Malignant Fibrous Histiocytoma (MFH) Cells and Macrophages/Histiocytes Have a Common Antigen Recognized by a Monoclonal Antibody Risen against a Rat MFH-Derived Cloned Cell Line

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ABSTRACT. A monoclonal antibody (B9) was generated by using a rat malignant fibrous histiocytoma (MFH)-derived cloned cell line (MT-8) as the immunogen. Immunohistochemically, B9 reacted specifically with a cytoplasmic antigen of MT-8 cells. Furthermore, B9 immunolabeled another MFH-derived cloned cells (MT-9) and histiocytic sarcoma cells, as well as macrophages/histiocytes in normal and diseased tissues of rats. These findings suggest the presence of a common antigen recognized by B9 between MFH cells and macrophages/histiocytes. This suggests that MFH cells may express histiocytic nature.

NOTE Pathology

Malignant fibrous histiocytoma (MFH) is a tumor consisting of an admixture of fibroblastic, histiocytic and undifferentiated cells arranged in a storiform growth pattern [1]. The undifferentiated cells may be a progenitor with capacity to differentiate into histiocytic and fibroblastic cells [3]. However, the relationship between histiocytic cells in MFH and true macrophages/histiocytes belonging to the mononuclear phagocyte system (MPS) remains to be investigated [2, 9]. In contrast, Takeya et al. reported that histiocytic cells are not true histiocytes but infiltrating macrophages induced by chemotactic factors [9]. Thus, the histogenesis of MFH is still debatable [1, 3]. We established two different cloned cell lines (MT-8 and MT-9) from a rat transplantable MFH [10]. Depending on electron microscopical and immunohistochemical findings, MT-8 was regarded as undifferentiated mesenchymal cells and the induced tumors developed undifferentiated sarcoma, whereas MT-9 cells had both nature of fibroblasts and histiocytes and the induced tumors showed a storiform growth pattern typical of MFH [10]. These cell lines are useful experimental models for the histogenesis of MFH [10, 13]. In order to pursue the features of MFH-constituting cells, we have attempted to establish monoclonal antibodies against rat MFH cells [4]. The present study describes that a monoclonal antibody (named B9), generated by using MT-8 cells as the immunogen, immunolabeled macrophages/histiocytes, suggesting the presence of a common antigen between MFH and MPS cells. This may shed some light on nature of histiocytic cells present in MFH.

The methods of producing B9 were described in detail in a previous paper [4]. Briefly, MT-8 cells were inoculated several times into BALB/c mice. The spleens removed from these mice were dispersed and fused with mouse myeloma cells (line: P3-X63-Ag8.653) in the presence of 50% polyethylene glycol 1500 (Boehringer Mannheim, Gmbh, Germany). The hybridomas producing monoclonal antibodies reactive specifically with MT-8 cells were selected and consecutively cloned twice. The culture supernatant was named B9. The isotype of B9 was IgG3, which was determined by the monoclonal antibody isotyping Kit (Serotec, Tokyo).

Besides MT-8 and MT-9, HS-P was examined as cultured cell lines. HS-P cells were established from a rat transplantable histiocytic sarcoma (HS-J), of which original cells were derived from the MPS [5]. Subcutaneous tumors induced in syngeneic rats by inoculating these cell lines (10⁶ cells/rat) were also examined. Because these cell lines were established from F344 rat tumors, we examined the following normal and diseased tissues in F344/DuCrj rats (Charles River Japan, Hino, Shiga): as normal tissues, all organs/sites in adult rats over 10 weeks old, in neonatal rats aged 1, 4 and 8 days, and in fetal rats on gestation days 15, 18 and 20; as diseased tissues, CCL2-induced hepatic or cispilatin-induced renal lesions [11, 12]. All animals were euthanatized under ether anesthesia. Cryostat tissue sections, 7 µm-thick, and cultured cells on tissue culture glass slides were fixed in acetone for 10 min. Then, they were immunohistochemically stained by the indirect immunoperoxidase method [4]. Briefly, after treatment with 1.5% skimmed milk for 30 min at room temperature, the slides were incubated for 1 hr at 4°C with B9. Subsequently, they were incubated for 1 hr at 37°C with a peroxidase-conjugated sheep anti-mouse Ig F(ab')2 fragment antibody (Amersham Int., Buckinghamshire, UK) diluted 1/100. Diaminobenzidine tetrahydrochloride (DAB) was used as the chromogen and the slides were counterstained with hematoxylin. As negative controls, non-immunized mouse sera were used instead of B9.

In addition to immunopositive reaction of MT-8 cells (Fig. 1) used as an immunogen, MT-9 cells were immunolabeled with B9. Neoplastic cells of subcutaneous tumors...
Fig. 1. Cultured MT-8 cells. Positive reactions against B9 is seen as fine granules in the cytoplasm (arrows). Immunocytochemistry, counterstained with hematoxylin. ×300.

Fig. 2. MT-9 tumor tissue. Neoplastic cells show positive reactions against B9 (arrows). Immunohistochemistry, counterstained with hematoxylin. ×300.

Fig. 3. Cultured HS-P cells. Most of the cells react positively to B9 (arrows). Immunocytochemistry, counterstained with hematoxylin. ×400.

Fig. 4. The digestive tract of an adult rat. A large number of B9-positive macrophages are seen in the lamina propria mucosa (arrowheads). Immunohistochemistry, counterstained with hematoxylin. ×300.

Fig. 5. The lung of an adult rat. Alveolar macrophages are labeled by B9 (arrowheads). Immunohistochemistry, counterstained with hematoxylin. ×200.

Fig. 6. The spleen of an adult rat. Macrophages within follicles and red pulps are labeled by B9 (arrows). F: follicle. Immunohistochemistry, counterstained with hematoxylin. ×300.
induced by MT-8 and MT-9 (Fig. 2) cells were also stained with B9. The positive reactions appeared as fine granules in the cytoplasm (Fig. 1), apparently lysosome-like structures; but the organelles should be investigated further. Interestingly, B9 also labeled HS-P (Fig. 3) and its induced tumor cells, indicating that B9 can react with rat macrophages/histiocytes. In normal tissues of fetal, neonatal and adult rats, cells immunoreactive for B9 were detected in the dermis, Glisson’s sheath of the liver and the interstitium of kidneys and heart as well as in the lamina propria mucosa of the digestive tract (Fig. 4). These cells were round or oval in shape, and had relatively abundant cytoplasm. Depending on the distribution pattern and cell morphology, these positive cells were regarded as resident macrophages (histiocytes). Alveolar macrophages (Fig. 5), and macrophages within follicles and red pulps in the spleen were also immunopositive for B9 (Fig. 6). In the lymph node, thymus and bone marrow, diffusely distributed macrophages, round or ovoid in shape, were labeled by B9. B9 also reacted with cells in fetal liver with hematopoiesis; presumably, these cells might be cells differentiating toward monocytes/macrophages. However, Kupffer cells located along the sinusoids in the adult liver did not react to B9. At inflammatory sites in the liver and kidneys induced by chemicals, infiltrating macrophages were positive for B9.

It has been reported that human MFH cells expressed antigens specific for human macrophages [7]. The present study showed that B9 immunolabeled MT-8, MT-9 and macrophage-like HS-P cells as well as macrophages/histiocytes in normal and diseased tissues of rats. These findings suggest that MFH cells may express histiocytic nature; these cells may be related to the lineage of MPS [6, 7]. MPS cells are considered to be originally derived from stem cells in the bone marrow [8]. Macrophages consist of heterogeneous cell populations such as monocytes, exudate macrophages, resident macrophages and dendritic cells; furthermore, they differ in ontogeny, functions and immunophenotypes depending on anatomical location, differentiation and maturation [8]. However, there has been no evidence that pluripotent mesenchymal cells can differentiate into true macrophages/histiocytes, like bone marrow stem cells. Thus, at present, histiocytic cells in MFH should be considered to be due to transient phenotypic modulation of MFH-constituting cells under some conditions [9, 13, 14].

In conclusion, our study demonstrated the presence of common antigen detected by B9 between MFH cells and macrophages/histiocytes of rats. This gives us a clue to the histogenesis of MFH, in particular the presence of histiocytic cells. Furthermore, macrophage populations immunoreactive with B9 and biochemical functions of B9 should be investigated, in order to clarify the nature of histiocytic cells in MFH.

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