CD31 (JC70) expression in plasma cells: an immunohistochemical analysis of reactive and neoplastic plasma cells

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Abstract

Aims—To investigate the immunohistochemical expression of CD31 (JC70) in normal and neoplastic plasma cells.

Methods—Plasma cells in bone marrow biopsies and extramedullary locations were examined. All extramedullary biopsies were formalin fixed and paraffin embedded. The bone marrow biopsies were fixed in formal acetic acid and embedded in paraffin wax. Twenty multiple myelomas (12 bone marrow and eight extramedullary deposits), 10 extramedullary plasmacytomas, and 30 biopsies with reactive plasma cells (10 bone marrow, 20 extramedullary biopsies) were stained with anti-CD31 (JC70) using the streptavidin-biotin detection system with dianaminobenzidine as a chromogen. Antigen retrieval in bone marrow biopsies was achieved by pressure cooking. In all other biopsies, antigen retrieval was achieved by microwave pretreatment.

Results—All 20 extramedullary cases with reactive plasma cells showed intense membrane staining. Focal staining was detected in reactive plasma cells in bone marrow biopsies. Five of 10 plasmacytomas showed membrane staining. None of the cases of multiple myeloma, either medullary or extramedullary, showed any immunoreactivity for CD31.

Conclusions—CD31, a member of the immunoglobulin supergene family of cell adhesion molecules, is strongly expressed in extramedullary reactive plasma cells, focally in bone marrow reactive plasma cells, and occasionally in extramedullary plasmacytomas.

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Keywords: CD31; cell adhesion molecules; plasma cells

Platelet-endothelial cell adhesion molecule-1 (PECAM-1 or CD31) belongs to the immunoglobulin supergene family (IgSF) of adhesion molecules. Molecules belonging to the IgSF share the immunoglobulin structural homology unit.1

CD31 is expressed abundantly on endothelial cells, and a monoclonal antibody to CD31 has been used as a marker of these cells. Antibody JC70 detects a formalin resistant epitope which is widely distributed on a variety of benign endothelial cells. In detailing the range of tissues stained by the antibody, it was noted that in fixed tissue sections JC70 staining is restricted to endothelial cells and occasional bone marrow plasma cells.2

Although several cell adhesion molecules have been identified in normal and neoplastic plasma cells, CD31 expression has not previously been reported in plasma cells in formalin fixed, paraffin embedded tissues outside of the bone marrow.

We noticed during diagnostic use that reactive (polyclonal) plasma cells showed intense membrane staining with the anti-CD31 antibody, JC70. This prompted an immunohistochemical study of CD31 expression in reactive and neoplastic plasma cells.

Methods

The study consisted of a cohort of 60 cases which comprised 20 cases of multiple myeloma, 10 cases of plasmacytoma, and 30 cases with reactive plasma cells. Conditions in which reactive plasma cells were encountered included Castleman’s disease, Hodgkin’s disease, and mixed chronic inflammation.

Formalin fixed, paraffin wax embedded tissue blocks of cases with reactive and neoplastic plasma cells were obtained from the archives of the department of anatomical pathology, University of Natal Medical School. These consisted of eight extramedullary deposits of multiple myeloma, 10 plasmacytomas, and 20 cases with reactive plasma cells. There were biopsies from various extramedullary sites, including soft tissue abscesses, granulation tissue, reactive lymph nodes, perivascular chronic inflammatory infiltrates in skin, and so on. Formal acetic acid fixed, paraffin wax embedded tissue blocks of bone marrow trephine biopsies were retrieved from the files of the university department of cellular science, University of Oxford. These comprised 12 cases of multiple myeloma and 10 cases with reactive plasma cells, including five cases that had normal haematological indices. None of the 12 multiple myeloma cases had evidence of concurrent extramedullary deposits.

Tissue sections of 2 μm thickness were mounted onto poly-L-lysine coated glass slides (Sigma Diagnostics, St Louis, Missouri, USA). The sections were then heat fixed for 10 minutes at 60°C and deparaffinised. Antigen retrieval was achieved by microwave pretreatment (H2500 microwave processor, Energy Beam Sciences, Agawam, Massachusetts, USA) in a 0.01 M trisodium citrate solution (pH 6.0) for 10 minutes at 85°C. The bone...
marrow sections were mounted on silane coated glass slides. They were processed in a similar way to that described above, but antigen retrieval in these cases was facilitated by pressure cooking.

Endogenous peroxidase activity was blocked using an aqueous solution of 3% hydrogen peroxide. Sections were stained with monoclonal anti-CD31 antibody (JC70: Dako A/S, Copenhagen, Denmark; dilution 1/30) using the peroxidase labelled streptavidin-biotin kit (Dako). The reaction was visualised using diaminobenzidine (Liquid DAB, Dako) as a chromogen. Endothelial cells and megakaryocytes in the tissue sections were used as positive controls. Negative controls, in which the primary antibody was withheld, were also run simultaneously. In addition, all bone marrow sections were stained with monoclonal antibody VS38c (Dako, High Wycombe, UK; prediluted) to assess the number of plasma cells present. In all cases, light chain immunostaining was reassessed using kappa and lambda light chains (Signet Laboratories, Dedham, Massachusetts, USA; prediluted, polyclonal).

**Results**

The main results are shown in table 1. All 20 extramedullary biopsies with reactive plasma cells showed intense cytoplasmic membrane staining with CD31 (fig 1). The number of reactive plasma cells that stained varied from 75% to 100% of at least 100 cells counted per slide. In contrast, reactive plasma cells in the 10 bone marrow sections showed focal membrane staining only.

Five of the 10 plasmacytomas also showed membrane immunoreactivity for CD31. The remainder were negative. None of the cases of multiple myeloma, including both bone marrow sections and extramedullary deposits, showed any reactivity (fig 2). Internal controls in these cases were positive.

When applying χ² and Fisher's exact test to compare CD31 expression in reactive (polyclonal) and neoplastic (monoclonal) plasma cells, statistically significant p values of < 0.01 and < 0.05 respectively, were obtained.

Monoclonality was confirmed in all plasmacytomas and multiple myelomas. In reactive plasma cells, polytypic light chain expression was noted. All reactive plasma cells and myeloma cells in the bone marrow stained with VS38c.

**Discussion**

The molecules belonging to the immunoglobulin supergene family show diversity in cellular distribution and function. All members of the IgSF share a common structure, the immunoglobulin homology unit. Molecules belonging to this family include the major histocompatibility molecules, the T cell receptor, the platelet derived growth factor receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony stimulating factor-1 receptor, colony 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endothelial cells is immunopositive for extramedullary deposit of IN 1..t (JC70). Once downregulated, blasts and material has been implicated in cellular contact between osteoblasts and myeloma cells. Loss of CD56 expression has often been postulated to play a role in the dissemination of myeloma cells out of the bone marrow.20 21

CD31 expression on plasma cells in surgical biopsy material has not been reported previously. We have shown that CD31 is expressed strongly in all extramedullary reactive plasma cells, focally in bone marrow reactive plasma cells and in some extramedullary plasmacytomas, but not at all in multiple myeloma cells in either medullary or extramedullary locations. The loss of this molecule most probably occurs during the process of neoplastic transformation. In the inflammatory process, CD31 may participate in heterotypic interactions with other adhesion molecules and this may be a reason for the high CD31 expression in reactive plasma cells. It is uncertain whether other mechanisms—including chemical mediators of inflammation— influence CD31 expression. The precise role of CD31 in plasma cells is presently unclear but further investigation may reveal its function in these settings.

The reason for the decreased CD31 immunostaining in reactive plasma cells in bone marrow is unknown, but we speculate that it may be due to the local environmental milieu of the bone marrow, which is different from that of extramedullary sites. However, this postulate does not hold true for the extramedullary plasmacytomas, where five CD31 positive cases were encountered. The decreased staining of reactive plasma cells in the bone marrow cannot be ascribed to the acid fixation of bone marrow specimens because the internal controls were positive for CD31.

This is the first study to look at JC70 staining in plasma cells. We have added to the list of cells known to be immunoreactive for the JC70 antibody and shown that reactive extramedullary plasma cells in routinely fixed tissues strongly express CD31. Finally, we are not advocating the use of anti-CD31 to differentiate between reactive and neoplastic plasma cells but emphasise the intense immunoreactivity shown by extramedullary reactive plasma cells, and the immunohistochemical presence of another cell adhesion molecule in plasma cells. Further investigation is necessary to determine the reasons for the staining differences of plasma cells for CD31 in reactive neoplastic conditions, such as monoclonal gammopathy of uncertain significance and neoplastic states.

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