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Association of Activated T Cells and Dendritic Cells in Atherosclerotic Lesions in Apo E-Deficient Mice

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Abstract. Background: Antigen-specific T cell activation plays an important role in the initiation and progression of atherosclerosis. T cell activation can occur only if antigen is presented to T cells by antigen-presenting cells expressing co-stimulatory signal molecules. Apo E-deficient mice are widely used for investigating the role of antigen-presenting dendritic cells in atherosclerosis. Although the accumulation of dendritic cells in developing atherosclerotic lesions in apo E-deficient mice has been shown, it is still unknown whether dendritic cells might activate T cells directly within atherosclerotic lesions. Aims: The present study was undertaken to examine this question. We investigated whether the marker T cell activation IL-2 receptor beta chain and the dendritic cell co-stimulatory molecules CD80 and CD86 were co-expressed in atherosclerotic lesion areas that contained co-localized T cells and dendritic cells. Methods: Aortic atherosclerotic plaques obtained from apo E knockout mice were studied using an immunohistochemical approach. Results: We found a co-localized expression of CD3, CD11c, CD80, CD86 and IL-2 receptor beta chain in all analyzed tissue specimens. This observation suggests that primary activation of T cells might occur directly within atherosclerotic lesions in apo E-deficient mice.

Keywords: Dendritic cells, T cells, Atherosclerosis, Apo E-deficient mice

1. Introduction

Dendritic cells (DCs) are the most potent antigen-presenting cells with the unique ability of primary immune response initiation [1, 2]. DCs are known to originate from bone marrow progenitors circulating in the peripheral blood and subsequently penetrate peripheral tissues [1, 2]. DCs were identified in human arteries in 1995 [3, 4] and since then, further knowledge has been gained about the peculiarities of these vascular-associated DCs [5]. Mapping of the distribution of DCs in human atherosclerotic plaques has revealed that DCs are most frequently present in areas enriched with T cells and, particularly so, within inflammatory infiltrates [6]. DCs in human atherosclerotic plaques have been shown to cluster with T cells [6]. DCs, clustering with T cells in atherosclerotic lesions, have been found to display the intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1), interactions of which with leukocyte function-associated antigen-1 (LFA-1) and very late activation antigen-4 (VLA-4), respectively, are essential for T cell activation [6]. In DC/T cell interactions, DCs have been shown to display CD40 and express high levels of HLA–DR and CD1 molecules [6].

The observations made during examination of human arterial tissue specimens and theoretical speculations about the key importance of DCs in atherosclerosis have received support from experimental

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murine studies which focused on the elucidation of the functional significance of DCs in atherogenesis [5, 7, 8]. Initially, the presence of DCs was shown in atherosclerotic lesions in apo-E deficient mice [9-11]. Ludewig et al. [12] were first to report a link between immune-mediated arterial inflammation and cholesterol-induced atherosclerosis in a transgenic mouse model, mediated by DCs. Angeli et al. [13] showed that dyslipidemia associated with atherosclerotic disease systemically alters DC function. Although oxidized low density lipoproteins (LDL) could be potentially dangerous for the survival and functioning of DCs, Packard et al. [14] demonstrated that DCs can maintain antigen-processing and antigen-presentating capabilities allowing them to efficiently prime T cells under hypercholesterolemic conditions associated with atherosclerosis. In an experimental study, Liu et al. [15] showed that DC accumulation in arterial lesions is associated with plaque growth and inflammation. Further understanding of the impact of DCs in atherosclerosis has been achieved in a study by Gautier et al. [16] which unambiguously demonstrated that DCs can be considered as central to the atherosclerotic process because they are directly implicated in both cholesterol homeostasis and in the immune response. Recent studies utilizing DCs for vaccination in mouse models of atherosclerosis strengthened the concept that DCs are functionally significant in atherogenesis [17-19].

Although the presence of DCs in atherosclerotic lesions in apo E-deficient mice has been shown, it is still not certain whether DCs might activate T cells directly within atherosclerotic lesions or whether T cell activation in atherosclerosis can occur only in the lymph organs. The present study was undertaken in order to investigate this question.

2. Materials and Methods

2.1. Aortic Tissue Specimens

The present study used aortic tissue specimens, some characteristics of which were reported previously [9, 10]. The aortic samples used in the study were obtained from 13 apo-E deficient C57BL:6X129 mice maintained in microisolator cages on the PMI Autoclavable Rodent diet (No. 5010) for 8 months [9, 10]. The mice were anaesthetized with methoxyflurane. The protocol details were reported previously [9, 10].

2.2. Immunohistochemical Analysis

Aortic tissue specimens were processed for immunohistochemistry as described previously [9, 11]. Consecutive sections of the aortic wall segments were immunostained with the following antibodies: anti-CD11c (Pharmingen; HL3), anti-CD3 (Pharmingen; 145-2C11), anti-CD80 (BD Pharmingen; B7.1; 16-10A1), anti-CD86 (Pharmingen; B7.2; GL1), and anti-IL-2 receptor beta chain (Pharmingen; CD122; TM-β1). Antibodies binding to the antigens was visualized using horseradish peroxidase-conjugated antibodies and diaminobenzidine (DAB). Controls were carried out as described previously [9, 11]. No negative control showed immunopositivity.

A computerized quantitative analysis of the expression of the proteins was carried out at ×400 magnification using the Image-Pro Plus image analysis program (Media Cybernetics, Bethesda, MD). Expression of the proteins in each consecutive section was measured in pixels per 7 standard microscopic fields (0.04 mm^2 each) and the results were presented as means from all sections of the sample. Statistical analysis was performed using Prism® 5 (GraphPad Software, San Diego, CA).

3. Results

$CD3^{(+)}$ T cells were abundant in all atherosclerotic plaques analyzed. Although $CD11c^{(+)}$ DCs were detected in all atherosclerotic plaques, this cell type was less frequently observed than T cells. In plaques, the primary marker of T cell activation IL-2 receptor-beta chain was consistently displayed in some intimal areas which were also characterized by the expression of CD80 and CD86, as this was shown by examination of consecutive tissue sections. Examination of consecutive tissue sections immunostained with anti-CD3 and anti-CD11c suggested frequent co-localization of DCs with T cells as well. The relative expression of CD3, IL-2 receptor-beta chain, CD11c, CD80 and CD86 in atherosclerotic plaques are presented in Figure 1.

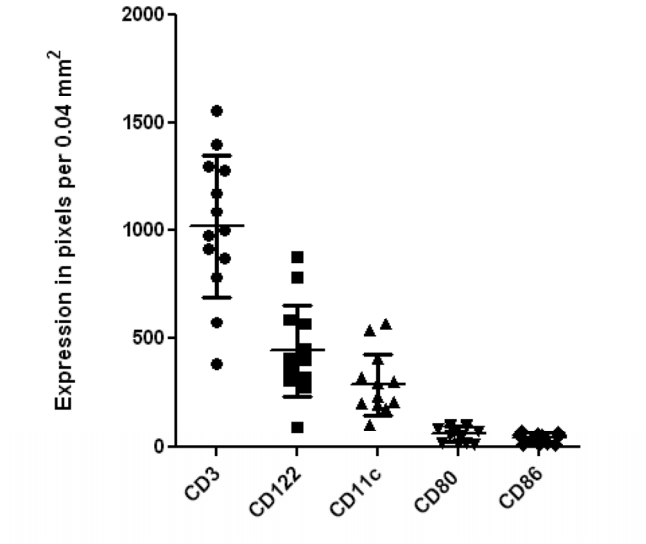

Fig. 1: The relative expression of CD3, IL-2 receptor-beta chain (CD122), CD11c, CD80 and CD86 in atherosclerotic plaques of apo-E deficient mice.

In contract to atherosclerotic plaques, no expression of CD3, IL-2 receptor-beta chain, CD11c, CD80 or CD86 was detected in aortic sites which did not show signs of atherosclerotic alteration of the intima.

4. Discussion

The study showed that, in aortic atherosclerotic plaques in apo-E deficient mice, activated T cells were co-localized with DCs that co-expressed co-stimulatory molecules CD80 and CD86. This observation is in agreement with publications of other investigators who demonstrated that activated T cells are present in apo-E deficient mice and that all essential components for local lymphocyte activation that could trigger plaque inflammation can also be detected in murine atherosclerotic plaques [13-28].

By utilizing genetically-modified mice, it has been demonstrated that DCs play an important role in the initiation and promotion of immune-inflammatory reactions in atherosclerotic lesions [13-28]. However, it is necessary to note here that the relevance of experimental murine studies to human atherosclerosis is not well understood [5]. It is well known that early atherosclerotic events in human atherosclerosis occur in the tunica intima which is composed of several layers of intimal smooth muscle cells. In contrast to human arteries, the intima in the aorta and large arteries in mice is composed only of a monolayer of endothelial cells which are located along the practically acellular subendothelial matrix, which is separated from the tunica media by the internal elastic lamina. Thus, in mice, the formation of atherosclerotic lesions completely depends on the focal invasion of medial smooth muscle cells and blood cells into the intima. In human arteries, increased proliferation of intimal smooth muscle cells and other intimal resident cell types can be sufficient to produce the atherosclerotic lesions. Because of the complexity of the human tunica intima, the information obtained in experimental murine models cannot be easily translated to the pathogenesis of atherosclerosis in humans. Nevertheless, the findings of the present work can be considered as further support to the concept that the activation of T cells in atherosclerotic lesions critically depends on the DC/T cell interaction.

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