The Role of Pro-and Anti-Apoptotic Mediators in Patients with Multiple Sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic neurological disorder characterized by myelin destruction and a variable degree of oligodendrocyte death. Programmed cell death (apoptosis) is critical for the normal development and homeostasis of the immune system. Apoptosis of autoreactive T cells in the CNS is likely to be important in preventing the development of MS. The death receptor (Fas) and its ligand (Fas-L) interaction results in activation-induced apoptosis and their abnormal expression together with nuclear factor (NF-\textsuperscript{kb}) and Bcl-2 may be involved in the pathogenesis and the clinical course of MS. Objective: This work aim at clarifying the role of pro- and anti-apoptotic mediators in pathogenesis of MS. Methods: We studied the level and expression of Fas, Fas-L, NF-\textsuperscript{kb} and Bcl-2 using RT-PCR, morphological changes of apoptosis in peripheral blood mononuclear cells (PBMCs), DNA fragmentation in 24 patients with MS divided into 3 groups: in relapse, in remission and secondary progressive cases. In addition, a group of 16 healthy cases served as controls. Results: We found that Fas & Fas-L were significantly decreased in patients with multiple sclerosis compared with healthy control subjects. While NF-\textsuperscript{kb} and Bcl-2 were significantly increased in patients compared with healthy control subjects. Also, DNA fragmentation showed significant decrease in patients versus control. Conclusion: Impaired apoptosis detected by Fas, Fas-L, NF-\textsuperscript{kb} and Bcl-2 mediators and DNA fragmentation, play an important role in the pathogenesis of MS. (Egypt J Neurol Psychiat Neurosurg. 2010; 47(2): 261-266)

Key words: multiple sclerosis, pathogenesis, Apoptosis, peripheral blood mononuclear cells (PBMC), DNA fragmentation, death receptor (Fas), nuclear factor (NF-\textsuperscript{kb}), Bcl-2.

INTRODUCTION

There are two principally different and partly opposite ways in which apoptosis could affect an auto immune attacks on a target organ. First, the induction of apoptosis in B-cells of the langerhans islets\textsuperscript{1,2} or in oligodendrocytes\textsuperscript{12} may contribute to disease in diabetes or MS. Secondly, activation of T cells is normally followed by “activation–induced cell death” (AICD), which is a physiologic mechanism to limit an immune response. Thus failure in this process, i.e. decreased apoptosis, may lead to inappropriate persistence of activated T cells, thereby contributing to autoimmunity\textsuperscript{2}.

Apoptosis is the effect of complex chain of intracellular events leading to the activation of a number of pathways\textsuperscript{3}. These pathways are triggered by a number of cell-surface receptors, the most important of which belong to the tumor necrosis factor (TNF) receptor family, of these Fas (CD95) together with its ligand Fas-L (CD95L)\textsuperscript{4}.

Fas mediate apoptosis of T cells. T cells apoptosis contributes to resolution of the central nervous system inflammation and clinical recovery form attacks of experimental autoimmune encephalomyelitis [EAE], animal model of multiple sclerosis (MS)\textsuperscript{5}. Apoptosis of autoreactive T cells in the CNS is likely to be important in preventing the development of MS\textsuperscript{6}. NF-\textsuperscript{kb} represents a family of dimeric transcription factor that play a central role in inflammatory responses by regulation of gene expression and inhibition of apoptosis\textsuperscript{7}.

Bcl-2 family proteins include the death antagonists Bcl-2 and Bcl-X(L), and death agonists Bax and Bad the commitment of T lymphocytes to die is partly regulated by the Bcl-2 family proteins and altered expression of these families in T lymphocytes is involved in promoting cellular resistance to apoptosis in patients with MS\textsuperscript{8,9}.

This work aims at clarifying the role of pro- and anti-apoptotic mediators in pathogenesis of MS.

PATIENTS AND METHODS

This study included 24 patients with multiple sclerosis selected from neurology clinics, Tanta University hospital and 16 healthy individuals as control. Patients were divided into 3 groups: Group I: Included 8 patients with remitting-relapsing multiple sclerosis in remission. Group II: Included 9 patients with remitting-relapsing multiple sclerosis in relapse. Group III: Included 7 patients with secondary progressive multiple sclerosis.
Inclusion criteria:
All patients had clinically definite MS patients according to McDonald criteria. Relapsing, remitting, and secondary progressive courses based on criteria of Lublin and Reingold.

Exclusion criteria:
1. Patients aged less than 15 years or more than 55 years.
2. Patients administrating any medications that suppress the immune system as corticosteroids and immune- suppressant drugs within two months from inclusion in the study.
3. Patients suffering from any neurological or medical problems including e.g. diabetes, liver, kidney or other autoimmune diseases.

Patients and controls were subjected to the following:
I. Morphological assessment of apoptosis by Giemsa, acridine orange and propidium iodide stain at time 0, 24, 48 and 72 hours.
II. Morphological assessment of apoptosis by determination of DNA fragmentation using agarose gel electrophoresis.
III. Immunological studies of apoptotic and antiapoptotic factors.

RESULTS

The percentage of apoptotic PBMCs was significantly lower in patients versus controls, with no difference between patients groups (Table 1 and Figs. 1, 2, 3).

Apoptotic factors [Fas and Fas ligand] showed highly significant decrease in patients versus control with no significant correlation between patient’s groups (Table 2 and Figs. 4, 5). DNA fragmentation showed significant increase in control versus patients (Table 3; Figure 6). Antiapoptotic factors [Bcl-2 and NF-κB] showed significant increase in patients than control (Table 4 and Fig. 7).

<table>
<thead>
<tr>
<th>Type of stain</th>
<th>Giemsa</th>
<th>Acridine orange</th>
<th>Propidium iodide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.18±3.06</td>
<td>57.7±6.0</td>
<td>27.82±4.6</td>
</tr>
<tr>
<td>Group I (RRMS in remission)</td>
<td>4.43±1.19</td>
<td>13.42±1.13</td>
<td>5.27±1.48</td>
</tr>
<tr>
<td>Group II (RRMS in relapse)</td>
<td>4.1±0.6</td>
<td>13.06±0.64</td>
<td>5.1±2.19</td>
</tr>
<tr>
<td>Group III (SPMS)</td>
<td>4.35±0.47</td>
<td>12.6±1.4</td>
<td>5.2±1.97</td>
</tr>
</tbody>
</table>

P value comparing control to all patients groups < 0.0001* < 0.0001* < 0.0001*

RRMS relapsing remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis
*Statistically significant at p<0.01

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fas</th>
<th>FasL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>54.45 ±12.24</td>
<td>54.319 ±12.255</td>
</tr>
<tr>
<td>P value (80/13131)</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>12.75 ±0.807</td>
<td>14.03 ±7.039</td>
</tr>
<tr>
<td>P value (80/13131)</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

RRMS relapsing remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis
*statistically significant at p<0.01
Table 3. Assessment of apoptosis of the studied by DNA electrophoresis patients with multiple sclerosis and controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>No apoptosis</th>
<th>Mild apoptosis</th>
<th>Moderate apoptosis</th>
<th>Severe apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Group I (RRMS in remission)</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II (RRMS in relapse)</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group III (SPMS)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RRMS relapsing remitting multiple sclerosis, SPMS secondary progressive multiple sclerosis

Table 4. Antiapoptotic factors (Bcl-2 and NF-kB) determined by ELISA in μg/mL and RT-PCR in patients with multiple sclerosis and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Group I RRMS in remission</th>
<th>Group II RRMS in relapse</th>
<th>Group III SPMS</th>
<th>Group I RRMS in remission</th>
<th>Group II RRMS in relapse</th>
<th>Group III SPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA Mean ±SD</td>
<td>23.21±2.73</td>
<td>80.9±3.81</td>
<td>80.76±4.71</td>
<td>81.77±2.42</td>
<td>26.74±2.33</td>
<td>82.16±3.56</td>
<td>77.16±3.52</td>
</tr>
<tr>
<td>P value (8(I,II,III))</td>
<td>&lt; 0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>PCR Mean ±SD</td>
<td>14.11±12.92</td>
<td>80.72±3.78</td>
<td>80.73±4.52</td>
<td>80.8±3.51</td>
<td>17.5±14.06</td>
<td>78.7±11.34</td>
<td>77.16±3.69</td>
</tr>
<tr>
<td>P value (8(I,II,III))</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

RRMS relapsing remitting multiple sclerosis, SD standard deviation, SPMS secondary progressive multiple sclerosis

*Statistically significant at p<0.01
DISCUSSION

The pathogenesis of MS is under strong genetic control involving several genes each of modest effect. It has been hypothesized that either decreased apoptosis of auto reactive T cells in the CNS, or increased apoptosis of oligodendrocytes may play an important role.

Abnormal expression of many apoptosis-related molecules, including TNF, Fas, and their corresponding receptors, has been observed in MS lesion. The data vary, however, regarding the extent of apoptosis associated with this pattern of expression. Whereas some authors have observed little evidence for apoptosis of oligodendrocytes, others report intense DNA fragmentation as in apoptosis both in lymphocytes and oligodendrocytes.

Our study revealed highly significant decrease in the percentage of apoptotic PBMCs in patients versus control subjects as demonstrated morphologically by Giemsa stain, Acradin orange stain, propidium iodide stain and by DNA fragmentation.

In MS, spontaneous apoptosis of unfractionated peripheral mononuclear cells was significantly reduced, and activated intrathecally, and peripheral T cells were found to be predominantly resistant to independent apoptosis. These results indicate that (clinically active MS) the reduced susceptibility of cells to apoptosis is partly due to impairment of Fas-independent apoptotic pathway. An alternative explanation is that increased expression levels reflect a constitutional over activity of apoptosis-inducing molecules. This, however, would be contrary to the hypothesis that MS may be influenced by a genetically determined failure of activation-induced apoptosis of auto reactive T cells. This view is supported by experimental autoimmune encephalitis data which show that CNS apoptosis is more prevalent in effector (lymphoid) cells than in target cells (oligodendrocytes).

Our study revealed highly significant decrease in the expression of apoptotic factors (Fas & Fas L) in patients versus control and highly significant increase in the expression of anti-apoptotic factors (Bcl-2 & NF-κB) in patients compared to control.

Ichikawa found that the expression rate of Fas antigen on T cells in peripheral blood (PB) in MS patients was higher than that of healthy control and the expression rate in MS patients was higher in CSF than in peripheral blood, the results suggested that there is acceleration or impairment of apoptosis on activated T cells in MS.

Zipp, Dianzani et.al. and Gomes et.al. are in agreement with our results about Fas and suggested that development of autoimmune lymphoproliferative patterns may involve several alterations hitting the Fas system, but might also involve alterations in other systems contributing to the switching-off or proliferation of lymphocytes. Moreover Lopatins Kaya et al. found that Fasl mRNA was increased prior to exacerbation in relapsing remitting (RR) MS and decreased during clinical activity which may be due to the migration of inflammatory cells to the central nervous system.

Sharief et al. suggested that altered expression of Bcl-2 family proteins in T lymphocytes is involved in promoting cellular resistance to apoptosis in MS. However, the relationship between these alterations in Bcl-2 proteins expression and clinical disease activity has not yet been evaluated. They observed a significant reduction in the expression ratios of the pro- to anti-apoptosis Bcl-2 members in peripheral lymphocytes from patients with active MS when compared to corresponding ratios in patients with stable MS or other controls. This imbalance in the expression ratios of pro- and anti-apoptosis proteins was functionally active in reducing cellular susceptibility to apoptosis and may allow for continuing cellular proliferation and tissue destruction within the central nervous system in active MS. It also correlated with clinical features of disease activity. This finding indicate that dysregulated expression of Bcl-2 family proteins in peripheral lymphocytes is a feature of clinically active MS. Heessen detected the same results also found that the cellular expression of Bcl-2, Bcl-X(L),Bax or Bad in MS patients was independent of the expression of other apoptotic regulatory molecules, such as Fas receptor protein.

Seidi suggested that cellular over expression of apoptosis-inhibitory proteins in patients with relapsing MS may promote apoptotic resistance of potentially pathogenic, auto reactive B lymphocytes and...
consequently, may allow for continuing autoimmune tissue destruction.

Bonetti and JoAnn detected increased expression of NF-κB in multiple sclerosis with minimization of initial innate cytokine and chemokine responses and prevention of further propagation of the inflammatory response after using NF-κB antagonist. Moreover, activation of NF-κB may exert anti-apoptotic effects and contribute to the absence of an apoptotic response by oligodendrocytes in MS.

The MS plaque microenvironment is able to activate the NF-κB pathway in oligodendrocytes and microglia, this in concert with other protective mechanisms such as Bcl-2 and ciliary neurotrophic factor, activation of NF-κB may exert anti–apoptotic effects and contribute to the absence of an apoptotic response by oligodendrocytes in MS.

Many recent studies have utilized NF-κB inhibitors in attempts to control various inflammatory diseases, including multiple sclerosis.

Conclusion: decreased expression of Fas and FasL- and increased expression of Bcl-2 and NF-κB may serve as indicators of multiple sclerosis susceptibility and further studies are required for evaluation of apoptosis in MS.

REFERENCES

10. Sharief MK, Mathew SH, Noori MA. expression of ratios of the Bcl-2 family proteins and disease activity in MS. Mult Scler. 2003; 192: 64-7