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ORIGINAL ARTICLE

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Macrophages, smooth muscle cells, endothelial cells, and T-cells express CD40 and CD40L in fatty streaks and more advanced human atherosclerotic lesions

Colocalization with epitopes of oxidized low-density lipoprotein, scavenger receptor, and CD16 (FcγRIII)

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Abstract CD40–CD40L receptor–ligand interaction plays a central role in antigen presentation, immunological reactions, and in T-cell and macrophage activation. Since all these mechanisms are important for the pathogenesis of atherosclerosis, we studied the expression profile of CD40–CD40L in different types of human atherosclerotic lesions using double immunostaining techniques with cell type-specific antibodies. Normal human intima did not contain CD40 or CD40L immunoreactivity. From type-II lesions (fatty streaks) to advanced type-VI lesions (complicated plaques), colocalization of CD40 and CD40L was observed in T cells ($CD3^+$ cells), macrophages ($CD68^+$ cells), and smooth muscle cells ($HHF35^+$ cells). No correlation was found between the lesion type and CD40–CD40L expression. Positive lesions had dense infiltrations of macrophages and macrophage-derived foam cells together with T cells. The most intensive immunoreactivity for the CD40 receptor and its ligand CD40L was found in macrophage- and T-cell-rich pockets, where both cell types were in close contact with each other. The majority of macrophages, and especially those of macrophage-derived foam cells, were positive for both CD40 and CD40L. A small subset of the lesion macrophage population (10–20%) consisted of cells positive only for either CD40 or CD40L, suggesting the presence of a subpopulation of macrophages more active in inflammatory processes than in lipid uptake. Intimal smooth muscle cells in and around the macrophage-rich areas as well as some of the medial smooth muscle cells near the lesions stained positive for CD40 and CD40L. Moderate to faint expression of these proteins was also found in endothelium. In addition, CD40–CD40L immunoreactivity colocalized with epitopes characteristic of oxidized low-density lipoprotein, scavenger receptor class A, and CD16 (FcγRIII), thus suggesting the involvement of CD40–CD40L and these pathogenetic mediators in foam cell formation, progression of atherosclerotic lesions, and differentiation of immunologically active subsets of macrophages. These results support the hypothesis that CD40–CD40L interaction is involved in atherogenesis and that it might provide a target for future therapeutic interventions.

Keywords CD40-CD40L · Atherogenesis · T cells · Macrophages · Smooth muscle cells

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Introduction

Characteristic features for atherosclerotic lesions are proliferation of smooth muscle cells, accumulation of lipids and extracellular matrix, and infiltration of monocyte macrophages and T cells [24]. Macrophages and T cells are immunologically competent cells with a key role in chronic inflammation. Previous studies have established that activated endothelial cells [9], smooth muscle cells [26], and macrophages [3] express an immune mediator CD40 and its ligand CD40L (CD154 or gp39) [17], which were previously considered to be expressed only on activated $CD4^+$ T cells [6]. Ligation of CD40 in vitro in cells present in atherosclerotic lesions triggers proatherogenic alterations, such as induction of adhesion molecules [9, 12, 15] and expression of proinflammatory cytokines [10, 11, 17, 29], interstitial collagenase (MMP1), and stromelysin (MMP3) [2, 18, 21, 27], which has been shown to be present in vivo in human atherosclerotic plaques [27]. In addition, expression of tissue factor in macrophages is increased upon ligation of CD40 [18]. Disruption of the CD40–CD40L sig-

Table 1 Human samples used in immunocytochemistry studies. Lesions were classified according to Stary et al. [28]. – absent; + faint; ++ moderate; +++ strong positive immunocytochemistry signal with CD40 and CD40L antibodies

Age (years), gender, and location of samples	Lesion type	CD40 expression	CD40L expression
30/Male/coronary arteries	Normal	–	–
	I	+	+
	I	+	+
	II	++	++
	II	++	+
	IV	++	++
34/Male/abdominal aorta	Normal	–	–
	II	+	+
	II	++	+++
	III	++	++
36/Female/coronary arteries	II	++	+++
	III	+++	++
	IV	++	++
40/Male/thoracic aorta	I	+	+
	III	++	+++
	IV	+++	++
43/Male/thoracic aorta	VI	++	++
	VI	++	++
51/Male/coronary arteries	V	++	++
	V	++	++
	VI	+	+
59/Male/coronary arteries	VI	++	++
	VI	+	++

naling pathway in low-density lipoprotein receptor-deficient ($LDLR^{-/-}$) hyperlipidemic mice decreases expression of stromelysin within atherosclerotic lesions [27]. Inhibition by an antibody or genetic disruption of CD40 signaling in ApoE-deficient ($ApoE^{-/-}$) and $LDLR^{-/-}$ mice is reported to result in a reduced number of macrophages and T cells in atherosclerotic lesions, smaller lesion size and lower lipid content, as well as higher collagen content of advanced lesions [16, 19]. Due to previously mentioned potentially proatherogenic functions, it is anticipated that the CD40–CD40L signaling pathway may play a key role in atherogenesis.

The finding that both the CD40 receptor and its ligand CD40L are expressed in lesion cells other than T cells is of great importance, since this mode of expression allows T-cell-independent route of immune activation. However, no systematic data exists regarding the expression of CD40–CD40L proteins in various types of human atherosclerotic lesions. It was found that CD40–CD40L receptor-ligand proteins are expressed in different types of human atherosclerotic lesions in areas containing dense infiltrations of macrophages and T cells. CD40–CD40L immunoreactivity also colocalized with epitopes of oxidized LDL, scavenger receptor class A, and CD16 (FcγRIII). Endothelial cells and medial smooth muscle cells expressed both the CD40 receptor and its ligand CD40L in the vicinity of lesions.

Methods

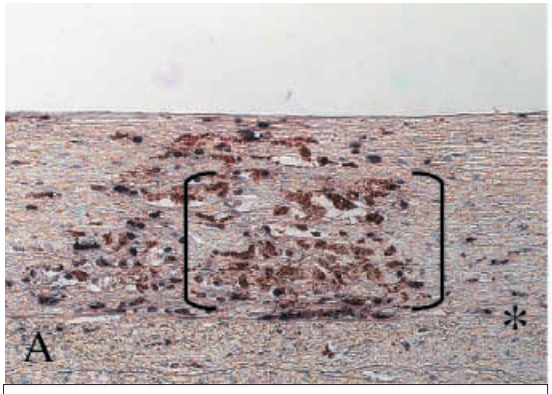
Tissue samples

Human aortic and coronary samples were obtained from seven medicolegal autopsies (six males and one female, age 30–59 years) 4–12 h post-mortem. Tissue samples were removed and immediately immersion fixed for 4 h in 4% formal sucrose [4% paraformaldehyde, 15% sucrose, containing 50 µM butylated hydroxy toluene (BHT) and 1 mM ethylene diamine tetraacetic acid (EDTA)], rinsed in 15% sucrose/50 µM BHT/1 mM EDTA for 12 h and embedded in paraffin [31]. Atherosclerotic lesions were classified according to Stary et al. [28] into type-I (initial lesions), type-II (fatty streaks), type-III (intermediate lesions), type-IV (atheroma), type-V (plaque), and type-VI (complicated) lesions. All human studies were approved by the ethics committee of the University of Kuopio.

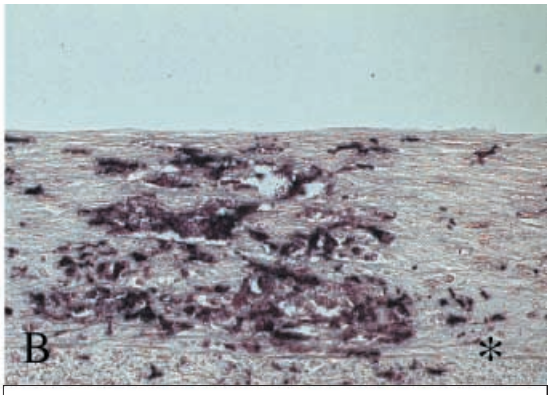
Immunocytochemistry

Serial paraffin-embedded 6- to 10-µm sections were used for immunocytochemistry using the following antibodies: mouse mAb against human CD68 (dilution 1:300; Dako, Copenhagen, Denmark); mouse mAb against muscle α- and γ-actin (HHF35; dilution 1:50; Enzo Diagnostics, Farmingdale, N.Y.); mouse mAb against CD3 (dilution 1:30; Novocastra Laboratories, Newcastle upon Tyne, UK); mouse mAb against CD16 (dilution 1:30; Novocastra Laboratories); rabbit polyclonal antibody raised against a peptide corresponding amino acids 258–277 mapping at the carboxy terminus of the CD40 precursor of human origin (dilution 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.); rabbit polyclonal antibody raised against a peptide corresponding amino acids 239–258 mapping at the carboxy terminus of CD40L of human origin (dilution 1:50; Santa Cruz Biotechnology); mouse mAb against HLA-DR (dilution 1:50; Zymed Laboratories, San Francisco, Calif.); mouse mAb against CD31 (dilution 1:50; Dako); polyclonal quinea pig antisera against MDA-modified LDL (MAL-2, dilution 1:500) [22]; and mouse antisera against scavenger receptor class A (SRKO8, dilution 1:250) [5].

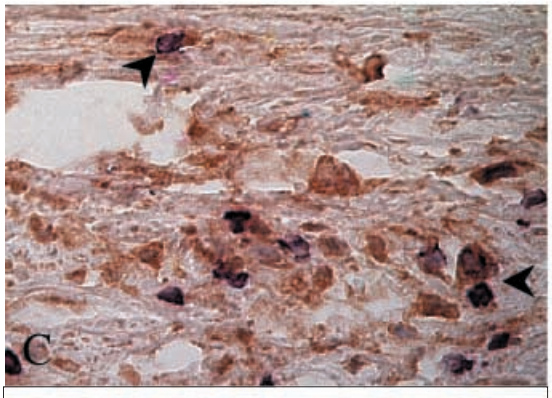
The avidin-biotin–horseradish peroxidase system (Histostain-Plus Kit, Zymed Laboratories, San Francisco, Calif.) was used for immunocytochemistry according to manufacturer's instructions, with either diaminobenzidine or aminoethyl carbazole as a color substrate. For double immunostainings, the first antibody was visualized using streptavidin-conjugated alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT) as a color



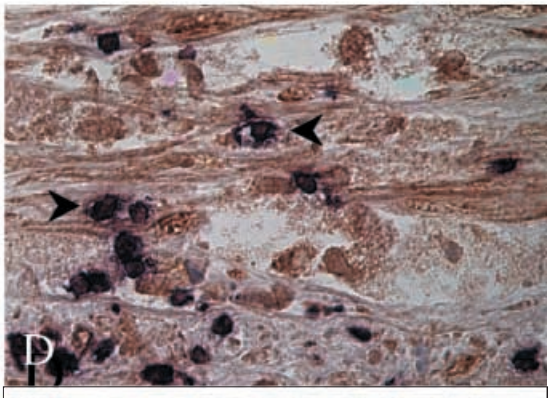
CD3: black CD68:red x19.9



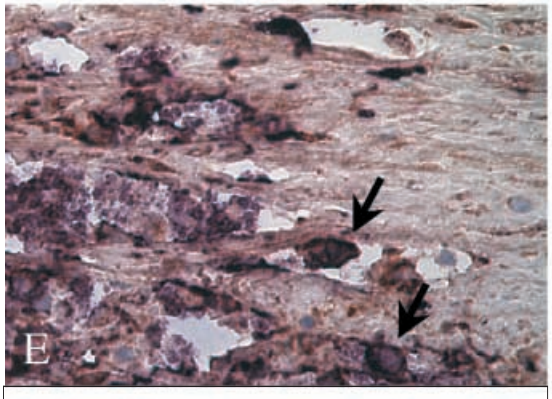
HLA-DR: black x19.9



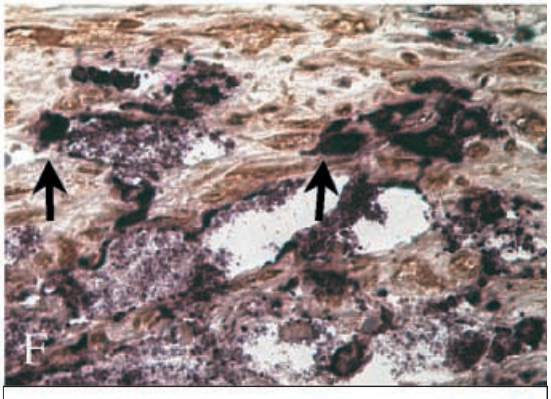
CD3: black CD40:brown x59.7



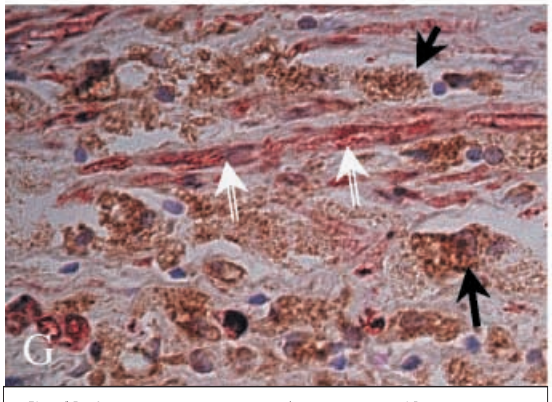
CD3: black CD40L:brown x59.7



CD68: black CD40:brown x59.7



CD68: black CD40L:brown x59.7



CD68: brown HHF:red x59.7

Nonimmune control x19.9

substrate followed by the second antibody which was visualized using horseradish peroxidase and either diaminobenzidine or aminoethyl carbazole as color substrates. Irrelevant class- and species-matched immunoglobulins as well as incubations without the primary antibody were used as controls for the immunostainings. Intensity of the immunostainings was classified from three stained sections per lesion as follows: – absent, + faint, ++ moderate, and +++ strong positive signal without knowledge of the origin of the samples.

Micrographs were taken using a digital camera (SenSys KAF1400-G2, Photometrics Ltd., Tucson, Ariz.), processed using digital image processing software (Image-Pro Plus, Media Cybernetics, Silver Spring, Md.), and printed using a sublimation printer (Kodak DS 8650, Eastman-Kodak, Rochester, N.Y.).

Results

The atherosclerotic lesions used for analysis included two normal aortic samples and 21 aortic and coronary lesions ranging from type I to advanced and ruptured type-VI lesions (Table 1). In all but the two normal samples and three type-I lesions studied, intima contained infiltrations of macrophages and macrophage-derived foam cells together with T cells and smooth muscle cells. In these areas, irrespective of the lesion type, double staining immunocytochemistry colocalized CD40 and CD40L proteins in these pockets of inflammatory cells (Fig. 1, Fig. 2, Fig. 3 and Fig. 5). In addition, positive staining for CD40 and CD40L was also seen in medial smooth muscle cells under the lesions (Fig. 4) in 7 of 18 samples ranging from type-II to type-VI lesions. Staining with CD31 revealed partly intact endothelium in grade-I lesions but only in 6 of 18 more advanced lesions. If present, endothelium showed moderate to faint staining for CD40 or CD40L. The normal aortic samples showed no expression for neither CD40 or CD40L (Table 1). An example of CD40–CD40L receptor-ligand profile in a type-II atherosclerotic lesion (fatty streak) is shown in Fig. 1. Double immunostaining reveals a dense macrophage-derived foam cell area (red) intermingled with abundant T cells (black) (Fig. 1A). Staining with anti HLA-DR shows that most of the cells in the lesion are activated (Fig. 1B). A high power view of the same area (serial sections, double staining) shows coexpression of CD40 receptor and its ligand CD40L in macrophages as well as in T cells (Fig. 1C–F). In Fig. 1G, a double staining for smooth muscle cells (white arrows/red stain) and macrophages (black arrows/brown stain) shows close contact between these cells and when compared with 1C-1F it can be deduced that the spindle shaped cells positive for CD40 and CD40L in those areas are intimal smooth muscle cells.

The same expression pattern was seen in type-III lesions (intermediate lesions). An example of a type-III lesion is shown in Fig. 2. The view in Fig. 2B–F is a higher magnification of the area shown inside brackets in Fig. 2A; thickened intima is invaded by T cells together with several macrophages. The colocalization of CD40 with T cells is shown in Fig. 2C (CD40/brown stain) and that of CD40L in Fig. 2D (CD40L/brown stain). In Fig. 2E and F, the staining pattern is seen for macrophages; overlapping staining pattern of CD40 and CD40L with that of CD68 suggests that macrophages express both proteins. The inserts in the right upper corners of the Fig. 2C–F are high power views (×119.4) showing CD40 and CD40L protein associated with solitary T cells and mac-

◀ **Fig. 1** Expression of CD40 and CD40L protein in a type-II (fatty streak) atherosclerotic lesion (human aorta; 34-year-old male). Immunostainings of serial sections (A–H). **A** Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and macrophages (positive cells are red, anti-CD68, dilution 1:300). The *area in brackets* is shown in higher magnification in **C**–**H**. An *asterisk* indicates the boundary between intima and media. **B** Antibody specific for HLA-DR (positive cells are black, dilution 1:50). **C** Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and CD40 (positive cells are brown, dilution 1:50). *Arrowheads* indicate T cells expressing CD40 protein. **D** Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and CD40L (positive cells are brown, dilution 1:50). *Arrowheads* indicate T cells expressing CD40L protein. **E** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40 (positive cells are brown, dilution 1:50). *Arrows* indicate macrophages expressing CD40 protein. **F** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40L (positive cells are brown, dilution 1:50). *Arrows* indicate macrophages expressing CD40L protein. **G** Double immunostaining with antibodies specific for smooth muscle cells (positive cells are red, HHF 35, dilution 1:50) and macrophages (positive cells are brown, anti-CD68, dilution 1:300). *White arrows* indicate smooth muscle cells and *black arrows* macrophages. **H** Non-immune control for the immunostaining. Hematoxylin counterstain. Original magnification ×19.9 (**A**, **B** and **H**) and ×59.7 (**C–G**)

Fig. 2 Expression of CD40 and CD40L protein in a type-III (intermediate) atherosclerotic lesion (human coronary artery; 36-year-old female). Immunostainings of serial sections (**A–H**). **A** An overall view of the lesion. Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and macrophages (positive cells are red, anti-CD68, dilution 1:300). The *area in brackets* is shown in higher magnification in **B**–**F**. An *asterisk* indicates the boundary between intima and media. The *inserts* in the right upper corner of the **C**–**F** show CD40 and CD40L protein associated with solitary T cells and macrophages. **B** Double immunostaining showing T cells (positive cells are black, anti-CD3, dilution 1:30) and macrophages (positive cells are red, anti-CD68, dilution 1:300). *Arrowheads* indicate T cells and *arrows* indicate macrophages. **C** Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and CD40 (positive cells are brown, dilution 1:50). *Arrowheads* indicate T cells expressing CD40 protein. **D** Double immunostaining with antibodies specific for T cells (positive cells are black, anti-CD3, dilution 1:30) and CD40L (positive cells are brown, dilution 1:50). *Arrowheads* indicate T cells expressing CD40L protein. **E** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40 (positive cells are brown, dilution 1:50). *Arrows* indicate macrophages expressing CD40 protein. **F** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40L (positive cells are brown, dilution 1:50). *Arrows* indicate macrophages expressing CD40L protein. **G** Antibody specific for HLA-DR (positive cells are brown, dilution 1:50). **H** Non-immune control for the immunostaining. Hematoxylin counterstain. Original magnification ×3.9 (**A**), ×39.8 (**B–H**), and ×119.4 (*inserts* in **C–F**) ▶

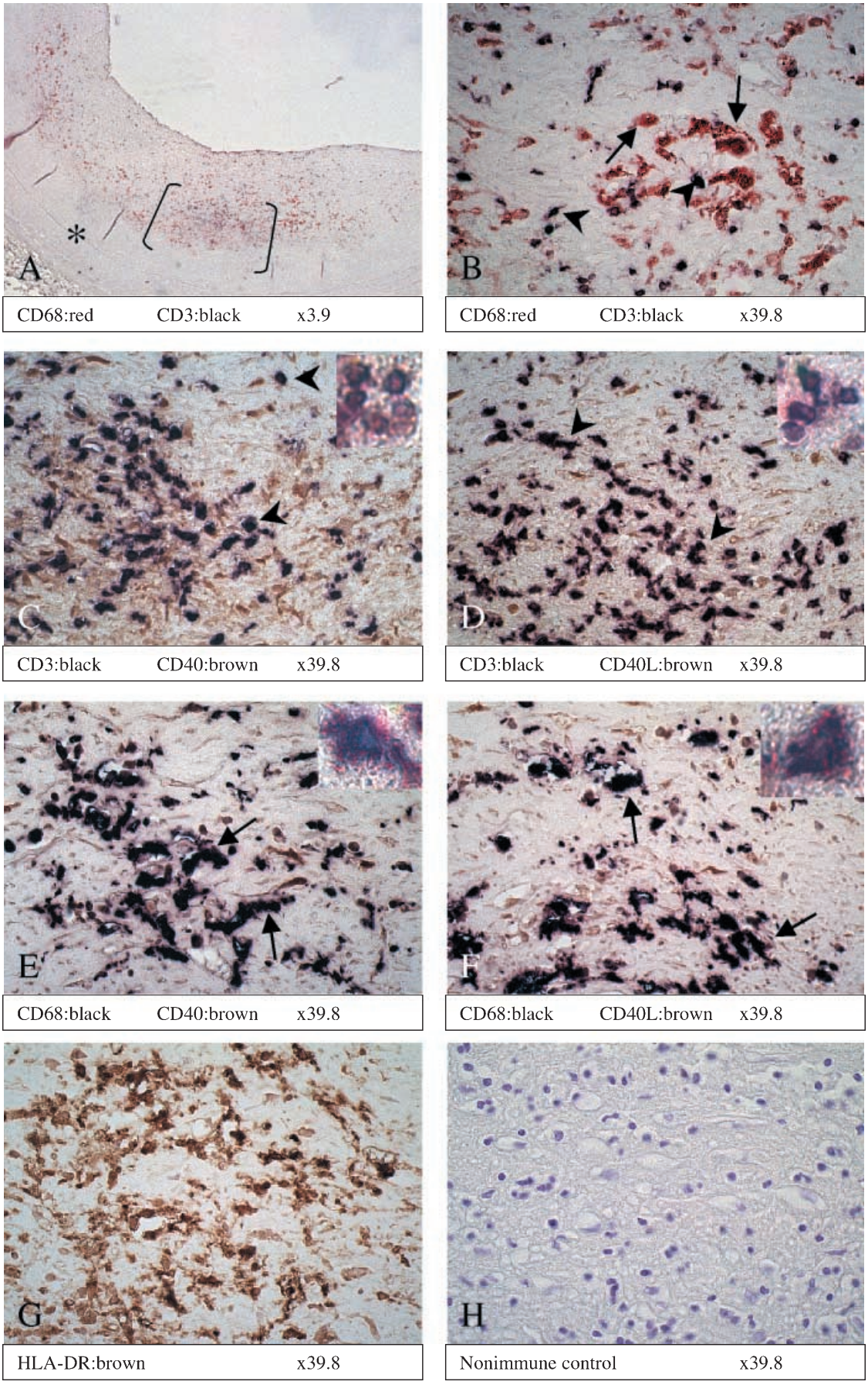

A
CD68:red CD3:black x3.9
B
CD68:red CD3:black x39.8
C
CD3:black CD40:brown x39.8
D
CD3:black CD40L:brown x39.8
E
CD68:black CD40:brown x39.8
F
CD68:black CD40L:brown x39.8
G
HLA-DR:brown x39.8
H
Nonimmune control x39.8

Fig. 2

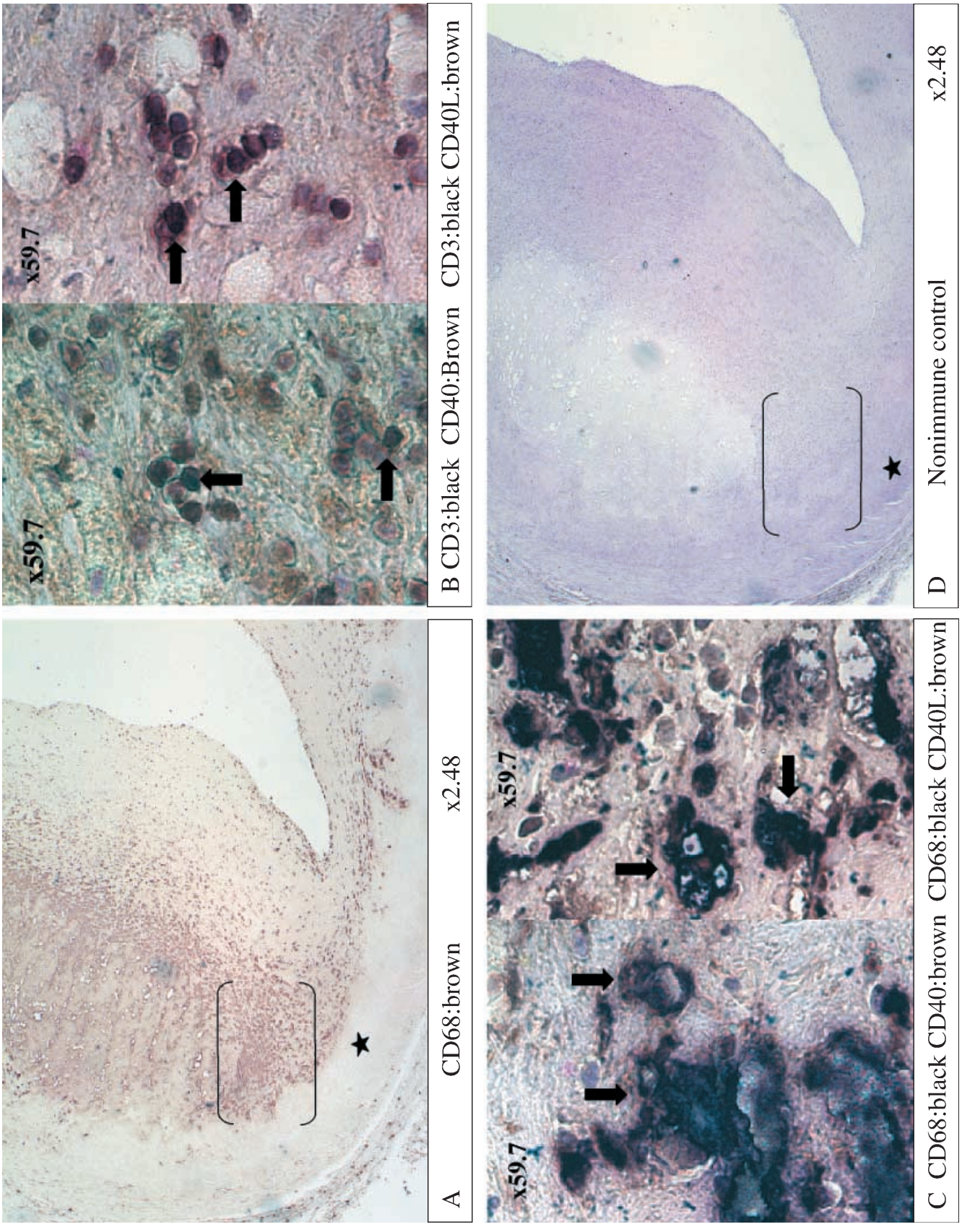

x59.7
x59.7
x59.7
x59.7
A CD68:brown x2.48
B CD3:black CD40:Brown CD3:black CD40L:brown
C CD68:black CD40:brown CD68:black CD40L:brown
D Nonimmune control x2.48

Fig. 3

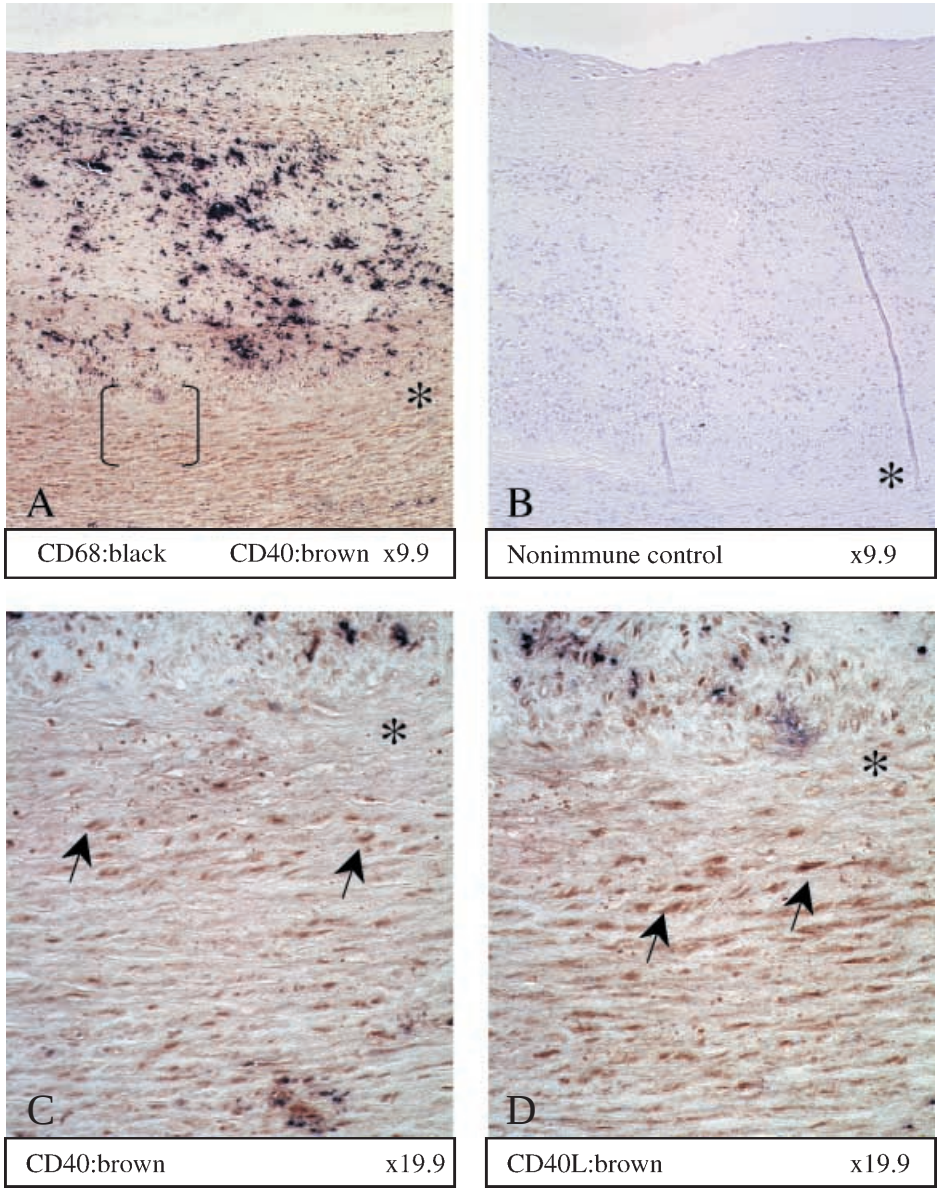


Fig. 4 Expression of CD40 and CD40L protein in the medial smooth muscle cells. **A** An overall view of the lesion. Combined anti-CD68 and anti-CD40L staining. Type-III lesion in human aorta (40-year-old male). **B** Non-immune control for the immunostaining. **C** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40 (positive cells are brown, dilution 1:50). *Arrows* indicate medial smooth muscle cells expressing CD40 protein. **D** Double immunostaining with antibodies specific for macrophages (positive cells are black, anti-CD68, dilution 1:300) and CD40L (positive cells are brown, dilution 1:50). *Arrows* indicate medial smooth muscle cells expressing CD40L protein. Hematoxylin counterstain. Original magnification ×9.9 (**A** and **B**) and ×19.9 (**C**, **D**)

◀ **Fig. 3** Expression of CD40 and CD40L protein in advanced type-V (fibroatheroma type plaque) atherosclerotic lesion (human coronary artery; 51-year-old male). Immunostainings of serial sections (**A–D**). **A** An overall view of the lesion area. Antibody specific for macrophages (positive cells are brown, anti-CD68, dilution 1:300). **B** Double immunostaining with antibodies specific for T cells (*left* and *right* half of the picture; positive cells are black, anti-CD3, dilution 1:30), CD40 (*left half* of the picture; positive cells are brown, dilution 1:50) and CD40L (*right half* of the picture; positive cells are brown, dilution 1:50). **C** Double immunostaining with antibodies specific for macrophages (*left* and *right* half of the picture; positive cells are black, anti-CD68, dilution 1:300), CD40 (*left half* of the picture; positive cells are brown, dilution 1:50) and CD40L (*right half* of the picture; positive cells are brown, dilution 1:50). **D** Non-immune control for the immunostaining. Hematoxylin counterstain. Original magnification ×2.48 (**A** and **D**) and ×59.7 (**B** and **C**)

rophages. In addition, Fig. 2C–F show smooth muscle cell-like spindle-shaped cells showing positive staining for CD40 and its ligand CD40L. As in type-II lesions, most of the cells in type-III lesions are activated (Fig. 2G, HLA-DR$^+$ cells shown brown). In general, the majority of macrophages were positive for both CD40 and CD40L. However, a small subset of macrophages (10–20%) consisted of cells positive only for either CD40 or CD40L, suggesting the presence of a subpopulation of macrophages more active in inflammatory processes than in lipid uptake.

The expression pattern of CD40 and CD40L in more advanced lesions followed that of the earlier type-II and -III lesions. In Fig. 3A, an overall view of an advanced type-V atherosclerotic lesion stained for macrophages (CD68$^+$ cells) is shown. This lesion has a large atheromatous core and a thick fibrous cap above it. The brack-

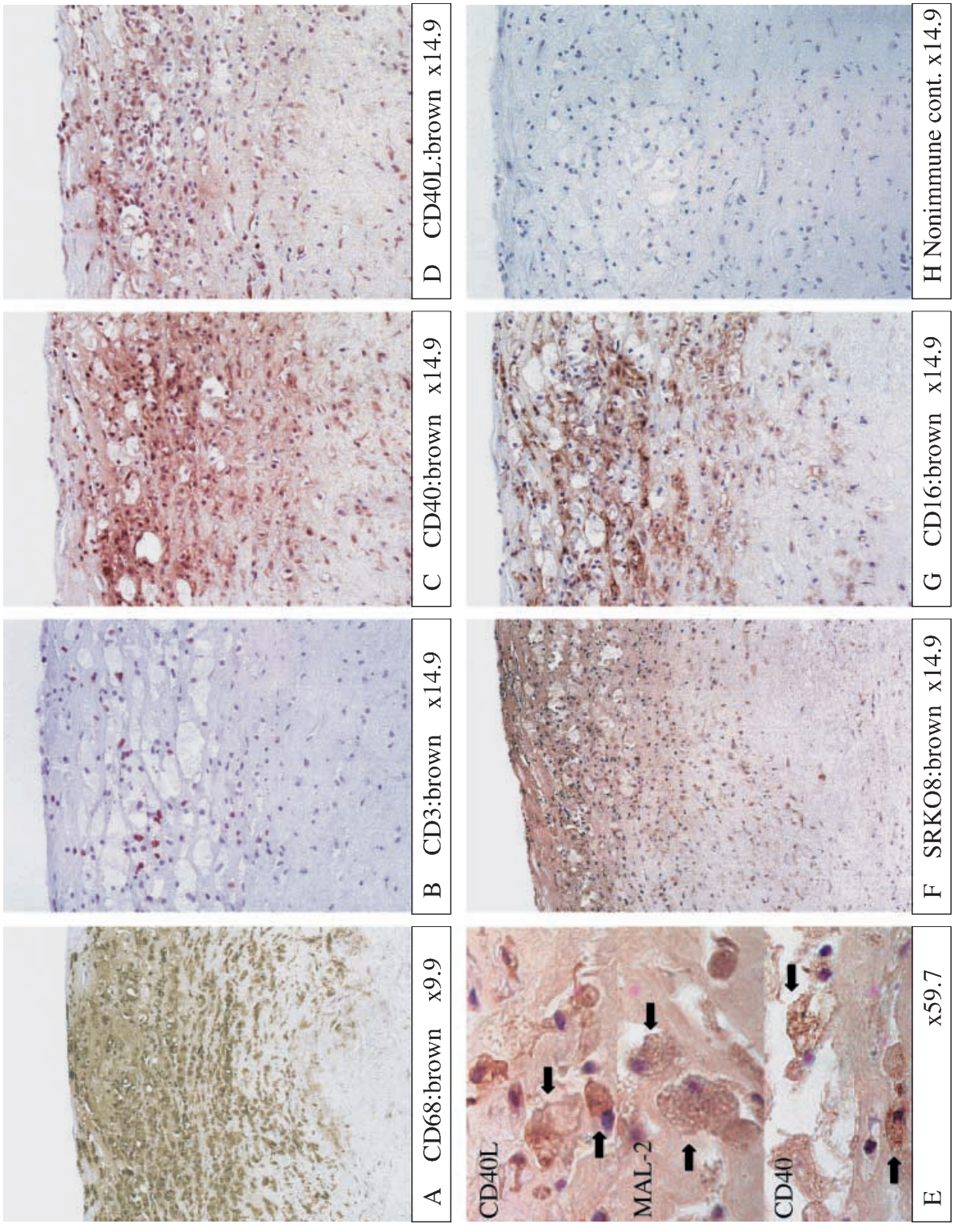


Fig. 5 CD40–CD40L expression colocalizes with epitopes characteristic of oxidized LDL, scavenger receptor, and CD16 protein (FcγRIII) in human atherosclerotic lesions. Immunostainings of serial sections (type-IV atherosclerotic lesion from human aorta; 40-year-old male). **A** Antibody specific for macrophages (positive cells are brown, anti-CD68, dilution 1:300). **B** Antibody specific for T cells (positive cells are brown, anti-CD3, dilution 1:300). **C** Antibody specific for CD40 (positive cells are brown, dilution 1:50). **D** Antibody specific for CD40L (positive cells are brown, dilution 1:50). **E** Close-ups of serial sections stained with antibodies specific for CD40L, MDA-modified LDL and CD40 (positive cells are brown, MAL-2 dilution 1:500, CD40L and CD40 dilution 1:50). **F** Antibody specific for class-A scavenger receptor (positive cells are brown, SRKO8, dilution 1:250). **G** Antibody specific for CD16 (FcγRIII) (positive cells are brown, dilution 1:30). **H** Non-immune control for the immunostainings. Hematoxylin counterstain. Original magnification ×9.9 (**A** and **F**), ×14.9 (**B–D** and **G–H**), and ×59.7 (**E**)

ets indicate the area from where the high power views of the serial sections shown in Fig. 3B and C were taken. The close-ups show overlapping staining pattern of CD40 and CD40L with that of CD3 and CD68 suggesting T cell and macrophage expression of both proteins.

Not only in lesion areas but also in the media, under and nearby the lesions, smooth muscle cells showed positive CD40 and CD40L staining. In Fig. 4A, a general view of an intermediate human aortic lesion is shown with macrophages (black) and CD40 (brown). The view in Fig. 4C–D is a higher magnification of the area shown inside brackets in Fig. 4A. The expression of CD40 in medial smooth muscle cells is shown in 4C and that of CD40L in 4D (black arrows indicate positive cells/brown).

In the lesions studied, the CD40–CD40L immunoreactivity colocalized with epitopes characteristic of oxidized LDL, scavenger receptor class A, and CD16 (FcγRIII) (Fig. 5). In Fig. 5A, a general view of a macrophage-rich area ($CD68^+$ cells/brown) of a type-IV atherosclerotic lesion is shown. Figure 5B–D are higher magnifications of the area showing immunostainings for T cells (brown), CD40 (brown) and CD40L (brown), respectively. In Fig. 5E, close-ups of foam cells from serial sections of the same lesion show that epitopes characteristic of oxidized LDL ($MAL\text{-}2^+$ cells/brown) associate with those of CD40 and CD40L. Figure 5F and G show expression of scavenger receptor (brown) and CD16 (FcγRIII; brown) in the macrophage-rich area, respectively. In all the cases, non-immune control stainings were negative (Fig. 1H, Fig. 2H, Fig. 3D, Fig. 4B and Fig. 5H).

Discussion

The present data show that the CD40–CD40L receptor ligand pair was predominantly associated with macrophages and T cells in different types of human atherosclerotic lesions. Expression of CD40 and CD40L in smooth muscle cells and endothelium was also detected. This is in accordance with previous data by Mach and co-workers [17], who established that human endothelial cells, smooth muscle cells, and macrophages constitutively express biologically active CD40L in vitro and that in human atheroma all the aforementioned three cell types express CD40L, and often at the same time its receptor CD40. As we systematically analyzed different types of atherosclerotic lesions, there was, however, no correlation between the lesion type and the profile of CD40–CD40L expression.

As atherosclerosis has features of a chronic inflammatory disease, it may be in part because CD40 ligation in macrophages triggers several functions relevant to this process [19, 20]. Production of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8 [10, 11, 17, 29], is upregulated and antigen presentation by macrophages is enhanced [12]. However, IL-1β and TNF-α have been shown to reciprocally increase CD40L expression in lesion-associated cells [17]. Synthesis and secretion of matrix metalloproteinases which can weaken the lesion matrix is stimulated, as well as the expression of tissue factor [2, 18, 21, 27]. The last two factors are directly related to the propensity of plaques to cause acute clinical manifestations.

In endothelium, the ligation of CD40 induces the expression of adhesion molecules E-selectin, vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM) [9]. This facilitates increased influx of T cells and macrophages into the vessel wall.

CD40–CD40L interaction mediates the signaling that has an effect on the activation state of both the antigen-presenting cells and T cells, leading to T-cell expansion and increased cytokine production (IL-2, IL-4, IL-5, IL-10, and IFN-γ) by T cells [23]. T cells are also able to secrete metalloproteinases after CD40 ligation [1]. Previous studies have shown that smooth muscle cells are activated by CD40–CD40L interaction, including augmented expression of adhesion molecules and secretion of proinflammatory cytokines [17, 20, 26]. In the lesion pockets with macrophages and T cells, CD40–CD40L immunostaining colocalizes with epitopes of oxidized LDL, scavenger receptor class A, and CD16 (FcγRIII). This finding is of special interest since it suggests that, among other atherogenic processes, the CD40–CD40L signaling pathway could be associated with LDL oxidation, foam cell formation, and macrophage differentiation into an immunologically active subset.

CD40 ligation activates macrophages which in turn stimulates the production of factors such as nitric oxide (NO) [15], 15-lipoxygenase, and the superoxide anion participating in LDL oxidation [30]. Activation also promotes macrophage expression of IL-1 and TNF-α [15], which, besides upregulating CD40–CD40L expression, has been shown to also induce scavenger receptor expression on smooth muscle cells and macrophages in vitro [7, 8]. Scavenger receptor facilitates formation of foam cells, i.e., uptake of oxidized LDL into activated macrophages, and is not downregulated by the cholesterol content of the cell [4, 14]. It has been shown that inhibition or genetic disruption of CD40 signaling in $ApoE^{-/-}$ and $LDLR^{-/-}$ mice results in smaller plaques with less lipid, more stable plaques with increased collagen, and reduced number of T cells and macrophages [16, 19].

The expression of CD16 in macrophages is indicative of a functional subset of cells able to participate in humoral and cellular immune responses. CD16 protein is expressed only in a minor subset of peripheral blood monocytes [25, 32] and the level of expression seen in atherosclerotic lesions may be connected to upregulation due to CD40–CD40L signaling. Binding of LDL-immune complexes to Fc receptors on monocyte macrophages activates responses that promote atherosclerotic processes [13]. It is concluded that (1) lesion macrophage populations are probably heterogeneous and may contain subsets active in different tasks, e.g., lipid uptake, cytokine secretion, and inflammatory responses; and (2) CD40–CD40L interaction is involved in atherogenesis and might provide a new target for future therapeutic interventions.

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