Myxofibrosarcomas Contain Large Numbers of Infiltrating Immature Dendritic Cells

Elizabeth J. Soilleux, MA, MB, BChir, PhD,1,2 Brian Rous, MA, MB, BChir, PhD,2 Karl Love, MB, BS,2 Sarah Vowler, MSc,3 Lesley S. Morris,1 Cyril Fisher, MD, FRCPATH,4 and Nicholas Coleman, MBChB, PhD, FRCPATH1,2

Key Words: Myxofibrosarcoma; Dendritic cell; DC-SIGN; Dendritic cell–specific intercellular adhesion molecule-3 grabbing nonintegrin; Lectin

DOI: 10.1309/JEB7DGHH01J11VUM

Abstract

Myxofibrosarcoma is a malignant tumor with distinctive histologic features and is believed to be derived from fibroblasts. The function of infiltrating myeloid cells in myxofibrosarcoma is poorly understood. It previously has been shown that a combination of dendritic morphologic features and expression of the C-type lectin, dendritic cell–specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), is useful for identifying DC populations in tissue sections. In the present study, we found that 3% to 61% (median, 22%) of cells in myxofibrosarcomas express DC-SIGN and have dendritic morphologic features. These DC-SIGN–positive cells are not in cell cycle and are consistent with infiltrating DCs. The percentage of DCs in myxofibrosarcomas is independent of tumor grade. It previously has been shown that DC-SIGN–positive cells are either immature DCs or DCs that predominantly activate T<sub>H</sub>2 cells, both subsets likely to give rise to ineffective antitumor responses. The DC-SIGN–positive DCs that we have identified in myxofibrosarcoma may, therefore, be involved in the induction of ineffective immune responses or even tolerance to tumor antigens.

Myxofibrosarcomas are malignant tumors with distinctive histologic features and are believed to be derived from fibroblasts. They commonly show a nodular growth pattern and are composed of round or stellate tumor cells with indistinct cell margins, pale eosinophilic cytoplasm, and hyperchromatic atypical nuclei embedded in a myxoid matrix. Myxofibrosarcomas vary from a hypocellular, mainly myxoid, and purely spindle-cell appearance (low-grade neoplasms) to a high-grade, pleomorphic (malignant fibrous histiocytoma–like) tumor with multinucleated giant cells, high mitotic activity, and areas of necrosis. The presence of myeloid cells in myxofibrosarcoma is well described, although the function of such cells remains unclear. Myxofibrosarcoma may therefore be regarded as a useful model for studying tumor infiltration by myeloid cells.

Determination of whether infiltrating myeloid cells in a neoplasm are macrophages or dendritic cells (DCs) may have important consequences for our understanding of antitumor immunity. DCs are specialized antigen-presenting cells capable of activating naive T lymphocytes and, thus, initiating an immune response. Macrophages, on the other hand, are likely to perpetuate only an existing immune response. Furthermore, the phenotype of a DC may vary, and this variation may permit the initiation of functionally different immune responses ranging from a cytotoxic response to immune tolerance.

It has proven difficult to identify a molecule that labels the majority of DCs but does not immunostain macrophages. The recently identified C-type lectin, DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), has emerged as a useful marker of DCs, and recent work has suggested that a combination of DC-SIGN immunostaining...
and dendritic morphologic features identifies the majority of DCs in peripheral tissues. DC-SIGN also may be present on specialized populations of macrophages in the lung and placenta, but we have failed to identify expression of DC-SIGN by macrophages at any other location, including sites of inflammation.

DC-SIGN expression also provides information about DC function. DC-SIGN is present predominantly on immature DCs, which express few costimulatory molecules and are likely to be poor activators of T lymphocytes. Several DC lineages have been identified in humans, and recent work has demonstrated that DC-SIGN also is expressed by the DCs that predominantly activate TH2 cells (DC2) lineage. This finding is consistent with data demonstrating that DC-SIGN expression is up-regulated by both interleukin-4 and interleukin-13, cytokines intimately involved in the TH2 axis of immunity. Both immature DCs and DC2s are likely to lead to relatively ineffective antitumor immune responses.

In the present study, we demonstrated extensive DC-SIGN expression on cells with dendritic morphologic features in all grades of myxofibrosarcoma. By using antibodies against the cell cycle marker minichromosome maintenance protein-2 (Mcm2), we performed double-labeled fluorescent confocal microscopy to show that the DC-SIGN–positive cell population is not in cell cycle, even in high-grade myxofibrosarcoma, and is, therefore, consistent with an infiltrating DC population. Our findings raise pertinent questions about the role of DC-SIGN–positive DCs in myxofibrosarcoma and potentially in other malignant neoplasms.

Materials and Methods

Selection of Tissue Samples

Archival paraffin blocks were retrieved from 43 cases of myxofibrosarcoma from the Department of Histopathology, Royal Marsden Hospital, London, England. Sections were reviewed histologically, and tumors were stratified by grade, using the criteria of Trojani and colleagues. There were 23 low-grade tumors, 8 intermediate-grade tumors, and 12 high-grade tumors. Lymph node tissue was obtained from Addenbrooke’s Hospital, Cambridge, England. Tissue was obtained after receiving approval from the Local Research Ethics Committee.

Single Immunostaining of Paraffin Sections

We stained 5-µm paraffin sections by the indirect immunoperoxidase technique using rabbit anti–DC-SIGN polyclonal serum and preimmune serum on serial sections as a negative control, as described previously.

Immunofluorescent Staining for Confocal Microscopy

To perform double immunostaining for confocal microscopy, paraffin sections were immunostained with rabbit polyclonal anti–DC-SIGN serum and mouse monoclonal anti-Mcm2 antibody. Pressure cooking was used for antigen retrieval, as described previously. Sections were incubated in both primary antibodies overnight in a mixture of tris(hydroxymethyl)aminomethane (Tris)-buffered saline (TBS), 1% bovine serum albumin, and 10% normal goat serum. Following rinsing in TBS, fluorescein isothiocyanate–conjugated goat antimouse antibody (Sigma, Poole, England) and Alexa 594 conjugated goat antirabbit antibody (Molecular Probes, Eugene, OR) were used for detection. Sections were mounted in fluorescence mounting medium (DAKO, Glostrup, Denmark), and images were obtained using serial scanning techniques with a confocal laser scanning microscope TCS 4D (Leica Lasertechnik, Heidelberg, Germany). Lymph node tissue was used as a positive control for both antibodies. As a negative control, serial sections of each tumor were immunostained with preimmune rabbit serum and/or omission of the anti-Mcm2 mouse primary antibody.

Cell Counting and Statistical Analysis

For each tumor, the number of cells positive for DC-SIGN and the number of cells negative for DC-SIGN were recorded by each observer (K.L., B.R.) in 5 microscopic fields (×400 magnification) representative of the tumor as a whole, in terms of overall appearance and cellularity. No areas showing acute or chronic inflammation were included. We counted 600 to 1,500 cells in each case. For each tumor, the percentage of DC-SIGN–expressing cells was calculated by dividing the total number of cells positive for DC-SIGN by the total number of cells counted. All counting was performed by 2 independent observers who were blinded to tumor grade and to the findings of the other observer. The overall DC-SIGN labeling indices represented the means of the counts of the 2 observers. The Kruskal-Wallis test (Sigma Stat, version 10, SPSS, Chicago, IL) was used to determine whether there was a difference in the percentage of DC-SIGN–expressing cells between different grades of myxofibrosarcoma.

Results

Cells expressing DC-SIGN were present in myxofibrosarcomas of all grades. DC-SIGN–positive cells were present in areas showing a range of growth patterns, including pleomorphic, storiform, and myxoid regions (Image 1), and were observed in areas of high and low cellularity within each tumor. However, the percentage
of cells that were DC-SIGN–positive differed widely between cases, with a range of 3% to 61%. Figure 1. The median frequency of DC-SIGN–positive cells was 22%.

The majority of the DC-SIGN–positive cells had dendritic morphologic features, although some appeared more spindle shaped or occasionally oval. No DC-SIGN–positive cells showed cytologic features of malignancy. Consequently, in the high-grade tumors, DC-SIGN–positive cells represented a subset morphologically separate from the DC-SIGN–negative cells. In contrast, in the low-grade tumors the DC-SIGN–positive and the DC-SIGN–negative cells were difficult to distinguish morphologically.

To confirm that all the DC-SIGN–expressing cells represented infiltrating DCs rather than a neoplastic component of the myxofibrosarcoma, we reasoned that many of the malignant cells would be likely to be in cell cycle and, therefore, would express Mcm2. Accordingly, double immunostaining of the myxofibrosarcoma cases for confocal microscopy was performed with a monoclonal antibody...
against Mcm2. Fewer than 1% of the cells that immuno-stained with anti–DC-SIGN expressed nuclear Mcm2 in myxofibrosarcomas of any grade. In contrast, there was a high frequency of expression of Mcm2 by the DC-SIGN–negative cells. Confocal microscopy also confirmed the dendritic morphologic features of the DC-SIGN–positive cells (Image 2).

The percentage of DC-SIGN–positive cells as a function of the grade of myxofibrosarcoma is shown in Figure 1. The Kruskal-Wallis test showed no difference in the percentage of cells expressing DC-SIGN between the 3 grades of tumor (P = .93).

**Discussion**

We investigated whether infiltrating myeloid cells in myxofibrosarcomas are DCs. Myxofibrosarcomas contained between 3% and 61% (median, 22%) DC-SIGN–positive cells, and the percentage was independent of tumor grade. The DC-SIGN–positive cells had largely dendritic morphologic features, and fewer than 1% expressed the cell cycle marker Mcm2, regardless of tumor grade. Although only a proportion of neoplastic cells in myxofibrosarcoma would be expected to be in cell cycle at any given time, the lack of Mcm2 expression by the large majority of DC-SIGN–positive cells supports the notion that such cells represent infiltrating DCs rather than neoplastic cells. DC-SIGN–positive DCs are likely to represent a substantial fraction of the cells in myxofibrosarcoma previously reported as expressing myeloid lineage markers. At present, it is not possible to determine the relative numbers of DC-SIGN–positive and DC-SIGN–negative DCs in myxofibrosarcomas, as there is no good pan-DC marker (or marker of DC-SIGN–negative DCs) that does not also label macrophages.

To our knowledge, this is the first report describing the presence of DC-SIGN–positive cells in any neoplasm. Because such large numbers of DC-SIGN–positive DCs are seen in myxofibrosarcoma, it is important to establish their function within the tumor. The primary role of DCs is antigen presentation to T lymphocytes. Antigen presentation usually occurs only in specialized lymphoid tissues at some distance from a primary tumor. Therefore DC-SIGN–positive DCs are likely to be important in the uptake of antigen in tumors. DC-SIGN may contribute to this process by binding to mannosylated molecules and possibly by binding to one of its known ligands, intercellular adhesion molecule-3 (ICAM-3; CD50). Of note in this regard is that ICAM-3 is expressed at high levels on apoptotic cells, which are frequent finding in neoplasms.

The reasons for the presence of such large numbers of DC-SIGN–positive DCs in myxofibrosarcoma compared with normal tissues or sites of inflammation remain unclear. One possibility is that myxofibrosarcomas have specific mechanisms by which they recruit DCs, which also may determine the exact phenotype of DCs recruited. Such mechanisms could involve the release of chemotactic factors and/or the induction of various endothelial molecules that are important for macrophage-DC vascular transmigration. In support of this notion, macrophage chemotactic protein-1 (MCP-1) has been detected in cultures of myxofibrosarcoma cell lines. MCP-1 is known to act as a potent chemoattractant for DCs.

In addition, specific stimuli may be required to induce DCs to leave tissues and migrate to regional lymph nodes. Such stimuli may include proinflammatory cytokines, products of various pathogens and other “danger signals” such as tissue damage and inflammation, acting via the induction of mediators such as chemokines. It is possible that myxofibrosarcomas (and other tumors) may, to varying degrees, lack appropriate stimuli for DC migration, causing large numbers of DCs to remain within the tumor. Further studies of DC populations in local lymph nodes in relation to tumor grade, tumor behavior, and clinical outcome seem warranted.
The functions of the DC-SIGN–positive DCs in myxofibrosarcoma remain unclear. It previously has been shown that the more mature CD83+ DCs are almost universally negative for DC-SIGN in vivo, suggesting that DC-SIGN is expressed rarely by DCs that are competent to activate T lymphocytes. Moreover, DC-SIGN is expressed only on the blood dendritic cell antigen–2–positive subset of peripheral blood DC precursors, which have been shown to have a pre-DC2 phenotype. DC-SIGN–positive cells in nasal polyps also demonstrate a DC2 phenotype. DC2 cells are reported...
to stimulate naive T lymphocytes to become Th2 rather than Th1 effector cells and also may render CD4+ T cells anergic. It therefore is possible that DCs in tumors such as myxofibrosarcomas may induce an inappropriate immune response or even tolerance to tumor antigens.

We have shown that 3% to 61% (median, 22%) of cells within myxofibrosarcomas are infiltrating DCs and that the percentage is independent of tumor grade. We confirmed that the DC population in myxofibrosarcomas is not in cell cycle and, therefore, unlikely to be neoplastic. At present, the role of these DCs within the tumors remains largely unexplained.

From the 1Medical Research Council Cancer Cell Unit, Hutchison/ Medical Research Council Research Centre, Cambridge, England; 2Department of Histopathology, Addenbrooke’s Hospital, Cambridge; 3Cambridge University Centre for Applied Medical Statistics, Institute of Public Health, Robinson Way, Cambridge; and 4Department of Histopathology, Royal Marsden Hospital, London, England.

Supported by a Medical Research Council Clinical Training Fellowship and the Sackler Foundation, Cambridge, England (Dr Soilleux), and by the Medical Research Council and Cancer Research UK, London, England (Dr Coleman).

Address reprint requests to Dr Soilleux: Medical Research Council Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge CB2 2XY, England.

Acknowledgment: We are most grateful to Kate Bird for assistance with cutting and immunostaining the histologic sections.

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