Reed-Sternberg Cells in Hodgkin’s Disease Contain Fibronectin

By Gene D. Resnick and Ralph L. Nachman

Reed-Sternberg cells in the lymph nodes from five patients with Hodgkin’s disease were studied. Indirect immunofluorescence on fixed sections with a monospecific antisera to fibronectin revealed abundant cytoplasmic fibronectin in approximately 90% of the Reed-Sternberg cells. In addition, the cells were shown by immunofluorescence to contain polyclonal IgG; however, factor VIII antigen, albumin, fibrinogen, alpha-2-macroglobulin, antithrombin III, and ceruloplasmin were not present. The abundant cytoplasmic fibronectin suggests that Reed-Sternberg cells are derived from tissue macrophages.

THE REED-Sternberg cell, morphologically described over a century ago1 and considered vital for the histologic diagnosis of Hodgkin’s disease, remains of uncertain cellular origin. Although much of the lymphocytic infiltrate in lymph nodes involved with Hodgkin’s disease has been found to bear thymic-dependent (T-cell) markers,2 conflicting evidence has argued for either a lymphocytic3 or monococyte-macrophage origin4 of the Reed-Sternberg cell.

Fibronectin, or plasma cold insoluble globulin, is a cell surface and plasma protein that functions as a major nonimmune particulate opsonin and is also important in cell adhesion and connective tissue structure.5–8 Studies presented here demonstrate that fibronectin is present in Reed-Sternberg cells.

MATERIALS AND METHODS

Antigens and Antibodies

Rabbit antibodies to human fibronectin were prepared with fibronectin purified by gelatin affinity chromatography followed by fractionation of DEAE cellulose, a minor modification of the method of Engvall et al.5 Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis10 of the purified antigen in the unreduced state yielded a single band of 450,000 daltons. With reduction the band moved in to the gel, with a molecular weight of 220,000. Purified fibronectin was mixed with an equal volume of Freund’s complete adjuvant (Difco Laboratories, Detroit, Mich.) and used to raise an antiserum of high titre in white rabbits. Rabbit anti-human fibronectin IgG fraction was absorbed with human plasma that had been previously passed on a gelatin-sepharose column and thus rendered free of fibronectin.9

An IgG fraction of goat anti-rabbit IgG (GAR IgG) was prepared from goat antiserum kindly provided by Dr. Carl Becker (Cornell University Medical College), and conjugated to crystalline fluorescein isothiocyanate (FITC), as previously described.12

Preparation of Tissue for Immunofluorescence

Samples of surgical lymph node specimens from five untreated patients were obtained within 2 hr of operation. These were divided into approximately 1-g samples, rapidly frozen in liquid nitrogen after mounting in O.C.T. compound (Lab-Tek Products, Naperville, Ill.), and stored at –70°C. Pathologic analysis of surgical specimens with usual histologic techniques revealed all nodes to be involved with Hodgkin’s disease, nodular sclerosis type. In addition, lymph nodes and tumor tissue from nine patients with malignant lymphoma were studied. These included 3 with nodular poorly differentiated lymphocytic lymphoma, 3 with diffuse, poorly differentiated lymphocytic lymphoma, and 3 with diffuse histiocytic lymphoma.

For immunofluorescence, tissue sections were mounted on glass slides previously coated with 1% agarose, fixed in 100% acetone at 4°C for 10 min, and stored at 4°C up to 36 hr before use.

Fixed sections were prepared for immunofluorescence with two 10-min washes in PBS. Excess saline was removed and 20 μl of rabbit antiserum, or control nonimmune R IgG, was applied. The sections were incubated at 37°C in a moist chamber, followed by three 15-min changes in PBS. Excess saline was blotted from the coverslips and 20 μl of F-GA R IgG was applied to cover the sections. After further incubation and rinsing, the sections were coverslipped over a glycerol-phosphate buffered saline (9:1) solution. Antibodies and control rabbit IgG were used in dilutions of 1:20 to 1:60. Additional controls included substitution of PBS for rabbit antiserum or use of rabbit antifibronectin pretreated with purified human fibronectin (188 μg/ml). Acetone fixed confluent cultures of human endothelial cells, kindly provided by Dr. Eric A. Jaffe.

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Frozen sections of normal lymph node, and sections of liver and spleen obtained at postmortem examination, prepared as described, served as additional controls. Peripheral blood smears were fixed in methanol, and treated by the above methods for immunofluorescence.

**Immunofluorescence Microscopy and Photomicrography**

Immunofluorescence microscopy and photomicrography were performed with a Leitz-Ortholux II Photomicroscope and an Orthomat W microscope camera with 35 mm Kodak Tri-X Pan or Ektachrome 400 film, at 800 ASA.

**Histology**

Following immunofluorescence and photomicrography, the tissue sections were stained with hematoxylin and eosin. Coverslips were floated off the sections in tap water; fixation with activated Zenker's solution (Zenker's solution plus 5% glacial acetic acid) was followed by a thorough tap water rinse and storage in 70% ethanol.

Sequentially, the sections were rinsed in distilled water, treated with Gram's iodine and 5% aqueous sodium thiosulfate, rinsed and then stained with Delafield-hematoxylin and eosin by usual histologic methods. Sections were coverslipped with Pro-Texx mounting medium (Scientific Products, McGaw Park, Ill.) and photomicrographs made using Kodak Ektachrome 160 Tungsten film.

**RESULTS**

Typical Reed-Sternberg cells were identified in tissue sections from five patients with nodular sclerosing Hodgkin's disease. By conventional histology and phase optics, these were identified as large binucleate cells with characteristic nucleoli (Fig. 1A and C). Immunofluorescence analysis of the distribution of fibronectin in the tissue sections revealed bright staining of Reed-Sternberg cells (Fig. 1B). The staining was diffuse throughout the cytoplasm with nuclear shadows generally well defined. This pattern was noted in approximately 90% of all typical Reed-Sternberg cells examined simultaneously by phase optics and immunofluorescence.

In addition, a diffuse, web-like extracellular pattern of fibronectin staining was noted throughout the nodes, in areas of lymphoid channels and sinusoids. Heavy staining was seen in the node capsule. No fibronectin was detected by immunofluorescence in any of the non-Reed-Sternberg cells infiltrating the node section. Positive staining for fibronectin was seen in the connective tissue of vascular adventitia and in vascular endothelial basement membrane.

Normal peripheral blood monocytes and lymphocytes were not stained with antifibronectin. In addition, no cellular staining was seen in sections of normal lymph nodes, liver, or spleen. A network of stromal fluorescence was observed as previously described.\(^{13}\)

Additional immunofluorescent staining in Reed-Sternberg cells was observed with various antisera as shown in Table 1. Bright immunoglobulin staining with anti-Ig-\(\kappa\) and anti-Ig-\(\lambda\) was seen diffusely throughout the cytoplasm of most Reed-Sternberg cells. The small lymphocyte infiltrate present throughout the node sections demonstrated diffuse, scattered staining with antisera to immunoglobulin light chains. Reed-Sternberg cells did not stain with anti-factor VIII:AGN. Staining with anti-factor VIII:AGN was
seen only in nodal vessel endothelium in a punctuate, granular pattern.

Staining of both Reed-Sternberg cells and the fibronectin-containing node stroma was abolished by pretreatment of rabbit anti-fibronectin with purified fibronectin. No staining was seen in control sections stained with nonimmune rabbit IgG or with PBS as a substitute for rabbit antibody.

Fibronectin was not detected in the cells of the tumor infiltrates in the tissue studied from nine patients with non-Hodgkin's lymphoma. Stromal staining for fibronectin was observed in the three types of lymphoma tissues.

**DISCUSSION**

These studies indicate that Reed-Sternberg cells in Hodgkin's disease contain abundant fibronectin. Fibronectin is a glycoprotein of high molecular weight, present in the plasma and tissue stroma of vertebrates. It is immunologically cross-reactive with cold insoluble globulin, first described in 1948 as a plasma co-precipitate of fibrinogen in the cold. The cell and tissue associated forms of fibronectin have been variously termed large, external transformation sensitive (LETS) protein, cell surface protein, and fibroblast surface antigen. In addition, antigenic and biochemical identity of plasma fibronectin and opsonic \( \alpha_2 \)-SB protein has been demonstrated. Fibronectin has a wide distribution in normal human tissue, most notably in vascular basement membrane, connective tissue stroma, and lymphoid sinusoids, corresponding to histologically demonstrable reticulin. Fibronectin has been found in the connective tissue stroma of solid tumors, but demonstration of cell-associated fibronectin has been limited to cultured fibroblasts of various species including man, astroglial cells in culture, human endothelial cells, and human platelets. Intracellular fibronectin has been demonstrated in fibroblasts, endothelial cells, and macrophages.

The origin of the Reed-Sternberg cell remains controversial; immunologic characterization of the Reed-Sternberg cell has been reported by several investigators, with conflicting results. Intracellular immunoglobulin, notably Ig-\( \lambda \) and Ig-\( \lambda \), and less commonly IgM, has been detected in Reed-Sternberg cells by immunohistochemical and by immunofluorescence techniques. Some have reported intracellular immunoglobulin as evidence of lymphocytic origin of Reed-Sternberg cells, while others have cited polyclonal immunoglobulin in fresh tissue and enzyme and surface marker characteristics of cells in culture as evidence for a macrophage-monocyte origin. Further evidence supporting the macrophage origin of Reed-Sternberg cells comes from studies demonstrating the cell's ability to internalize aggregated IgG.

Acetone fixation renders cell membrane permeable to antibody permitting detection of intracellular components. Thus our studies suggest that fibronectin is present in the cytoplasm of Reed-Sternberg cells. We found no evidence for the presence of factor VIII:AGN, albumin, fibrinogen, \( \alpha_2 \)-macroglobulin, antithrombin III, or ceruloplasmin in Reed-Sternberg cells, suggesting that the presence of fibronectin was not due to indiscriminate uptake of protein by the Reed-Sternberg cells. Others have found intracellular albumin in the Reed-Sternberg cell using the immunoperoxidase technique, but this has not been confirmed in our studies or those of Kadin et al. using immunofluorescence. Whether this is due to the use of paraffin-fixed tissue in the immunoperoxidase technique or other causes is not clear.

The presence of fibronectin in the Reed-Sternberg cell...
cell suggests a nonlymphocytic origin for the cell. Although macrophages synthesize fibronectin there is no evidence for the synthesis of fibronectin by lymphocytes. In addition, no fibronectin was detected in the tumor cells of the non-Hodgkin's lymphoma tissue. These tumors are considered to be of lymphocytic origin. It is possible that the Reed-Sternberg cell is a transformed, macrophage-derived cell of endothelial or fibroblast origin. The lack of factor VIII:AGN by immunofluorescence argues against an endothelial origin and the presence of intracellular immunoglobulin argues against a fibroblast origin. We conclude that the Reed-Sternberg cell is derived from the macrophage cell line.

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