Increased mast cell density and microvessel density in the thymus of patients with myasthenia gravis

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Abstract

There were investigated 15 cases with normal thymus removed during cardiac surgery and nine cases with clinical signs of myasthenia gravis. Four patients with myasthenia gravis had thymoma (three invasive, one non-invasive). Specimens were fixed in buffer formalin, embedded in paraffin and slides were stained with Hematoxylin–Eosin and Alcian blue–Safranin. Additional slides were stained for factor VIII in order to estimate microvessel density. Mast cell density was performed at magnification ×400, and microvessel density at ×200, using the “hot spot” method. There were found intralobular mast cells in all cases, located mainly in the cortex (6.53 in the normal thymus, 21.4 in patients with myasthenia gravis, and 10 in thymoma-associated myasthenia gravis). A significant increase in the number of intralobular mast cells was noticed in patients with myasthenia gravis without thymoma (p < 0.001), and a moderate increase in patients with thymoma-associated myasthenia gravis (p < 0.023). Values of microvessel density were 10.3 for the normal thymus, 33 for myasthenia gravis without thymoma and 21.8 for myasthenia gravis with associated thymoma. A strong correlation was found between the number of mast cells and microvessel densities in all three conditions.

Keywords: mast cell, microvessel density (MVD), thymus, myasthenia gravis, thymoma.

Introduction

Mast cells are normally found in the connective tissue, usually in large number around blood vessels. The presence of mast cells in the human thymus was reported many years ago [1]. They are noticed in the connective septa and perivascular spaces as scattered cell that stain with Toluidin blue and Alcian blue–Safranin [2]. Few data are available about the mast cell density in the active human thymus [3–5], specific tumors, and to the best of our knowledge, they were not investigated in the thymus of patients with myasthenia gravis.

The location of the mast cells and the content of specific granules in angiogenic factors maybe speculated as an involvement of these cells in angiogenesis [6, 7].

Despite this process was extensively investigated in malignant tumors a consensus regarding the intimate mechanism was not yet reached. For some years the only method to estimate angiogenesis was the simply count blood vessels (microvessel density).

The criticism of this method is specifically related to the method used by different authors (subjectivism in choosing the hot spot fields, magnification, and endothelial marker). Our purpose was to investigate the relationship between the number of mast cell and microvessel density in patients with myasthenia gravis in comparison with the normal human thymus.

Material and methods

There were investigated 25 cases with normal thymus (n = 16) and myasthenia gravis (n = 9). Specimens of normal thymus were taken from 15 cases during surgical procedures on the heart. There were six females and nine males, with age ranging from 7 days till 20 years.

In five cases with myasthenia gravis the thymus was removed with therapeutic intention. In the other four cases myasthenia gravis was associated with thymoma (one invasive, three non-invasive cases).

The ratio female/male was 7/2, and age ranged between 15 and 58 years. Specimens from the primary tumor and surrounding tissues were analyzed, in order to estimate invasion and the pattern of remaining thymic normal tissue. Tumors of the thymus were typed according the WHO Classification [8].

Specimens were fixed in 4% buffer formalin (pH 7.2), and embedded in paraffin. Five µm thick sections were stained with routine Hematoxylin–Eosin and Alcian blue–Safranin pH 1.42 methods.

Mast cells identified with this method (that seems to be the most sensitive cytochemical method for mast cells) were stained in blue (that corresponds to the bone
marrow borne mast cells), red-orange (connective tissue mast cells), and violet (mix forms). Mast cells were counted using the “hot spot”-method with ocular grid, at magnification ×400. Three “hot spots” were chosen for each case, and the arithmetical media was reported as final result.

Mast cells were separately counted for the septa and intralobular areas. Microvessel density was performed using the hot spot method at magnification ×200, on slides stained for factor VIII (ready-to-use primary antibody, LSAB2, diaminobenzidine as chromogen). Dako, Denmark provided reagents for immunohisto-chemistry. Statistic analysis was performed with the commercially available SPSS10.0.

**Results**

According WHO’s classification, thymoma was type B1 in three cases, and B2 in one case. In all cases, proliferating epithelial cells were intensely positive for high molecular weight cytokeratin (Figure 1).

In cases with normal thymus, the general architecture was preserved, with lobulated parenchyma and segregation between cortex and medulla. In only one case a clear distinction between cortex and medulla was not possible. Hassall’s bodies were found in all cases, usually in large number with different size and central debris. Lymphoid follicles were found in one case (three years old) without clinical signs of myasthenia gravis. Islands of thymic tissue suggesting involution were noticed in four cases (six months two cases, three years, and 20 years).

Mast cells were found in all cases in the connective tissue septa and within the lobules. They were stained in deep blue with alcian blue, in red-orange with safranin, or more rarely, in violet (mix forms). On magnification ×400 the mast cell density in the septa ranges from 2 to 23, with an average of 7.06. Few isolated granules were noticed around septal mast cells in four cases (signifying mild degranulation). Intralobular mast cells were stained mainly with alcian blue. They were usually smaller and less stained than septal mast cells. Intralobular mast cells were located mainly in the cortex and rarely in the medulla. The mast cell density ranges from 2 to 19/field (×400), with an average of 6.53. The number of intralobular and septal mast cell is shown in Figure 2. Microvessel density (MVD) in the normal thymus had a media of 10.3/field ×200.

In patients with myasthenia gravis without thymoma (n = 5, 15 to 25 years old) the general architecture was preserved, but segregation between cortex and medulla was noticed in only three cases. Hassall’s bodies were found in all cases and thymic tissue of involution was noticed in four cases (especially in cases of 24 and 25 years old). Lymphoid follicles were found in four cases, developed in an enlarged medulla.

The number of septal mast cells was not significantly different from the normal thymus (3 to 7, average 4.4), but the number of intralobular mast cells significantly increased (8 to 31, average 21.4/field ×400). Almost all intralobular mast cells were intensely stained with alcian blue, and degranulation was weak or absent (Figure 3a). Safranin-positive mast cells were also noticed, but reduced in number (Figure 3b).

There were four cases with thymoma-associated myasthenia gravis. Hassall’s bodies were found in two cases, and lymphoid follicles were not found in two cases in the tumor or in the surrounding thymic tissue. The number of septal mast cells slightly decreased (ranges from 1 to 5, average 3). The intralobular mast cell density showed a moderate increase in comparison with the normal thymus (from 3 to 16, average 10) (Table 1, Figures 3 and 4).

### Table 1 – Mast cell density and MVD in the normal thymus and myasthenia gravis

<table>
<thead>
<tr>
<th>Thymus</th>
<th>Septal MCD</th>
<th>Intralobular MCD</th>
<th>MVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 15)</td>
<td>7.06</td>
<td>6.53</td>
<td>10.3</td>
</tr>
<tr>
<td>MG (n = 5)</td>
<td>4.4</td>
<td>21.4</td>
<td>33</td>
</tr>
<tr>
<td>MG + thymoma (n=4)</td>
<td>3</td>
<td>10</td>
<td>21.8</td>
</tr>
</tbody>
</table>

MG – myasthenia gravis; MCD – mast cell density; MVD – microvessel density

A slight but not significant decrease of MCD with aging was noticed in patients with myasthenia gravis (Figure 5).

The immunoreaction for factor VIII showed that only the cytoplasm of endothelial cells was stained in brown (Figure 6, a and b). Microvessel density (MVD) in patients with myasthenia gravis without thymoma was significantly higher than in the normal thymus (33 vs. 10.3/×200 field). An intermediate value of 21.8 vessels/field was noticed in cases with myasthenia gravis and thymoma. In both subgroups of patients with myasthenia gravis (with and without thymoma) we found a strong correlation between the intralobular mast cell density and MVD (p < 0.002). No correlation was found between septal mast cell density and MVD.

**Discussions**

Mast cells are residents of the normal thymus, and usually are found in the connective tissue septa and mainly in perivascular spaces; they are rare in the cortex and somewhat more in the medulla, near Hassall bodies [3, 9, 10].

The precise functional significance of mast cells in the thymus is not known, but it is clear that the thymus plays a role in the development of some mast cells. This was experimentally demonstrated in athymic mice, and interleukin 3 seems to be responsible for their development from specific precursors found in the thymus, [11, 12].

Interleukin 3 induces the “mucosal” phenotype of the mast cell, [13–15] and this could explain the high number of alcian blue positive mast cells we found in patients with myasthenia gravis. It may also explain the number of mast cells is significantly lower in patients with myasthenia gravis with associated thymoma than in cases without thymoma. The increased number of mast cells was noticed also by other authors [16]. They mentioned the presence of numerous mast cells only in cases with myasthenia gravis associated with follicular hyperplasia (33.3% of cases), but their density was not compared with the normal thymus in this study.
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Figure 1 – Thymoma type B2, strong positive reaction for high molecular weight cytokeratin in epithelial cells (×400)

Figure 2 – Septal and lobular mast cell density in cases with normal thymus

Figure 3 – Alcian blue positive mast cell in the medulla (a), ×900. Alcian blue positive and safranin positive mast cells in the cortex (b), ×40
Figure 4 – The correlation between septal and intralobular mast cells with age in patients with myasthenia gravis

Figure 5 – Few septal and numerous intralobular mast cells. Alcian blue–Safranin stain, ×200

Figure 6 – Blood vessels with positive reaction for factor VIII. Numerous blood vessels in a patient with myasthenia gravis without thymoma (a). Few blood vessels in serous lakes in a patient with thymoma (b)
Our results showed that mast cell were numerous in all cases, the density was not related with lymphoid hyperplasia, and their number was significantly higher than in the normal thymus.

Moreover, mast cell hyperplasia in patients with myasthenia gravis is given by the intralobular but not septal mast cells. It may be speculated that in myasthenia gravis could be a stimulation of mast cell progenitors that are thought to be present in the thymus [17].

Precursors for mast cells were clearly demonstrated only in cell culture of rat thymus cells and until now there are no data available about their presence in human [18, 19].

Concentration of mast cell precursors in the thymus of mice is less than 0.1% of the concentration found in the bone marrow [20].

These data indicate that differentiation of the precursor cells does not depend on T-lymphocytes, but on the other hand nothing is known about their behavior in the human thymus of patients with myasthenia gravis.

Both histochemical (e.g. Toluidin blue, Alcian blue–Safranin) and immunohistochemical methods (e.g. tryptase, CD117) are not enough sensitive to detect mast cell precursors.

We noticed the presence of many cells with very few alcin blue positive granules in the cytoplasm that could be mastoblasts, but they were not included in the counting procedure.

Angiogenesis in the thymus and specific lesions was less investigated than in other organs. Only two articles are available in the literature on this subject [21, 22].

In the first study, angiogenesis was investigated by microvessel count and expression of specific growth factor and it was found a correlation between these factors and stage of thymoma.

In the other it was shown that in the pathogenesis of myasthenia gravis angiogenesis could play an important role. On the other hand, mast cells were demonstrated to be a source for angiogenic factors, secreting the most powerful angiogenic factor, the vascular endothelial growth factor [23].

Our results support data published by other authors, indicating that in myasthenia gravis there is a significant increase in the number of mast cells, correlated with high microvessel density.

Conclusions

The study of the mast cell density and microvessel density in myasthenia gravis showed a marked increase in both parameters (mast cells and blood vessels), compared with the normal thymus.

The increased number of mast cells was noticed in the parenchyma of the thymus and strongly correlates with high values for microvessel density.

References


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