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Light chain (κ/λ) ratio of GM-CSF autoantibodies is associated with disease severity in autoimmune pulmonary alveolar proteinosis☆☆

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Abstract Previous studies demonstrated that antigranulocyte colony-stimulating factor autoantibody (GMAb) was consistently present in patients with autoimmune pulmonary alveolar proteinosis (aPAP), and, thus, represented candidature as a reliable diagnostic marker. However, our large cohort study suggested that the concentration of this antibody was not correlated with disease severity in patients. We found that the κ/λ ratio of GMAb was significantly correlated with the degree of hypoxemia. The proportion of λ-type GMAb per total

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λ-type IgG was significantly higher in severely affected patients than those in mildly affected patients, but the proportion of κ-type was unchanged. The κ/λ ratio was significantly correlated with both KL-6 and SP-D, which have been previously reported as disease severity markers. Thus, the light chain isotype usage of GMAb may not only be associated with the severity of aPAP, but may also represent a useful disease severity marker.

1. Introduction

Detection of granulocyte macrophage-colony stimulating factor (GM-CSF) autoantibody (GMAb) is known to be an excellent test with almost 100% sensitivity and specificity for the serological diagnosis of autoimmune pulmonary alveolar proteinosis (aPAP), which comprises 90% of all acquired PAP cases. This indicates its potential value in the routine clinical diagnosis of the disease; however, the test has neither approval nor commercial availability for clinical use [1]. On the other hand, our previous report demonstrated that serum GMAb levels were not correlated with the degree of hypoxemia [2] (according to disease severity score; partial pressure of oxygen in arterial blood, PaO_2; and alveolar–arterial oxygen difference, $AaDO_2$), but were moderately correlated with serum surfactant protein-A, -D, Krebs von den Lungen (KL)-6, and carcinoembryonic antigen (CEA) levels [1]. Because the autoantibodies were polyclonal, thereby recognizing multiple target epitopes on GM-CSF molecules with variable binding avidity, the loss of GM-CSF bioactivity in the lungs of patients with aPAP was thought to be affected not only by the concentration but also by multiple properties of GMAb such as binding avidity, neutralizing capacity, or targeting epitope. Thus, no characteristic correlation has been demonstrated between the properties of GMAb and the degree of hypoxemia.

Each B lymphocyte expresses only one isotype of light chain, κ or λ, which remains fixed for the life of the B lymphocyte. While immunoglobulin synthesis is matured and continually stimulated, the λ chain immunoglobulin concentration reaches a plateau by one year after birth and is maintained throughout the child's life [3]. On the other hand, the concentration of the κ chain, which increases gradually until 20 years of age, reflects the concentration of immunoglobulins as a whole [3,4]. The κ/λ ratio of immunoglobulin in normal adults ranges from 0.85 to 1.86 [5,6], from which it then becomes divergent in patients with monoclonal gammopathy or some autoimmune diseases. Studies suggest a selective preference for either κ- or λ-light chains in autoantibody formation, such as rheumatoid factor (RF) [7,8], anti-cardiolipin antibodies [9], anti-neutrophil antibodies [10], several thyroid-stimulating antibodies [11,12], anti-lamin B antibody [13], and circulating immune complexes in juvenile idiopathic arthritis [14]. Thus, measuring κ/λ ratios in some autoantibodies may be useful to identify the state of activation of B cells involved in some autoimmune diseases. Studies suggest selective preference for either κ or λ chains in autoantibody formation.

During a previous study on characterization of GMAb in patients with aPAP in comparison to pharmaceutical immunoglobulin (IVIG), which was produced from pooled normal sera of more than 1000 normal subjects, we measured concentrations, binding avidities, and κ/λ ratios of GMAb. We noticed that the κ/λ ratio was much higher for GMAb than for whole IgG in both IVIG and aPAP groups, but it decreased as the disease severity of aPAP increased. The aim of this study was to assess the potential use of the κ/λ ratio as a disease severity marker in aPAP. In addition, we discuss the possibility that a selective preference in light chain isotype usage might be associated with the pathogenesis of aPAP.

2. Materials and methods

2.1. Subjects

Forty-six patients with aPAP were enrolled in this study. All patients were diagnosed with PAP by computed tomography findings and lung biopsy or bronchoalveolar lavage findings, and diagnosis was confirmed by the existence of GM-CSF autoantibodies in sera according to the diagnostic criteria (http://www.pap-guide.jp/en/). The median age, gender, proportion of symptomatic individuals, mean arterial blood oxygen pressure, and mean percent vital capacity were comparable to those in our previous large cohort study [2]. All serum and plasma samples were gathered in our institution to measure the level of GM-CSF autoantibodies after written informed consent to collect samples. All participants provided written informed consent; minors provided consent in accordance with the Declaration of Helsinki. Healthy volunteers were also enrolled into the study as healthy subjects (HS) after agreement with written informed consent. All patients with aPAP were categorized by disease severity score (DSS) at enrollment, as previously described [2], from least severe (DSS-1) to most severe (DSS-5).

2.2. Pharmaceutical immunoglobulin (IVIG)

Eight different batches of pharmaceutically-prepared immunoglobulin, Venoglobulin-IH™, were kindly provided by Mitsubishi Pharma Corporation (Tokyo, Japan).

2.3. Enzyme-linked immunosorbent assay (ELISA)

2.3.1. Whole IgG

The serum concentration of whole IgG was measured by using a human IgG ELISA quantitation kit (Bethyl, Montgomery, TX, USA) according to the manufacturer's instructions.

2.4. GM-CSF autoantibody

Serum and culture medium GM-CSF autoantibody levels were measured using direct ELISA as previously reported [15–17]. In brief, micro-ELISA plates (Maxisorp™ flat-bottom, clear, 96-well plates; Nunc, Roskilde, Denmark) were coated with