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Widespread distribution of HLA-DR-expressing cells in macroscopically undiseased intima of the human aorta: A possible role in surveillance and maintenance of vascular homeostasis

Yuri V. Bobryshev[a,b,*], Michael M. Moisenovich[c], Olga L. Pustovalova[c], Igor I. Agapov[d], Alexander N. Orekhov[a,e,1]

[a] Institute for Atherosclerosis Research, Russian Academy of Natural Sciences, Moscow, Russia
[b] Faculty of Medicine, University of New South Wales, Sydney, Australia
[c] Biological Department, Moscow State University, Moscow, Russia
[d] Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russia
[e] Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia

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ABSTRACT

The architectonics and cell composition of the human large arteries are not sufficiently understood. The present study is the first to undertake an analysis of the distribution and quantities of HLA-DR-expressing cells in grossly undiseased human intima using immunohistochemical and immunofluorescent analysis, complemented by the advantages of confocal microscopy. The study revealed a widespread distribution of HLA-DR-expressing cells throughout the intimal space where the cells were integrated into continuous networks via long cell processes. Numbers of HLA-DR+ cells were found to be significantly larger in the middle third of the intima than in the superficial and deep intimal portions. We speculate that a widespread distribution of HLA-DR-expressing cells in the intima of normal human aorta might play a role in the surveillance and maintenance of vascular homeostasis.

Introduction

HLA-DR represents a molecule of major histocompatibility complex of class II (MHCII) (Handunnetthi et al. 2010; Nielsen et al. 2010). This molecule is characteristically expressed by antigen-presenting cells (APCs) (Geissmann et al. 2010; Handunnetthi et al. 2010; Nielsen et al. 2010). HLA-DR is crucially involved in the presentation of protein antigens to CD4+ T lymphocytes. Antigen presentation by means of MHCII is a key event of specific immune response that is necessary for the initiation of the production of antigen-specific antibodies as well as for the activation of cell-dependent cytotoxic mechanisms (Geissmann et al. 2010; Lotze and Thomson 2001). The family of APCs includes macrophages, B cells and dendritic cells (Geissmann et al. 2010; Lotze and Thomson 2001). Dendritic cells are the most powerful APCs, often referred to as professional APCs, while macrophages and B cells are described as semi-professional APCs (Geissmann et al. 2010; Nielsen et al. 2010; Lotze and Thomson 2001). Macrophages, B cells and dendritic cells are of haematogenous origin (Geissmann et al. 2010; Lotze and Thomson 2001). Apart from these haematogenous origin cells, epithelial cells of the thymus, which are not of haematogenous origin, also serve as specialized APCs (Handunnetthi et al. 2010; Nielsen et al. 2010). Other non-haematogenous cells, such as fibroblasts and endothelial cells which, in a steady state, do not express MHCII, can become capable of displaying MHCII in some circumstances *in vivo* and also of responding to IFN-γ *in vitro* (Handunnetthi et al. 2010; Nielsen et al. 2010; Lotze and Thomson 2001). Whereas the secondary lymphoid organs such as lymph nodes and spleen, as well as organ-associated lymphoid tissues, are characterized by the highest density of APCs, APCs are also constantly present in non-lymphoid organs and tissues, though in much smaller numbers (Geissmann et al. 2010; Lotze and Thomson 2001). All types of APCs as well as T cells have been identified in the internal layer of human large arteries as normal in atherosclerosis, supporting a concept that immune surveillance is a constitutive attribute of the arterial wall with immune reactions being involved

* Corresponding author at: Faculty of Medicine, University of New South Wales, Kensington NSW 2052, Australia. Tel.: +61 2 93851217; fax: +61 2 93851217.
E-mail addresses: y.bobryshev@unsw.edu.au (Y.V. Bobryshev), inat@inat.ru, office@inat.ru (A.N. Orekhov).
[1] Institute for Atherosclerosis Research, Russian Academy of Natural Sciences, Osennyaya Street 4-1-207, 121609 Moscow, Russia. Tel.: +7 903 169 08 66; fax: +7 495 415 95 94.

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in atherosclerotic alteration of arteries from a very early stage of the disease or even, perhaps, from a pre-disease stage (Hansson 2009; Hansson and Jonasson 2009; Wick et al. 2004).

The intima of arteries represents the internal layer of the vascular wall that is separated from the blood by an endothelial monolayer located on a continuous basal membrane and by the internal basal membrane that separates the intimal space from the underlying tunica media. The intima of large arteries in humans is characterized by notably more complex architectonics (Kolpakov et al. 1996; Orekhov et al. 2010; Rekhter et al. 1991, 1992) than in the arteries of animals such as rats and mice which are widely used for studies of intimal alterations of the arterial wall during atherosclerosis (Bentzon and Falk 2010; Daugherty et al. 2009; McNeill et al. 2010; Singh et al. 2009). In contrast to human large arteries, the intima of the aorta and large arteries of mice and rats consists only of the endothelium separated from the internal elastic lamina by a very thin layer of stroma matrix which practically does not contain cell elements (Bobryshev et al. 1999, 2001; Guyton et al. 1983; Ozmen et al. 2002). Human aortic intima as well as the intima of other human large arteries consists of several layers of cells with long processes by which cells form a continuous cellular network in the intimal space (Rekhter et al. 1991). The major cell components of healthy arterial intima include smooth muscle cells (Bochaton-Piallat and Gabbiani 2005; Hao et al. 2003) and stellate-shaped cells with pericyte-like properties, with ganglioside 3G5 being characteristically expressed by the latter cell type (Andreeva et al. 1998). Both smooth muscle cells and pericyte-like cells contain smooth muscle α-actin which is commonly used as a specific marker for the identification of smooth muscle cells (Andreeva et al. 1998; Bochaton-Piallat and Gabbiani 2005; Hao et al. 2003). While the major functional significance of smooth muscle cells in the tunica media has been attributed to the regulation of the arterial wall tonus (Bochaton-Piallat and Gabbiani 2005; Hao et al. 2003), the functional predisposition of intimal smooth muscle α-actin-expressing cells is far less understood, even though an important contribution of intimal smooth muscle α-actin-expressing cells in the production and maintenance of the extracellular matrix, mostly collagen and elastin, has been acknowledged (Andreeva et al. 1997; Bochaton-Piallat and Gabbiani 2005; Hao et al. 2003; Rekhter et al. 1993). Apart from smooth muscle α-actin-expressing positive cells, most of which have been established as originating in embryogenesis from the mesoderm and mesenchyme (Bochaton-Piallat and Gabbiani 2005; Hao et al. 2003; Kolpakov et al. 1996; Wright 1963), the human arterial intima also contains cells of haematogenous origin, all of which consistently express CD45 antigen (Trowbridge and Thomas 1994; Vanderlaan and Reardon 2005). In the human arterial intima, CD45+ cells are represented by monocytes/macrophages, lymphocytes, dendritic cells and mast cells (Bobryshev 2006, 2010; Orekhov et al. 2010; Vanderlaan and Reardon 2005). Wick and colleagues (Wick et al. 2004, 1997) have suggested that in the normal arterial intima, immune and immune-competent cells that represent a relatively small cell population, compared with resident smooth-muscle α-actin expressing cells, play an important role, forming vascular-associated lymphoid tissue (VALT) that continuously tests the arterial tissue environment for the presence of "danger signals". The key integrative element of VALT is dendritic cells (Millonig et al. 2001a,b,c; Wick et al. 1997, 2004). In the normal arteries, dendritic cells have been found to mostly reside in the superficial space of the intima where they, by means of their long cell processes, form continuous local cell networks (Bobryshev and Lord 1995; Millonig et al. 2001a). A comparison between atherosclerosis-resistant and atherosclerosis-predisposed areas of the normal human aorta demonstrated that cellular interactions between dendritic cells and other intimal cells are altered in

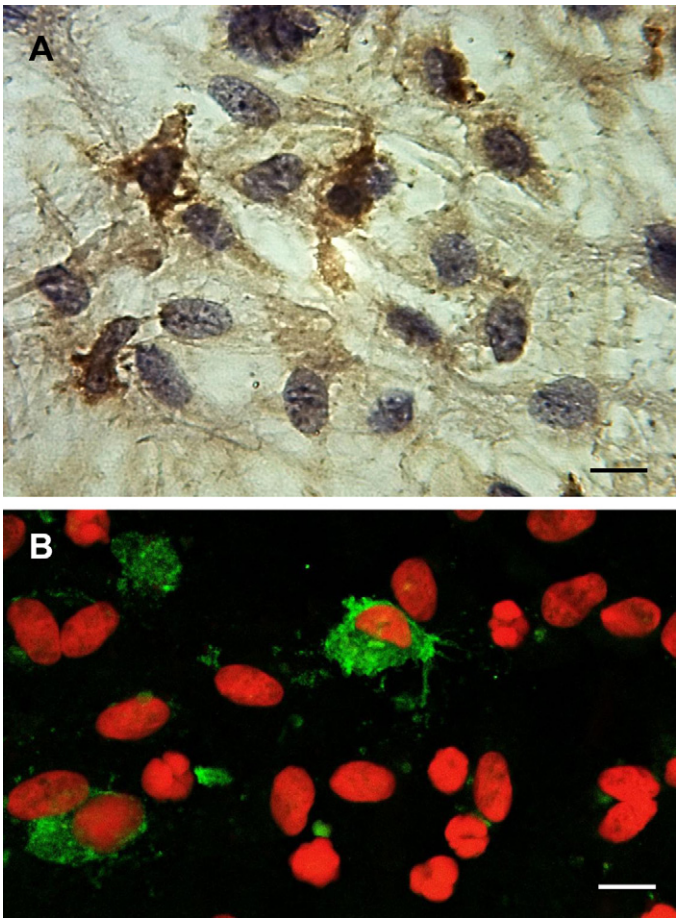

Fig. 1. HLA-DR+ cells, identified by immunohistochemical (A) and by immunofluorescent (B) techniques in human aortic intima. (A): "Hautchen" specimen of the intima. Immunoperoxidase visualization of HLA-DR with the use of DAB as a chromatogen. The nuclei were counterstained with haematoxylin. (B) An "individual" section obtained by the confocal examination of the intima. Scales = 10 μm (A and B).

atherosclerosis-predisposed areas (Bobryshev and Lord 1995), supporting the possibility that dendritic cells might be crucially involved in the development of atherosclerosis from very early moments of the alteration of vessels at a pre-diseased stage (Bobryshev and Lord 1995; Bobryshev 2010; Wick et al. 1997, 2004).

In addition to HLA-DR expression, vascular-associated dendritic cells are known to specifically express CD1a antigen (Bobryshev 2010; Bobryshev and Lord 1995, 2000; Millonig et al. 2001a,b; Wick et al. 1997; Yilmaz et al. 2004) that represents an antigen-presenting molecule involved in the presentation of antigen of lipid nature (Porcelli 1995). In our earlier studies (Bobryshev et al. 1996; Bobryshev and Lord 1996) we noted that in normal arteries, apart from the expression of HLA-DR by CD1a+ dendritic cells, other cell type(s) that were negative for CD1a expressed HLA-DR as well.

Despite the knowledge about antigen-presenting properties of arteries being of interest and important, no study has yet been undertaken to evaluate the distribution and quantities of HLA-DR-expressing cells in the normal intima. The present study was carried out with the aim to fill this particular gap in the existing knowledge. In the present study we also examined a possible association of HLA-DR-expressing cells with apo-B which represents the characteristic protein of low density lipoproteins (LDLs) that are the key elements in the initiation and progression of atherosclerosis (Kruth 2001; Ross 1999).