of experimental arteritis, however inhibitory mechanism against arteritis has been still unclear. The present study aimed to elucidate the effect of TNF(a) drug on the process of development of arteritis.

Methods.—Candida albicans Water-Soluble Fraction (CAWS) was used as inocula. Mice were injected CAWS for five consecutive days. They were sacrificed on the 6, 12, 24th hour and 2, 5, 8, 11, 14, 28th day after injection of CAWS. Etanercept (ETA) was used as anti-TNF a drug. ETA (20 mg/kg) was administered subcutaneously twice weekly, total 8 times.

Results.—In control (no treatment) group, endoarteritis was observed in mice which were sacrificed on 6th hour, 2nd day. After 2nd day, inflammation of adventitia was observed in addition to endoarteritis. Panarteritis was observed in mice, which were sacrificed on 11, 14, 28th day. On the other hand, no endoarteritis was observed until 11th day after inoculation in ETA Group. And the size of vasculitis observed on 28th day was smaller than that of control.

Discussion.—Anti TNF-a drug could inhibit the development of vasculitis by control of endoarteritis. It is known that TNF a directly and indirectly promotes adhesion between endothelial cells and inflammatory cells, especially neutrophils. These findings support our present results. However, our study were unable to clarify whether the involvement of TNF a was limited to just the onset and progression of endoarteritis, or extends even to the process of establishment of panvasculitis following endoarteritis.

Conclusion.—It is suggested that TNF-a plays an important role in initial process of development of vasculitis. We declare no potential conflicts of interest.

Further readings

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P18
The protective role of NADPH oxidase in ANCA-induced vasculitis
A. Schreiber, S. Krueger, C. Luft, R. Kettritz
Experimental and Clinical Research Center (ECRC), Berlin, Germany

Introduction.—ANCA-activated neutrophils and monocytes cause necrotizing crescentic glomerulonephritis (NCGN). In vitro studies suggest...
that both reactive oxygen species (ROS) and serine proteases mediate the disease, but their in vivo role is unclear. Generation of ROS involves activation of the NADPH oxidase. We tested the hypothesis that ROS generation is essential to induce NCGN in ANCA disease.

Methods.—To induce NCGN, we immunized MPO-deficient mice with murine MPO followed by irradiation and BM transplantation from either wild-type (WT) or NADPH−/− mice.

Results.—WT BM-transplanted mice developed NCGN, whereas the gp91phox−/− BM-transplanted mice developed a significant stronger renal phenotype (13.32.5% vs. 60.7 ± 8.9% glomerular crescents). The aggravated NCGN was confirmed in a second independent experiment using a different NADPH knock-out (p47phox−/−). In addition to necrosis and crescents, NADPH−/− mice showed markedly stronger glomerular inflammatory cell influx and higher levels of IL-1β generation in their kidneys (75 ± 120 vs. 1708 ± 360 pg/mg). We hypothesized that ROS generated by ANCA-stimulated NADPH oxidase down-regulates IL-1β generation by inhibition of the NLRP3-inflammasome and caspase-1, the classical pathway of IL-1β generation. Stimulation of WT murine monocytes with murine anti-MPO IgG resulted in IL-1β generation that was significantly accelerated in both gp91phox−/− and p47phox−/− monocytes. The increase observed in gp91phox−/− cells was reduced by pretreatment with a specific caspase-1 inhibitor.

Finally, we treated p47phox−/− BM transplanted mice with the specific caspase-1 inhibitor. Regulating the inflammatory cascade by blocking caspase-1 and caspase-1, the classical pathway of IL-1β generation. Stimulation of WT murine monocytes with murine anti-MPO IgG resulted in IL-1β generation that was significantly accelerated in both gp91phox−/− and p47phox−/− monocytes. The increase observed in gp91phox−/− cells was reduced by pretreatment with a specific caspase-1 inhibitor.

Discussion.—Our data strongly suggest that reactive oxygen species generated by ANCA-stimulated NADPH oxidase are important for down-regulating the inflammatory cascade by blocking caspase-1- and NLRP3-dependent IL-1β generation.

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P19
Investigating Annexin-A1 in ANCA vasculitis and glomerulonephritis

P. Jukes1, F. D’Acquisto2, R. Pepper1, Y. Ghani1, M. Perretti2, A. Salama1, M. Little1
1. UCL, London, United Kingdom
2. QMUL, London, United Kingdom
3. Trinity College Dublin, Dublin, Ireland

Introduction.—Annexin-A1 (AnxA1) is a key mediator of inflammation resolution. It is naturally cleaved by Proteinase 3 (PR3), which is released during neutrophil degranulation and thus may be of relevance in the persistence of inflammation in ANCA vasculitis. A novel human recombinant PR3-cleavage-resistant form of AnxA1, “SuperAnxA1” (SAnxA1), has been developed, which has been shown to accelerate resolution of inflammation in in vivo studies. We aimed to investigate the role of AnxA1 and SAnxA1 in ANCA vasculitis and in nephrotoxic nephritis (NTN), a mouse model of crescentic glomerulonephritis.

Methods.—Firstly, we investigated whether the activation of primed human neutrophils by patient ANCA is modulated by SAnxA1 in vitro. Next, we tested whether SAnxA1 had an effect in vivo in a mouse model of accelerated nephrotoxic nephritis (NTN). Pre-immunised C57BL/6 mice were injected with a mixture of nephrotoxic serum and LPS (0.1 μg) IV at day 0. SAnxA1 (1 μg) or vehicle was administered IP daily from day −2 to day 7, at which point mice were culled. Lastly, to assess the effect of AnxA1 loss on glomerular inflammation we induced NTN in Anxa1−/− and WT mice.

Results.—The activation of human neutrophils by patient MPO and PR3-ANCA was inhibited in a dose-dependent manner by increasing concentrations of SAnxA1 (figure 1).

However, there was no observed pathological effect on disease severity following the administration of SAnxA1 in NTN, but there was a trend towards a decrease in CD11b expression in the peripheral blood monocytes in the SAnxA1 group. AnnexinA1-deficient mice with NTN had a 73% increase in the degree of glomerular thrombosis compared to WT controls.

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Conclusion.—Exogenous administration of SAnxA1 inhibits ANCA-induced neutrophil activation and loss of AnxA1 exacerbates murine glomerulonephritis. These results support the investigation of AnxA1 as a pro-resolution mediator in ANCA vasculitis.

P20
Immunization of NOD mice with recombinant mouse proteinase 3 causes immune complex not pauci-immune crescentic glomerulonephritis

P. Hu, H. Xiao, R. Falk, C. Jennette
UNC-Chapel Hill, Chapel Hill, USA

Introduction.—Antineutrophil cytoplasmic autoantibodies (ANCA) specific for proteinase 3 (PR3) and myeloperoxidase (MPO) are closely associated with pauci-immune crescentic glomerulonephritis (CGN) and small vessel vasculitis (SVV). A pathogenic role for MPO-ANCA has been well established in animal models; however, animal models of PR3-ANCA disease are controversial. We tried to reproduce a recently reported model of PR3-ANCA disease in NOD mice and observed contradictory results.