CONCISE REPORTS

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.

(Ann Rheum Dis 2001;60:1137–1140)

Department of
Internal Medicine,
Saga Medical School,
5-1-1 Nabeshima, Saga
849-8501, Japan
Y Kikuchi
S Koarada
Y Tada
O Ushiyama
F Morito
N Suzuki
K Nagasawa

Department of
Nursing, Saga Medical
School
A Ohta

Department of
Immunology, Saga
Medical School
K Miyake

First Department of
Internal Medicine,
Faculty of Medicine,
Kyushu University,
3-1-1 Maidashi,
Higashi-ku, Fukuoka
812-8582, Japan
T Horiuchi

Correspondence to:
Dr Kikuchi
kikuchi@post.saga-med.ac.jp

Accepted 3 May 2001

suggest a humoral immune mediated mechanism.¹⁻⁴

RP105 was first described as the leucine-rich repeat molecule expressed on B cells. It was found to transmit an activation signal for B cell proliferation and resistance against apoptosis in mice.⁵ The extracellular domain of RP105 is structurally similar to that of Toll-like receptors, suggesting that RP105 senses pathogen invasion and activates B cells.⁶ Mice devoid of RP105 have recently been generated, and it has been shown that RP105 regulates lipopolysaccharide signalling in B cells.⁷ In contrast with these findings in mice, little is known about RP105 in humans.

We have recently shown that although virtually all B cells in the peripheral blood of normal subjects have RP105, patients with systemic lupus erythematosus have an increased number of RP105 negative B cells in the peripheral blood.⁸ We defined the phenotype of RP105 negative B cells as CD95 positive, CD86 positive, and CD38 bright, which is consistent with that of activated B cells or germinal centre B cells.⁸

These prompted us to investigate whether DM might be distinguished from PM by B cell activation—that is, the surface expression of RP105 on circulating B cells. We found that the percentage of RP105 negative B cells (activated B cells) was distinctly increased in the peripheral blood of patients with DM as compared with patients with 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.*⁹ Table 1 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

Dermatomyositis (DM) and polymyositis (PM) have been presumed to belong to the same category of connective tissue disease with the involvement of muscular system, though the former includes characteristic skin lesions as well as myositis. Autoimmune mechanisms are thought to have an important role in the pathogenesis of both types of myositis. Recent immunohistochemical studies, however, suggest a different pathogenesis between DM and PM. In contrast with PM, in which cell mediated cytotoxicity with major infiltration of CD8+ T cells may be the mechanism, the infiltration of CD4+ T cells together with B cells and the deposition of complement components in the perivascular area of muscular or cutaneous lesions in DM

Table 1 Clinical characteristics of the patients tested

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

*CK = creatine kinase (normal range M = 60–240, F = 40–160); LDH = lactate dehydrogenase (normal range 120–230).

†Patient 12 died of alcoholic pancreatitis.

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)¹⁰ 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 cells was found in the patient with DM (fig 1A). Figure 2 shows

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.

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).

the percentages of RP105 negative B cells from seven patients with DM and 11 with PM. RP105 negative B cells from all patients with PM were within the normal range (5.8 (SD 2.4)%). In contrast, patients with DM had much greater number of RP105 negative B cells in the peripheral blood (33.0 (6.9)%) ($p < 0.05$). There was no significant difference in the percentages of RP105 negative B cells between patients with PM with IP (5.8 (2.7)%) and those without IP (5.1 (1.9)%), indicating that IP itself may not affect the increase in RP105 negative B cells. Moreover, neither ANA or antibodies to Jo-1 were associated with the number of RP105 negative B cells.

We also assessed RP105 staining of 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.¹⁰ 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.⁸ 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).⁸ 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.^{2,11} In contrast, however, recent investigations have suggested that immune mechanisms in the inflammation sites are distinctly different between DM and PM. Immunohistochemical studies indicate that muscle damage may be caused by predominantly infiltrating CD8+ T cells in PM.^{1,2} 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.^{1,2} 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*.⁴ This indicates that T cell dependent B cell activation and humorally mediated muscle fibre damage may play a part in the pathogenesis of DM.¹⁻⁴ 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 peripheral blood because their level was low in patients with PM, irrespective of the presence of IP. It was also shown that autoantibodies such as ANA and antibodies to Jo-1 were rarely found in the serum of patients with DM as compared with PM. This seems incompatible with the result that increased numbers of RP105 negative activated B cells were seen in the peripheral blood of patients with DM but not in PM. Although the role of RP105 negative B cells in DM is uncertain, they may either be responsible for the development of inflammation in the muscle, lung, or cutaneous tissues or play a part in the production of, as yet unknown, autoantibodies.

In conclusion, we emphasise that activated B cell involvement in DM may be pathogenetically different from that in PM.

- 1 Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I. Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. *Ann Neurol* 1984;23:193–208.
- 2 Sugiura T, Murakawa Y, Nagai A, Kondo M, Kobayashi S. Fas and Fas ligand interaction induces apoptosis in inflammatory myopathies: CD4+ T cells cause muscle cell injury directly in polymyositis. *Arthritis Rheum* 1999;42:291–8.
- 3 Hausmann G, Herrero C, Cid MC, Casademont J, Lecha M, Mascaro JM. Immunopathologic study of skin lesions in dermatomyositis. *J Am Acad Dermatol* 1991;25:225–30.
- 4 Cambridge G, Faith A, Saunders C, Dubowitz V. A comparative study of in vitro proliferative responses to mitogens and immunoglobulin production in patients with inflammatory muscle disease. *Clin Exp Rheumatol* 1989;7:27–34.
- 5 Miyake K, Yamashita Y, Hitoshi Y, Takatsu K, Kimoto M. Murine B cell proliferation and protection from apoptosis with an antibody against a 105-kD molecule: unresponsiveness of X-linked immunodeficient B cells. *J Exp Med* 1994;180:1217–24.
- 6 Miyake K, Yamashita Y, Ogata M, Sudo T, Kimoto M. RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family. *J Immunol* 1995;154:3333–40.
- 7 Ogata H, Su I, Miyake K, Nagai Y, Akashi S, Mecklenbrauker I, *et al*. The Toll-like receptor protein RP105 regulates lipopolysaccharide signaling in B cells. *J Exp Med* 2000;192:23–30.
- 8 Koarada S, Tada Y, Ushiyama O, Morito F, Suzuki N, Ohta A, *et al*. B cells lacking RP105, a novel B cell antigen, in systemic lupus erythematosus. *Arthritis Rheum* 1999;42:2593–600.
- 9 Bohan A, Peter JB, Bowman RL, Pearson CM. A computer assisted analysis of 153 patients with polymyositis and dermatomyositis. *Medicine (Baltimore)* 1977;56:255–86.
- 10 Miura Y, Shimazu R, Miyake K, Akashi S, Ogata H, Yamashita Y, *et al*. RP105 is associated with MD-1 and transmits an activation signal in human B cells. *Blood* 1998;92:2815–22.
- 11 Sugiura T, Kawaguchi Y, Harigai M, Takagi K, Ohta S, Fukasawa C, *et al*. Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40-CD40 ligand interaction in IL6, IL8, IL15, and monocyte chemoattractant protein-1 production. *J Immunol* 2000;164:6592–600.

What's in the next issue

Future content

See which articles have just been accepted for publication and preview the table of contents for the next issue a month before it is published

www.annrheumdis.com