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 (757 ± 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 IL-1-receptor antagonist Anakinra. Untreated mice developed NCGN, whereas Anakinra-treated mice were rescued from the aggravated renal phenotype (14.8 ± 3.2% vs. 60.7 ± 9.8% crescents), whereas Anakinra-treated mice were rescued from the aggravated renal phenotype (13.3 ± 2.5% vs. 60.7 ± 8.9% crescents). The aggravation of 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 compared to WT controls.

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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.

**Figure 1**

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

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**Investigating Annexin-A1 in ANCA vasculitis and glomerulonephritis**

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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.

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

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

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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.
Methods.—NOD/LtJ, C57BL6 (B6), 12956 mice were immunized with 100 μg of rPR3 or BSA in CFA and boosted × 4 with the same dose. Anti-PR3 response was confirmed by ELISA. Mice were sacrificed at day 75.8 × 10^7 anti-rPR3 or anti-BSA splenocytes from NOD mice were transferred into NOD/SCID mice. Mice were sacrificed on day 40. Anti-rPR3 was injected into NOD mice. Anti-rPR3 IgG (750 μg/mL) purified from NOD mice was used for in vitro activation of NOD-neutrophils.

Results.—After immunization with rPR3, all NOD mice developed high levels of anti-PR3, whereas B6 and 12956 developed very lower levels of anti-PR3. Anti-PR3 reacted with native PR3 in the cytoplasm of neutrophils. No anti-PR3 was detected in BSA immunized mice. NOD and 12956 mice injected with rPR3 developed proteinuria, leukocyturia and hematuria, whereas no control mice had urine abnormalities. CGN developed in all rPR3-immunized NOD (average 25.6% crescents) and one out of four 129S6 mice (22.0% crescents). Glomeruli in all mice that developed CGN had granular capillary wall IgG. C3 and PR3. There were no glomerular lesions and no immune deposits in rPR3-immunized B6 and BSA-immunized control mice. NOD/SCID mice that received anti-rPR3 splenocytes developed circulating anti-rPR3 but no glomerulonephritis. Injection of anti-rPR3 IgG at 100 μg/g body weight did not induce CGN in NOD mice. Anti-PR3 IgG did not activate murine neutrophils in vitro.

Conclusion.—NOD mice immunized with recombinant PR3 develop circulating anti-PR3 and CGN, but the CGN is not pauci-immune.

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Behçet’s disease

P21

Infliximab for Behçet disease with aortic involvement

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Introduction.—Behçet disease (BD) is a multisystem inflammatory disorder, there have been only few case reports or small studies showing efficacy of infliximab (IFX) in combination with corticosteroids and/or immunosuppressants. Here, we describe novel cases of BD with aortitis successfully treated by IFX without corticosteroids or immunosuppressants.

Methods.—We had three cases of vascular BD, who were successfully treated with infliximab monotherapy. We describe the clinical courses of the patients.

Results.—A 43-year-old woman with recurrent oral ulcers, acneiform nodules, transient arthritis of the knees, anterior uveitis and HLA A-26 was diagnosed as BD was made. Computed tomography (CT) angiography revealed diffuse wall thickening of the ascending aorta, aortic arch, and left common carotid artery, with stenosis of the left subclavian artery. The patient, having refused corticosteroids due to the fear of side effects, was administered 5 mg/kg of IFX monotherapy. The patient’s symptoms improved immediately after the first infusion. Laboratory abnormalities normalized within 2 months and remained normal afterward. The second case is a 31-year-old woman with a 10-year history of BD with aphthous stomatitis, genital ulcers, erythema nodosum, acneiform skin lesions, colitis and posterior uveitis. CT imaging disclosed a diffuse aortic wall thickening from root to bifurcation. Given severe posterior uveitis, 5 mg/kg of IFX was started. Follow-up CT scan showed marked improvement of aortic wall thickening with normalization of CRP and ESR. She continued to receive IFX every 8 weeks and has remained in remission for 12 months without additional therapy.

Discussion.—IFX monotherapy rapidly improved clinical symptoms, laboratory abnormalities, and imaging findings in both patients.

Conclusion.—This strongly suggests that IFX is beneficial for treating BD with major vessel involvement.

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P22

Infliximab treatment of resistant uveitis in Behçet disease

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Introduction.—Behçet disease is a rare multiorgan vascular condition which has often a severe eye manifestation. The aim was to analyse the effect of infliximab (INF) on uveitis in patients with Behçet disease resistant to conventional treatment.

Methods.—Observation of patients with uveitis in Behçet disease resistant to combination of corticosteroids with two systemic immunosuppressive drugs, being azathioprine and cyclosporine A. Treatment included INF (5 mg/kg body weight) was administered on week 0, 2 and 6. Blood tests and complete ophtalmologic examination were performed at the infusion day and every 6 weeks afterwards. Changes in corticosteroid dosage and adverse events were registered. There was no change in immunosuppressive treatment.

Results.—Two female patients (age 23 and 42 years) with Behçet disease, one had had bilateral chronic panuveitis and one had unilateral chronic anterior uveitis. Previously, both patients had received topical treatment, oral corticosteroid equivalent to 1 mg/kg/day of prednisone, cyclosporine A and azathioprine.

Both patients improved at 100% from the visual acuity registered before the first INF infusion and reached a normal vision in one of their eyes. Anterior Tyndall became negative at third infusion in both cases, and vitreous Tyndall became also negative. One patient relapsed. She remained asymptomatic for 8 months. The three infusions were repeated with the same response. The other one did not have any flare following 24 months. We could taper corticosteroid dosage below 10 mg/day of prednisone or equivalent in both patients. There has been observed no adverse event.

Conclusion.—Infliximab could be a good option in management of Behçet disease associated uveitis resistant to conventional treatment.

Further readings


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