Exaggerated neutrophil extracellular trap formation in Kawasaki disease: a key phenomenon behind the outbreak in western countries?

We read with great interest the article by Pouletty et al recently published in your journal. The authors described a series of 16 cases with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the Paris area. While the affected children exhibited, in a complete or incomplete form, clinical features of Kawasaki disease (KD), they also presented several features distinct from KD, such as an older age at onset and a higher frequency of myocarditis and/or pericarditis, and of resistance to first treatment with intravenous immunoglobulin (IVIG). Clusters of similar cases have been identified in the USA and other European countries since April 2020. However, it is still a matter of debate whether PIMS-TS and KD share aetiology and/or pathophysiology, or represent two distinct clinical entities. Herein, we explore the cause behind its outbreak in relation to neutrophil extracellular traps (NETs), a novel killing mechanism of neutrophils.

KD is a multisystem vasculitis that primarily affects coronary arteries of young children, especially in Japan. Although

Figure 1  Exaggerated NET formation in patients with KD and model mice. (A, B) The effect of human KD serum on NET formation. Human neutrophils were incubated with the serum from KD cases with serious (A) or mild (B) illness at acute (left panel) or convalescent (right panel) phase for 3 hours. NET formation was visualised using laser scanning fluorescence confocal microscopy. Representative micrographs are shown. Scale bars represent 100 µm. (C) H&E staining of longitudinal sections of hearts from CAWS-treated (upper panel) and control (lower panel) mice. Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Scale bars represent 1 mm. Arrow indicates panvasculitis at the aortic roots of CAWS-treated mice. (D) Immunoperoxidase staining with anti-Ly-6B.2 (neutrophil) (left panels), anti-F4/80 (macrophage) (middle panels) and CD3 (T-cell) (right panels) in CAWS-treated mice. The lower panels represent high-power views of the boxed areas in the corresponding upper panels. Ao, aorta. Scale bars represent 500 µm (upper panels) and 50 µm (lower panels). (E–G) Immunofluorescence staining with anti-PAD4 (green) and anti-Ly-6B.2 (neutrophil) (red) in CAWS-treated (E, F) and control (G) mice. Nuclei were counterstained with DAPI (blue). Photographs in (F) are high-power views of the boxed areas in (E). Scale bars represent 50 µm (E, G) and 10 µm (F).
the aetiology of KD remains unclear, it may be triggered by infectious agents, leading to exaggerated activation of immune systems in genetically susceptible children. We first investigated whether KD patients’ serum stimulates NET formation in human neutrophils. NETs are extracellular structures primarily composed of DNA fibres, histones, and antimicrobial granule proteins such as neutrophil elastase and myeloperoxidase. Although NETs can fight diseases, excessive NET formation is associated with the pathogenesis of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, as well as atherosclerosis and thrombosis. The study was approved by the Institutional Review Board at Kyoto University Hospital, and written informed consent was obtained from their parents. Nine cases were analysed at the acute phase before IVIG treatment and convalescent phase 5–10 days after treatment. Serious cases were determined by the risk score proposed by Kobayashi et al, and given corticosteroids in addition to IVIG. In all cases, healthy neutrophils stimulated with KD sera in the acute phase produced NETs. Serum from serious cases (figure 1A) stimulated formation of spider-like NETs to a greater extent than milder cases (figure 1B). Serum in the convalescent phase failed to stimulate NET formation (figure 1A and B). NETs were not induced by serum from patients with infectious diseases such as upper respiratory infection or gastroenteritis (data not shown).

The role of NETs in the pathogenesis of KD was explored using a mouse model of vasculitis that mimics that of human KD, elicited by Candida albicans water-soluble fraction (CAWS). In CAWS-treated mice, a large number of neutrophils infiltrated the aortic root and coronary artery, leading to severe pannascularitis, consistent with KD autopsy findings (figure 1C and D). However, macrophages and/or T cells were scarcely detected (figure 1D). Next, we examined whether infiltrative neutrophils expressed peptidylarginine deiminase 4 (PAD4). NET formation requires conversion of histone arginine residues to citrulline and vice versa. Excessive NET formation is associated with endothelial injuries, vascular inflammation, and cardiovascular symptoms, such as hypertension and thromboembolism. Zuo et al reported high levels of NETs in patients with COVID-19. NET formation induces endothelial injury and vice versa. Excessive NET formation is associated with pathogenesis of thrombosis. Therefore, NETs may play a crucial role in development of cardiovascular defects in patients with COVID-19. This study demonstrated that NET formation is significantly increased in patients with KD, irrespective of pathogen, and mice model. Taken together, we speculate that KD and Kawasaki-like PIMS-TS share the pathophysiology, thus SARS-CoV-2 infection may trigger the development of Kawasaki-like symptoms at least in part via exaggerated NET formation in relatively non-susceptible children in western countries. Kawasaki-like PIMS-TS may represent a more severe form of KD. Findings may provide insight into development of therapeutic strategies to treat SARS-CoV-2-induced Kawasaki-like PIMS-TS.

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