Difference in B cell activation between dermatomyositis and polymyositis: analysis of the expression of RP105 on peripheral blood B cells

Y Kikuchi, S Koarada, Y Tada, O Ushiyama, F Morito, N Suzuki, A Ohta, T Horiuchi, K Miyake, K Nagasawa

Abstract

Background—It has previously been shown that RP105, a new B cell surface protein, is lost in activated human B cells. Objective—to investigate whether there is a difference in B cell activation between patients with dermatomyositis (DM) and those with polymyositis (PM) using RP105 as a marker.

Methods—The population of RP105 negative B cells (activated B cells) in the peripheral blood mononuclear cells of seven patients with dermatomyositis (DM) and 11 with polymyositis (PM) was analysed by flow cytometry.

Results—the percentage of RP105 negative B cells in the peripheral blood of patients with PM was low (5.8 (SD 2.4)%), similar to that of normal subjects. In contrast, all patients with DM showed increased RP105 negative B cell populations (33.0 (6.9)%). Bronchoalveolar lavage fluid from a patient with DM and active interstitial pneumonitis contained a large number of RP105 negative B cells.

Conclusion—These findings suggest that the expansion of RP105 negative B cells is a hallmark of DM, and that B cell activation in DM may be pathogenetically different from that in PM.

Patients and methods

Blood samples from seven untreated patients with DM and 11 patients with PM with active disease who were admitted to our hospital between January 1999 and September 2000 were evaluated in this study. DM and PM were diagnosed by the criteria of Bohan et al. Patients and methods—Table I gives the clinical characteristics of the patients. The DM group included four women and three men, ranging in age from 27 to 70 (mean 46). The PM group included seven women and five men, ranging in age from 29 to 68 (mean 52).

Samples of peripheral venous blood from patients with DM and PM, and bronchoalveolar lavage fluid (BALF) from a patient with
DM and interstitial pneumonitis (IP) were collected into tubes containing heparin. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) after centrifugation. Cells suspended in staining buffer (phosphate buffered saline supplemented with 2.0% fetal calf serum) were incubated for 20 minutes on ice with FITC conjugated antihuman RP105 monoclonal antibody (mAb) and phycoerythrin conjugated antihuman CD19 mAb (PharMingen). Labelled cells were analysed on a FACScan (Becton Dickinson, Mountain View, CA) using CellQuest software, and 25 000–50 000 events were analysed.

Wilcoxon’s signed rank test was used to compare the RP105 negative B cells (%) in DM and PM. A value of p<0.05 was taken to indicate significance.

Results
Table 1 summarises the clinical backgrounds of all the patients. IP was present in 6/7 (86%) patients with DM and 7/11 (64%) patients with PM. The IP in the six patients with DM was progressive and caused death in two patients (Nos 1 and 6) despite intensive treatment. Serum levels of lactate dehydrogenase were higher than normal in all the patients. On the other hand, creatine kinase levels were markedly raised in patients with PM in contrast with patients with DM in whom the increase was mild or even absent. Antinuclear antibodies (ANA) were detected in 2/7 (29%) patients with DM as compared with 6/11 (55%) patients with PM (not significant) and none of patients with DM had antibodies to Jo-1 whereas these were found in three patients with PM.

PBMC were prepared from patients with DM and PM as described in “Patients and methods” and were stained with mAb against CD19 and RP105. Figures 1A and B show representative results of staining from a patient with DM and PM, respectively. In the patient with PM, most of the CD19 positive B cells were also positive for RP105 (fig 1B), which is a similar staining pattern to that found in normal subjects (not shown). On the other hand, a distinctly increased percentage of RP105 negative and CD19 positive B cell was found in the patient with DM (fig 1A).

Table 1  Clinical characteristics of the patients tested

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age/sex</th>
<th>Interstitial pneumonitis</th>
<th>CK* (IU/l)</th>
<th>LDH* (IU/l)</th>
<th>Antinuclear antibody</th>
<th>Antibodies to Jo-1</th>
<th>Treatment outcome</th>
<th>RP105 negative B cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DM</td>
<td>44/M</td>
<td>+</td>
<td>198</td>
<td>499</td>
<td>−</td>
<td>−</td>
<td>Dead</td>
<td>31.2</td>
</tr>
<tr>
<td>2</td>
<td>DM</td>
<td>47/M</td>
<td>−</td>
<td>333</td>
<td>402</td>
<td>+</td>
<td>−</td>
<td>Alive</td>
<td>35.6</td>
</tr>
<tr>
<td>3</td>
<td>DM</td>
<td>45/F</td>
<td>+</td>
<td>78</td>
<td>381</td>
<td>+</td>
<td>−</td>
<td>Alive</td>
<td>27.4</td>
</tr>
<tr>
<td>4</td>
<td>DM</td>
<td>27/F</td>
<td>+</td>
<td>99</td>
<td>345</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>40.8</td>
</tr>
<tr>
<td>5</td>
<td>DM</td>
<td>42/M</td>
<td>+</td>
<td>231</td>
<td>414</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>39.5</td>
</tr>
<tr>
<td>6</td>
<td>DM</td>
<td>70/F</td>
<td>Dead</td>
<td>452</td>
<td>653</td>
<td>−</td>
<td>−</td>
<td>Dead</td>
<td>23.5</td>
</tr>
<tr>
<td>7</td>
<td>DM</td>
<td>45/F</td>
<td>+</td>
<td>120</td>
<td>784</td>
<td>−</td>
<td>−</td>
<td>Dead</td>
<td>20.0</td>
</tr>
<tr>
<td>8</td>
<td>PM</td>
<td>66/F</td>
<td>−</td>
<td>2435</td>
<td>496</td>
<td>+</td>
<td>−</td>
<td>Alive</td>
<td>7.1</td>
</tr>
<tr>
<td>9</td>
<td>PM</td>
<td>50/M</td>
<td>−</td>
<td>4298</td>
<td>836</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>6.2</td>
</tr>
<tr>
<td>10</td>
<td>PM</td>
<td>52/F</td>
<td>−</td>
<td>1504</td>
<td>643</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>2.9</td>
</tr>
<tr>
<td>11</td>
<td>PM</td>
<td>62/F</td>
<td>−</td>
<td>555</td>
<td>254</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>4.2</td>
</tr>
<tr>
<td>12</td>
<td>PM</td>
<td>68/M</td>
<td>+</td>
<td>2532</td>
<td>522</td>
<td>+</td>
<td>−</td>
<td>Dead†</td>
<td>4.0</td>
</tr>
<tr>
<td>13</td>
<td>PM</td>
<td>42/F</td>
<td>+</td>
<td>1224</td>
<td>307</td>
<td>+</td>
<td>−</td>
<td>Alive</td>
<td>4.3</td>
</tr>
<tr>
<td>14</td>
<td>PM</td>
<td>29/M</td>
<td>+</td>
<td>8580</td>
<td>659</td>
<td>+</td>
<td>+</td>
<td>Alive</td>
<td>3.6</td>
</tr>
<tr>
<td>15</td>
<td>PM</td>
<td>47/M</td>
<td>+</td>
<td>7203</td>
<td>998</td>
<td>+</td>
<td>+</td>
<td>Alive</td>
<td>7.4</td>
</tr>
<tr>
<td>16</td>
<td>PM</td>
<td>54/F</td>
<td>+</td>
<td>3841</td>
<td>631</td>
<td>−</td>
<td>−</td>
<td>Alive</td>
<td>7.4</td>
</tr>
<tr>
<td>17</td>
<td>PM</td>
<td>48/F</td>
<td>+</td>
<td>1232</td>
<td>454</td>
<td>−</td>
<td>+</td>
<td>Alive</td>
<td>10.5</td>
</tr>
<tr>
<td>18</td>
<td>PM</td>
<td>49/M</td>
<td>+</td>
<td>9810</td>
<td>1501</td>
<td>+</td>
<td>−</td>
<td>Alive</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*CK = creatine kinase (normal range M = 60–240, F = 40–160); LDH = lactate dehydrogenase (normal range 120–230).
†Patient 12 died of alcoholic pancreatitis.

Figure 1  Expression of RP105 on peripheral blood B cells from (A) a patient with dermatomyositis (No 4) and (B) a patient with PM (No 15). Peripheral blood mononuclear cells were stained with monoclonal antibody against RP105 and CD19.
B cell activation in DM and PM

In this study, we have shown that they are hyperactive and well differentiated B cells. This subset of B cells has also been found to produce class switched immunoglobulin (IgG). They have been shown to produce class switched immunoglobulin (IgG). In this study we have shown that DM is another autoimmune disease that shows an increase in RP105 negative B cells in the peripheral blood. Of note is that this population of B cells was not increased in PM whether or not autoantibodies, such as ANA and antibodies to Jo-1, were present.

DM and PM are inflammatory skeletal muscle diseases in which autoimmune mechanisms are presumed to play a part. Both diseases clinically resemble each other except for the presence of characteristic cutaneous manifestations, seen only in DM. Some investigations have suggested a similar immunological basis in both diseases demonstrating that the Fas-Fas ligand system or CD40-CD40 ligand interaction may participate in the development of muscle damage in DM as well as PM. In contrast, however, recent investigations have suggested that immune mechanisms in the inflammation sites are distinctly different between DM and PM. Immuno-histochemical studies indicate that muscle damage may be caused by predominantly infiltrating CD8+ T cells in DM. On the other hand, in DM, infiltrating cells mainly consist of activated CD4+ T cells accompanied by B cells and, moreover, deposition of immunoglobulin and C5b-9 complement membrane attack complex has been shown in the intramuscular blood vessels. Cambridge et al also suggested a peripheral blood B cell activation in patients with DM demonstrating that PBMC from DM, but not from patients with PM, spontaneously produced a significant amount of immunoglobulin in vitro. This indicates that T cell dependent B cell activation and humorally mediated muscle fibre damage may play a part in the pathogenesis of DM. Our results showing that activated B cells were increased in the peripheral blood of all patients with DM and in the BALF of one patient with DM corroborate these previous reports. These accumulated results indicate that activated B cells may have a crucial role in the pathogenesis of DM, which differs distinctly from the pathogenesis of PM.

In this study, IP was found in all the patients with DM and tended to be progressive, in contrast with patients with PM, of whom 64% had IP and it was responsive to treatment. However, IP did not seem to affect directly the number of RP105 negative B cells in the BALF of a patient with DM (No 1) who had severe and progressive IP. The BALF contained as much as 82% RP105 negative B cells, far higher than in the peripheral blood of the same patient (31.2%) (data not shown). This result suggests that accumulated RP105 negative B cells in the lung may be associated with IP in DM.

Discussion

It has been shown that RP105 is expressed on virtually all mature B cells and might be one of the B cell markers in humans as well as in mice. We have shown that there are few RP105 negative B cells in the peripheral blood of normal subjects, but the number is significantly increased in patients with systemic lupus erythematosus, a disease known for hyperactivation of B cells. RP105 negative B cells have been phenotypically defined as CD95 positive, CD86 positive, and CD38 bright, indicating that they are hyperactive and well differentiated B cells. This subset of B cells has also been found to produce class switched immunoglobulin (IgG). In this study we have shown that DM is another autoimmune disease that shows an increase in RP105 negative B cells in the peripheral blood.

Figure 2 Proportion of RP105 negative B cells from patients with dermatomyositis (DM) and polymyositis (PM). RP105 expression on B cells from DM and PM was analysed by flow cytometry. The percentage of RP105 negative B cells in patients with DM was higher than in patients with PM (p<0.05). Bars show the mean (SD).

www.annrheumdis.com


Difference in B cell activation between dermatomyositis and polymyositis: analysis of the expression of RP105 on peripheral blood B cells

Y Kikuchi, S Koarada, Y Tada, O Ushiyama, F Morito, N Suzuki, A Ohta, T Horiuchi, K Miyake and K Nagasawa

Ann Rheum Dis 2001 60: 1137-1140
doi: 10.1136/ard.60.12.1137

Updated information and services can be found at:
http://ard.bmj.com/content/60/12/1137

These include:

References
This article cites 11 articles, 4 of which you can access for free at:
http://ard.bmj.com/content/60/12/1137#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Muscle disease (160)
- Musculoskeletal syndromes (4951)
- Immunology (including allergy) (5144)
- Connective tissue disease (4253)
- Interstitial lung disease (145)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/