Recombinant Antibodies to an Oxidized Low-Density Lipoprotein Epitope Induce Rapid Regression of Atherosclerosis in Apobec-1−/−/Low-Density Lipoprotein Receptor−/− Mice

Alexandru Schiopu, MD, PhD,* Björn Frendéus, PhD,† Bo Jansson, PhD,† Ingrid Söderberg, BSI,* Irena Ljungcrantz, BSI,* Zufan Araya, PhD,† Prediman K. Shah, MD, FACC,‡ Roland Carlsson, PhD,† Jan Nilsson, MD, PhD,* Gunilla Nordin Fredrikson, PhD*§

Malmö and Lund, Sweden; and Los Angeles, California

Objectives

The present study tested the hypothesis that treatment with human recombinant immunoglobulin G1 (IgG1) antibodies against a specific oxidized low-density lipoprotein (oxLDL) epitope will induce regression of existing atherosclerotic lesions in LDL receptor-deficient mice expressing apolipoprotein B-100 (Apobec-1−/−/LDLR−/−).

Methods

Apobec-1−/−/LDLR−/− mice were fed a high-fat diet until they were 24 weeks and were subsequently transferred to chow. Starting at 25 weeks, mice were given 3 weekly injections of either of 2 recombinant human IgG1 antibodies (IEI-E3 or 2D03) against a malondialdehyde-modified apoB-100 peptide sequence.

Results

At 25 weeks, atherosclerotic lesions covered 10.3 ± 3.7% of the descending aorta. Transfer to chow diet resulted in a modest regression of atherosclerosis over a 5-week period (8.28 ± 4.36%; p = NS). Antibody treatment induced additional regression of atherosclerosis by 50% (2D03; p = 0.001) and 36% (IEI-E3; p = 0.004) compared with control IgG1. The 2D03 treatment also reduced plaque inflammation, enhanced plaque expression of the adenosine triphosphate–binding cassette transporter A1, and inhibited expression of monocyte chemotractant protein-1 in cultured monocytes.

Conclusions

Human IgG1 against a specific oxLDL epitope can induce rapid and substantial regression of atherosclerotic lesions, possibly by stimulating lipid efflux and inhibiting macrophage recruitment. These recombinant human antibodies could represent a novel strategy for rapid regression/stabilization of atherosclerotic lesions. (J Am Coll Cardiol 2007;50:2313–8) © 2007 by the American College of Cardiology Foundation

Oxidized low-density lipoprotein (oxLDL) is one of the most important targets for the immune system in atherosclerosis (1,2). Oxidation of LDL results in formation of neoepitopes such as oxidized phospholipids and aldehyde-modified breakdown fragments of apolipoprotein B-100 (apoB-100), leading to escape from self-tolerance (3). Immunization of hypercholesterolemic rabbits with oxLDL inhibits the development of atherosclerosis, suggesting that immune responses against oxLDL may have an atheroprotective effect (4,5). We have characterized the apoB-100 peptide sequences in oxLDL that give rise to autoantibody formation in humans (6) and demonstrated that immunization with some of these peptide sequences significantly reduces the development of atherosclerosis in ApoE−/− mice (7,8). Peptide 45, corresponding to the sequence between amino acids 661 and 680 of apoB-100, was...
found to be one of the most effective peptides in these studies. The presence of autoantibodies against this peptide sequence was recently shown to be associated with reduced risk for development of acute myocardial infarction in humans (9). We have developed human recombinant immunoglobulin G1 (IgG1) antibodies specific for this aldehyde-modified peptide (10). Antibody treatment was found to reduce early lesion development by up to 50% in ApoE/−/− mice. The aim of the present study was to test the hypothesis that human recombinant IgG1 against the peptide 45 sequence in apoB-100 induces regression of existing atherosclerotic plaques in Apobec-1/−/−/LDLR/−/− mice and to explore the mechanisms through which this regression is achieved.

**Methods**

Antibody treatment and tissue preparation. Antibodies were produced against the malondialdehyde (MDA)-modified human apoB-100 p45 sequence as previously described (10). Antibodies were produced against the human sequence rather than the mouse sequence because the long-term aim of these studies is to develop an antibody-based therapy that can be used in humans. Both the IEI-E3 and 2D03 antibodies raised against the MDA-modified apoB-100 p45 specifically recognized MDA-modified LDL but not native LDL. Fluorescein isothiocyanate-8 (FITC-8), the control IgG1 directed to FITC did not bind to MDA–LDL or native LDL (data not shown). The binding of 2D03 to MDA–apoB-100 was 10 times higher than that of IEI-E3, as determined by the Biacore technique (3 × 10−9 vs. 3 × 10−8, respectively).

We used male Apobec-1/−/−/LDLR/−/− mice with C57BL/6 background from Jackson Laboratories (Bar Harbor, Maine). These mice express full-length apoB-100 in their LDL particles and have 3-fold higher plasma levels of apoB-100 than LDLR/−/− mice (11). From 4 weeks of age, the mice were fed a high-cholesterol diet (0.15% cholesterol, 21% fat; Lactamin AB, Kimstad, Sweden) provided ad libitum. One week before the first immunization, the diet was changed to normal chow. At 25 weeks of age, the mice were injected intraperitoneally with 1 mg (0.5 ml) of 2D03, IEI-E3, or FITC-8 antibodies. The injections were repeated 2 times at 1-week intervals, and the mice were sacrificed 2 weeks after the last injection (Table 1). For a detailed description of tissue preparation and staining for lipids, macrophages, and ATP-binding cassette transporter A1 (ABCA-1), please see the Online Appendix.

Quantitation of monocyte chemoattractant protein (MCP)-1 and ABCA-1 expression. The effect of antibodies on MCP-1 gene expression, MCP-1 secretion, and ABCA-1 protein expression in cultured human monocytes/macrophages was measured by real-time reverse transcrip-
tion, enzyme-linked immunosorbent assay, and flow cytometry, respectively. Please see the Online Appendix for a detailed method description.

**Statistical analysis.** Analysis of all the in vivo data was performed using the 2-tailed Mann-Whitney test and is presented as box plots demonstrating median and 25th and 75th percentiles.
75th percentiles, with whiskers showing the highest and lowest values. Analysis of the in vitro data was performed using parametric analysis of variance with Tukey post test and is presented as mean ± SD. Results were considered statistically significant at $p \leq 0.05$.

**Results**

**Effect of antibody treatment on the extent of atherosclerosis.**

The design of the experiments and the different treatment groups are outlined in Table 1. A modest decrease in plaque area was observed in animals transferred to chow diet alone and sacrificed at 29 weeks of age ($8.28 \pm 4.36\%$ vs. $10.31 \pm 3.73\%$) (Fig. 1). The remaining groups received intraperitoneal injections of 1 mg of 2D03, IEI-E3, or control FITC-8 antibodies at 25, 26, and 27 weeks. There were no significant differences among the treatment groups and the chow-fed control group with respect to weight and the plasma levels of cholesterol and triglycerides (data not shown). Treatment with the FITC-8 control antibody had no effect on atherosclerosis compared with transfer to chow diet alone, whereas treatment with the 2D03 antibody resulted in a more than 50% regression of atherosclerosis ($3.91 \pm 1.83\%$) compared with treatment with control IgG1 ($p = 0.001$) (Fig. 1). A less pronounced regression was observed in mice treated with the IEI-E3 antibody ($5.16 \pm 1.07\%$, $p = 0.004$).

**Effect on ABCA-1 expression.**

The reduction in oil red O staining of neutral lipids in the aorta of 2D03- and IEI-E3–treated mice compared with that in the FITC-8 group suggested a stimulation of lipid efflux from the plaques. To study the effect of the antibodies on pathways mediating reverse cholesterol transport, we analyzed the expression of ABCA-1 in aortic plaques of antibody–treated mice and in cultured human monocytes. The fraction of total plaque area demonstrating positive ABCA-1 immunoreactivity was found to be increased by 30% ($p < 0.02$) in the atherosclerotic plaques of 2D03–treated mice compared with that in the FITC-8 control group (Figs. 2A and 2B). There was a close colocalization between ABCA-1 and macrophage immunoreactivity in the lesions, suggesting that ABCA-1 was primarily expressed by macrophages (Fig. 3). Monocytes cultured in oxLDL-containing human serum in the presence of 2D03 for 48 h showed increased ABCA-1 expression.
compared with FITC-8–treated or –untreated cells as measured by flow-cytometry (Figs. 2C and 2D).

**Effect on inflammatory activity.** Treatment with the 2D03 antibody resulted in a 38% reduction of macrophage immunoreactivity compared with the FITC–8 control group (Fig. 4). A similar trend was also observed in mice treated with the IEI–E3 antibody.

To study the possible mechanisms involved in the anti-inflammatory effect of antibody treatment, we examined the effect of 2D03 and IEI–E3 on macrophage expression of MCP-1. Freshly isolated CD14-positive human monocytes were cultured in oxLDL-containing human serum in the presence or absence of the antibodies for 48 h. Both 2D03 and IEI–E3 inhibited monocyte MCP-1 release and mRNA expression in a dose-dependent manner, whereas the FITC–8 control antibody had no effect (Fig. 5).

**Figure 5** Antibodies to MDA–apoB-100 Peptides Block oxLDL-Induced Monocyte MCP-1 Production In Vitro

(A) MCP-1 levels in conditioned medium from monocytes incubated with titrated concentrations of IEI–E3 (shaded squares), 2D03 (solid squares), or FITC–8 control IgG1 (open squares) in the presence of oxLDL-containing human serum. The graph shows mean ± SD of triplicate values from a representative experiment. (B) MCP-1 messenger ribonucleic acid (mRNA) relative to 18S mRNA levels in human monocytes incubated with 60 μg/ml 2003, IEI–E3, or FITC–8 in the presence of oxLDL-containing human serum. Graphs show mean ± SD of triplicate values using cells obtained from 3 different donors (n = 9). ***p < 0.001 versus FITC–8. IgG = immunoglobulin G; MCP = monocyte chemoattractant protein; oxLDL = oxidized low-density lipoprotein; other abbreviations as in Figure 2.

**Discussion**

This study demonstrated for the first time that treatment with recombinant human IgG1 antibodies against an oxLDL-specific antigen induces regression of advanced pre-existing atherosclerotic plaques in the aorta of ApoE–/−/LDLR–/− mice by more than 50% over a 4-week period. The present observation that 2D03 antibodies stimulated the expression of ABCA-1, a key protein in reverse cholesterol transport, identifies an important mechanism likely to be involved in plaque regression. Moreover, our results showed that 2D03 treatment inhibits macrophage release of the chemoattractant chemokine MCP-1, leading to a less inflammatory plaque phenotype in vivo.

**Antibody-induced plaque regression and the role of ABCA-1.** Transfer of cholesterol-fed ApoE−/−/LDLR−/− mice to a low-fat diet halted further progression of atherosclerosis but was alone not sufficient to induce a significant regression of aortic lesions. In contrast, a marked reduction in aortic lipid-rich plaques was found in 2D03 and IEI–E3 antibody–treated mice, suggesting that the antibodies facilitated net removal of lipids from the aorta by activation of reverse cholesterol transport. Our findings that 2D03 stimulated the expression of ABCA-1 in cultured monocytes and that 2D03 treatment in mice resulted in increased expression of ABCA-1 in atherosclerotic plaques point to a possible mechanism responsible for this effect. ABCA-1 has an important role in reverse cholesterol transport by controlling the efflux rate of cholesterol and phospholipids to apoA-I (12), and overexpression of ABCA-1 has been shown to inhibit atherosclerosis in hypercholesterolemic mice (13). The macrophage expression of ABCA-1 is strongly induced by lipid loading (14) and IgG1 against MDA-modified apoB-100 promotes uptake of oxLDL in macrophages (10). These observations suggested that 2D03 antibodies increase lipid efflux from plaques by enhancing macrophage uptake of oxLDL from the surrounding extracellular matrix. This subsequently resulted in increased macrophage expression of ABCA-1 and stimulation of apoA-I–mediated reverse cholesterol transport.

**Possible mechanisms of antibody-induced plaque macrophage depletion.** Our in vitro results demonstrated the ability of MDA–apoB-100 antibodies to inhibit macrophage expression of the chemokine MCP-1. Expression of the potent chemokine MCP-1 is enhanced by oxidized lipids, and MCP-1 deficiency reduces development of atherosclerosis in hypercholesterolemic mice (15). Accordingly, blocking of monocyte MCP-1 release by anti-oxLDL IgG1 would provide an attractive means of selectively neutralizing MCP-1 in atherosclerotic plaques where oxLDL and macrophages colocalize.

**Study limitations.** The mouse homology for the human p45 sequence is 85%, suggesting that the affinity of the antibodies may be lower for mouse than for human oxLDL. Because the present results suggested that antibodies with higher binding affinities are more effective, it is likely that...
this will limit the effectiveness of the antibodies when used in mice. A second limitation is that the functional studies of possible mechanisms mediating the protective effects of the IEI-E3 and 2D03 antibodies were performed on human macrophages. Accordingly, these observations cannot be directly extrapolated to the animal experiments. On the other hand, they add support to the possibility that the anti-inflammatory effects of the antibodies can also be achieved in humans. A third limitation of the present study is the generation of mouse anti-human IgG1 antibodies (10). These antibodies may reduce the effectiveness of human IgG1 treatment in mice by blocking their binding sites or by inducing their clearance from the circulation.

Conclusions

The present findings showed that treatment with human IgG1 against a specific oxLDL antigenic epitope can induce a rapid and substantial regression of atherosclerotic lesions, possibly mediated by the macrophage reverse cholesterol transport, as indicated by increased ABCA-1 expression. Our studies also indicated that 2D03 treatment reduces macrophage MCP-1 release, leading to a less inflammatory plaque phenotype. These recombinant human IgG1 represent a potentially novel approach for treatment of atherosclerosis in humans. However, in this respect, the present study needs to be interpreted with due caution because the atherosclerotic disease process of ApoB-100/HDLC/LDL/ mice is not likely to be identical to that of human atherosclerosis.

Reprint requests and correspondence: Dr. Alexandru Schiopu, Transplantation Immunology Unit, Nuffield Department of Surgery, Level 6, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, United Kingdom. E-mail: alexandru.schiopu@med.lu.se.

REFERENCES


APPENDIX

For a detailed description of tissue preparation and staining for lipids, macrophages, and ABCA-1, please see the online version of this article.