Autophagy inhibition in adult-onset Still's disease: still more space for hydroxychloroquine?

Sirs,

Adult-onset Still's disease (AOSD) is an interleukin 1β (IL-1β)-mediated autoinflammatory disorder (1). Neutrophils are a significant source of bioactive IL-1β which externalise it on Neutrophil Extracellular Traps (NETs), extracellular chromatin fibres decorated with various neutrophil-derived proteins in an autophagy-dependent process (2, 3). In the following AOSD case, we provide initial proof of concept that administration of hydroxychloroquine (HCQ) is associated with inhibition of autophagy-mediated NET release preventing disease relapses and reducing the needs for glucocorticoids and IL-1 inhibition.

A 66-year-old woman with a history of AOSD in remission for several years presented with a flare of fever, rash and arthritis of the hands. Initially, she responded favourably to methylprednisolone, but, as the fever relapsed with methylprednisolone tapering, anakinra was added with a quick resolution of fever. However, due to injection site reactions caused by anakinra and after obtaining her consent and authorities approval, she was switched to canakinumab 150 mg/8 weeks. Subsequently, the patient improved further allowing for complete glucocorticoid withdrawal. Gradually, though, the symptoms started to recur shortly before each canakinumab injection requiring re-introduction of glucocorticoids. As by that time we had no approval to shorten the canakinumab dose intervals, the patient was switched to tocilizumab 8 mg/kg/4 weeks. With tocilizumab, however, the patient's symptoms persisted and the need for glucocorticoids grew even further (Fig. 1A).

We have previously shown that during attacks of familial Mediterranean fever and AOSD, neutrophils spontaneously release NETs decorated with bioactive (mature) IL-1β (2, 3). We, therefore, examined our patient's neutrophils during a disease relapse (Fig. 1A, week 106) and found high rates of NET release (Fig. 1B, C) and abundance of IL-1β on NETs (Fig. 1B, D), confirming our previous data (3). Furthermore, NET release was associated with increased levels of neutrophil autophagy (Fig. 1E, F).

Since autophagy induction is required for NET generation (2-4), we assumed that the inhibition of autophagy may prevent NET release and, therefore, externalisation of active IL-1β. HCQ is an autophagy inhibitor acting through entering lysosomes and raising their pH probably impairing both the autophagosome-lysosome fusion and the degradation of the autophagosome contents (5). It has been successfully used to treat AOSD in combination with glucocorticoids and/or other disease-modifying antirheumatic drugs (6). We, thus, prescribed HCQ 200 mg bid with a short initial boost of anakinra (Fig. 1A, week 106). Indeed, over the following 12 months the patient remained afebrile, the inflammatory markers normalised and the need for glucocorticoids was minimal (Fig. 1A). Moreover, analysis of peripheral blood after 6 months of treatment with HCQ (week 130), showed a significant reduction of neutrophil autophagy levels, NET release and NET-expressed IL-1β to levels similar to controls, in line with the clinical and laboratory observations of low disease activity (Fig. 1B-F).

HCQ by deactivating NETosis, apart from IL-1β, might also inhibit the activity of other cytokines of the IL-1 family, such as IL-18 and IL-36 (7), which after their potential expression on NETs could be involved in AOSD pathogenesis. HCQ could also indirectly affect the production of IL-1β by

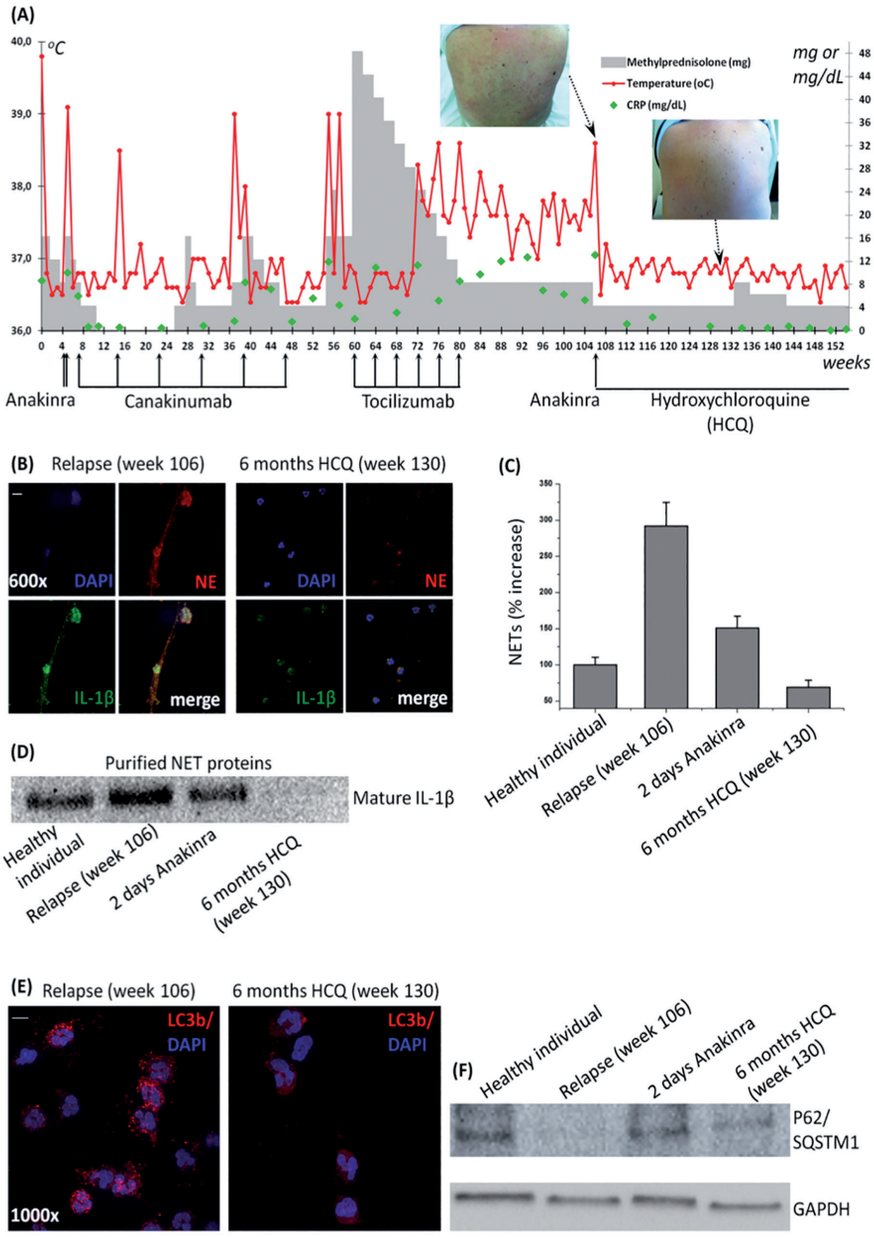

Fig. 1. **(A)** Timeline of the patient's course showing body temperature, C-reactive protein values and therapeutic approach. Photos show active Still's rash before HCQ initiation (left) and rash remission during HCQ treatment (right). **(B)** NET-release is decreased after HCQ treatment as assessed by immunofluorescence and **(C)** MPO-DNA complex ELISA (means of 3 different samples at the same time point). **(D)** HCQ treatment reduces the amount of mature IL-1β in NETs as assessed by immunoblotting. **(E)** Reduction of neutrophil autophagy levels after HCQ treatment as shown by immunofluorescence staining of LC3b, and **(F)** consumption of p62/SQSTM1 in immunoblotting of neutrophil lysates. Bar in panels B, E: 0.3 μm. DAPI: 4',6-diamidino-2-phenylindole, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, LC3b: Microtubule-Associated Protein 1 Light Chain Beta, MPO: Myeloperoxidase, NE: Neutrophil Elastase, SQSTM1: Sequestosome 1.

monocytes given that IL-1β bearing NETs are implicated in the production of mature IL-1β by peripheral blood mononuclear cells (2, 3).

In conclusion, we suggest a plausible mechanism that may explain the benefits of HCQ in AOSD. As autophagy is involved in NET formation, inhibition of autophagy with HCQ reduces the delivery of IL-1β by NETs, promising a decrease in the dependency on glucocorticoids and/or IL-1β inhibitors. Further confirmation of our observations in larger studies, may change the place of HCQ, a low-toxicity and low-cost drug, in the treatment of this disease as well as other diseases with crucial involvement of neutrophils/NETs in their pathogenesis (8, 9).

Acknowledgements

Immunofluorescence experiments were performed at the CIBIT facility of Democritus University of Thrace, Alexandroupolis, Greece.

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Funding: this study was funded by the Scientific Committee of Democritus University of Thrace grant no. 80895.

Competing interests: none declared.

Received on April 5, 2017; accepted in revised form on July 11, 2017.

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