

Phenotypical analysis of lymphocytes using flow cytometry in dermatomyositis with and without interstitial pneumonia

Y. Shimojima , M. Matsuda, W. Ishii, T. Gono, K. Tazawa, T. Yoshida,
N. Kato, S. Ikeda

Department of Medicine (Neurology and Rheumatology), Shinshu University School of
Medicine, Matsumoto, Japan

Summary

Phenotypic analysis of peripheral blood lymphocytes (PBL) was performed using flow cytometry in dermatomyositis (DM) with (n=8) and without interstitial pneumonia (IP) (n=5). Nine healthy subjects served as controls. Both DM patients with and without IP showed significant decreases in CD8+CD25+ (p<0.05), CD4+IFN- γ + (p<0.01 and p<0.05, respectively) and CD4+IL-4+ cells (p<0.05) compared with controls. There was no significant difference in these subpopulations between DM with and without IP. CD8+IFN- γ + (Tc1) cells were significantly decreased in DM patients with IP compared with those without IP (p<0.05). Both helper and cytotoxic T cells are activated in patients with active DM. Tc1 cells in PBL may be key markers suggestive of associated IP in DM.

Introduction

Dermatomyositis (DM) is an autoimmune disorder mainly involving the skeletal muscles and skin. Interstitial pneumonia (IP) complicates DM at a rate ranging from 10 to 50%, and significantly affects the prognosis of this disease, particularly in cases of an amyopathic form. Activated T lymphocytes are considered to play an important role in the pathogenesis of DM [1]. To find out the clinical marker suggestive of associated IP in

DM, we investigated intracellular cytokines and surface antigens of peripheral blood lymphocytes (PBL) using flow cytometry.

Patients and Methods

We studied thirteen patients with active DM with (six women and two men; age range, 21-65 years; mean age, 42.5±15.4 years) or without IP (four women and one man; age range, 25-83 years; mean age, 52.4±24.9 years). Nine healthy subjects (six women and three men; age range, 25-65 years; mean age, 41.3±16.0 years) were enrolled in this study. Clinical profiles of the patients are summarized in Table 1. Six patients with IP and two without IP showed the clinical features of amyopathic DM with regard to typical skin lesions with no or little involvement of skeletal muscles [2]. Other patients were diagnosed as having DM according to the classification criteria of Bohan and Peter [3]. Existence of IP was confirmed by computed tomography (CT) in all the patients with IP. No abnormal findings suggestive of associated malignancies could be found in systemic survey of any patients.

Heparinized whole blood samples were taken from the patients and controls, and mononuclear cells were separated by the Ficoll-Hypaque gradient method. To detect surface markers of lymphocytes, cells were stained with monoclonal antibodies (mAbs) to CD3, CD4, CD8, CD25, HLA-DR, CD19, CD23 and CD80. For intracellular staining of cytokines, cells were incubated at 37°C for 4 hours in 5ml of RPMI 1640 containing 5% fetal bovine serum, 2mM glutamine, 2µM monensin, 40 ng/ml of phorbol 12-myristate 13-acetate and 500 ng/ml of ionomycin. After blocking Fc receptors and fixation, appropriate mAbs, including anti-IFN-γ and anti-IL-4, were added for staining. The labeled cells were analyzed by two- or three-color flow cytometry.

Results

Results of phenotypical analysis of PBL are summarized in Figure 1. Both DM patients with and without IP showed significant decreases in activated CD8-positive T cells (CD8+CD25+ T cells) ($p<0.05$) compared

with controls. Both DM patients with and without IP showed significant decreases in CD4+IFN- γ + T cells (Th1 cells) ($p<0.01$ and $p<0.05$, respectively) and CD4+IL-4+ T cells (Th2 cells) ($p<0.05$) and a significant increase in the Th2/Th1 ratio ($p<0.01$ and $p<0.05$, respectively) compared with controls. There was no significant difference in these subpopulations between DM with and without IP. CD8+IFN- γ + cells (Tc1 cells) were significantly decreased in DM patients with IP compared with those without IP ($p<0.05$).

Discussion

In our study the patients with DM showed a significant decrease in activated CD8-positive T cells and a significant increase in the Th2/Th1 ratio irrespective of associated IP compared with controls. Several recent reports have also demonstrated that the percentage of CD8-positive (cytotoxic) T cells in PBL is decreased in active DM [1, 4]. IFN- γ -positive T cells and the Th2/Th1 ratio have been shown to significantly decrease and increase in active DM compared with controls, respectively [1].

In addition to these results Tc1 cells in PBL were significantly decreased in DM patients with IP than in those without IP in our study. The CD4+/CD8+ ratio in PBL has been reported to show significantly higher values in DM patients with rapidly progressive IP than in those with chronic IP [5]. There are several pieces of direct evidence suggesting that Tc1 cells may play an important role in development of IP in DM: an increase in CD8-positive T cells in the bronchoalveolar fluid (BALF) [6, 7], infiltration of CD8-positive T cells in the alveolar wall [8], and detectable mRNA of IFN- γ in BALF [7]. Tc1 cells in PBL may be a key marker suggestive of associated IP in DM, and a further study in a larger set of patients is required.

References

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