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INTERFERON-Λ1/IL-29 INHIBITS C5A-INDUCED NEUTROPHIL EXTRACELLULAR TRAPS-ASSOCIATED THROMBOTIC ANTIPHOSPHOLIPID SYNDROME

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ABSTRACT

Objective: This study aims to determine whether C5a activation in the inflammatory environment of thrombotic antiphospholipid syndrome (APS) drives polymorphonuclear neutrophils (PMNs) to release neutrophil extracellular traps (NETs), and whether interferon-λ1/interleukin 29 (IFN-λ1/IL-29) has a function of inhibiting thrombotic inflammation. Methods: The expression of NETs and autophagosome markers were assessed by confocal microscopy. Western blotting was performed to investigate the autophagy-related proteins. Flow cytometry was performed to detect the expression of NET-related markers on PMNs. The thrombin-antithrombin (TAT) complex level was determined by enzyme-linked immunosorbent assay (ELISA). Results: Serum-derived C5a activation in APS patients induced the expression of NETs in PMNs. C5a promoted autophagy and NET-dependent thrombin generation in PMNs. IL-29 reduced the production of NETs and NET-associated thrombin by inhibiting autophagy. Conclusion: IL-29 has an effect of inhibiting NET-associated thrombotic APS induced by C5a activation.

Introduction

Antiphospholipid syndrome (APS) is a kind of autoimmune disease for unknown reasons. Arterial and venous thrombosis and fetal abortion are the main clinical manifestations (1). Studies have shown that anti-B2-GPI antibody-stimulated neutrophils promote more neutrophil extracellular traps (NETs) release and thrombin generation (2). Furthermore, various prothrombin mechanisms associated with APS have been demonstrated, including enhanced blood coagulation and impaired fibrinolysis (3). Therefore, it is necessary to elucidate the prothrombin mechanism of thrombotic APS, and develop a new antithrombotic strategy accordingly. Polymorphonuclear neutrophils (PMNs) release extracellular chromatin-based structures through a process called NETosis, forming NETs, which are the main mediators and emerging therapeutic targets for thrombotic inflammation. Recent studies have shown that the DNA component of NETs can activate the intrinsic coagulation cascade, while the histones component promotes thrombin generation through platelet-dependent mechanisms (4). In antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis, NETs might promote thrombotic inflammation and a disease-associated thrombotic status (5).

The present study investigates the involvement of NETs in the development of thrombotic APS, and the potential regulatory functions of C5a activation and IFN-λ1/IL-29 in the formation of NETs, to elucidate the role of immunological mechanisms in APS thrombosis.

Literature review

Autophagy is a newly found cell self-degradation process. It is a key regulator of cell survival and death, and is crucial in cellular homeostasis. It has been shown that autophagy plays a key role in the regulation of leukocyte response and the release of NETs (6). In addition, autophagy is involved in the extracellular delivery of tissue factor (TF) in NETs, and the subsequent coagulation cascade activation (7). The process of autophagy can be observed

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through specific protein markers, such as autophagy-associated protein light chain 3 (LC3, an autophagosome marker) and p62/SQSTM1, and autophagic flux indicator (8).

It has been demonstrated that complement plays a role in the procoagulant pathway. In APS, complement activation is triggered by antiphospholipid (aPL) antibodies. It leads to the induction of TF expression, which is a key initiating component of the blood coagulation cascade and TF-dependent coagulant activity *via* C5a receptors in neutrophils (9). Furthermore, the serum levels of complement components C3a, C4a and C5a become upregulated in thrombotic APS patients and complement activation is reduced after the oral administration of anticoagulant rivaroxaban (10). In addition, when the components of fibrin clots in thrombotic APS were analyzed, it was found that the clots contained proteins other than blood coagulation components, such as proteoglycan 2 (PRG2), complement C4-C9, platelet glycoprotein, myeloperoxidase enzyme (MPO) and histones (HD), in large amounts (11). These pro-inflammatory proteins may be involved in thrombosis and affect its properties, and the presence of some NETs that form associated proteins appears to provide additional evidence for the enhanced release of NETs in thrombotic APS.

Human IFN-λs comprises of four members (IFN-λ1/IL-29, IFN-λ2/IL-28A, IFN-λ3/IL-28B, and IFN-λ4), while murine IFN-λs comprise of two members (IFN-λ2/IL-28A and IFN-λ3/IL-28B).

IFN-λ has been mainly considered as an epithelial cytokine, which restricts viral replication in epithelial cells and constitutes an added layer of protection at mucosal sites. Further studies have indicated that IFN-λs directly modulate the function of immune cells. Therefore, this may be a cytokine that directly controls the inflammatory response at mucosal sites. Increasing evidence has shown that IFN-λs are pleiotropic in immune regulation and differentiate helper T cells (12). These have the function of forming a frontier for antiviral defense without affecting host adaptability. (13) However, the role of IFN-λs in other inflammatory aspects of neutrophil function, such as NET release, has been rarely investigated.

Materials and Methods

Patients and healthy controls

A total of 13 APS patients in the active stage were enrolled from the Department of Rheumatology and Immunology of the Second Affiliated Hospital of Soochow University. According to the 2006 Sydney classification criteria, all active patients met the APS laboratory classification criteria (14). Among these patients, eight patients had primary APS and five patients had secondary APS. Peripheral blood samples from 10 healthy blood donors were used as experimental controls. The present study was conducted in accordance with the Helsinki Declaration and was approved by the Clinical Research Ethics Committee of the Second Affiliated Hospital of Soochow University. Each study participant provided written informed consent.

Isolation of PMNs

The PMNs were separated from the heparinized venous blood obtained from healthy volunteers by density gradient centrifugation using a polymorphonuclear separation reagent (Biovision), according to the manufacturer's instructions. Then, the erythrocyte lysis was performed using an erythrocyte lysis buffer (Gibco). Giemsa staining was used to assess the neutrophil purity, which was >98%. The trypan blue staining demonstrated that neutrophil viability was >95%. In addition, the May-Grünwald-Giemsa (MGG) staining revealed no adhesion of platelets to neutrophils, which suggests that the contamination of platelets was <1%. Next, the PMNs were washed in Hanks balanced salt solution without $Ca^{2+}$and Mg^{2+}, and cells were adjusted to 2×10^6/mL in RPMI 1640 medium.

Western blotting

The PMNs were lysed by resuspending in RIPA buffer (50 mM of Tris pH 7.4, 150 mM of NaCl, 2 mM of EDTA, 1% Triton-X, and Roche protease inhibitor) for one hour on ice. After removing the debris by centrifugation at 14,000 g for 10 minutes at 4°C, the protein concentration was measured using a BCA protein assay kit (Pierce). The protein sample (which contained approximately 80 µg of protein per lane) was diluted at 1:1 in 2× SDS loading buffer, and heated at 100°C for five minutes. Then, the sample was separated by 12% SDS-PAGE under denaturing conditions, and transferred onto a 0.45-µm nitrocellulose membrane. Afterwards, the membrane was blocked in TBST solution (Biyuntian), containing 3% skim milk, for two hours, and incubated with the following primary antibodies at 4°C overnight: mouse anti-human TF mAb (1:1,000 dilution, Sekisui diagnostics), rabbit anti-human LC3B polyclonal antibody (1:1,000 dilution, Sigma-Aldrich), mouse anti-human p62/SQSTM1 polyclonal antibody (1:500 dilution, Santa Cruz), and rabbit anti-human GAPDH polyclonal antibody (1:4,000 dilution, Trevigen). After thorough washing in TBST, HRP-conjugated donkey anti-mouse (1:4,000 dilution, Santa Cruz) or goat anti-rabbit polyclonal antibody (1:4,000 dilution, R&D) was incubated with the membrane for 1.5

hours at room temperature. The ECL detection system (Supersignal West Pico Chemiluminescent Substrate, Pierce) was used to detect the immunoreactive proteins, and these were exposed to the X-ray film. A gel imaging analyzer was used for the optical density scanning of the bands on the film.

Stimulation and inhibition

The PMNs in RPMI medium were seeded at a concentration of 1.5×10^6 cells/mL in a 6-well culture plate placed with polylysine-treated sterile slides. The serum from healthy controls or APS patients was added into the plates to a final concentration of 6%, which is the optimal concentration to stimulate PMNs and avoid NET degradation. This was incubated for three hours at 37°C with 5% CO_2 (15). To investigate the autophagy-dependent NET release, the PMNs were pretreated with autophagy inhibitor 3-methyladenine (3-MA) (5 mM, Calbiochem) for 30 minutes. Then, various autophagy regulators were added and incubated for one hour, followed by the detection of autophagy-related proteins LC3b and p62/SQSTM1 by immunoblotting and immunofluorescence. Phorbol-12-myristic acid-13-acetate (PMA 50 nM, Sigma) was used as a positive control for the NET release assay. The NADPH oxidase inhibitor diphenylphosphonium iodide (DPI, 10 μM; Tocris Bioscience) was used as the negative control. These controls were pre-incubated with PMNs prior to stimulation with various serum. To determine whether IL-29 is a potentially potent inhibitor of NET release, recombinant IL-29 (50 ng/ml, R&D) was pretreated with neutrophils for 30 minutes. To explore the possible role of C5a in serum of APS patients, the PMNs were first treated with the C5a receptor blocker anti-CD88 blocking antibody (1:50 dilution, Abcam) for 30 minutes on ice. Then, to confirm the release of NETs on C5a-activated PMNs at various concentrations, the PMNs were treated with the recombinant C5a (BioVision) for 15 minutes. Phosphate buffered saline (PBS) was used as the negative control. All reagents used in the present study were free of endotoxin.

Immunofluorescence staining and quantification of NETs

Fixation and permeabilization: The cell slides were removed from 6-well plates and fixed overnight at 4°C with 4% paraformaldehyde (PFA). Then, the slides were washed with PBS, followed by permeabilization using PBS containing 0.5% Triton X-100.

Sample staining: The PMNs were stained with mouse anti-MPO monoclonal antibody (1:200 dilution, Abcam), mouse anti-TF monoclonal antibody (1:200 dilution, Sekisui diagnostics), and rabbit anti-LC3B polyclonal antibody (Sigma-Aldrich), respectively. An anti-CD19 monoclonal antibody with the IgG isotype (1:200 dilution, BD Biosciences) was used as the isotype control. A polyclonal goat anti-mouse Alexa Fluor 488 antibody (1:1,000, Abcam) or polyclonal goat anti-rabbit Alexa Fluor 647 antibody (1:1,000, Abcam) was used as the secondary antibody. Then, the DNA was counterstained by 4',6-diamino-2-phenylhydrazine (DAPI, 1:2,000; Sigma-Aldrich) and imaged by laser confocal microscopy. The percentage of NET released cells was determined by detecting 200 cells during the double-blind experiments.

TAT complex ELISA assay

In brief, 1.5×10^6 *in vitro* stimulated PMNs in RPMI 1640 medium were seeded in 6-well plates (Corning) and incubated at 37°C with 5% CO_2 for three hours. Then, each well was washed twice with RPMI 1640 medium. To separate the NET structures, 1 ml of RPMI was added to each well and collected by centrifugation at 300 g for five minutes after vigorous stirring. The collected NET supernatant was mixed with healthy human plasma at a ratio of 1:4, and incubated at 37°C for 30 minutes. The TAT complex levels were detected using a TAT ELISA kit (Assaypro), with a minimum detectable dose of 300 pg/ml.

Flow cytometry

The PMNs were isolated from heparinized venous blood and stimulated according to the experimental requirements. To quantitate the NETs by flow cytometry, 2×10^6 of the stimulated PMNs were incubated in PBS containing 3% BSA to block the non-specific binding of antibodies. Then, 5 μg/ml of anti-human MPO antibody (Abcam) was added, and the samples were incubated at room temperature in the dark for 30 minutes. Afterward, the cells were washed with PBS and resuspended in PBS containing 3% BSA. Next, 4 μg/ml of PE-labeled anti-mouse IgG antibody (Biolegend) was added, and the samples were incubated at room temperature in the dark for 30 minutes. After washing with PBS, the cells were stained with a plasma membrane-impermeable DNA-binding dye, SYTOX Green (Invitrogen). The intracellular TF expression in PMNs was detected using a FITC-labeled mouse anti-human TF antibody (Sekisui diagnostics) after fixation and permeabilization using a FIX and PERM kit (Caltag Laboratories). FITC-labeled mouse IgG1 (BD Biosciences) was used as the isotype control. All the above assays were detected on a FACScan cytometer with the Cell Quest PRO software (BD Biosciences). In the flow cytometry, the PMNs were identified through the properties of forwarding and side scatter.

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Circulating DNA quantification

The above stimulating solutions were diluted at 1:10 (v:v) in PBS. The diluted solutions were mixed with 50 µl of propidium iodide (1 µg/ml), and used to label the circulating DNA. Fluorescence was detected using a spectrofluorophotometer (Thermo). The excitation and emission wavelengths were 488 nm and 620 nm, respectively. The concentration of the circulating DNA was deduced from the DNA standard curve (the dynamic range was 0.1-8.0 µg/ml). Autofluorescence was measured by PBS containing 1 µg/ml of propidium iodide.

Statistical analysis

The test samples were analyzed for normal distribution. The comparison among multiple groups was performed using one-way analysis of variance (Scheffe test was used for post-matching comparison), while the Mann–Whitney test was used for comparisons between two groups. All statistical analyses were performed using SPSS 13.0 software. The results were reported as mean ± standard deviation (x ± SD). A *P*-value of <0.05 was considered statistically significant.

Results

APS patient serum induced the release of TF expressing NETs by PMNs via C5a

To investigate serum-derived C5a activation on the formation of NETs in the inflammatory environment in APS, PMNs from healthy donors were stimulated with APL-positive patient serum. Comparison of healthy PMNs incubated with healthy donor serum, confocal microscopy and flow cytometry showed increased TF expressing NETs release cells (Fig. 1a, 1b, 1c).

We found that the circulating DNA levels were significantly increased when healthy PMNs were stimulated with APS patient serum (Figure 1d). Further, we evaluated the role of C5a. The C5a receptor (CD88) function on healthy PMNs was blocking with an anti-CD88 antibody by incubation on ice for 30 minutes. Then the samples were incubated with the serum from APS patients. It was shown that blocking the C5a receptor resulted in a significant decrease in the expression of NETs (Fig. 1a, 1b,1c).

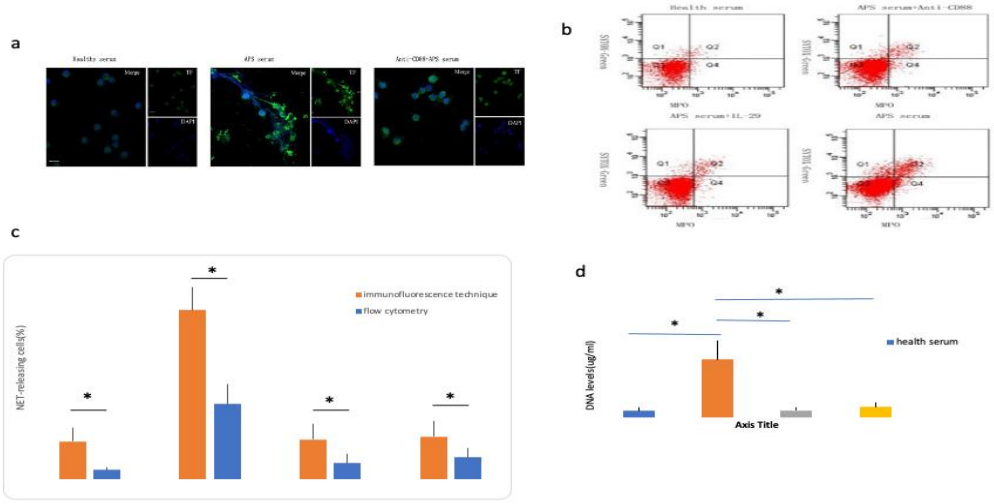

Figure 1 C5a-activated APS patient serum induces TF-expressed NETs release by PMNs from healthy donors. (a) In the presence and absence of anti-CD88 blocking antibody, healthy serum or APS patient serum were incubated with and normal PMNs, respectively, for 3 h. Confocal microscopy was used to assess the extracellular NETs structure using co-staining of TF (green) and DAPI (blue). Original magnification: 600X. Ruler:5 µm. (b) NETS was quantitively determined by flow cytometry through double staining of SYTOX green and MPO on PMNs. (c) The percentage of NETS-released cells. (d) The circulating DNA levels in the simulation solution. Data are shown as one of six independent experiments and are expressed as mean ± standard deviation (*P<0.05).

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IL-29 inhibits the formation of TF-expressed NETs by PMNs induced by APS patient serum-derived C5a.

Since C5a activation induces intracellular accumulation of TF and release of NETs by neutrophils in the inflammatory environment in APS, Given the potent anti-inflammatory effects of IL-29 on PMNs (17), we sought to investigate whether this cytokine affects the release of NETs. Healthy PMNs were pretreated with IL-29 and then stimulated with APS patient serum. We observed a significant reduction in intracellular TF, as shown by immunofluorescence (Fig. 2a), immunoblotting (Fig. 2b), and flow cytometry (Figure 2c). The pretreatment of IL-29 abolished the formation of TF-expressed NETs induced by APS serum-stimulated C5a activation, assessed by immunofluorescence (Fig. 2a, 1c), flow cytometry (1b) and circulating DNA quantitation (Figure 1d).

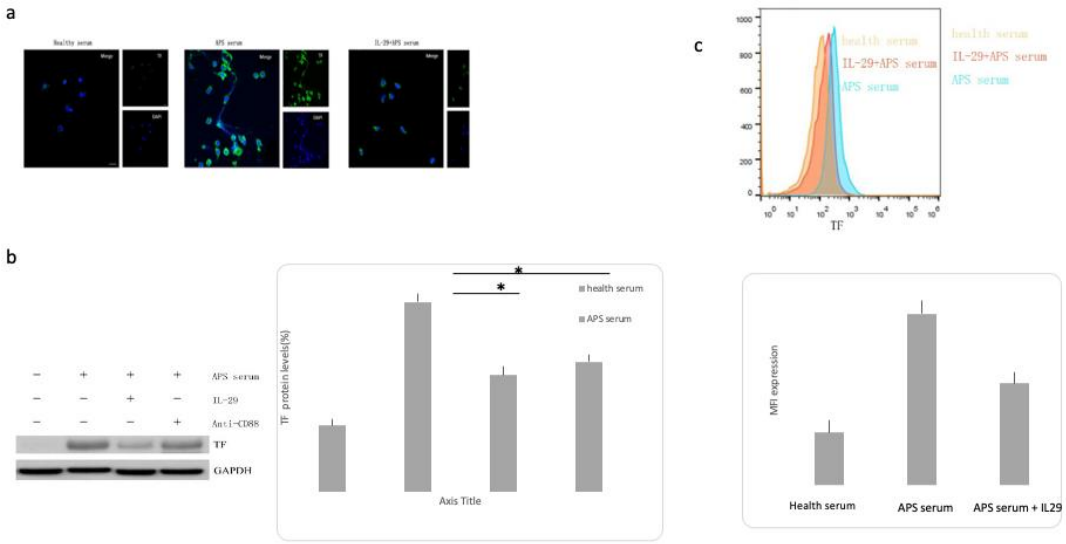

Figure 2 IL-29 regulates the release of TF-expressed NETs induced by C5a in thrombotic APS. (a) Formation of NETs in PMNs treated with APS patient serum in the presence or absence of IL-29 (50 ng/ml) (Confocal microscopy, green: TF; blue: DAPI/DNA; Original magnification: 600×, Ruler: 5□m). (b) Immunoblotting was used to detect the expression of TF. (c) Flow cytometry was used to detect TF expression in APS serum-treated PMNs. Data are shown as one of six independent experiments and are expressed as mean ± standard deviation (*P < 0.05).

IL-29 and C5a regulate PMNs release NETs by autophagy

How C5a and IL-29 governs the formation of NETs and the interaction mechanism between them are still unclear. Autophagy was reported to be involved in the release of NETs (18). So, we are going to investigate whether autophagy plays a role in the NETosis regulated by C5a and IL-29.

To further explore the role of autophagy, we used immunofluorescence assay to detect the expression of LC3B and found that autophagy was increased in the healthy PMNs stimulated by APS patient serum. Autophagy was found to decrease when the C5a receptors on PMNs were blocked with anti-CD88 antibody as evidenced by immunoblotting for LC3B and p62/SQSTM1.

These results strengthen our hypothesis that autophagy participates in the C5a-mediated NETs release. Using 50 ng/ml IL-29 as the threshold dose for autophagy inhibition, we observed significant

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attenuation of autophagy-driven NETs and autophagy-related proteins in APS patient serum C5a-induced healthy PMNs (Fig. 3a,3b). Inhibition of IL-29 was not reversed by continuous C5a stimulation, even at higher concentrations (Fig. 3c).

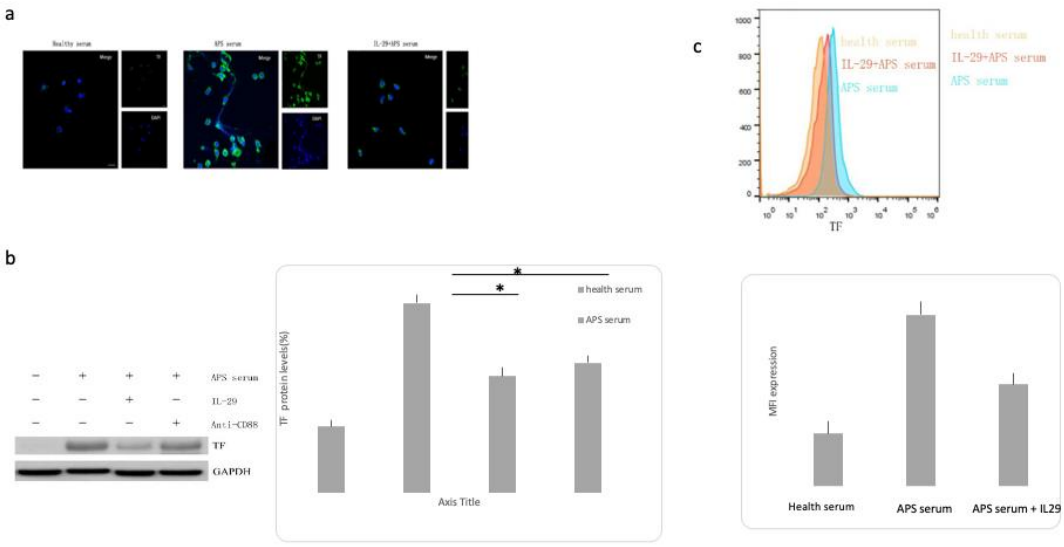

Figure 3. The effects of C5a and IL-29on the level of autophagy in PMNs and NETs release from PMNs. (a) Autophagy-induced NETs release assessed by LC3B immunostaining in APS serum-treated PMNs in the presence or absence of IL-29 (50 ng/ml) or anti-CD88 antibody (Confocal microscopy, green: TF; red: LC3B; blue: DAPI/DNA; Original magnification: 600 ×, Ruler: 5 um). (b) Immunoblotting of LC3B and p62/SQSTM1 in normal PMNs treated with APS serum in the presence or absence of IL-29 (50 ng/ml). (c) Circulating DNA levels in supernatants of recombinant C5a -treated PMNs in the presence or absence of IL-29 (50 ng/ml). Data are shown as one of six independent experiments (*P<0.05, N.S. =non-significant). Taken together, these data suggest that the opposite effects of IL-29 and C5a onthe formation and/or activities of NETs are caused by the opposite effects of them on autophagy-associated proteins, which regulate the induction of autophagy on NETs accordingly.

IL-29 inhibits the production of NETs-associated thrombin in thrombotic APS

We found that APS patient serum induced the generation of thrombin and complement C5a in the serum played an important role. APS patient serum was used to stimulate the release of NETs from healthy PMNs and then the thePMNs-derived NETs supernatant was mixed with the plasma from healthy controls. It was observed that the generation of thrombin increased as evidenced by the elevated level of TAT complex in the mixture and such effect was weaken when the mixture was treated with an anti-C5a receptor (CD88) antibody (Figure 4a). These results indicated that complement C5a was one of the components in APS patient serum inducing the generation of thrombin through PMNs-released NETs.

We also confirmed that the generation of thrombin-induced by APS patient serum was NETs dependent. DNase I can digest and remove the chromatin scaffolds of NETs, which leads to the degradation of NETs. When DNase I was added

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into the above mixture, the TAT complex level in the mixture was remarkably reduced (Figure 4a). It suggested that PMNs-released NETs induced by APS patient serum were necessary for the generation of thrombin.

Further, we found a critical role of TF in the thrombin generation induced by PMNs-derived NETs. 10 μg/ml mouse anti-TF monoclonal antibody (IgG) was used to block the function of TF expressed on NETs by incubating with the above mixture for 30 min at room temperature. We observed significant declination of the level of TAT complex in the mix (Figure 4a).It indicated that the TF expressed on NETs mainly mediated the thrombin generation induced by PMNs derived NETs.

Asit has already demonstrated that IL-29 could be a regulator of TF expression on PMNs and an inhibitor of NETs. We tried to investigate the effects of IL-29 on the thrombin generation mediated by PMNs derived NETs. Our results showed that IL-29 treatment down-regulated the thrombin level induced by PMNs derived NETs as assessed by TAT complex ELISA assay in the above mixture (Figure 4a).

In summary, when stimulated with APS serum, PMNs were induced by the complement C5a in the serum to produce TF and release NETs, leading to the generation of thrombin. This process can be inhibited by C5a receptor blockers, anti-TF antibody, DNase I, or IL-29. It suggests that a mechanism leading to hypercoagulability which was not clearly elucidated previously exists in thrombotic APS and the active molecules such as IL-29 process a novel function to inhibit thrombotic inflammation against NETs.

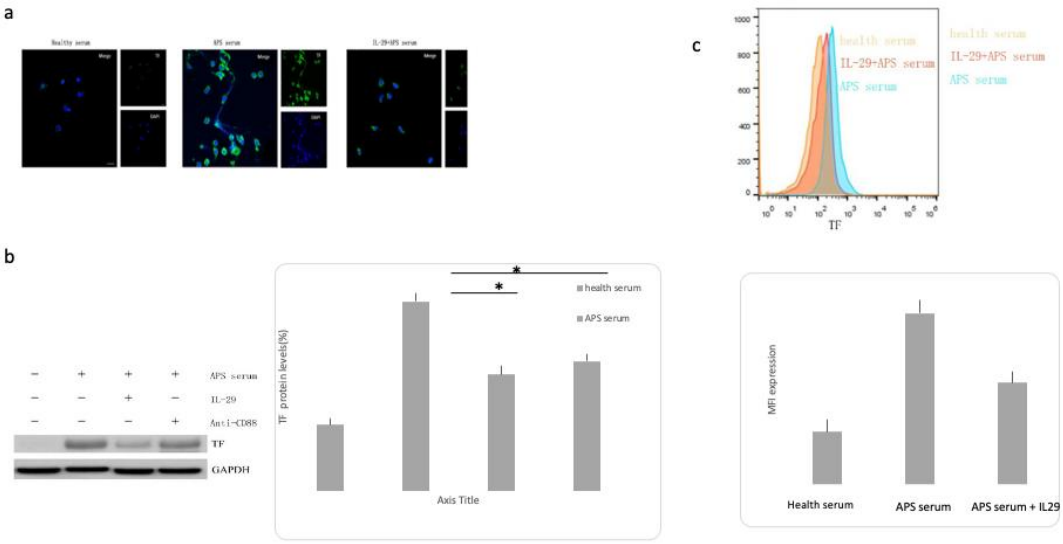

Figure 4 IL-29 inhibits the effect of APS-related stimulation on NETs-dependent thrombin generation. The level of TAT complex was determined by the NETs supernatant formed 3 hours after PMNs were incubated with serum from healthy donors and APS patients and/or IL-29, anti-CD88 blocking antibody, anti-TF and DNase I. Data are shown as one of six independent experiments and are expressed as mean ± standard deviation (*P < 0.05).

Discussion

In recent years, the potential interaction between immune regulation, inflammatory response, and thrombosis has attracted increasing research interest. NETosis is a newly recognized mode of neutrophil death and plays an important role in the innate immunity of capturing and killing microorganisms. To date, although C5a has been

extensively studied as a pro-inflammatory factor and a procoagulant-responsive molecule, its role as an inducer of NET production has been poorly reported. The present study revealed that the treatment of PMNs obtained from healthy blood donors with C5a-activated APS patient serum resulted in the elevation of TF-expressed NETs and TAT complex levels, and the C5a receptor antagonist could abolish this. This suggests that in thrombotic APS, the pro-inflammatory factor C5a activates the coagulation system by TF-expressed NETs. Anti-TF mAb can reduce the level of TAT complexes. It was elucidated through antibody neutralization studies that TF is involved in Net-dependent thrombin generation, indicating that TF binding on NETs can also activate the blood coagulation system. The decreased level of TAT complexes treated with DNase I further confirmed that the thrombin generation process was dependent on NETs. The critical role of C5a in the formation of thrombotic NETs makes this molecule a promising therapeutic target. Hence, it may be feasible to treat complement-associated thrombotic diseases by reducing the production of NETs through complement inhibitors.

Recently, some thrombotherapeutic strategies against NETs have been successfully applied in mouse models (19-21). However, these NET inhibitors have certain limitations. Considering the established antithrombotic strategy defect, such as blood bleeding risk (22), searching for new and safe NET inhibitors is urgently needed. Previous studies have shown that IL-29 exerts anti-inflammatory effects by inhibiting neutrophil infiltration and IL-1β production (17). Most importantly, IL-29 does not appear to affect normal hemostatic function (23). The present study found that IL-29 reduces thrombotic inflammation by regulating the neutrophil signaling pathway. It was observed that IL-29 blocked the release of TF-expressed NETs from PMNs trigged by C5a in human thrombotic APS *in vitro*.

The following were found in the literature: (1) The complement C5a/C5aR pathway promotes the production of endoplasmic reticulum stress (ER), which is an inducing factor of autophagy (24). (2) Reactive oxygen species (ROS) is an important activator of autophagy (25). *In vitro* and *in vivo* experiments have shown that complement C5a can stimulate the release of a large amount of ROS (26). (3) Through its receptor, complement C5a enhances the production of TGF-β1. TGF-β1 and autophagy can promote each other (27). (4) Bcl-2 inhibits autophagy by reducing the production of autophagy-related protein Beclin-1 (28), while the complement C5a/C5aR pathway attenuates the effect of Bcl-2. The above evidence suggests that complement C5a may be positively correlated to the level of autophagy. This suggestion was confirmed through the *in vitro* experiments, in which healthy PMNs were stimulated with thrombotic APS patient serum, and immunofluorescence and immunoblotting were used to detect the level of autophagy.

Since autophagy is a key step in the release of NETs (29), and IL-29 attenuates the formation of NETs, the investigators attempted to determine whether there was any relationship between IL-29 and autophagy. The results revealed that IL-29 inhibits the expression of autophagy-associated protein LC3B on C5a-induced PMNs and upregulates autophagy-degrading protein p62/SQSTM1. That is, IL-29 has the function of inhibiting autophagy in thrombotic APS.

Study implications

We demonstrated that in the inflammatory microenvironment of thrombotic APS, complement C5a induces PMNs to express NETs, which are biological activities, and this process is mediated by autophagy. IL-29 attenuates the formation of NETs by inhibiting autophagy-associated proteins and subsequently reduces the generation of NET-dependent thrombin to achieve antithrombotic effects. IL-29 and C5a have opposite effects on the release of TF-expressed NETs, and on the formation of TAT complexes by affecting autophagy. In addition, the effect of C5a on autophagy suggests that the formation of NETs may be regulated by targeting C5a. IL-29 is an immunomodulatory cytokine with anticoagulant activity and has high potential in antithrombotic therapy by inhibiting the formation of NETs. This can be used at lower doses, and produce better antithrombotic effects with less bleeding. As a pleiotropic cytokine, IL-29 is of great value for exploring the biological activities of other stimulating immune cells.

Limitation and Future perspective

There are several limitations to the current study. The current study involves data from a small population of the patient. Further studies are required to confirm the effect of IL-29 in a larger population. Moreover, a multi-center study will further benefit this research direction. Furthermore, it remains to be studies whether IL-29 has an immunogenic effect and alters the patient's total blood count and serum chemistry.

Conflict of interest

None

Funding

None

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