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Possible linkage between microscopic polyangiitis and thrombosis via neutrophil extracellular traps

Running title: NETs link MPA and thrombosis

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Sirs,

Microscopic polyangiitis (MPA) is included in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (1, 2). In MPA patients, ANCA for meyloperoxidase (MPO) is usually present in the serum. It is reported that patients with AAV, including MPA, have an increased risk of thrombosis (3). However, it remains elusive why AAV patients are prone to develop thrombosis.

After exhibition of phagocytic activity against invading microbes, neutrophils undergo cell death. Neutrophils can then release chromatin fibers with decoration of intra-cytoplasmic antimicrobial proteins, such as MPO, outside of the cells (4). This process, called neutrophil extracellular traps (NETs), is an innate immune system to trap and kill the microbes that survive phagocytosis. Interestingly, NETs have been detected in the glomerular crescents of AAV patients regardless of the absence of infection (5, 6). MPO-ANCA has shown to induce NETs on neutrophils independent of infectious agents.

On the other hand, NETs are critically associated with thrombosis because histones within NETs can bind platelets and blood coagulants (7). Although the synergy of antimicrobial and pro-thrombotic functions of NETs is valuable concerning the inclusion of microbes in the NETs, an excessive formation of NETs unexpectedly causes thrombosis. Recently, Nakazawa et al. reported the abundant NETs formation in the venous thrombus of a patient with MPA (6).

In the present study, the amount of NETs in the thrombus was compared between MPA patients and controls. Autopsy materials from 3 MPA patients (M1-M3) and 3 controls (C1-C3) were applied. M1 is a 79-year-old male with MPA, who died of pulmonary thromboembolism. M2 is a 71-year-old male with MPA, who died of sepsis due to *Candia albicans* and *Pseudomonas aeruginosa*. M3 is a 73-year-old female with MPA, who died of sepsis due to methicillin-resistant *Staphylococcus aureus*. C1 is a

49-year-old male with lung adenocarcinoma, who died of disseminated intravascular coagulation. Autopsy revealed thrombus in the inferior vena cava. C2 is a 51-year-old male with acute myocarditis, who died of diffuse alveolar damage. Autopsy revealed thrombus in the superior vena cava. In C1 and C2, acute splenitis suggested the presence of sepsis. C3 is an 83-year-old female with diffuse large B cell lymphoma, who died of pulmonary thromboembolism. Severe alveolar pneumonia was present in C3. Immunohistochemistry for citrullinated histone 3 (Cit H3) was conducted on specimen sections with thrombi. Since citrullination of histones is an essential process for generation of NETs (8), this method accurately reflects the amount of NETs (6). Results demonstrated that the amount of NETs in thrombus was significantly greater in MPA patients than controls (Figure 1). The collective evidences suggest that MPO-ANCA induces excessive NETs, and the excessive NETs induce thrombosis in MPA patients.

Under physiological condition, NETs are adequately digested by serum DNase I (9). Therefore, it seemed likely that the amount of NETs in the thrombus was kept in a relatively low level in controls, though infection was present in these patients. On the other hand, the large amount of NETs in the MPA thrombus regardless of presence or absence of infection suggested the high ability for NETs induction by MPO-ANCA and/or low ability for NETs degradation by DNase I in MPA patients. Although further studies are needed to clarify the disorders of NETs formation and regulation in MPA patients and if the feature is specific to MPA among autoimmune diseases, this study has demonstrated that NETs could link MPA and thrombosis. Since the excessive NETs are involved not only in thrombogenesis, but also in production of MPO-ANCA and subsequent development of MPA (10, 11), active regulation of NETs could be a promising strategy to treat MPA, as well as thrombosis complicated with MPA.

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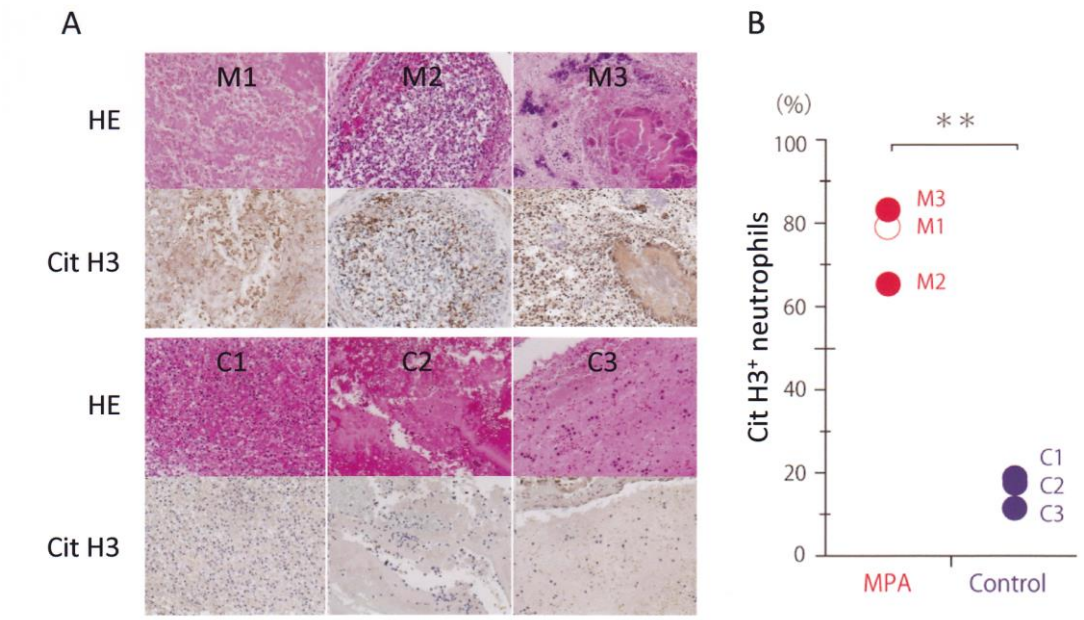

Figure 1. Cit H3$^+$ neutrophils in thrombus

Formalin-fixed paraffin-embedded sections with thrombi were allowed to react with 1:100 dilution of anti-Cit H3 antibody (Abcam, Tokyo, Japan) for 60 min at room temperature. Immunohistochemistry was performed by the conventional labeled-streptavidin biotin method. The Cit H3$^+$ cells in fresh thrombi were counted, and then the data was standardized by the number of neutrophils in the serial section with hematoxylin and eosin (HE) staining. (A) Representative figures are shown. M1-M3 and C3: thrombus in pulmonary artery. C1: thrombus in inferior vena cava. C2: thrombus in superior vena cava. Original magnification ×200. (B) Comparison of the rate of Cit H3$^+$ neutrophils between MPA patients and controls. Closed and open circles represent the presence and absence of infection, respectively. **p<0.01 in Student's *t*-test.