerosis With Decreased Inflammation in Mice Lacking CD4+ Cells**

The development of atherosclerosis was significantly reduced in CD4/apoE dKO mice, with less advanced lesions in the proximal aorta (Figure 2a). At 18 weeks of age, CD4/apoE dKO mice showed a 70% reduction in lesion size as compared with apoE KO mice. The cellular composition of the lesions was similar, with a predominance of lipid-laden.
CD68+ macrophages (Figure 2b). However, expression of the activation markers I-A <sup>+</sup>, reflecting IFN-γ signaling, and VCAM-1, which depends on NF-κB activation, were substantially reduced in CD4/apoE dKO mice (Figure 2b). These findings imply parallel reductions in both atherosclerotic lesion size and local inflammation in the mice lacking CD4.

**Decreased Titers of Circulating Autoantibodies to Modified LDL**

ELISA of serum antibodies to MDA-LDL was used to assess autoimmune responses to oxidized lipoproteins, which are known to be associated with atherosclerosis. The titers of T cell–dependent IgG1 and IgG2a anti–MDA-LDL were significantly decreased in untreated CD4/apoE dKO mice (Figure 3b and 3c), indicating a role for CD4+ T cells providing help to B cells in the production of such autoantibodies. IgG2b antibodies to MDA-LDL did not differ between groups (Figure 3d), whereas IgM antibody titers were significantly reduced in mice lacking CD4 (Figure 3a).

**Reduced IFN-γ and CD68 Expression in CD4/apoE dKO Mice**

In comparison with apoE single-KO mice, the CD4/apoE dKO mice showed significantly reduced expression of IFN-γ mRNA in spleen cells, confirming an important role for CD4+ cells in IFN-γ production (Figure 4a). No significant changes were observed with regard to IL-4 or TNF-α expression (Figure 4b and 4c). Similarly, the splenic expression of scavenger receptors SR-A and CD36 did not differ significantly between strains, whereas CD68/macrosialin was re-
duced in mice lacking CD4 (Figure 4d through 4f). This implies differential effects of the CD4 deficiency on genes involved in scavenger functions. Meanwhile, the number of CD11b per cell appeared to be slightly lower in mice lacking CD4 (Figure 1g).

**MDA-LDL Immunization Protects Against Atherosclerosis in the Absence of CD4+ T Cells**

To determine the role of CD4 in immunoprotection against atherosclerosis, apoE KO and CD4/apoE dKO mice were immunized with homologous, MDA-modified LDL every 2 weeks, starting at 6 weeks of age. Immunization of fully immunocompetent apoE KO mice resulted in a 23% reduction in mean lesion size at 18 weeks of age as compared with PBS/adjuvant immunized controls and in a 39% reduction as compared with untreated mice (Figure 2a). This is in agreement with previous findings that oxLDL immunization ameliorates atherosclerosis. As mentioned, CD4/apoEdKO mice displayed reduced lesion size when compared with apoE KO mice. Surprisingly, CD4/apoE dKO mice exhibited a 39% decrease in lesion development after MDA-LDL immunization as compared with PBS-immunized controls and in a 39% reduction as compared with untreated mice (Figure 2a). This is in agreement with previous findings that oxLDL immunization ameliorates atherosclerosis. As mentioned, CD4/apoEdKO mice displayed reduced lesion size when compared with apoE KO mice. Surprisingly, CD4/apoE dKO mice exhibited a 39% decrease in lesion development after MDA-LDL immunization as compared with PBS-immunized controls and in a 39% reduction as compared with untreated mice (Figure 2a). This is in agreement with previous findings that oxLDL immunization ameliorates atherosclerosis. As mentioned, CD4/apoEdKO mice displayed reduced lesion size when compared with apoE KO mice. Surprisingly, CD4/apoE dKO mice exhibited a 39% decrease in lesion development after MDA-LDL immunization as compared with PBS-immunized controls and in a 39% reduction as compared with untreated mice (Figure 2a). This is in agreement with previous findings that oxLDL immunization ameliorates atherosclerosis. As mentioned, CD4/apoEdKO mice displayed reduced lesion size when compared with apoE KO mice. Surprisingly, CD4/apoE dKO mice exhibited a 39% decrease in lesion development after MDA-LDL immunization as compared with PBS-immunized controls and in a 39% reduction as compared with untreated mice (Figure 2a).

**Adjuvant-Induced Protection Against Atherosclerosis Is Extinguished in CD4/apoE dKO Mice**

Injection with Freund adjuvant has been found to reduce atherosclerosis in hypercholesterolemic mice even in the absence of antigen. Because all immunization experiments were performed after emulsification of antigen in Freund adjuvant and controls received PBS/Freund adjuvant emulsions, we could evaluate the effect of adjuvant alone comparing lesion size in untreated mice with that in mice receiving PBS/adjuvant. As shown in Figure 2a, lesions in apoE single-KO mice were significantly reduced after injection of Freund adjuvant. However, no reduction was observed in CD4/apoE dKO mice (Figure 2a). Therefore, CD4+ cells are required for inhibition of atherogenesis by Freund adjuvant.

**MDA-LDL Immunization as well as Freund Adjuvant Alone Stimulates Anti–MDA-LDL Antibody Production**

Antibody responses were analyzed in mice immunized with MDA-LDL/adjuvant or with PBS/adjuvant. Because immunization led to dramatic increases in the levels of specific antibodies to MDA-LDL, a series of dilutions were used to assess the responses. At 18 weeks of age, ie, 12 weeks after the start of immunization, MDA-LDL antibodies of all subclasses were significantly elevated in MDA-LDL immunized apoE KO and CD4/apoE dKO mice as compared with PBS-immunized ones, except for IgM antibodies on the
IgG antibodies were not changed (data not shown).

The results of the present study provide new information about the role of CD4+ cells in atherosclerosis, by showing that (1) the absence of CD4+ cells in apoE KO mice leads to reduced atherosclerosis, and (2) the atheroprotective effect of LDL immunization does not depend on CD4+ cells, whereas (3) the atheroprotective effect of adjuvant injection depends on CD4+ cells. Together, these data indicate that CD4+ cells constitute a major proatherogenic cell population but also that MDA-LDL immunization–induced atheroprotection but not adjuvant-induced atheroprotection largely depends on other mechanisms.

CD4+ cells are the dominating type of T cells in atherosclerotic lesions, in humans as well as mice. A significant proportion of them respond to oxidized LDL and most are IFN-γ-producing Th1 cells. Analysis of compound knockout mice and cytokine injection experiments have identified a deleterious role of IFN-γ in atherosclerosis and cell culture studies have demonstrated several potentially atherogenic effects, including macrophage activation with upregulated scavenger receptor CXCL16 and reduced (cholesterol efflux mediating) ABCA-1 expression, radical production, metalloproteinase, and TNF-α secretion, as well as smooth muscle inhibition with reduced collagen, α-actin expression, and blocked proliferation, and also endothelial activation with adhesion molecule expression. The present data showing attenuated IFN-γ expression in lymphoid cells in parallel with reduced intraplaque inflammation in CD4+ KO mice strongly support the notion that CD4+ T cells promote atherosclerosis through IFN-γ secretion, possibly a result of antigen-specific activation.

Because the immune activity, and thereby cytokine levels, are impaired in CD4-deficient mice, the underlying mechanism between the difference in lipoproteins could depend on cytokine-dependent regulation of enzymes involved in lipoprotein metabolism as well as regulation of lipoprotein receptors. In the triglyceride profile analysis, the observation of a higher glycerol peak in the CD4+ dKO mice suggests that the increased triglyceride levels in these mice are probably not the result of a reduced hydrolysis of triglyceride-rich lipoproteins but of an increased secretion. Thus, the increase observed in the HDL-cholesterol fraction could possibly be consequence of an increased synthesis of pre-β-HDL. The increased synthesis would be the result of a condition in which an increase in the secretion of triglyceride-rich lipoproteins is paralleled by a maintained hydrolysis of these particles. This may contribute to the reduced atherosclerosis seen in these dKO animals.

It was believed for a long time that interactions between B cells and CD4+ T cells were necessary for the induction of IgG antibody responses to thymus-dependent antigens. In the immunocompetent individual, CD4+ T cells play a key role for efficient Ig isotype switching from IgM to IgG.

**Discussion**

The results of the present study provide new information about the role of CD4+ cells in atherosclerosis, by showing that (1) the...
of high-titer IgG antibodies during MDA-LDL immunization can take place in the absence of CD4+ T cells. In this situation, it is possible that Ig class switching in the B cell receives T cell help from CD4-CD8-TCRβ+ cells or γδT cells. In addition, MHC class II restriction has recently been reported in CD8-positive cells in CD4 deficient mice, which implies that there may be a "compensatory" mechanism in other compartments during immunological development to partly take over some of the functions that would normally be the function of CD4+ cells. It is not likely that the knockout mice "leak" CD4+ T cells, because no such cells were detected by flow cytometry of lymphoid organs in the CD4/apoE dKO mice.

In the present study, immunization with MDA-LDL in apoE KO mice reduced lesion size in parallel with the formation of high-titer IgG antibodies to MDA-LDL, which is in line with previous observations. Interestingly, this was the case also in CD4/apoE dKO mice, with increased titers of IgG antibodies and reduced lesions after MDA-LDL immunization. This shows that CD4+ cells are not necessary for the atheroprotective effect. If antibodies are primarily responsible for the atheroprotective effect, these data suggest that even lower titers of the specific antibodies may be enough for this effect.

**Figure 4.** Real-time quantitative RT-PCR analysis of cytokine and scavenger receptor mRNA expression in the spleens of untreated CD4/apoE dKO mice and apoE KO mice. Top, bottom, and line through the middle of the box correspond to the 75th, 25th, and 50th percentile (median), respectively. Ranges at the top and bottom of the box extend from the 90th and 10th percentile, respectively. n = 8 per group. HPRT (Hypoxanthine phosphoribosyl transferase) was used as a reference housekeeping gene. N.S. indicates no statistical difference.

**Figure 5.** MDA-LDL antibodies in CD4/apoE dKO and apoE KO mice after immunization with MDA-LDL. Comparison of antibody formation after immunization in both CD4/apoE dKO mice and apoE KO mice. Statistical values (P) are regarding the linear part of the curve and represent the difference between MDA-LDL immunized apoE KO mice and MDA-LDL immunized CD4/apoE dKO. n = 7 per group. OD indicates optical density.
Interestingly, the MDA-LDL immunization results in a strong IgG1 production with a weak IgG2a response, which is in line with the findings by Binder et al.40 In mouse, production of IgG1 depends on help by Th2-type cells, whereas the switch from IgM to IgG2a requires help from IFN-γ-secreting Th1 cells. Such strong bias may reflect immunomodulatory effects of immunization.

Several lines of evidence support the hypothesis that humoral immunity protects against atherosclerosis. First, injection of immunoglobulin preparations inhibits atherosclerosis.41 Second, removal of the B cell–rich spleen enhances disease, whereas B-cell transfer from diseased, hence autoimmunized, apoE KO mice to young, disease-prone mice reduces the lesion development.42 Third, “natural antibodies” to phospholipids inhibit uptake of oxidized LDL in macrophages and induction of such antibodies by pneumococcal immunization reduces disease in LDLR KO mice.26 Antibodies to modified LDL particles may reduce lesion development by removing lipoproteins through Fc and/or CR1 receptors on macrophages and other cells in spleen and other lymphoid organs and/or by blocking their uptake by lesion foam cells.

Several investigators studying antiatherosclerotic effects of immunization have observed that injection of adjuvant alone reduces disease development in hypercholesterolemic animals.25,26 This contrasts with the studies by Wick and Xu, who reported increased disease after parenteral administration of rabbits with Freund complete and no effect of incomplete adjuvant.43 The discrepancy may be attributable to immunization protocols or the precise composition of adjuvant. Our present data clearly show that injection of Freund adjuvant alone leads to reduced disease in apoE KO mice. Interestingly, injection of the same adjuvant does not inhibit atherosclerosis in CD4/apoE dKO mice, although it increases antibody levels to MDA-LDL in both apoE KO and CD4/apoE dKO mice. This implies a crucial role of CD4+ cells in the atheroprotective response to adjuvant. Apart from CD4+ T cells, other cells including mono/macrophages, dendritic cells, and hematopoietic cells can also express varying levels of CD4. Although the function of the CD4 molecule in these cells is largely unknown, it cannot be excluded that the function of any of these cells may be affected because of lack of CD4 molecules.

The CD4-dependent adjuvant protection does not necessarily mean that the adaptive immune system is directly responsible for this effect. Instead, B cells or components of the innate immune system that are dependent on factors secreted by or costimulation provided by the CD4+ cells may be the true actors. One such example is the elevation of MDA-LDL–specific IgM antibodies found after immunization with adjuvant alone. Because IgM but not IgG subclass antibodies to MDA-LDL were induced by adjuvant alone, we speculate that adjuvant immunization can raise T cell–independent antibodies, which is supported by data from Binder et al.26 The production of T cell–independent antibodies is enhanced by T cell cytokines, which may explain the lower levels of IgM antibodies in the CD4 KO mouse. If these antibodies are protective, the lower titers in the CD4/apoE dKO mouse may explain the lack of adjuvant-related effect on atherosclerosis. Immunization with oxLDL enhanced production of such protective antibodies.

Various cell types can be activated by immunization and confer protection against atherosclerosis. Freigang et al23 reported that immunization with native LDL inhibited atherosclerosis in LDL receptor knockout mice, almost to the same extent as MDA-LDL, despite the fact that they did not develop high titer antibodies to oxidized neoepitopes. This would suggest a role for cellular immune responses, possibly involving regulatory T cells. The generation and the effects of these cells depend largely on their production of the antiinflammatory/immunosuppressive cytokines interleukin-10 and TGF-β.44,45 Binder et al40 have shown that there are more IL-4/5/10/13, but less IFN-γ is secreted after immunization. Indeed, we have observed dramatically increased atherosclerosis in mice that are deficient in interleukin-10 and also in mice with abrogated TGF-β signaling in T cells.46,47 Disease protection after immunization with MDA-LDL as well as the effect of Freund adjuvant alone may therefore depend on activation of regulatory T cells, many of which are CD4+.

In summary, lack of CD4 substantially decreases the development of atherosclerosis in apoE KO mice. The CD4 defect also extinguishes the protective effect of adjuvant. However, CD4+ T cell help is not obligatory to elicit the atheroprotective effect after immunization with MDA-LDL. These results indicate that the adjuvant-induced atheroprotection is CD4-dependent, whereas immunization with oxidized LDL confers disease protection through mechanisms operating also in CD4-deficient mice. These findings demonstrate complex roles of immune cell–cell interactions in the regulation of the atherosclerotic process.

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References


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