Methods. — Five thousand sera of 3095 patients from three French hospitals were found IIF-ANCA positive from 2004 to 2012. PR3 and MPO specificities were assessed by ELISA, immunodot or ALBIA.

Results. — Twenty-eight IIF-ANCA positive patients had ANCA specific for both PR3 and MPO by at least one of the solid phase test (0.7% of serum, 0.9% of patients). None of the 23 patients with an available file had a necrotizing systemic vasculitis. No relevant clinical association was noticed.

Discussion. — We described 23 documented patients with both PR3 and MPO-ANCA. Only case reports or incidentally reported cases in serological studies appear in the literature [1,2,3].

Animal models

P13
Synergistic effect of GCSF and LPS in ANCA Vasculitis

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Introduction. — Granulocyte colony stimulating factor (GCSF) is a cytokine that is important in mobilizing neutrophils from the bone marrow and has proinflammatory effects. We have previously shown that serum GCSF is raised in patients with ANCA vasculitis and exacerbates disease in an established murine model [1]. LPS was given to all mice in the previous study, though it was not known if this was required with GCSF.

We set out to investigate the relative role of GCSF and LPS in this model. Methods. — Purified murine MPO was used to immunise MPO knockout mice to generate anti-MPO antibody. Four groups of wild type C57BL/6 mice were used (n=5–6/group). They were given GCSF or control subcutaneously starting 8 days before the disease induction with anti-MPO (day 0). LPS or control was administered intraperitoneally on day 0 and 3. Serum, urine and histology was assessed on day 7.

Results. — The group which received both LPS and GCSF had significantly higher serum creatinine levels compared to mice with administration of neither LPS nor GCSF, administration of LPS alone or GCSF alone (12.3 ± 0.8 compared to 9.0 ± 0.5, 8.3 ± 0.6, 9.5 ± 0.4 μmol/L respectively, P < 0.001). This group also had significantly more albuminuria compared to the other three groups (106 ± 18.24 compared to 15.47 ± 1.43, 27.21 ± 4.33, 21.75 ± 2.75 mgc in 24 hrs respectively, P < 0.0001). Furthermore, this group had significantly more glomerular crescents compared to the other three groups (31.6 ± 5.2 compared to 0 ± 0, 0.3 ± 0.3, 0.2 ± 0.2 per 100 glomeruli respectively, P < 0.0001) and glomerular macrophages (15.3 ± 1.3 compared to 0.5 ± 0.1, 2.2 ± 0.3, 4.1 ± 0.8 cells per glomerulus respectively, P < 0.0001).

Discussion. — This study shows that both LPS and GCSF are required to obtain robust disease in this model, with a strong synergistic effect on both histological and biochemical disease parameters.

Conclusion. — These findings have implications for investigators using this model and for our understanding of disease pathogenesis. They suggest that endogenous GCSF may be a therapeutic target.
P14 Pathogenic leukocytosis and their susceptibility QTLs for vasculitis and crescentic glomerulonephritis in a model of SCG/Kj mice

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Introduction.— The spontaneous crescentic glomerulonephritis-forming/Kinjo mouse (SCG/Kj), a model of human crescentic glomerulonephritis (CrGN) and systemic vasculitis, is characterized by the production of MPO-ANCA and marked leukocytosis. This study was done to identify the specific population(s) of leukocytes associated with CrGN, and their susceptibility loci on SCG/Kj genome. Methods.— Four hundred and twenty female (C57BL/6 (B6) × SCG/K) F2 intercross mice were subjected to serial flow cytometry examination of the peripheral blood (PB) and serum titer of autoantibodies including MPO-ANCA. Kidney granulocytes and monocytes were histopathologically examined with anti-Gr-1 and anti-F4/80 antibodies. Linkage analyses were done with 102 polymorphic microsatellite markers. Results.— Correlation studies revealed that increase of the Gr-1+ granulocyte, F4/80+ macrophages/monocytes, CD3+CD4+CD8- T cells, and dendritic cells (DCs) in peripheral blood were significantly associated with CrGN, crescent formation, and renal vasculitis. In kidney sections, F4/80low cells were observed in crescent, while F4/80high cells were around the Bowman’s capsules and in the interstitium. Numbers of inflammatory macrophages via blood stream. Discussion.— T cells reactive with rat vascular endothelial cells (RECs) were extracted from the vasculitis-prone rats by repeated REC-stimulation. A T cell clone reactive with RECs was established and then named VASC-1. Characterization of VASC-1 identified these cells as invariant NKT (iNKT) cells. Interaction of VASC-1 and RECs was determined by in vitro co-culture experiments, and pathogenicity of VASC-1 was elucidated by in vivo cell transfer experiments. Results.— The T cell receptor genotype with Vα14, CD4 CD8 double negative phenotype, and characteristic cytokine profile with production of IL-4 and IFN-γ but not IL-2 or IL-10 indicated VASC-1 as an iNKT cell clone. In vitro co-culture experiments of VASC-1 and RECs demonstrated the proliferation of VASC-1 interacted with RECs. Moreover, VASC-1 was activated to shed off CD62L from the cell surface and to produce proinflammatory cytokines, such as IL-2, IL-5, IL-6, and IL-17, after interaction with RECs. On the other hand, RECs were also activated to produce eotaxin by interacting with VASC-1. These findings clearly indicated the reaction and reciprocal activation of VASC-1 and RECs. Furthermore, small vessel vasculitis (SVV) similar to the original rat model was induced in normal rats by intravenous injection of VASC-1. Discussion.— Invariant NKT cells can react with glycolipid antigens presented by CD1d. Recent studies revealed that CD1d can also present self peptides to iNKT cells. Since RECs express CD1d, the possibility that VASC-1 recognizes the CD1d-restricted REC antigen via TCR is worthy of consideration. Clarification of the antigen recognition mechanism of VASC-1 is the next important subject. Conclusion.— REC-reactive iNKT cells could be involved in the pathogenesis of SVV in the rat model.

References

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P16 Anti TNF-α drug inhibits initial process of vasculitis in animal model of Kawasaki disease

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Introduction.— Kawasaki disease (KD) is one of the vasculitis syndrome in childhood. Recently, anti TNF-α drugs are administered for some KD patients who are resistant to IVIG therapy. Previously we revealed histologically that TNF-α drug could inhibit the development

Reference

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