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Original Article

NETosis may play a role in the pathogenesis of Hashimoto's thyroiditis

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Abstract: Neutrophil extracellular traps (NETs) are shown to play important roles in the progression or development of systemic autoimmune diseases. However, implication of NETs or NETosis in the pathogenesis of non-systemic autoimmune diseases such as Hashimoto's thyroiditis (HT), a chronic inflammatory organ-specific autoimmune disease, has not been previously reported. In the present study, our results demonstrate that the concentration of NET products, neutrophil elastase (NE) and proteinase 3 (PR3) in plasma, are significantly higher in the patients with HT than in healthy controls, respectively. In addition, PR3 concentration in plasma was positively associated with the titers of autoantibodies against thyroglobulin (TGAb) and thyroid peroxidase (TPOAb) in serum, respectively. Consistently, NETosis was more markedly induced in neutrophils derived from the HT patients than controls. Concomitantly, IL-6 production in the NETosis induction system in the neutrophils from the patients was significantly higher than those in controls. Moreover, serum from HT patients but not healthy controls induced more pronounced NETosis in neutrophils. Meanwhile, our immuno-fluorescence staining results showed that NETs from the HT patients contained autoantigens. These findings together indicate roles for NETs and/or NETosis in autoantibody generation as well as pathogenesis of HT. Therefore, the underlying mechanisms of NETs in the pathogenesis of HT warrant further study.

Keywords: Neutrophil extracellular traps, Hashimoto's thyroiditis, autoimmunity, neutrophil elastase, proteinase 3

Introduction

Hashimoto's thyroiditis (HT), also known as an autoimmune thyroiditis, is the most common clinical thyroid inflammation characterized by thyroid enlargement and acquired hypothyroidism [1]. At present, genetic and environmental factors that predispose or contribute to HT development have been discussed [2], however, the etiology and pathogenesis of this disease are still largely unclear. Severe filtration of lymphocytic cells in the glad and biased cellular immune response and tissue atrophy, have been previously described [3]. Anti-thyroid antibodies, especially against thyroid peroxidase (TPOAb) and thyroglobulin (TGAb) can be detected in plasma or serum of HT patients. Several studies have claimed that TPOAb and TGAb might play a role in increased inflammatory reaction of the thyroid parenchyma [4-6]. Specifically, anti-thyroid autoantibodies may be involved in the pathogenesis of the disease through inducing follicular cell degeneration by interacting with complements and compliment-mediated cytotoxic effects [7].

Neutrophil extracellular traps (NETs) are web-like structural DNA-protein fibers that are initially released by the neutrophils to wrap, restrict, and kill pathogens at sites of infection [8]. A variety of organic or inorganic stimuli such as microbes, inflammatory factors, immune complexes, or synthetic PKC family of enzymes (i.e., phorbol myristate acetate: PMA, a potent mitogen and inducer of NET production), can activate neutrophils to generate NETs [9]. Reactive oxygen species (ROS) production and histone deimination by peptidylarginine deiminase 4 (PAD4) are prerequisites for NETs formation, a process known as NETosis [10]. Distinct

from necrosis and apoptosis, NETosis promotes the externalization of various autoantigens and the granular enzymes (e.g. the serine protease neutrophil elastase (NE), myeloperoxidase (MPO), proteinase 3 (PR3) and deiminated histones), and at present NE as well as PR3 are used as markers to identify NETs or to reflect NETosis [11, 12].

A vicious cycle is established when activated neutrophils express and release a large array of pathogenic pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor (TNF) superfamily members [13], which subsequently induce NETosis. Exposure of cellular components to extracellular matrix during NETosis qualifies as a major source of autoantigens. Defects in the release and removal of NETs have recently been suggested to play a role in the pathogenesis and progression of autoimmune diseases such as systemic lupus erythematosus (SLE) [14, 15], rheumatoid arthritis (RA) [16], and vasculitis [17, 18]. These studies highlight that NETs play important parts in neutrophil-mediated immune responses by capturing curable and pathogenic factors which present in the circulation and tissues, by which they contribute to autoimmune disease development. In this study, our results demonstrate a positive correlation between auto-antibodies (anti-TPOAb and anti-TGAb) and NET (using NE and PR3 as NET markers) concentrations in plasma, and NETosis was significantly induced in neutrophils derived from HT patients compared with controls, implicating a role of NETosis in the generation of auto-antibodies as well as in the pathogenesis of HT.

Materials and methods

Study cohort

30 diagnosed female patients with HT, with ages ranging from 25 to 57 years, and with a median age of 35 years, as well as 30 age-matched healthy female controls were recruited between 2015 and 2016 at department of Endocrinology and Metabolism, and Physical Examination Center of the Second Hospital of Shandong University. Patients with fever, cancer, and other autoimmune diseases were excluded from this study. This study was approved by the Ethics Committee of the Second Hospital of Shandong University, and written consent was obtained from all the participants.

Neutrophil isolation

Neutrophils were isolated using a human peripheral blood neutrophil separation kits according to the manufacturer's instructions (TBD Bio. Co., Tianjin, China). Briefly, heparinized peripheral blood was collected and carefully aspirated with a pipette into the liquid level of the separation solution. After centrifugation at 550 g for 25 min, the lower white cell layer containing neutrophils was acquired and the red cells were removed by erythrocyte lysis buffer. The entire process was performed at room temperature within 6 h after samples were collected. Neutrophils were resuspended in phenol red-free RPMI-1640 supplemented with 2 mM L-glutamine, and adjusted to 2×10^6 cells/mL before seeding in 12-well plates. Cell viability in all the experiments was higher than 96%, as determined by the trypan blue dye exclusion method.

Neutrophil activation test

Neutrophils were seeded in 96-well culture plates with the density of 1×10^5 cells per well. To determine the appropriate stimulation time and concentration of PMA (Sigma-Aldrich), cells were activated with 25, 50, 75, 100, and 150 nM PMA for 2 h or 25 nM PMA incubation for 10 min, 1 h, 2 h, and 4 h. NETs formation was analyzed after 0.5 μM SYTOX Orange dye (Molecular Probes, Invitrogen Life Technologies) addition to the cells, vortex mixing, and incubation for more than 10 min. Fluorescence was measured using Varioskan Flash (Termo Scientific, USA) at 547/570 nm and NET formation was expressed as the fold-increase in fluorescence relative to unstimulated cells [19]. To visualize NETs, purified neutrophils were seeded onto poly-L-lysine-coated coverslips in 12-well plates at a density of 2×10^6 cells per well and allowed to adhere for 30 min at 37°C. Neutrophils were stimulated with PMA at indicated incubation time points and concentrations, washed twice with PBS, followed by fixation with 4% paraformaldehyde and permeabilization with 0.2% Triton-X 100 for 15 min. Cells were rinsed with PBST (PBS with 0.1% Tween-20) and blocked with goat serum for 1 h. 0.5 μM SYTOX Orange dye was added to the cells and incubated for more than 10 min. The cells were then washed and counterstained with a 1:1000 dilution of DAPI (1 mg/mL; Sigma) in PBS for 15 min at room temperature. Cells were imag-

ed with an Olympus IX-73 inverted fluorescence microscope and analyzed using Image-Pro Plus software v 7.0 (Olympus).

Quantification of NETs

NETs were quantified using a previously reported flow cytometry assay [20]. Briefly, activated neutrophils were fixed with 2% paraformaldehyde at 4°C for 20 min. After washing in cold PBS, the cells were first incubated with a 1:200 dilution of the primary rabbit anti-histone H3 citrulline antibody (Abcam), and then washed in cold PBS followed by incubation with PE-conjugated donkey anti-rabbit secondary antibody (Biolegend) or a FITC-conjugated anti-myeloperoxidase antibody (Abcam) at 1:200 dilution for 30 min at 4°C, respectively. The cells were resuspended in PBS buffer, and analyzed using a FACS Calibur Flow cytometer (BD, US) with FlowJo X software (BD, US).

Detection of autoantigens

Purified neutrophils were seeded onto poly-L-lysine-coated coverslips in 12-well plates at a density of 2×10^6 cells/mL and allowed to adhere for 30 min at 37°C. Neutrophils were stimulated with 25 nM PMA for 1 h, and the cells were washed twice with PBS, followed by fixation with 4% paraformaldehyde, and permeabilization with 0.2% Triton-X 100 for 15 min. Cells were rinsed with PBST (PBS with 0.1% Tween-20) and blocked with goat serum for 1 h at room temperature. To test whether NETs derived from HT patients' neutrophils contained potential antigens for autoantibody, serum from a HT patient, or a healthy controls was added to the cells, and incubated in a humidified chamber at 37°C for 1 h. Coverslips were washed three times with PBS and then incubated with an Alexa Fluor 488-conjugated goat anti-human IgG antibody (1:400 dilution) (Invitrogen) at 37°C for 30 min. Cells were washed and then counterstained with a 1:1000 dilution of DAPI (1 mg/mL; Sigma) in PBS for 15 min at room temperature. NETs were visualized under an Olympus IX-73 inverted fluorescence microscope and analyzed using ImagePro Plus software v 7.0 (Olympus).

Quantification of NETosis in plasma

Heparinized peripheral blood was collected from HT patients and healthy controls. After centrifugation at 3000 rpm for 10 min, plasma samples were harvested and divided into aliquots, and then frozen at -80°C until use. PR3 and NE concentrations in plasma were quantified using ELISA kits from BioVendor (Asheville, NC) and eBioscience (San Diego, CA) according to the manufacturers' protocols, respectively. Briefly, Plates were washed and test supernatants were added and assayed in duplicates at 100 µl per well. After washing, the plates were incubated with biotinylated anti-human PR3 or NE antibody, followed by HRP-conjugated avidin. Finally, substrate was added and the color reaction was measured in an ELISA reader at 450 nm (Multiskan™ FC, Termo Scientific, USA). The results were calculated by reference to a standard curve and expressed as ng per ml.

Detection of IL-6 in supernatants of NETosis cultural system

Neutrophils isolated from controls and patients with HT were seeded in 96-well plates at a density of 2×10^5 cells per well and stimulated with or without 25 nM PMA. Supernatants of the culture medium were collected after 1 and 4 h stimulation of PMA, respectively, and IL-6 concentration was measured using a ELISA kit following the procedures provided by the manufacturer (Abcam, UK). The absorbance of each well was measured at 450 nm using a spectrophotometer (Multiskan™ FC, Termo Scientific, USA).

Quantification of TPOAb and TGAb in serum

Serum *TPOAb and TGAb* concentrations were determined by radioimmunoassay using UniCel DxI 800 analyzer (Beckman Counter. Inc, USA) and following the protocols provided by the manufacture enclosed in the Access TPO Antibody and Access Thyroglobulin Antibody II kits (Beckman Counter. Inc, USA), respectively. The detection thresholds for anti-TPO and anti-Tg were 0.25 U/mL and 0.37 U/mL, respectively. The assay was performed at Laboratory of Biochemistry, Diagnostics Laboratory of the Second Hospital of Shandong University.

Statistical analysis

SPSS version 21.0 was used for all analyses. Comparisons between two groups were performed using Student's t tests. Linear correlations were assessed using Pearson's r coefficient. Statistical significance was defined when $P < 0.05$.

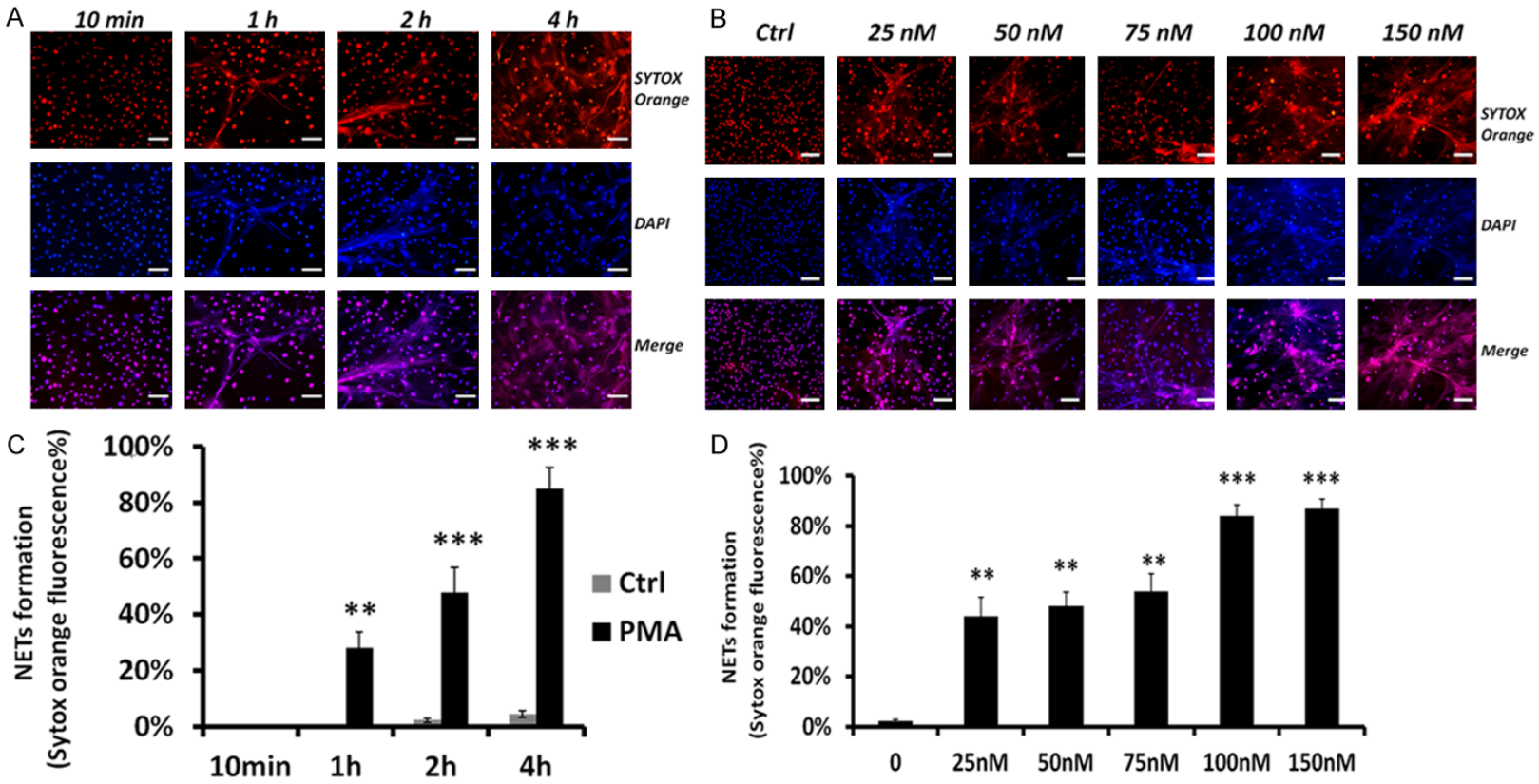

Figure 1. PMA induces NETosis in neutrophils derived from healthy controls. Isolated neutrophils from peripheral blood of healthy controls were cultured in the presence of PMA with various doses and time points indicated, the cells were then stained with SYTOX Orange and DAPI, and analyzed under Olympus IX-73 inverted fluorescence microscope (A) NETosis was induced with 25 nM PMA at the time points indicated. (B) NETosis was induced with the indicated concentrations of PMA for 2 h, representative fluorescence staining results from 6 independent experiments are shown, Scale bar: 100 μm. (C) NETs formation induced by PMA in a time dependent manner. (D) NETs formation induced by PMA in a dose dependent manner. Data (C and D) are presented as the mean ± SD of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.

Results

Neutrophils from patients with HT underwent NETosis more markedly than from controls after PMA stimulation in vitro

We first established the NETosis system in healthy controls using PMA as an inducer. Our results showed that PMA induced NETs generation in a dose-dependent and time-dependent manner. After 1 h of stimulation with 25 nM PMA, approximately 28.2% of neutrophils underwent NETosis compared with controls (neutrophils without PMA stimulation) (0.8%). This phenomenon became more obvious with a prolonged observation time. After a 4 h incubation with PMA, cells showed an approximately 19.7-fold increase in the rate of NETosis (**Figure 1A** and **1B**). We then tested the activation effects of various concentrations of PMA for 2 h and identified similar trends, as the NETosis rate increased from 44.1% in the cells treated by 25 nM PMA to 87.6% by 150 nM PMA (**Figure 1C** and **1D**).

To attempt to find differences in NETosis induced by PMA between the patients and controls, we used lower dose PMA (25 nM) and a shorter stimulation time (1 h) as a NETosis induction system in the following experiments. To confirm the network of extracellular chromatin fibers, the cells were stained with antibody against H3Cit, a specific antibody related to the process of NET formation [12, 21], robust NET networks were visualized (**Figure 2A**). To more precisely quantify NETs, a flow cytometry-based method [20] was applied. Our results demonstrate that after 1 h of treatment with PMA, the proportion of sorted $H3Cit^+$ and MPO^+ NETs was significantly higher in the patients (80.7%) than controls (66.1%) (**Figure 2B**). In line with this finding, fluorescence staining with SYTOX Orange and DAPI showed a similar trend (**Figure 2C**). Moreover, we compared the effect of serum derived from HT patients and healthy controls on NETosis induction, using PMA stimulation as a positive control. Our results demonstrated that serum from patients with HT, but not from healthy controls induced pronounced NETosis in neutrophils derived not only from HT patients but also healthy controls (**Figure 2D**), indicating that auto-antibodies in serum from HT patients may be the factor or patients 'serum may contain other molecules such as cytokines triggering NETosis.

IL-6 levels are significantly increased in NETosis in patients with HT

IL-6 is an important proinflammatory cytokine and NETs could augment inflammatory response by induction IL-6 release (15). As expected, PMA stimulation significantly induced IL-6 secretion by neutrophils from HT patients and controls, respectively. Moreover, neutrophils derived from the HT patients produced significantly higher levels of IL-6 after PMA stimulation at 1 h and 4 h when compared with controls, respectively (**Figure 3**).

Levels of circulating NET-associated components are increased and positively associated with TGAb/TPOAb titers in the patients with HT

According to previous studies, levels of NET-associated biomarkers, such as proteinase 3 (PR3), histones, cell-free DNA (cfDNA) and NE, might increase in patients with various autoimmune diseases [22, 23] and non-immunological diseases [24]. In this study, our results showed a dramatic increase in NE concentration in plasmas from HT patients (273.86 ± 114.05 ng/mL; $P < 0.001$) compared with healthy controls (141.45 ± 52.14 ng/mL) (**Figure 4A**). Meanwhile, the circulating PR3 level was also significantly elevated in the patients (74.99 ± 25.56 ng/mL; $P = 0.0015$) compared to controls (57.28 ± 10.52 ng/mL) (**Figure 4B**). We next explored the relationship between plasma levels of NET-associated biomarkers NE and PR3 with main autoantibodies presented in the serum of the HT patients. Our results demonstrated a strong correlation between the levels of the PR3 protein and TGAb titers ($r = 0.3924$, $P = 0.0319$) (**Figure 5A**). Likewise, TPOAb titers were also positively correlated with PR3 levels ($r = 0.5098$, $P = 0.0040$) (**Figure 5B**). However, a significant correlation was not observed between the circulating NE level and TGAb ($r = 0.2674$, $P = 0.1531$) or TPOAb titer ($r = 0.0294$, $P = 0.8773$) (**Figure 5C** and **5D**). These data suggest that PR3 may represent a new biomarker for NETosis as well as disease activity of HT.

NETs derived from patients with HT contained autoantigens

NETs have been reported to contain auto-antigens in patients with various autoimmune diseases [25, 26]. HT is also characterized by a

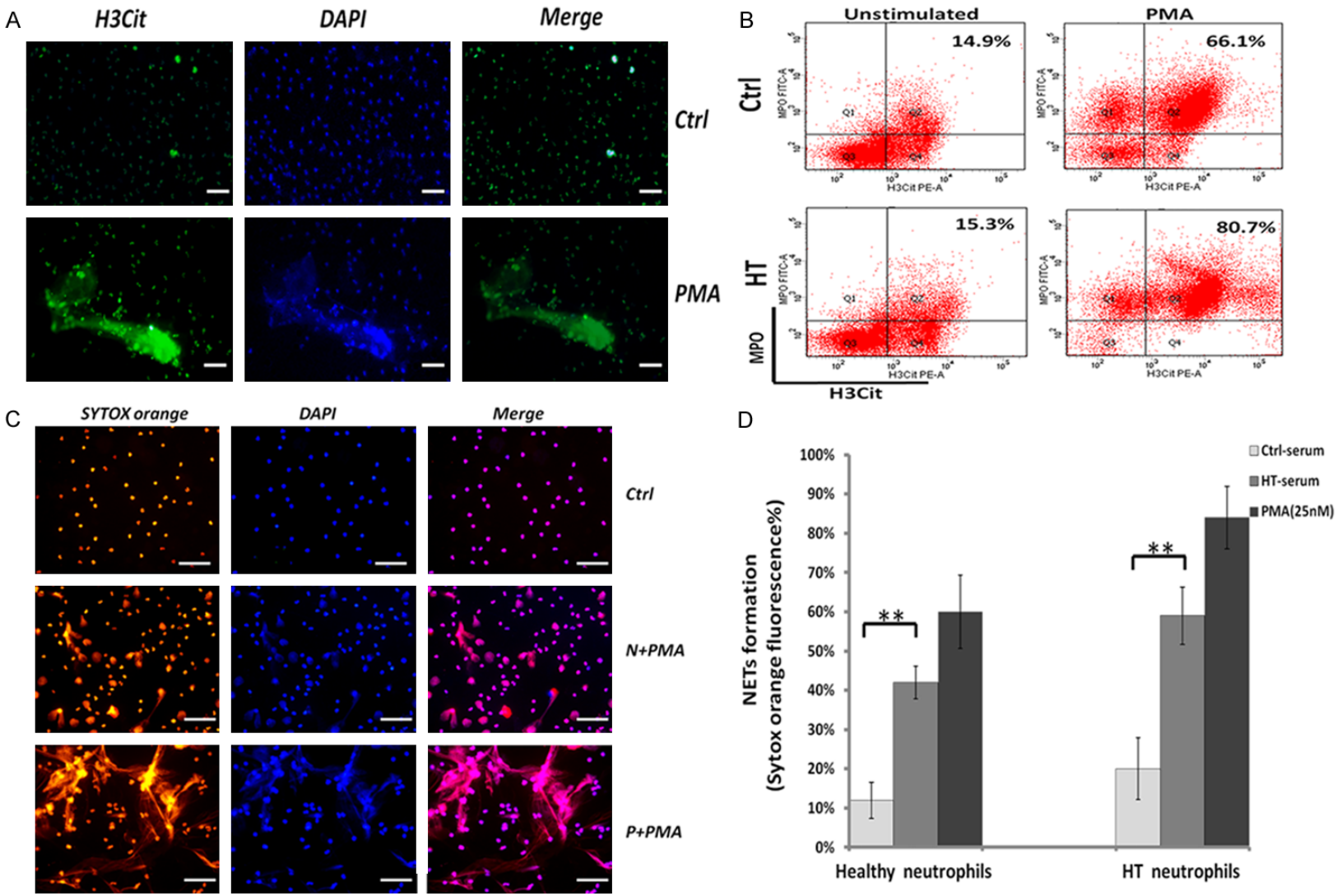

Figure 2. Neutrophils derived from patients with HT are more likely to develop NETs. (A) Immunofluorescence staining for H3Cit and DAPI to identify NETs after treatment with t 25 nM PMA for 1 h. Scale bar: 100 μm. (B) NETs were quantified by flow cytometry. Representative flow cytometry data showing the percentage of H3Cit and MPO double-positive cells, which represent NETs. (C) Representative micrographs of NETs formation induced by a 1 h stimulation with 25 nM PMA in neutrophils stained with SYTOX Orange and DAPI. Scale bar: 100 μm. Ctrl, unstimulated; N-PMA, PMA-treated neutrophils from normal individuals; P-PMA, PMA-treated neutrophils from subjects with HT. Neutrophils derived from healthy controls and patients with HT were treated with serum from healthy controls (Ctrl serum), serum from patients with HT (HT serum) or PMA (25 nM) for 1 h as positive control, and then the NETosis rate was quantified by flow cytometry of the proportion of $H3Cit^+$ and MPO^+ double-positive NETs. Representative images (A, C) or Flow data (B) were presented from 4 independent experiments, respectively. Results (D) were presented as mean ± SD of 4 independent experiments. $^{**}P < 0.01$

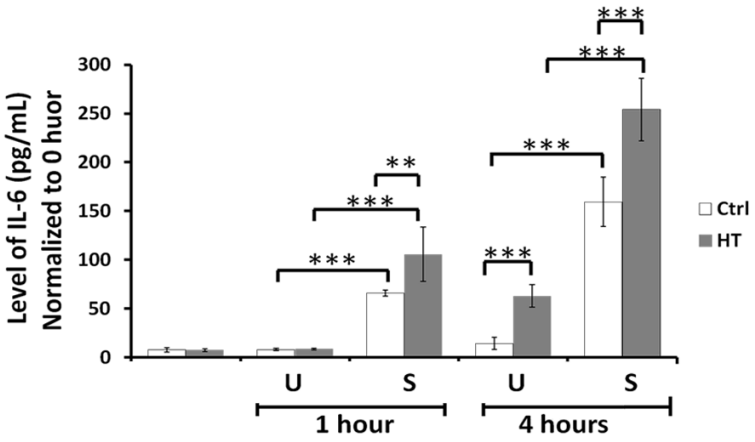

Figure 3. IL-6 concentration in supernatants of NETosis culture. Neutrophils from controls and patients with HT were cultured in the presence or absence of 25 nM PMA for 0, 1, and 4 h. U, unstimulated; S, stimulated. IL-6 levels in the supernatants were measured using ELISA. Results were presented as mean ± SD $^{**}P < 0.01$ and $^{***}P < 0.001$. Scale bar: 100 μm.

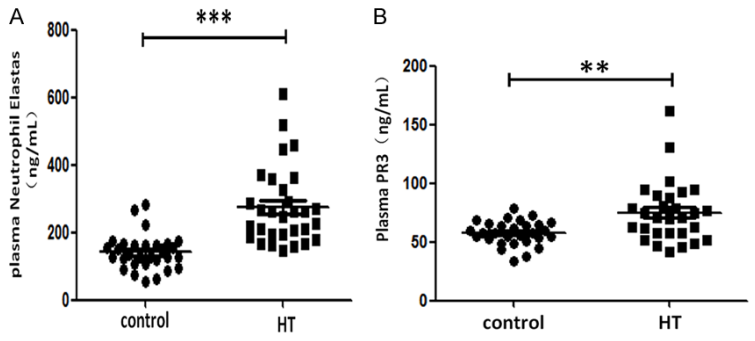

Figure 4. Levels of NET-associated components NE (A) and PR3 in plasma of HT patients. (A) NE concentration in plasma of HT patients was significantly higher than that of controls. (B) PR3 in plasma markedly increased. Data are shown as scatter plots ± errors of the means (SEM) (n = 30). $^{***}P < 0.001$, $^{**}P < 0.01$.

large number of many different types of auto-antibodies that primarily target the thyroid tissue [27]. We hypothesized that NETs from HT patients may contain autoantigens responding to auto-antibodies in the serum of the patients. We used serum from patients with HT and healthy controls as sources of primary auto-antibodies, respectively, to see whether we could identify patient's serum and NETs associated antigen-antibody reaction. Unstimulated neutrophils from different sources (patients with HT or healthy individuals) both displayed similar antigen-antibody responses, regardless of whether the primary antibodies originated from the patients or healthy controls when neutrophils were activated by PMA stimulation. As expected only serum from the patient with HT markedly reacted with the web-like fibers of NETs (**Figure 6**), indicating that patient's serum may contain autoantigens.

Discussion

Hashimoto's thyroiditis is one of the most common organ-specific autoimmune diseases, caused by inflammation of organs due to production of antibodies against self-structures and cytotoxic action of T cells. Our novel findings show that the levels of circulating NET products, NE and PR3, are significantly higher in the patients with HT than in healthy age matched-controls and that PR3 concentration in plasma increases progressively with the elevation of titers of TGAb and TPOAb.

Anti-TPO antibodies are more commonly presented in HT patients than anti-Tg antibodies, and more indicative for thyroid disease [28]. Anti-TPO antibodies are inductors of oxidative stress evidenced by decreased antioxidant potential, advanced glycosylation products and oxygen metabolites in blood [29]. Moreover, anti-TPO antibodies from patients with autoimmune thyroid disease (AITD) could fix complement resulting in thyrocytes damage [30].

NE and PR3, the two major neutrophil serine proteases, are released into the blood stream during the process of NETosis, marking NET formation. According to previous studies, NE and PR3 levels are markedly elevated in patients with type 1 diabetes mellitus (T1DM), particularly in patients who were diagnosed with the disease less than 1 year prior to the analysis, supporting an early role for neutrophil activation and augmented neutrophil serine protease activities in the pathogenesis of β-cell autoimmunity [23]. HT is also an autoimmune disease

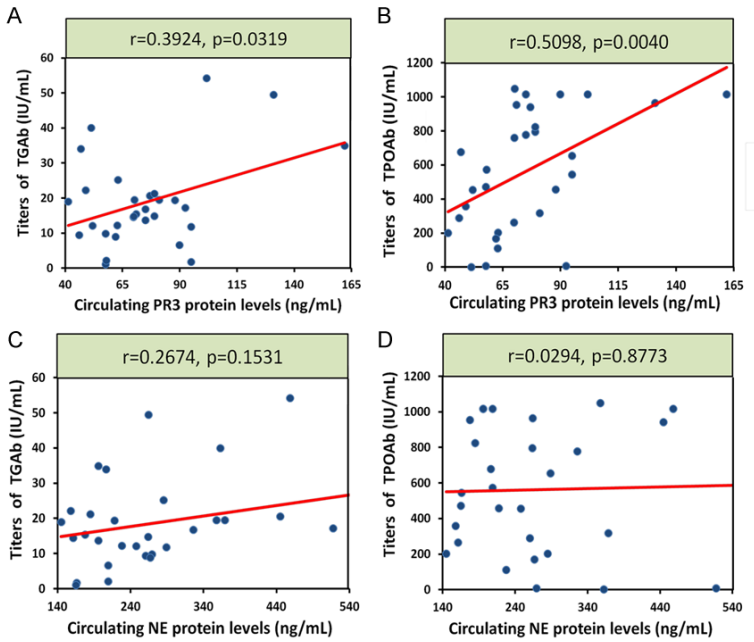

Figure 5. PR3 is positively associated with auto-antibody titers in serum. A, B. Titers of TGAb and TPOAb were significantly correlated with circulating PR3 levels, respectively, but not with NE. C and D. Linear correlation analysis between TGAb and TPOAb and either with PR3 or NE are given. Pearson's coefficient and statistical significance are indicated by r and *P* respectively.

involving a specific immune attack on the thyroid gland. TGAb and TPOAb are ubiquitous and are often detected in the plasma or sera of patients with HT. Moreover, a remarkable elevation of the titers of these antibodies (more than 3 times the normal range) serves as one of the criterion for HT diagnosis. These antibodies are capable of binding target proteins and promoting cell phagocytosis mediated by complements. These autoantibodies are also cytotoxic, for example, TPOAb interacts with complement to form complement-activated products, such as the membrane attack complexes, causing thyroid cell damage. In addition, the interaction between TPOAb and TPO inhibits TPO activity and reduces the synthesis of thyroid hormones [31]. In a mouse model of spontaneous immune thyroiditis, TGAb and TPOAb titers showed two distinct phases in thyroid autoimmunity. The former reflected the early stage of autoimmunity, whereas the latter reflected the adaptive immune response, which was a gradual escalation of the immune phase [32]. Previous study demonstrated that NETs could affect dendritic cell maturation and promote more Th2 cell polarization [33] by which autoantibody production or generation could be facilitated in HT. Together with our findings showing significantly increased NETosis and a significant positive association of NETs marker PR3 concentration with titers of TGAb and TPOAb in plasma suggest that NETosis may be in part regulating auto-antibody production involved in the pathogenesis of HT.

Cytokine are also important mediators for the HT development through direct cytotoxic activity by TNF-alpha and IFN-gamma and amplification of inflammation response by IL-6 in the gland [34]. It is known that NETs can directly prime T cell activation in a NETs/cell direct manner. In addition, NETs could also promote T cell response to specific antigens [35]. Therefore, NETs release from NETosis could play roles in the HT pathogenesis by shaping both the innate and adaptive immune response.

NETosis is induced by a variety of stimuli through several mechanisms. The compound most frequently are used to induce NETosis is PMA, a synthetic activator of the PKC family of enzymes [8, 9]. PKC activation depends on elevated cytoplasmic calcium levels and is directly responsible for activating NADPH oxidase and inducing ROS production.

T cell-mediated apoptosis of thyroid follicular cells constitutes a major thyroid damage machinery, but cytokines are also important effectors causing gland damage [30]. IL-6, is one of the key cytokines in inflammation process, and targeting on IL-6 signaling has been successfully applied in clinic to treat certain autoimmune disease and severe inflammation [36, 37]. In the current study, our findings of higher concentration of IL-6 in the supernatants of PMA stimulated-NETosis culture system observed in neutrophils derived from the HT patients suggest that IL-6 may be implicated in the pathogenesis through inducing more NETosis, trigger and amply inflammation in thyroid tissue of the gland.

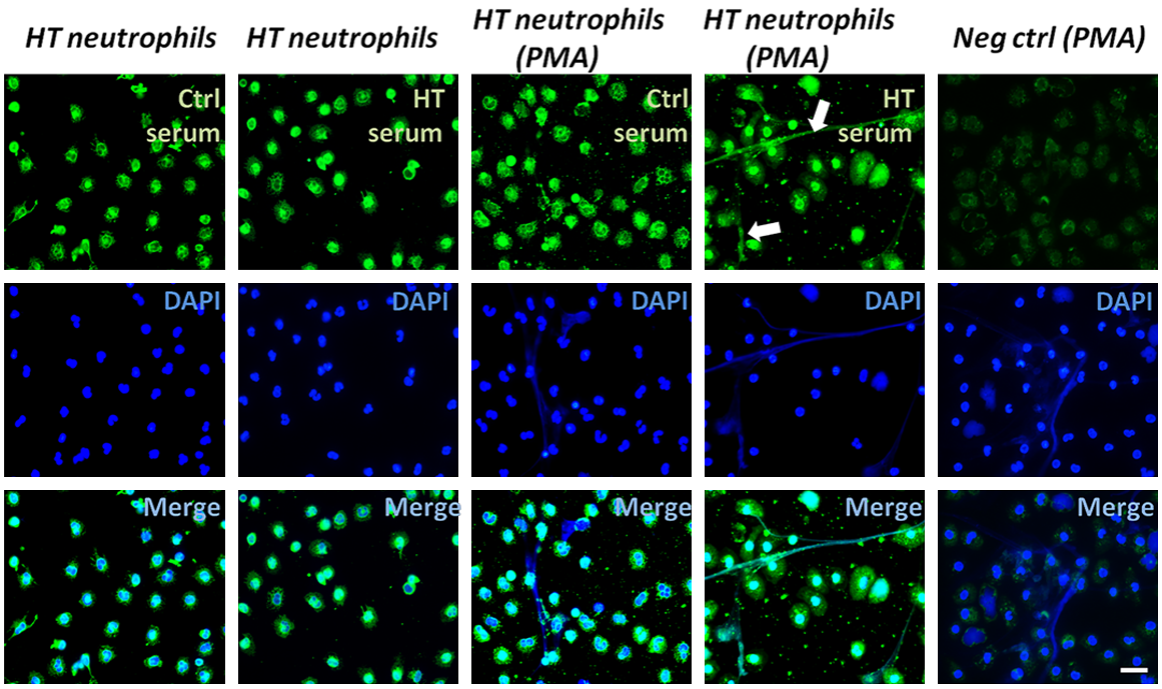

Figure 6. NETs derived from patients with HT contained autoantigens. Images of immunofluorescence staining of neutrophils derived from the peripheral blood of patients with HT following stimulation with or without PMA. Neutrophils were stimulated with 25 nM PMA for 1 h at 37°C. After fixation, the cells were incubated with serum from one control (Ctrl) or one HT patient (HT) serum, followed by incubation with secondary antibody Alexa Fluor 488-conjugated goat anti-human IgG antibody. Stimulated cells that were only incubated with the secondary antibody were used as negative controls (Neg Ctrl). After all the steps of staining, the cells were stained DAPI. White arrow indicated antigens in the NETs. Scale bar: 10 μm. The representative results from 4 independent experiments were presented.

With long DNA-protein network-like fishing nets, NETs have recently been shown to be a major source of autoantigens in the body, and catch all the possible curative and pathogenic factors in the circulation and tissues in patients with autoimmune diseases. The polypeptide antibiotic LL-37 protects nucleic acids from degradation. In patients with SLE, an autoimmune disease in which the immune system mistakenly attacks healthy tissues in many parts of the body, large quantities of immune complexes containing DNA and LL-37 are released by neutrophils, leading to massive auto-antibody production [14, 38]. In addition, ANCA induces NETosis, and PR3 or MPO antigens in NETs are detected in the blood and renal biopsy samples from majority of patients with ANCA-associated small vasculitis. The deposition of NETs in the diseased kidney and circulating levels of the MPO-DNA complex suggest that NETs trigger vasculitis in patients and promote an autoimmune response to the anti-neutrophil components [39]. In an organ-specific disease, such as T1DM, levels of the circulating NE were significantly higher in patients and non-obese diabetic (NOD) mice with autoimmune insulitis than in controls. Immunohistochemistry of islet tissues from newborn to 2-week-old NOD mice showed neutrophil invasion and NET formation [40, 41]. In line with our results demonstrating that NETs biomarkers PR3 and NE in plasma are significantly increased, PR3 concentration is positively associated with TGAb and TPOAb, and in parallel neutrophils of the patients presented more pronounced NETosis companied with higher IL-6 release and antigens exposure suggest that excess NETosis may constitute one common pathogenesis machinery in system- and organ-specific autoimmune diseases such as HT.

To our knowledge, this is the first report to link NETosis to HT pathogenesis. More studies on NETosis in HT are warranted to clarify a potential role and the underlying mechanisms for NETs in HT development.

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Disclosure of conflict of interest

None.

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