Autoantibodies for several antigens in neutrophil cytoplasm other than PR3 and MPO also promote release of NETs from neutrophils

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Introduction.– Neutrophil extracellular traps (NETs) were first described as web-like structures that trap and neutralize microbes at sites of infection. NETs are comprised of chromatin components and neutrophil cytoplasmic proteins, and it has been reported that NETs are involved in autoimmunity such as SLE or ANCA-associated vasculitis (AAV). It has also been reported that autoantibodies themselves for important cytoplasmic autoantigens of neutrophils such as MPO and PR3 induce NETs production. However, the role of other neutrophil cytoplasmic antigens for the production of NETs is unclear. We investigated how autoantibodies for these antigens are involved in NETs productions.

Methods.– Human peripheral blood neutrophils were obtained from healthy donors. Neutrophils were cultured with PMA (control) or PMA plus several kinds of anti-neutrophil cytoplasmic antigens for 3 hours. Cells were stained with Hoechst 33,342, Sytox Green and anti-MPO antibody. The percentage of NETs producing cells and the quantitation of nuclear decondensation were analyzed using Image J software. We distinguished between cells that released NETS fiber and cells that just died of NETosis.

Results.– Anti-MPO antibody strongly promoted both NETosis induction and release of NETS fiber. Although anti-PR3 antibody promoted NETosis induction, it did not increase the release of NETS fiber. Antibody for cathespin G, found in the azurophil granule, also promoted NETosis induction only. There were some other antibodies, such as anti-lactoferrin and anti-neutrophil elastase, that promoted both NETosis and release of NETS fiber.

Discussion.– It has been thought that ANCA has an important role in the pathogenesis of AAV partly because ANCA promote NETS production by neutrophils at sites of vasculitis. We showed that not only anti-MPO and anti-PR3 but also antibodies for other neutrophil cytoplasmic antigens promote NETS production. The mechanism these antibodies induce NETosis and what they do in the pathogenesis of AAV are to be elucidated.

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Antibodies to LAMP-2 alter lysosome function and attenuate chaperone-mediated autophagy in human macrophages

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Introduction.– Lysosome-associated membrane protein-2 (LAMP-2) is membrane protein that traffics from the cell surface to lysosomes. It maintains lysosome integrity and is rate limiting for chaperone-mediated autophagy (CMA). Anti-LAMP-2 antibodies are frequent in patients presenting with ANCA-associated vasculitis (AAV) [1,2]; whether they affect LAMP-2 function is unknown. Here, we determine whether antibodies to LAMP-2 compromise lysosome function and affect cell survival of human macrophages.

Methods.– Human THP-1 cells and monocyte derived macrophages (MDM) incubated alone or together with monoclonal antibodies to LAMP-2 (H4B4) or isotype control (CD4). IgG uptake was measured by confocal microscopy and Western blotting (WB); and viability and apoptosis by trypan blue and caspase-3. Lysosomes were purified (lysosome kit) and their proteins quantified by WB and fluorescence. CMA was assessed by lysosomal acquisition of H5C70 and import of cytoplasmic proteins.

Results.– H4B4 was selectively taken up by THP-1 cells and significantly increased apoptosis and cell death after 24 hours. It accumulated in lysosomes and significantly reduced LAMP-2 but not LAMP-1; CD4 did not. Lysosomal HSc70 was also reduced and correlated with LAMP-2 and CMA. H4B4 abrogated of the stress-induced CMA response as shown by measuring the CMA substrate GAPDH and the reduced import of cytosolic proteins. H4B4 also increased lysosomal pre-pro-cathepsin D but not mature cathepsin D, suggesting lysosomal integrity was also affected. H4B4 had similar on MDM.

Discussion.– H4B4 is specifically taken up by macrophages and traffics to lysosomes where it reduces membrane LAMP-2 and disrupts function. It markedly inhibits basal CMA and abrogates its stress-induced augmentation. There are clear implications for macrophage function is obvious not least because CMA is critical for antigen presentation to T cells.

Conclusion.– H4B4-triggered CMA dysfunction provides the relevant molecular mechanism for cell death by antibodies to LAMP-2.

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Urinary HMGB1 levels are associated with CD4+ T-cells in urine in patients with ANCA-associated vasculitis and active nephritis

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References

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References

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