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Citation	Nephron, 138(4), 328-330 https://doi.org/10.1159/000485902
Issue Date	2018-04
Doc URL	https://hdl.handle.net/2115/73832
Rights	This is the peer-reviewed but unedited manuscript version of the following article: Nephron 2018;138:328-330 (DOI:10.1159/000485902). The final, published version is available at http://www.karger.com/?doi=10.1159/000485902 .
Type	journal article
File Information	Nephron_138(4)_328-330.pdf



**Vanishing immunoglobulins: the formation of pauci-immune lesions in
myeloperoxidase-antineutrophil cytoplasmic antibody-associated
vasculitis**

Running Title: Pathogenesis of pauci-immune lesion

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Dear editor,

Myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-associated vasculitis is characterized by necrotizing crescentic glomerulonephritis and small vessel vasculitis with a presence of MPO-ANCA in the serum. Although the pathogenic role of MPO-ANCA in the formation of necrotizing lesions has been demonstrated, only little deposition of immunoglobulins can be detected in the affected tissues. This histological feature is what we call “pauci-immune.”

Neutrophil extracellular traps (NETs) are web-like DNA decorated with antimicrobial proteins, such as MPO, which are extruded from activated neutrophils [1]. NETs can trap microorganisms by the web-like DNA and kill them using the antimicrobial proteins scattered on the extracellular DNA. Although NET formation is regarded as an essential event in innate immunity, recent studies have noted the adverse effects of NETs on the hosts, e.g., thrombogenic potential and cytotoxicity to vascular endothelial cells [2].

The involvement of NETs in the pathogenesis of MPO-ANCA-associated vasculitis was firstly reported in 2009 [3]. The following studies have demonstrated the presence of NETs in crescentic glomeruli with pauci-immune necrotizing lesions [4]. In MPO-ANCA-associated vasculitis, neutrophils primed by inflammatory cytokines, such as $\text{TNF-}\alpha$, express MPO on their surfaces, and MPO-ANCA can bridge the MPO and $\text{Fc}\gamma$ receptors on the cell resulting in the formation of NETs [5].

In order to reveal the pathogenesis of pauci-immune lesions in MPO-ANCA-associated vasculitis, we determined that neutrophil elastase released from NET-forming neutrophils, which was activated by MPO-ANCA, could digest immunoglobulins. When the anti-MPO antibody (FITC-conjugated) was applied to

ethanol-fixed neutrophils, the antibody binding to the neutrophils was detected as ANCA steadily for 16 h (**Figure 1a**). On the contrary, the anti-MPO antibody (MPO-ANCA) induced NETs on TNF- α -primed neutrophils and then disappeared simultaneously. Since MPO has been shown to distribute on the DNA of NETs [1], it can be considered that the vanishing MPO-ANCA reflect a disappearance of the antibody rather than the antigen. Based on these findings and previous reports on the secretion of neutrophil elastase from NET-forming neutrophils [3], we speculated that neutrophil elastase could digest MPO-ANCA. As expected, time and dose-dependent digestions of the IgG by neutrophil elastase were demonstrated (**Figure 1b**). In addition, the MPO-ANCA, which was exposed to neutrophil elastase, could no longer bind to neutrophils (**Figure 1c**). The collective findings suggest that neutrophil elastase released from NET-forming neutrophils, which was activated by MPO-ANCA, could be involved in the pathogenesis of pauci-immune lesions, at least in part, in MPO-ANCA-associated vasculitis via digestion of MPO-ANCA.

Funding

This work was supported by a grant for Japan Research Committee of the Ministry of Health, Labour, and Welfare for Intractable Vasculitis.

Disclosure statement

The authors declare that no conflict of interest exists.

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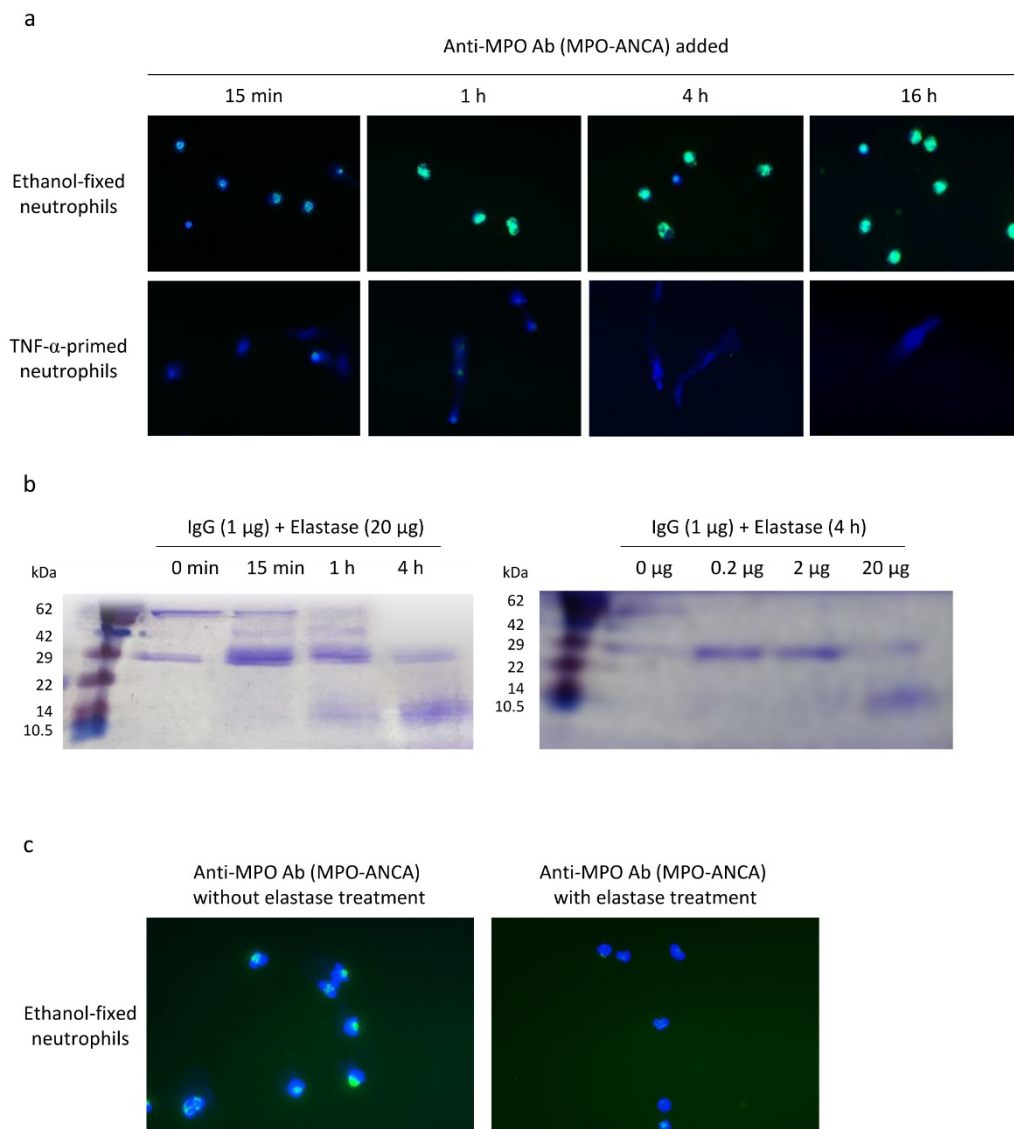


Figure 1. (a) Vanishing MPO-ANCA. Peripheral blood neutrophils from a healthy volunteer were seeded in slide chambers ($1 \times 10^6/\text{ml}$), incubated for 30 min at 37 °C, and then fixed with 100% ethanol for 15 min at room temperature or exposed to 2 ng/ml of TNF- α for 15 min at 37 °C. After washing with PBS, the cells were allowed to react with 1 $\mu\text{g}/\text{ml}$ of the FITC-conjugated anti-MPO antibody (Gene Tex, Irvine, CA) at 37 °C. After incubation for 15 min, 1 h, 4 h, and 16 h, the cells were washed with PBS and then mounted using the solution containing DAPI (Sigma-Aldrich, St. Louis, MO). Microphotographs (magnification, $\times 400$) were taken under a fluorescent microscopy. **(b)** Time and dose-dependent digestions of the IgG by neutrophil elastase. Mouse IgG (1 μg) (BD Biosciences, Tokyo, Japan) was exposed to 20 μg of neutrophil elastase (abcam, Cambridge, UK) at 37 °C for 0 min, 15 min, 1 h, and 4 h. Alternatively, 1 μg of

mouse IgG was exposed to 0, 0.2, 2, and 20 μg of neutrophil elastase at 37 °C for 4 h. Thereafter, the samples were boiled for 5 min in the sample buffer followed by electrophoresis through 12% SDS polyacrylamide gel. The gels were stained with Coomassie Brilliant Blue. (c) Effect of neutrophil elastase exposure on MPO-ANCA. Peripheral blood neutrophils from a healthy volunteer were seeded in slide chambers ($1 \times 10^6/\text{ml}$), incubated for 30 min at 37 °C, and then fixed with 100% ethanol for 15 min at room temperature. After washing with PBS, the cells were allowed to react with 1 $\mu\text{g}/\text{ml}$ of the FITC-conjugated anti-MPO antibody, which was treated without or with 20 $\mu\text{g}/\text{ml}$ of neutrophil elastase at 37 °C for 4 h. After 1 h incubation at room temperature, the cells were washed with PBS and then mounted using the solution containing DAPI. Microphotographs (magnification, $\times 400$) were taken under a fluorescent microscopy. All experiments were repeated more than twice. Since similar results were reproduced, the representative results were shown.