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/kinoh (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−CD8+ 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 around the Bowman’s capsules and in the interstitium. Numbers of F4/80low cells were observed in crescent, while F4/80high cells were in the interstitium. Numbers of inflammatory macrophages via blood stream.

Discussion. – This study discovered CrGN-associated leukocytes and susceptibility QTLs with their positional candidate genes. Immunohistochemistry findings suggest crescent-forming F4/80+ are trafficking inflammatory macrophages via blood stream.

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

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 IL-4 and IFN-γ but not IL-2 or IL-10 indicated VASC-1 as an iNKT cell clone. In vitro 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 IL-4 and IFN-γ, but not IL-2 or IL-10. We had established a rat model that developed medium-to-small vessel vasculitis [1]. Our earlier studies suggested that autoimmune T cell-mediated vasculitis injury could be involved in the pathogenesis [2].

Methods. – 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 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 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 TNF-α 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. – RE-reactive iNKT cells could be involved in the pathogenesis of SVV 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 – We had established a rat model that developed medium-to-small vessel vasculitis [1]. Our earlier studies suggested that autoreactive T cell-mediated vasculitis injury could be involved in the pathogenesis [2].

Methods – 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 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 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 TNF-α 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 – RE-reactive iNKT cells could be involved in the pathogenesis of SVV in the rat model.

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

Methods.—Candida albicans Water-Soluble Fraction (CAWS) was used as incula. 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-α 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-α drug could inhibit the development of vasculitis by control of endoarteritis. It is known that TNF-α 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-α 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-α plays an important role in initial process of development of vasculitis. We declare no potential conflicts of interest.

Further readings

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P17
The role of quantitative trait loci (QTL) in the pathogenesis of experimental autoimmune vasculitis (EAV)
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Introduction.—The genetic susceptibility to anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis is incompletely understood. We have recently discovered that the Wistar Kyoto (WKY) rat is susceptible to experimental ANCA associated vasculitis (EAV), but Lewis (LEW) rats are resistant. We have generated congenic rat strains to dissect out the role of selective quantitative trait loci (QTL) in EAV.

Methods.—Rats were immunised with human myeloperoxidase (MPO) in adjuvant to induce disease. Control rats were immunised with human serum albumin (HSA) in adjuvant. Disease progression was assessed by measuring proteinuria, haematuria, serum ANCA titre and by histology.

Double congenic WKY rats with introgression of QTL from LEW chromosomes 13 and 16 were used.

Results.—WKY rats developed proteinuria (figure 1), haematuria (+++ or ++ through weeks 3–8) and glomerular abnormalities (7.1% of glomeruli abnormal at week 8 in MPO immunised animals versus 0.3% in HSA immunised animals; P < 0.05). Double congenic WKY rats, introgressed with loci from the Lewis strain, did not develop proteinuria or haematuria and were protected from glomerular injury. Both WKY and double congenic strains developed equivalent ANCA titres.

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P18
The protective role of NADPH oxidase in ANCA-induced vasculitis
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Introduction.—ANCA-activated neutrophils and monocytes cause necro-tizing crescentic glomerulonephritis (NCGN). In vitro studies suggest