Mapping of vascular dendritic cells in atherosclerotic arteries suggests their involvement in local immune-inflammatory reactions

Yuri V. Bobryshev*, Reginald S.A. Lord
Surgical Professorial Unit, St. Vincent’s Hospital, University of New South Wales, Sydney, Australia
Received 5 June 1997; accepted 4 September 1997

Abstract

Objective: We previously demonstrated that vascular dendritic cells (VDCs) are present in the intima of large arteries and that their numbers are increased in atherosclerotic lesions. This study was undertaken to determine whether VDCs are involved in immune-mediated reactions in atherogenesis. Methods: Specimens of carotid artery and aorta were obtained at operation. VDCs were identified with anti-CD1a or with S-100. Co-localisation of VDCs with different intimal cells, including T-cells and macrophages, was studied using a double immunostaining procedure. In areas where the co-localising cells were detected, the peculiarities of expression of HLA-DR, ICAM-1, VCAM-1 were examined. Results: In all the atherosclerotic plaques, VDCs were seen in contact with T-cells, but these co-localising cells were irregularly distributed and were mainly found in zones of neovascularisation containing inflammatory infiltrates. In other areas, T-cell/VDC co-localisation was rarely detected but VDCs were often found in contact with macrophages. VDCs were detected also in the media beneath atherosclerotic lesions and in the adventitia, where they were mostly around vasa vasorum, especially in areas exhibiting signs of acute inflammation. In these areas VDCs expressed ICAM-1, VCAM-1 and were in contact with T-cells. In both plaques and in the adventitia, the areas with co-localising VDCs and T-cells corresponded to the areas with HLA-DR expression. Conclusions: The results suggest that VDCs are involved in T-cell activation in atherogenesis. There are two regions within the arterial wall where VDC/T-cell co-localisation mostly occurs, namely, in zones of neovascularisation containing inflammatory infiltrates located within atherosclerotic lesions, and in areas with inflammatory infiltrates around vasa vasorum in the adventitia. Possibly, some intimal VDCs migrate through the media and adventitia to adjacent lymph nodes where they present atherosclerosis associated antigens. We also speculate that VDC/macrophase contacts are essential in processing immune information in atherogenesis. © 1998 Elsevier Science B.V.

Keywords: Arteries; Atherosclerosis; T-cells; Vascular dendritic cell

1. Introduction

In previous publications we reported that cells from the family of dendritic cells reside in the intima of large arteries [1–3]. These vascular dendritic cells (VDCs) are common in atherosclerotic lesions but their mature forms are rare in normal intima [1–3]. VDCs exhibit typical ultrastructural characteristics of other dendritic cells and, similar to Langerhans cells and interdigitating cells [4–8], VDCs express both S-100 protein [1–3] and CD1a [2,3] which are markers for their immunohistochemical identification. Like other dendritic cells [4–8], VDCs express HLA-DR [2,3], display intercellular adhesion molecule (ICAM-1) [2] and vascular cell adhesion molecule-1 (VCAM-1) [2,9].

Dendritic cells are intimately associated with T-cells and through their direct contacts, dendritic cells control or regulate immune reactions [4–8]. The functional significance of VDCs in atherogenesis is unknown. As members of the dendritic cell family, VDCs can be expected to behave like other well-studied dendritic cells and thus, it is

* Corresponding author. Tel. (+61-2) 9361 2354; Fax (+61-2) 9360 4424.

0008-6363/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII S0008-6363(97)00229-0
reasonable to expect that a principal function of VDCs might be antigen processing/presentation.

Atherosclerotic plaques contain inflammatory infiltrates consisting of macrophages and activated T-cells [10–16]. Antigen specific T-cell activation depends on the interactions of T-cell receptors (TCR) with antigens presented by MHC molecules but how this is realised in atherogenesis is unclear [17–20]. Our previous observations that VDCs display ICAM-1 and VCAM-1 in atherosclerotic lesions [2,9] imply that VDCs might interact with T-cells leading to the activation of T-cells, since VCAM-1/VLA-4 interactions are critical in T-cell activation [21–23]. Indeed, in our previous electron-microscopic examination, direct contacts between T-cells and VDCs were detected [36] but it remained unclear whether this process is typical or only occasionally occurs. If co-localisation of T-cells and VDCs normally occurs in atherosclerotic lesions, this favours an association between VDC and T-cell function. This hypothesis was examined in the present study.

To examine whether VDCs usually contacted T-cells, analysis of the co-localisation of these cell types in atherosclerotic lesions was required and, therefore, a double immunostaining technique was used. Double immunostaining was used to estimate the frequency of VDC/T-cell co-localisation in different regions of atherosclerotic plaque. This approach for VDC investigation was used for the first time in the present examination. The data obtained were compared with the distribution of ICAM-1, VCAM-1 and HLA-DR expression using sets of serial consecutive sections. This enabled a determination of whether VDCs express cell adhesion molecules and whether VDCs can potentially present antigens in the same areas where they co-localise with T-cells. In the present work we also investigated VDC distribution in the media and adventitia which has not been studied before.

We now report how VDC-dependent T-cell activation in atherogenesis might occur and offer a scheme of the possible migratory pathways followed by VDCs.

## 2. Methods

### 2.1. Tissue specimens and routine histology

Arterial wall segments from 26 carotid arteries and 19 aortas were obtained from patients whose ages ranged from 32 to 71 years. The carotid specimens were obtained by endarterectomy [25] and the aortic specimens were collected during aortic reconstructions [26]. None of these specimens had been used in our previous studies [1–3,9,36]. Material was collected in accordance with the principles outlined in the Declaration of Helsinki [24] and the present study was approved by the institutional review board of St. Vincent’s Hospital, Sydney.

The arterial specimens selected for immunohistochemical investigations included atherosclerotic lesions and areas of the adjacent normal appearing arterial wall. Paraaortic lymph nodes and jugulodigastric lymph nodes near carotid bifurcations were also taken during the operations. For immunohistochemistry, some aortic samples were processed by standard formalin fixation and paraffin embedding. Other unfixed samples were immediately embedded in OCT compound, rapidly frozen in liquid nitrogen and stored at −70°C until cryostat sectioning. Paraffin and frozen sections were cut at 5–7 μm thickness and air dried.

### Table 1 Antibodies used in the study

<table>
<thead>
<tr>
<th>Designation</th>
<th>Type *</th>
<th>Clone</th>
<th>Specificity</th>
<th>Cell types identified</th>
<th>Source</th>
<th>Working dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>M</td>
<td>NA1/34</td>
<td>CD1a</td>
<td>thymocytes, Langerhans cells, interdigitating cells, vascular dendritic cells</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>S100</td>
<td>P</td>
<td>–</td>
<td>S-100A, S-100B</td>
<td>glial cells, ependyma, Schwann cells, Langerhans cells, interdigitating cells, vascular dendritic cells</td>
<td>DAKO</td>
<td>1:700</td>
</tr>
<tr>
<td>CD3</td>
<td>P</td>
<td>–</td>
<td>CD3</td>
<td>T-cells</td>
<td>DAKO</td>
<td>1:100</td>
</tr>
<tr>
<td>CD4</td>
<td>M</td>
<td>MT310</td>
<td>CD4</td>
<td>helper/inducer subtype of T-cells</td>
<td>DAKO</td>
<td>1:10</td>
</tr>
<tr>
<td>CD8</td>
<td>M</td>
<td>DK25</td>
<td>CD8</td>
<td>suppressor/cytotoxic subtype of T-cells</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>CD27</td>
<td>M</td>
<td>LT27</td>
<td>CD27</td>
<td>activated T-cells</td>
<td>BioSource Int</td>
<td>1:10</td>
</tr>
<tr>
<td>CD28</td>
<td>M</td>
<td>CD28.2</td>
<td>CD28</td>
<td>activated T-cells</td>
<td>Immunotech</td>
<td>1:25</td>
</tr>
<tr>
<td>EBM11</td>
<td>M</td>
<td>PG-MI</td>
<td>CD68</td>
<td>macrophages</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>M</td>
<td>F8/86</td>
<td>Factor VIII-related antigen</td>
<td>endothelial cells</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>SMA</td>
<td>M</td>
<td>I4</td>
<td>smooth muscle alpha-actin</td>
<td>smooth muscle cells</td>
<td>DAKO</td>
<td>1:400</td>
</tr>
<tr>
<td>HMA</td>
<td>M</td>
<td>HHF35</td>
<td>human muscle actin</td>
<td>smooth muscle cells</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>M</td>
<td>CR/43</td>
<td>MHC class II antigen (HLA-DR)</td>
<td>various cell types (antigen presenting cells)</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>M</td>
<td>84H10</td>
<td>CD54</td>
<td>various cell types</td>
<td>Immunotech</td>
<td>1:50</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>M</td>
<td>1G11</td>
<td>CD106</td>
<td>endothelial cells and other cell types</td>
<td>Immunotech</td>
<td>1:50</td>
</tr>
</tbody>
</table>

* M — monoclonal antibody; P — polyclonal antibody.
Table 2
Frequency of intimal cells in different areas of complicated atherosclerotic plaque (cells per vision field, 10×40 magnification)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Area subdivision</th>
<th>NA + II</th>
<th>NA</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDCs</td>
<td>+ + / + + +</td>
<td>+ + / + + +</td>
<td>+ + / + + +</td>
<td>+ + / + + +</td>
</tr>
<tr>
<td>T-cells</td>
<td>+ + / +</td>
<td>+ / +</td>
<td>+ + / + + +</td>
<td>+ + / + + +</td>
</tr>
<tr>
<td>Macrophages</td>
<td>+ / + / + +</td>
<td>+ / + / + +</td>
<td>+ / + / + +</td>
<td>+ / + / + +</td>
</tr>
<tr>
<td>SMCs</td>
<td>+ / + / + + +</td>
<td>+ / + / + + +</td>
<td>+ / + / + + +</td>
<td>+ / + / + + +</td>
</tr>
</tbody>
</table>

FC — fibrous cap; NA + II — neovascularisation areas associated with massive inflammatory infiltrates; NA — neovascularisation areas not associated with signs of inflammation; A — areas without neovascularisation underlying the necrotic core.

Semi-quantitative grading scale (— to ++++) for estimating cell numbers: For vascular dendritic cells (VDCs): — indicates that no cells were detected, + indicates 1–2 cells, ++ indicates 2–5 cells, +++ indicates more than 5 cells. For T-cells, macrophages and smooth muscle cells (SMCs): — means cell number less than 2% of the total cell population, + less than 20%, ++ about 50%, +++ more than 50%.

for 45 min. Sections were stained for analysis with Mayer’s haematoxylin.

2.2. Identification of intimal cells and examination of their antigen characteristics

Vascular dendritic cells in the arterial intima were identified with anti-CD1a and S-100. CD1a is a membrane associated marker and anti-CD1a antibody exclusively identifies VDCs in the arterial wall [2,3]. Antibody to nervous tissue protein S-100 is also convenient for identifying VDCs since the arterial intima does not contain neuronal or glial cells and other intimal cells do not stain positively with S-100 [1–3]. T-lymphocytes in the arterial intima were identified with anti-CD3 and the T-helper/inducer and suppressor/cytotoxic T-cell subsets were identified with anti-CD4 and anti-CD8. Since CD27 is a marker of mature lymphocytes and CD28 is known to prevent apoptosis in lymphocytes [27–29], T-cell activation was examined using anti-CD27 and anti-CD28. Intimal macrophages were identified with anti-CD68 antibody. Endothelial cells were identified by von Willebrand factor. Smooth muscle cells in the arterial intima were identified with antibody to alpha-smooth muscle actin and antibody to muscle actin as previously reported [30]. Anti-HLA-DR was used to estimate the capability of cells to present antigens. Anti-VCAM-1 and anti-ICAM-1 were used to evaluate the expression of cell adhesion molecules. The sources and working concentrations of antibodies used are given in Table 1.

2.3. Single staining immunoperoxidase procedure

An analysis was carried out using sets of consecutive parallel sections immunostained with antibodies to CD1a, CD3, CD4, CD8, CD27, CD28, CD54 (ICAM-1), CD68, CD106 (VCAM-1), HLA-DR, S-100, muscle actin (MA), smooth muscle alpha-actin (SMA), and von Willebrand factor (vWf). Although cells of different types were inter-
mingled in the plaques, in some places individual cells were located separately from others. Comparison of the consecutive sections thus enabled their immunogenic characteristics to be investigated as we previously reported [2,3,9]. In the present study, this approach was expanded by using sets of several serial consecutive sections. This
enabled the cell composition to be determined in different areas and ICAM-1, VCAM-1 and HLA-DR expression was examined in the same areas. For single immunostaining, after eliminating endogenous peroxidase activity by 0.3% H2O2 for 5 min and treatment when necessary, by 0.1% trypsin in PBS for 5–10 min, the consecutive sections were preincubated with normal goat serum and then tested with one of the antibodies by avidin-biotin complex using the ABC method [31]. The sections were incubated for 30 min with each primary antibody. After washing in tris-phosphate buffered saline, pH 7.6 (TPBS, 10 min), the sections were incubated for 20 min with the appropriate biotin-labelled secondary antibodies (horse anti-mouse — VECTOR BA-2000, or goat anti-rabbit — VECTOR BA-1000). The sections were then washed in TPBS for 5 min and treated with avidin-biotin complex (ELITE — ABC, VECTOR PK61000) for 30 min. After washing 10 min in TPBS, brown staining was produced by 5 min treatment with 3,3' diaminobenzidine (DAB). All the incubations were completed at room temperature. For negative controls, the first antibodies were omitted or the sections were treated with an immunoglobulin fraction of non-immune goat serum (VECTOR S-1000) as a substitute for the primary antibody. None of the negative control sections showed positive immunostaining. Counterstaining was performed with Mayer’s haematoxylin and sections were examined in a Olympus microscope at 10×10 and 10×40 magnifications. A semiquantitative analysis of the frequency of intimal cells in different areas was undertaken at 10×40 magnification.

2.4. Double immunostaining procedure

Differing combinations of antigens were analysed using paraffin (S-100/SMA, S-100/CD68) or frozen sections (CD1a/CD3, S-100/CD4, S-100/ICAM-1, S-100/VCAM-1) by a double immunostaining technique (DAKO-DOUBLYSTAIN™ Kit System 40, K665). This kit allows simultaneous staining for detection of two different tissue markers on one section by a combination of mono- and polyclonal antibodies and of the peroxidase–antiperoxidase (PAP) and alkaline phosphatase–antialkaline phosphatase (APAAP) technique. Using a rabbit primary antibody in the PAP system with DAB chromogen yields a brown reaction product at the site of the target antigen while a mouse primary antibody in the APAAP system with Fast Red chromogen results in a rose precipitate at the site of the identified antigen. This difference allows the topographical relationships between the two antigens to be observed. For double immunostaining, after eliminating endogenous peroxidase activity by 0.3% H2O2 for 5 min and treatment, if necessary, by 0.1% trypsin in PBS for 5–10 min, the consecutive tissue sections were preincubated with normal swine serum and then tested according to the manufacturer’s instructions (DAKO). The sections were incubated with the working solution of the two primary antibodies prepared by mixing equal volumes of each antiserum diluted to one-half of the established optimal dilution appropriate for single immunostaining procedure. The sections were then sequentially incubated with the mixture of anti-rabbit and anti-mouse link antibodies, the mouse APAAP immune complex, followed by the rabbit PAP immune complex. Positive and negative controls were carried out according to the DOUBLYSTAIN Kit System manufacturer’s instructions. None of the negative control sections showed positive immune staining. Counterstaining was performed with Mayer’s haematoxylin.

3. Results

3.1. Selection of specimens for study of VDC/T-cell co-localisation

VDCs were detected in all the samples studied and the pattern of their distribution in the atherosclerotic lesions was similar in the carotid and aortic specimens, consistent with our previous observations [1–3]. T-cells (CD3+) were seen in all atherosclerotic lesions studied. T-cell subpopulations were estimated in serial frozen sections stained with anti-CD4 and anti-CD8. Both these T-cell subtypes were present in the atherosclerotic lesions, with their proportions in individual lesions ranging from about equal numbers to three CD4+ cells per one CD8+ cell. Consecutive paraffin sections stained with anti-CD3, anti-CD68, S-100, and anti-SMA were compared to select specimens appropriate for further study of specific cell co-localisation by a double immunostaining procedure. This analysis showed that fatty streaks and non-complicated atherosclerotic plaques contained large numbers of CD68+ and CD3+ cells, intermingled with SMA+ cells. Some CD68+ and SMA+ cells were identified as foam cells. S-100+ cells were also seen between CD3+ cells.

Fig. 2. S-100+ cells (A, C) and CD3+ cells (B, D) in massive inflammatory infiltrate in an aortic plaque. A and B — consecutive paraffin sections. C — detail of figure A. D — detail of figure B. Asterisks indicate the necrotic core. ABC immunoperoxidase technique, counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×100, ×400, ×400.

Fig. 3. S-100+ cells (brown) located between CD4+ cells (rose) in large (A–C) and small (D) inflammatory infiltrates. B — detail of figure A. Aortic plaques. Frozen sections, double immunostaining (S-100+CD4: PAP+APAAP) technique, counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×400, ×400, ×400.
CD68⁺ macrophages and foam cells of different origin. Despite the high frequency of co-localisation of S-100⁺ cells and CD3⁺ cells, the different cell types were often intermingled and very closely apposed, which precluded determining whether these cells specifically contacted each other or were simply densely packed together.
In contrast, in the complicated plaques, specific co-localisation of different intimal cells could be analysed since these plaques contained areas where most of the intimal cells were separated by 10–30 μm, with only some cells seen in close apposition to each other. Even though the plaques contained a large number of CD3⁺, CD68⁺, and SMA⁺ cells, each of these cell types was distributed irregularly throughout the plaque, being prevalent in certain intimal areas (Table 2). Furthermore, these plaques were enriched with S-100⁺ cells.

Each plaque selected for the present analysis showed a typical structure with a necrotic core containing some calcified deposits of different sizes, plexuses of neovascularisation in the base, and a fibrous cap on the luminal side. To estimate the contacts between different intimal cells, uniform areas were selected for analysis, namely (i) fibrous cap, (ii) areas of neovascularisation not associated with notable inflammatory infiltration, (iii) inflammatory areas associated with neovascularisation underlying the necrotic core, and (iv) areas underlying the necrotic core and not associated with neovascularisation.

In all plaques studied, S-100⁺ cells were seen in different areas of the plaques but were always more frequent within inflammatory infiltrates and around the necrotic core, especially in areas beneath the necrotic core (Fig. 1). A semiquantitative method to indicate relative differences was applied and the peculiarities of the distribution of different cell types are summarised in Table 2.

### 3.2. Peculiarities of VDC / T-cell and VDC / macrophage co-localisation in plaques

#### 3.2.1. Inflammatory infiltrates associated with neovascularisation

Analysis of single immunostained sections demonstrated that about 90% of inflammatory infiltrates contained S-100⁺/CD1a⁺ cells, identified as VDCs, and that the cellular processes of these VDCs embraced round-shaped cells with the appearance of inflammatory cells (Fig. 2A, C), most of which were CD3 positive (Fig. 2B, D). Double immunohistochemical analysis in combinations such as anti-CD1a/anti-CD3 and S-100/anti-CD4 confirmed that in these inflammatory infiltrates, VDCs co-localised mostly (approximately 90%) with T-cells (Fig. 3). In contrast, analysis of the anti-CD68/S-100 combination showed that in these inflammatory infiltrates, CD68⁺ cells seldom co-localised with S-100⁺ cells.

#### 3.2.2. Areas underlying the necrotic core not associated with neovascularisation

These areas presented the best opportunity to study cellular co-localisation since most cells were located at least 10 μm apart. Analysis of double immunostained formalin sections showed that more than 60% of co-localising cells were macrophages (CD68⁺) and VDCs (S-100⁺) (Fig. 4), and that VDC/VDC close apposition was also prominent (10–30%). Only a few contacts between T-cells (CD3⁺) and VDCs (CD1a⁺) were detected in frozen sections of 5 aortic and 9 carotic specimens. The CD1a/CD3 examination required frozen sections which gave a much lower resolution than paraffin sections which meant that some T-cell/VDC contacts could have been missed. A few smooth muscle cells were also found in these areas but their close apposition with S-100⁺ cells were not apparent.

#### 3.2.3. Neovascularisation areas without signs of inflammation

A few co-localising CD68⁺ and S-100⁺ cells were detected in these areas. T-cells were seldom seen and they did not contact other intimal cells while the clustering of VDCs with each other was usual. Typically, these VDCs were located irregularly, even mosaically, around vessels formed by neovascularisation.

#### 3.2.4. Fibrous caps

S-100⁺/CD1a⁺ cells were distributed mostly without obvious contact with other cell types. Sometimes when VDCs were present in groups of 2–4 cells they were seen in close association with each other.

### 3.3. VDCs in the media

The present study extended our previous examination of VDC distribution in the arterial wall by including the media and adventitia. In the media of non-atherosclerotic arteries, a few S-100⁺/CD1a⁺ cells were detected between smooth muscle cells. Immunostaining with anti-CD68 demonstrated the presence of a few macrophages also. Capillaries in the media were identified by von Willebrand factor-positive immunostaining. Analysis of consecutive sections showed that CD68⁺ macrophages were always associated with capillaries and these capillaries were continuous with capillaries in the adventitia. Analysis of parallel sections showed that some medial

---

Fig. 4. S-100⁺ cells (brown) and CD68⁺ cells (rose) in the area underlying the necrotic core (A–D). Contacting cells are shown by arrows. Note that some VDCs and macrophages form close embracing contacts. Asterisk in figure A indicates the necrotic core. B — detail of figure A. Paraffin sections, double immunostaining (S-100+/CD68: PAP+APAAP) technique, counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×400, ×400, ×400.

Fig. 5. A group of S-100⁺ cells (brown) located between SMA⁺ cells (rose) in the media. B — detail of figure A. Aortic specimen, paraffin section, double immunostaining (S-100+/SMA: PAP+APAAP), counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×400.
Fig. 6. Inflammatory infiltrates in the adventitia containing S-100⁺ cells (brown) contacting CD4⁺ cells (rose) (A–D). In figure A, inflammatory infiltrate is shown by the open arrow while the solid arrow indicates an S-100⁺ nerve twig (brown). Frozen sections, double immunostaining (S-100 + CD4: PAP + APAAP) technique, counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×400, ×400, ×400.

Fig. 7. ICAM-1 expression in cells and cell clusters surrounding the necrotic core (asterisk). B — detail of figure A. Frozen section, ABC immunoperoxidase technique, counterstaining with Mayer’s haematoxylin. Original magnifications: ×100, ×400.
S-100+/CD1a+ cells were associated with these capillaries as well but other S100+/CD1+ cells were located independently from capillaries and were CD68 negative. Double immunostaining with antigen combinations such as S-100/CD68 and S-100/SMA clearly demonstrated that the S-100+ cells were CD68 negative and that in some parts of the media, these S100+ cells were surrounded by smooth muscle cells only (Fig. 5).

In the media underlying atherosclerotic plaques, the numbers of S100+/CD1a+ cells increased markedly (at least 2- to 3-fold) compared with the adjacent media of nonatherosclerotic areas of the specimens. These S100+/CD1a+ cells were often found in the diminished medial layer underlying complicated atherosclerotic plaques, frequently in direct contact with smooth muscle cells.

3.4. VDCs in the adventitia

Unlike the intima and media, the adventitia contains nerve endings which stain intensively with S-100 but not with anti-CD1a. The S-100+ nerve twigs with their fine endings and S-100+ cells of nervous origin allowed them to be easily distinguished from VDCs exhibiting a dendritic or flame-like shape (Fig. 6). Around the vasa vasorum in the atherosclerotic arterial wall, massive inflammatory infiltrates were present which usually (in more than 90% of infiltrates) contained S-100+/CD1a+ cells of typical dendritic cell appearance. Other S-100+/CD1a+ cells were dispersed around the inflammatory infiltrates, sometimes near the media.

3.5. Topography of cell adhesion molecules and molecules suggesting cell activation

The present study extended our previous investigation of ICAM-1 and VCAM-1 expression [2,9] by comparing the immunopatterns of ICAM-1 and VCAM-1 distribution in the arterial wall.

ICAM-1 and VCAM-1 expressing cells were irregularly distributed throughout the plaques, although in most specimens the patterns of VCAM-1 and ICAM-1 immunoreactivity were similar. In most sections, ICAM-1 and VCAM-1 were widely expressed on the endothelium lining the neovasculature as well as on nonendothelial cells, but few endothelial cells lining the vessel lumen stained positively with antibodies to ICAM-1 and VCAM-1. This expression was most intense in areas where inflammatory infiltrates were associated with neovascularisation and in cells and cell clusters surrounding the necrotic core (Fig. 7).

Analysis of serial sections showed that different cell types including VDCs (CD1a+, S-100+), macrophages (CD68+), and smooth muscle cells (SMA+) expressed ICAM-1 and VCAM-1, and that the areas populated by ICAM-1+ and VCAM-1+ cells were contiguous with areas containing mainly ICAM-1+ and VCAM-1+ cells. However, the areas of intensive ICAM-1 and VCAM-1

Fig. 8. S-100+ cells (A, B) and CD3+ cells (C, D) in a jugulodigastric lymph node adjacent to an atherosclerotic carotid artery. A and C — consecutive paraffin sections. ABC immunoperoxidase technique, counterstaining with Mayer’s haematoxylin. Original magnifications: × 100, × 400, × 100, × 400.
expression corresponded to those areas where VDCs contacted T-cells and macrophages.

In the media, S-100+/CD1a− cells were also ICAM-1 and VCAM-1 positive. Intensive ICAM-1 and VCAM-1 expression was detected in the adventitia and was mostly associated with vasa vasorum and inflammatory infiltrates.

In most specimens, the areas expressing cell adhesion molecules also strongly displayed HLA-DR. About half of the CD3+ cells also expressed HLA-DR suggesting that they were activated. Staining of serial sections with anti-CD3, anti-CD27 and anti-CD28 demonstrated that some CD3+ cells in inflammatory infiltrates displayed CD27 and CD28. These areas often corresponded to areas where CD1a+ cells co-localised with CD3+ cells. The assessment of serial sections also demonstrated that some CD68+ cells co-localised with CD3+ cells in areas which corresponded to sites of HLA-DR expression. Areas of the adventitia with inflammatory infiltrates also displayed intensive HLA-DR expression.

3.6. VDCs in adjacent lymph nodes

Analysis of lymph nodes removed from a site near the atherosclerotic arterial wall demonstrated a large number of S-100+ dendritic shaped cells (Fig. 8). These cells were located irregularly throughout the lymph nodes but were always found in T-cell associated zones expressing CD3 antigen.

4. Discussion

The family of dendritic cells consists of various members located in different organs [4–8,32]. Dendritic cells, whether in lymphoid or nonlymphoid tissues, constitute only a minor cell population. In blood, dendritic cells represent less than 0.1% of the white blood cells [6,33]. In the epidermis, Langerhans cells represent 3 to 5% of the total cell population [6,34] while in spleen cell suspensions 1–1.6% are dendritic cells [6,35]. Our previous investigations showed that mature vascular dendritic cells expressing S100 and CD1a are rarely found in normal (non-atherosclerotic) intima [1–3] even though immature VDCs can often be detected [36]. These immature VDCs can be unambiguously identified by their typical ultrastructural features [36] including a tubulo-vesicular apparatus unique to dendritic cells. They are regularly located along the subendothelial layer, and are often in close contact with endothelial cells [36]. These observations imply that expression of both S100 and CD1a is associated with the activation and maturation of VDCs. Although we did not perform a precise quantitative analysis of VDCs, we estimated that the number of VDCs (including both immature and mature forms) in the normal intima is similar to the number of Langerhans cells in the skin, namely, 2 to 5% [1,36]. However, in some areas of atherosclerotic lesions, VDCs were estimated to represent about 10% of the total cell population when viewed at 10×40 magnification.

Dendritic cells are intimately associated with T-cells [4–8]. Since VCAM-1/VLA-4 and ICAM-1/LEA-1 are critical in T-cell activation [21–23], the presence of cell adhesion molecules such as ICAM-1 and VCAM-1 on VDC surfaces implies that VDCs are capable of forming contacts responsible for T-cell activation. In the present work, co-localisation of VDCs and T-cells was demonstrated which suggests that VDCs may be instrumental in T-cell activation and that specific T-cell activation might depend on cell-to-cell contacts involving cell adhesion molecules. The observation that interacting VDCs and T-cells also express HLA-DR favours our speculation that VDCs might be involved in T-cell activation. Although the presence of HLA-DR and co-localisation of VDCs and T-cells suggests an interaction, physical proximity does not necessarily define a functional relationship and thus, the functional significance of VDC-T cell co-localisation requires further clarification.

T-cell involvement in immune responses in atherosclerosis is well recognised [10–13,16–20], but whether the T-cells in atherosclerotic lesions respond to a restricted set of antigens or recognise a wide range of epitopes is unclear. Different mechanisms may be involved in the activation of different subtypes of T-cells. Similar to other dendritic cells which typically express high levels of MHC class II molecules [4–8], VDCs express HLA-DR [2,3] which might suggest their involvement in antigen presentation. VDCs also display on their surfaces CD1a which has recently been recognised as an antigen presenting molecule in the same sense as the classical MHC class I and II molecules [37–39], and thus an involvement of CD1-restricted responses in atherosclerosis cannot be excluded. Furthermore, the association of VDCs with T-cells may be a secondary response rather than indicating a primary antigen presenting function for VDCs.

In the present examination, VDCs were seen to be irregularly distributed throughout the atherosclerotic lesions which suggests that their distribution and intercellular communications might depend on microenvironmental influences. In atherosclerotic plaques, more than 90% of VDCs co-localising with T-cells were located in neovascularisation areas associated with inflammatory infiltrates. Outside these areas, co-localising VDCs and T-cells were only occasionally or even rarely detected, and contacts between VDCs and macrophages were more usual.

Multiple and close contacts between VDCs and macrophages in atherosclerotic lesions were detected in our previous electron microscopic investigation [36]. The present study showed that the co-localisation of these cells mostly occurs in areas not associated with neovascularisation. The VDC/macrophage co-localisation was especially abundant around the necrotic core where most destruction processes occur. Possibly, this is where antigen presenting cells accept most arterial destruction antigens. The pres-
ence of a large number of VDC/macrophage contacts in this area also may imply that in atherogenesis, interactions between these cell types are essential for processing immune information. Dendritic cells lack endocytotic activity and lysosomal activity [4–8] and VDC/macrophage co-operation might compensate for these VDC deficiencies. The small number of VDCs contacting T-cells around the necrotic core suggests that to present antigens engulfed and collected within and around the necrotic core, VDCs migrate to other areas where the microenvironment allows T-cell/VDC interactions.

Based on our observations of where VDCs and T-cells frequently co-localised, we identified two arterial wall regions where VDC-dependent T-cell activation might mostly occur (Fig. 1). First are the zones of neovascularisation associated with inflammatory infiltrates within atherosclerotic lesions. The second region suspected of intensive presentation of immune information by VDCs to T-cells is the areas around the vasa vasorum in the adventitia where inflammatory infiltrates accumulate. That the adventitia might be involved in this process is further supported by our observations of the concentration and co-localisation of large numbers of T-cells and VDCs in these areas and that these areas are characterised by strong HLA-DR expression. The VDCs which were observed in the media may represent VDCs migrating from the intima to the adventitia (Fig. 1).

The presence of regions in the atherosclerotic intima containing immuno-competent cells together with antigen presenting cells supports the notion of a ‘vascular-associated lymphoid tissue’ proposed in 1995 by Kleindienst et al. [40]. Like the mucosa-associated lymphoid tissues (MALT) of respiratory and gastrointestinal tracts, vascular-associated lymphoid tissue (VALT) is thought to be designed to screen ‘vascular tissue’ for potentially harmful antigens [40].

We suggest that lymph nodes associated with the arterial wall are a third region where VDCs may activate T-cells. VDCs might share the origin and migratory routes with other well-studied dendritic cells such as Langerhans cells. Dendritic cells mature in different tissue sites from MHC class II (Ia) negative bone marrow-derived precursors [6] and develop region-specific characteristics which could reflect lineage differences [6,32]. Within nonlymphoid tissues, these precursors apparently develop into Ia+ cells with the capacity to take up and process antigens [4–7,41]. These cells can migrate into lymphoid tissues and mature into lymphoid dendritic cells with specialised co-stimulatory functions necessary for T-cell activation [4–7,42–44]. Dendritic cells can migrate via the blood to the spleen in addition to their documented migratory route via the lymphatics, as veiled cells, into lymph nodes where they are known as interdigitating cells [4–7,44].

Our present pilot study of lymph nodes associated with the arterial wall demonstrated a large number of S-100+ cells co-localised with T-cells in lymph nodes. We propose that some of these S-100+ dendritic shaped cells might be interdigitating cells of VDC origin. The distinctive nature of the lymph node interdigitating dendritic cell microenvironment allows interactions between a single presenting cell and numerous potential responses [5] which leads us to consider whether lymph nodes are involved in immune mechanisms in atherogenesis. This hypothesis needs further verification including comparing the numbers of interdigitating cells in lymph nodes located on the atherosclerotic arterial wall such as para-aortic and jugulodigastroic lymph nodes with those lymph nodes remote from nonatherosclerotic arteries. VDCs might migrate from the intima to the adventitia and thence to lymph nodes, but we cannot exclude an exchange of dendritic cells back and forth between the arterial wall and lymph nodes (Fig. 1). This possible to-and-fro traffic needs further study.

The results of the present study favour the hypothesis that VDCs are responsible for T-cell activation. Our observations lead us to speculate that the readiness of VDCs to present antigen to T-cells requires a primary interaction of VDCs with intimal macrophages. Some intimal VDCs migrate through the media to the adventitia where they interact with T-cells within inflammatory infiltrates around the vasa vasorum. Local lymph nodes may also be involved in VDC/T-cell interactions. Perhaps, only some VDCs migrate to lymphoid organs while others contact T-cells directly within the intima, since T-lymphocyte-like cells clustering with VDCs were detected in our electron microscopic study [36].

Acknowledgements

This research was supported by St. Vincent’s Clinic Foundation, Sydney.

References


