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Possible Implication of Disordered Neutrophil Extracellular Traps in the Pathogenesis of MPO-ANCA-associated Vasculitis

Short title: NETs disorder and MPO-AAV

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Abstract

Neutrophil extracellular traps (NETs) are characterized by the presence of extracellular DNA fibers studded with antimicrobial proteins, including myeloperoxidase (MPO). Although NETs play an important role in the innate immune system, the scattered extracellular enzymes, such as MPO, are risky for the host. Therefore, NETs are strictly regulated by DNase I in the serum, which prevents the persistence of NETs. Recent studies have demonstrated that dysregulation of NETs could be involved in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus. In this review, we interpret the association of disordered NETs with autoimmune diseases, especially propylthiouracil-induced MPO-ANCA-associated vasculitis.

Key words: NETs, PTU, MPO-ANCA, MPO-AAV

Neutrophil extracellular traps

In humans, neutrophils act as the first line of defense against invading microbes. They employ a wide spectrum of antimicrobial strategies, most notably phagocytosis, to eliminate microbes. Subsequent to phagocytosis, neutrophils exhibit a unique style of cell death, which is characterized by the presence of extracellular DNA fibers studded with antimicrobial proteins including myeloperoxidase (MPO). This mode of cell death, called neutrophil extracellular traps (NETs), is a defense mechanism of the host to trap and kill invading microbes [1]. NETs function even after the neutrophils are dead.

Dysregulation of NETs in systemic lupus erythematosus

Although NETs play an important role in the innate immune system, there is a risk that antimicrobial enzymes, such as MPO, in the NETs can cause injury to the host tissues. Thus, NETs are strictly regulated by DNase I in the serum, which digests DNA in NETs and prevents the persistence of NETs. A subgroup of patients with systemic lupus erythematosus (SLE) has been reported to poorly digest NETs because of the low activity of DNase I or the presence of DNase I inhibitors, such as anti-DNA antibodies, in the serum [2]. Hakkim *et al.* suggested that the genetic background with low activity of DNase I could induce the persistence of NETs, and the persistent extracellular DNA in the NETs could be recognized by the host immune system resulting in the production of anti-DNA antibodies. Furthermore, the anti-DNA antibodies could inhibit the activity of DNase I; therefore, the pathogenesis of SLE mediated by NETs becomes a vicious cycle.

Propylthiouracil and MPO-ANCA-associated vasculitis

Propylthiouracil (PTU) is an anti-thyroid drug used for the treatment of hyperthyroidism. Approximately 30% of patients administered with PTU produce

MPO-ANCA, and some of them develop MPO-ANCA-associated vasculitis (MPO-AAV). The majority of PTU is metabolized in the liver, but a part is modified by MPO in neutrophils [3].

Involvement of disordered NETs induced by PTU in development of MPO-AAV

Based on the evidences, we hypothesized that PTU can influence the formation or regulation of NETs and induce MPO-ANCA and subsequently MPO-AAV [4]. First, we examined whether the addition of PTU would create an impact on the formation or regulation of NETs *in vitro*. Widely extended DNA fibers studded with MPO were observed when human neutrophils were treated with phorbol myristate acetate (PMA, Figure 1a). On the contrary, the DNA fibers showed round-shape distribution; thus, the NETs were limitedly created surrounding the dead neutrophils treated by PMA with PTU (Figure 1b). Interestingly, although the PMA-induced normal NETs were digested by DNase I completely (Figure 1c), the abnormal NETs induced by PMA with PTU were hardly digested by DNase I (Figure 1d). These findings clearly indicated that PTU influenced the NETs formation and degradation by DNase I.

Next, we examined whether the disordered NETs would induce the production of MPO-ANCA *in vivo*. For this purpose, WKY rats were immunized with the abnormal NETs, which had been induced by PMA with PTU using rat neutrophils. WKY rats immunized with normal NETs induced by PMA hardly produced ANCA; however, WKY rats immunized with abnormal NETs induced by PMA with PTU produced ANCA [4]. The serum reactivity to neutrophils was markedly inhibited by pre-treatment of the neutrophils with anti-MPO antibodies; therefore, this indicated that the ANCA was MPO-ANCA.

Lastly, we attempted to establish a suitable model for MPO-AAV. For this purpose, WKY rats were given oral PTU with intra-peritoneal injection of PMA.

Contrary to the finding that widely extended NETs were observed in the peritoneal tissues of the PMA-injected WKY rats without PTU administration (PMA-treated rats), NETs in the PMA-injected WKY rats with PTU administration (PTU/PMA-treated rats) did not extend outward [4]. Furthermore, NETs area in the peritoneal tissues of PTU/PMA-treated rats was significantly larger than that of PMA-treated rats. These findings corresponded to the *in vitro* data indicating the abnormal conformation and impaired degradation of NETs induced by PMA with PTU. As expected, MPO-ANCA was produced in PTU/PMA-treated rats followed by development of pauci-immune glomerulonephritis and pulmonary bleeding due to alveolar capillaritis [4]. These findings clearly indicated that PTU induced NETs disorder and MPO-ANCA production and subsequent development of MPO-AAV.

Possible implication of disordered NETs in pathogenesis of MPO-AAV

Abundant NETs have been detected in the lesion of crescentic glomerulonephritis, and elevation of circulating MPO-DNA complexes in the serum, which might be derived from *in situ* NETs, has been shown in patients with MPO-AAV [5]. It has been reported that excessive formation of NETs was induced by influenza virus infection [6]. Although most patients with MPO-AAV are not administered with PTU, undetermined environmental factors that act similarly like PTU, e.g., infectious agents, can induce the disorder of NETs and trigger the MPO-ANCA production resulting in the development of MPO-AAV (Figure 2).

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References

1. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science*. 2004; 303:1532-5.
2. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, Herrmann M, Voll RE, Zychlinsky A. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci USA*. 2010; 107:9813-8.
3. Waldhauser L, Uetrecht J. Oxidation of propylthiouracil to reactive metabolites by activated neutrophils. Implications for agranulocytosis. *Drug Metab Dispos*. 1991; 19:354-9.
4. Nakazawa D, Tomaru U, Suzuki A, Masuda S, Hasegawa R, Kobayashi T, Nishio S, Kasahara M, Ishizu A. Abnormal conformation and impaired degradation of NETs induced by propylthiouracil: Implication of disordered NETs in MPO-ANCA-associated vasculitis. *Arthritis Rheum*. 2012; doi: 10.1002/art.34619.
5. Kessenbrock K, Krumbholz M, Schonermarck U, Back W, Gross WL, Werb Z, Grone HJ, Brinkmann V, Jenne DE. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*. 2009; 15:623-5.
6. Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA, Phoon MC, van Rooijen N, Chow VT. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am J Pathol*. 2011; 179:199-210.

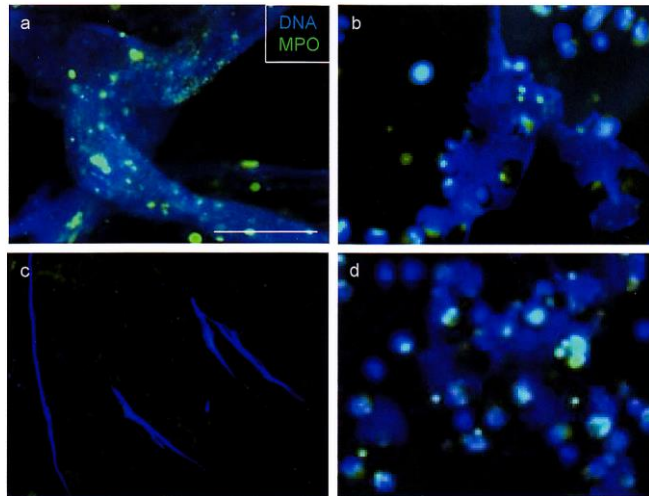


Figure 1. Abnormal conformation and impaired degradation of NETs induced by PMA with PTU

(a) Normal conformation of NETs induced by PMA. (b) Abnormal conformation of NETs induced by PMA with PTU. (c) Digestion of PMA-induced normal NETs by DNase I. (d) Resistance of abnormal NETs induced by PMA with PTU to DNase I. Bar: 100 μ m. Representative figures are shown.

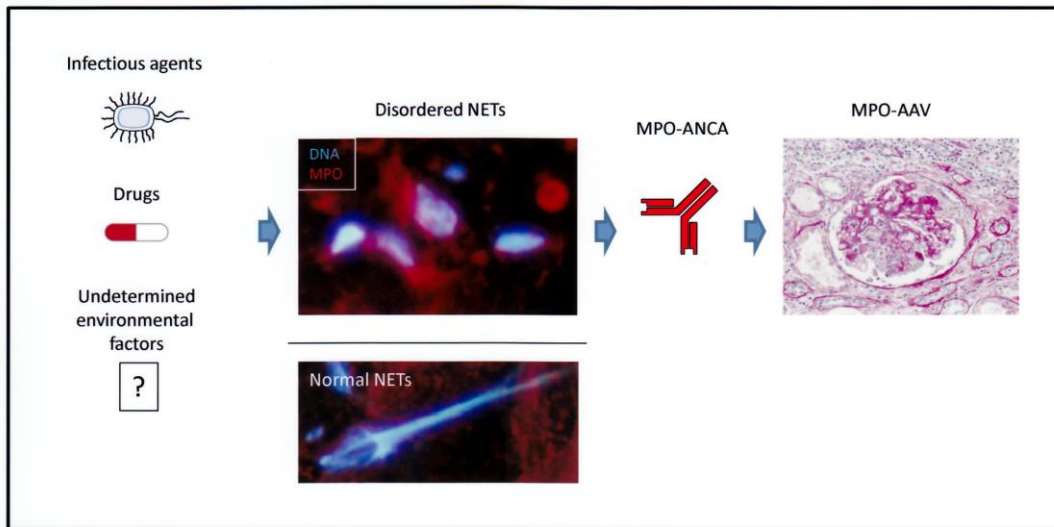


Figure 2. Possible implication of disordered NETs in pathogenesis of MPO-AAV
 Undetermined environmental factors, including infectious agents and drugs, could induce the disorder of NETs and trigger the MPO-ANCA production resulting in the development of MPO-AAV.