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. None of them had a SV, neither granulomatosis with polyangiitis nor microscopic polyangiitis nor Churg-Strauss syndrome.

To the best of our knowledge, no cohort studies focused on such PR3 and MPO-ANCA. Only case reports or incidentally reported cases in serological studies appear in the literature [1,2,3].

Conclusion.—This cohort study demonstrates that, unlike the monospecific PR3- or MPO-ANCA, ANCA with both anti-PR3 and anti-MPO activity, are not associated with SV, unlike few reports of sparse MPO and PR3-ANCA cases ascertained as SV with old laboratory tests.

References

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P12 Aklides® – a highly versatile imaging platform for detection of ANCA
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Introduction.—Here we describe a highly versatile microscopy platform – Aklides® – (A) for the automated pattern analysis of ANCA with neutrophils combined with (B) multiplex microbead-based detection of Proteinase 3 (PR3), Myeloperoxidase (MPO), and glomerular basement membrane (GBM) autoantibodies (aab). The manual interpretation of ANCA patterns is very subjective and prone to high variability. EIA results are objective but EIA have their limit in multiplex detection of specific ANCA such as PR3 and MPO.

Methods.—(A) ANCA were automatically analysed with ethanol (ethN) or formalin (formN) fixed neutrophils. Sera from 342 patients with AAV and other systemic rheumatic and infectious diseases were tested for ANCA patterns with Aklides® and results were compared to those of conventional routine ELISA.

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 umol/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 mcg 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 ± 0.3 ± 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 (SCG/Kj) mouse, 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/Kj) 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 T cells, and dendritic cells (DCs) in peripheral blood were significantly associated with GN, crescent formation, and renal vasculitis. In kidney sections, F4/80low cells were observed in crescent, while F4/80high cells were with GN, crescent formation, and renal vasculitis. In kidney sections, analyses were done with 102 polymorphic microsatellite markers.

Conclusion.— The results will provide some information for future development of genome-based drug discovery and molecular target drugs of human CrGN and vasculitis.

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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 of vascular endothelial cell-reactive T cells.

Methods.— We had established a rat model that developed medium-to-small vessel vasculitis [1]. Our earlier studies suggested that auto-reactive T cell-mediated vascular injury could be involved in the pathogenesis [2].

Results.— 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 coculture experiments, and pathogenicity of VASC-1 was elucidated by in vivo cell transfer experiments.

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 SV in the rat model.

References

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P15 Implication of vascular endothelial cell-reactive invariant NKT cells in pathogenesis of small vessel vasculitis in rats
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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 of vascular endothelial cell-reactive T cells.

Methods.— We had established a rat model that developed medium-to-small vessel vasculitis [1]. Our earlier studies suggested that auto-reactive T cell-mediated vascular injury could be involved in the pathogenesis [2].

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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 SV in the rat model.

References

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