Phenotypic Changes in Lipopolysaccharide-Treated Cloned Cells Derived from Transplantable Rat Malignant Fibrous Histiocytoma

Jyoji YAMATE, Masanori TAJIMA, Kazumoto SHIBUYA, Mitsuru KUWAMURA, Takao KOTANI, and Sadashige SAKUMA

Department of Veterinary Pathology, College of Agriculture, Osaka Prefecture University, 1–1 Gakuen-cho, Sakai, Osaka 593 and Nippon Institute for Biological Science, 2221–1 Shin-Machi, Ome, Tokyo 198, Japan

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ABSTRACT. To investigate a possible phenotypic modulation, MT-8L and MT-9L cells were induced by in vitro culture of undifferentiated MT-8 and fibrohistiocytic MT-9 cells, which had been established from a rat malignant fibrous histiocytoma (MFH), in the medium containing 10 µg lipopolysaccharide (LPS)/ml. MT-8L and MT-9L gave greater positive reactions for histiocytic lysosomal markers and showed ultrastructures of histiocytic natures. In MT-8L, α-smooth muscle actin-positive myofibroblastic cells also significantly increased in number. MT-8L expressed both histiocytic and myofibroblastic phenotypes. MT-8L-induced tumors consisted mainly of storiform type MFH, differing from undifferentiated sarcoma type induced by MT-8. MT-9L and MT-9 tumors showed a storiform pattern. A phenotypic modulation of MFH cells was easily induced by LPS treatment. — KEY WORDS: lipopolysaccharide, phenotypic modulation, rat MFH cell.


The histogenesis of malignant fibrous histiocytoma (MFH) is still debatable [2, 3, 5, 6, 13]. It has recently been speculated that MFH may be a tumor consisting of a population of primitive mesenchymal cells with multidirectional differentiation [3, 5, 7, 9, 11, 16]. Thus, a phenotypic modulation of MFH cells may easily be induced under certain conditions [7, 9, 10, 11, 16, 17]. Two cloned cell lines (named MT-8 and MT-9) established from a transplantable rat MFH by us are useful for studying the histogenesis of MFH [18, 19]. MT-8 cells were interpreted as undifferentiated mesenchymal cells, whereas MT-9 cells had both histiocytic and fibroblastic natures (fibrohistiocytes) [15, 18]. Lipopolysaccharide (LPS), endotoxins of Gram-negative bacteria, is an activator and immunomodulator to monocytes/macrophages [4]. Since it has been reported that MFH cells showed histiocytic phenotypes [2, 7, 9, 13, 15], we pursued a possible phenotypic modulation using LPS-treated MT-8 (MT-8L) and MT-9 (MT-9L) cells in order to clarify the histogenesis of MFH.

The derivation and characteristics of MT-8 and MT-9 cell lines have been described [18]. Both lines were subcultured in Dulbecco's minimum essential medium (MEM) with 10% fetal bovine serum, streptomycin (100 µg/ml) and penicillin (100 U/ml) in a humidified 5% CO₂ incubator at 37°C. At 24-post seeding hr, MT-8 and MT-9 cells grown in chamber culture slides (LAB-TEK, Miles, IL, U.S.A.) were incubated in MEM containing 10 µg LPS/ml (Difco Laboratories, Detroit, MI, U.S.A.) for 6, 12, 24 and 48 hr. At each incubation point, fixed cells were examined as outlined below. Cells, which were incubated in LPS-free MEM and harvested at the same time as above, served as controls. Harvested cells were fixed in 4% formol calcium for enzymaticochemical stainings (acid phosphatase (ACP), Gomori's method, pH 5.0; non-specific esterase (NSE), the alpha-naphthyl acetate method, pH 7.0) or in cold acetone for indirect immunoperoxidase stainings [14, 17, 18]. In the immunostainings, primary antibodies were 400-fold-diluted anti-rat macrophage/histioyte monoclonal antibodies (ED1, Chemicon International Inc., CA; ED2, Serotech, Tokyo) [1] and 500-fold-diluted anti-α-smooth muscle actin (SMA; DAKO, Tokyo) for myofibroblasts [12, 14, 15], and 400-fold diluted peroxidase-conjugated affinity purified goat-anti mouse IgG Fc fragment antibody was used as a secondary antibody. Cells reacting to these enzyme/immunochemical stainings were counted twice in 400 cells in two different chambers and expressed as a percentage of 1,600 cells counted [16, 17]. The statistical analyses were made by the χ² test [16, 17]. Cell growth in LPS-containing MEM or in LPS-free MEM was evaluated by counting viable cells using a hemocytometer, and doubling time was determined from cell numbers at 2 and 5 days after seeding [18]. For electron microscopy, after being fixed and processed in the routine methods, pelleted cells were examined in a Hitachi H-600 electron microscope at 75 kV [16].

For in vivo observations, four SPF-F344/DuCrj male rats (160–300 g and 6–15 weeks old) each were inoculated subcutaneously with 10⁶ cells of MT-8, MT-8L, MT-9 or MT-9L. MT-8L and MT-9L cells used for the inoculation were obtained by culturing MT-8- and MT-9 cells three times in LPS (10 µg/ml)-containing MEM. At 7 weeks after the inoculation, all rats were euthanatized under ether anesthesia. Excised tumors were weighed, and fixed in 10% neutral buffered formalin. They were stained with hematoxylin-eosin (HE) and by the indirect immunohistochemistry for ED1 and SMA by the methods described [14, 15]. Frozen sections from fresh specimens were also stained for ACP and NSE [15, 18, 19].

Table 1 shows the doubling time and staining patterns. The percentage of ACP- and NSE-positive cells in MT-8L and MT-9L cells were significantly higher (P<0.05) than those of parental MT-8 and MT-9 cells at the all the incubation times, but no time-dependent increase was seen. The
Table 1. Comparison of results of in vitro observations between lipopolysaccharide (LPS)-treated (MT-8L and MT-9L) and parental (MT-8 and MT-9) cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>DT (h)</th>
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<th>12</th>
<th>24</th>
<th>48</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
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<tbody>
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<td>3.2</td>
<td>63.5</td>
<td>56.4</td>
<td>64</td>
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<tr>
<td>MT-8L</td>
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<td>0.0</td>
<td>83.2</td>
<td>51.2</td>
<td>99.8</td>
<td>7.5</td>
<td>11.0</td>
<td>21.5</td>
<td>29.1</td>
<td>9.0</td>
<td>23.3</td>
<td>33.4</td>
<td>49.5</td>
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</tr>
<tr>
<td>MT-9</td>
<td>50.7</td>
<td>42.5</td>
<td>46.9</td>
<td>55.0</td>
<td>43.6</td>
<td>50.8</td>
<td>61.5</td>
<td>7.3 - 18.9</td>
<td>19.3 - 36.0</td>
<td>19.6</td>
<td>16.1</td>
<td>24.7</td>
<td>28.7</td>
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</tr>
<tr>
<td>MT-9L</td>
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<td>47.0</td>
<td>71.1</td>
<td>78.5</td>
<td>73.0</td>
<td>90.3</td>
<td>91.0</td>
<td>7.0 - 18.1</td>
<td>22.5 - 33.2</td>
<td>22.5</td>
<td>15.0</td>
<td>26.6</td>
<td>31.6</td>
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</tbody>
</table>

a) Doubling time determined from cell numbers at 2 and 5 post-seeding days. b) Percentage of cells positive for enzyme/imunocytochemical stainings was calculated by observing 1,600 cells. c) Acid phosphatase. d) Non-specific esterase. e) Rat macrophage/histocyte-specific antibodies. No significant differences were seen, and thus figures show the range of percentages determined at 6 to 48 hr. f) α-smooth muscle actin. * The positive percentages of MT-8L and MT-9L cells are significantly higher (P<0.05) than those of MT-8 and MT-9 cells, respectively.

The percentage of SMA-positive cells increased significantly (P<0.05) in MT-8L cells than in MT-8 cells at each point; the positive rates increased time-dependently in both MT-8 and MT-8L cells. On the contrary, no significant difference in SMA-staining was seen between MT-9 and MT-9L cells. There were no significant differences in ED1 and ED2 stainings between MT-8 and MT-8L cells or MT-9 and MT-9L cells. The staining patterns for NSE (Figs. 1A and 1B), ED2 (Fig. 1C), and SMA (Fig. 1D) are shown.

Ultrastructurally, MT-8L cells examined at each point had irregular cell surface, numerous lysosomes and well-developed rough surfaced endoplasmic reticulum (Fig. 2A),

Fig. 1. Enzyme/imunocytochemical stainings. (A) Non-specific esterase (NSE)-positive cells (arrowheads) are seen in MT-8L cells incubated in LPS-containing medium for 24 hr and these cells appear to have more abundant cytoplasm. × 550. (B) In contrast, no cells reacting to NSE are seen in MT-8 cells incubated in LPS-free medium. × 550. (C) ED2-positive cells (arrowheads) in MT-9L cells. Counterstained with hematoxylin, × 550. (D) Alpha-smooth muscle actin (SMA)-positive cells (arrowheads) expressing filamentous reactions in MT-8L cells. Counterstained with hematoxylin, × 450.
while cytoplasmic organelles of MT-8 cells were poorly differentiated with glycogen granules (Fig. 2B). Fine structures of MT-9 and MT-9L cells were generally similar to each other, and resembled histiocytic natures of MT-8L cells.

Mean weights of tumors induced by MT-8, MT-8L, MT-9 and MT-9L cells were 54.3, 62.7, 56.8 and 42.1 g, respectively. MT-8L tumors consisted mainly of the storiform type of MFH, and partly mixed with undifferentiated sarcoma type (Fig. 3A), whereas MT-8 tumors were uniformly of undifferentiated sarcoma type. MT-9L and MT-9 tumors showed a storiform growth pattern. The histological features of the storiform and undifferentiated sarcoma types have been described in detail [15–18]. Briefly, the undifferentiated sarcoma type was composed of round to fusiform cells with scanty cytoplasm and hyperchromatic nuclei, arranged in a compact sheet or organoid structure (Fig. 3A); SMA-positive cells and histiocytic cells positive for ACP, NSE and ED1 were rarely seen. On the contrary, the storiform type consisted of an admixture of histiocytic cells and elongated fibroblastic cells often arranged in a storiform pattern; a moderate amount of collagen fibers was present among neoplastic cells, and occasional SMA-positive myofibroblastic cells were seen (Fig. 3A).

Previously, we have reported that MT-8 and MT-9 cells enhanced expressions of histiocytic lysosomal (ACP and NSE) and antigenic (ED1 and ED2) markers by treating
them with cisplatin (anti-cancer drug) [16] or by incubating them with hyperlipemic rat serum [17]. Likewise, MT-8L and MT-9L cells gave greater positive reactions to ACP and NSE than did their parental cells, and they revealed fine structures of histiocytic cells. However, no significant differences were seen in ED1 and ED2 stainings between LPS-treated and their parental cells. Although the reason remains to be clarified, the immunoreactivities of MT-8 and MT-9 cells to ED1 and ED2 have been reported to be inconsistent [15, 16]. Thus, the relationship between rat MFH cells reacting to ED1 and ED2 and monocytes/macrophages should be elucidated by further studies [16].

Interestingly, SMA-positive cells significantly increased in number in MT-8L cells. This indicates that MT-8L cells also revealed myofibroblastic natures along with the enhanced histiocytic natures; similar phenomena have been observed in cisplatin-treated MT-8 cells [16]. Moreover, MT-8L tumors consisted mainly of the storiform type, differing from MT-8 tumors of undifferentiated sarcoma type. The storiform type was histology characteristic of MT-9 and MT-9L tumors. MT-9 cells have been regarded as fibrohistiocytes having originally both histiocytic and fibroblastic natures [15, 17, 18]. Moreover, pulmonary tumors, which developed by intravenous inoculation of MT-8 cells, showed predominant histology of the storiform type (Fig. 3B). Tumorigenic Friend erythroleukemia cells in DBA mice induced tumors of different differentiation stages depending on development sites, indicating that microenvironmental factors might influence cellular differentiation [8]. It was speculated that MT-8 cells might be at a more primitive stage and able to differentiate to cells similar to MT-9 cells under certain conditions [16]. It thus appeared that LPS is a possible factor responsible for phenotypic modulation of rat MFH cells. However, the exact mechanism, why rat MFH cells were changed their phenotypes by LPS treatment, remains to be investigated.

Roholl et al. [11] reported similar findings that human MFH cell lines expressed immunophenotypic and ultrastructural features specific for epithelial, smooth muscle and Schwannian cells, and that such a phenotypic modulation became more evident when these cells were adapted to culture conditions or grew in nude mice. MFH cells appear to have totipotential differentiations [3, 5, 7, 9, 11], resulting in changeable phenotypes depending on surrounding conditions [9, 10], as have been demonstrated by our present and previous studies on rat MFH cells [15–17]. Therefore, MFH should manifest a variety of histology, and the term “MFH” may be inappropriate for describing this tumor, because it does not appear to be a tumor derived from “true macrophages/histiocytes” [13]. This tumor may be categorized as multipotential, undifferentiated mesenchymal cell tumor. However, factors leading to phenotypic modulation of MFH cells in tumor tissues remain to be solved, although some investigators have surmised that such a modulation might be induced by conditions evoked by cell-to-cell and cell-to-matrix interactions [9, 10].

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REFERENCES