**L9. The role of genetic background in an animal model of ANCA-associated vasculitis**

**Introduction**

There is increasing evidence for genetic influences affect vasculitis and glomerulonephritis caused by antineutrophil cytoplasmic autoantibodies (ANCA) [1–21]. A genome-wide association study demonstrated a genetic basis for differences between diseases associated with ANCA specific for myeloperoxidase (MPO-ANCA) versus proteinase 3 (PR3-ANCA) [1].

A genome-wide association study demonstrated a genetic basis for differences between diseases associated with ANCA specific for myeloperoxidase (MPO-ANCA) versus proteinase 3 (PR3-ANCA) [1]. Additional evidence for genetic influences on ANCA-associated vasculitis (AAV) includes familial occurrences [2–6], prevalence in first-degree relatives of AAV patients [7], racial influences on incidence [7–11], and correlations between polymorphisms in genes that influence immune responses and the clinical and pathologic manifestations of AAV [12–21].

A genetic influence also has been demonstrated in a rat model of MPO-ANCA glomerulonephritis. Immunization of Wistar Kyoto (WKY) rats with human MPO results in anti-MPO antibodies that cross react with rat MPO and cause necrotizing and crescentic glomerulonephritis (NCGN) that resembles human ANCA NCGN [22]. Identical immunization of Lewis, Wistar Furth, and Brown Norway rats does not induce NCGN even though the rats have similar levels of circulating anti-MPO [22]. The authors propose that this difference in strain susceptibility is more likely caused by genetic influences on the innate immune response rather than the adaptive immune response.

A mouse model of AAV that closely mimics human disease, including the characteristic pauci-immune NCGN, is induced by injection of anti-MPO IgG derived from MPO knockout mice that have been immunized with murine MPO [23]. Investigations using this model demonstrate that neutrophils are the primary effector cells of acute injury, and that activation of the alternative complement pathway and engagement of leukocyte Fc receptors are important inflammatory mediators [24–28]. Intravenous injection of anti-MPO IgG into C57BL/6 (B6) mice consistently induces NCGN in all recipient mice with crescent formation in approximately 5% to 10% of glomeruli [23–25]. Although the individual murine glomerular lesions have a remarkable resemblance to human ANCA NCGN, the NCGN is less severe and less variable than NCGN in AAV patients who often have severe disease although there is substantial variability among patients, ranging from 100% to less than 5% crescents and averaging 50% [29]. A minority of patients who have systemic AAV have no NCGN. The homogeneous genetic background among B6 mice could explain the lack of variability in severity of anti-MPO GN among B6 mice, and, mice with different genetic background might have different susceptibility to and severity of anti-MPO NCGN. We confirmed this possibility by comparing NCGN induction by anti-MPO IgG in 13 mouse strains and performed genotyping to try to identify candidate loci that influenced disease severity [30]. We also used bone marrow chimeric mice and in vitro neutrophil activation assays to demonstrate that genetic differences were mediated primarily by effects on neutrophil function [30].

**Severity of anti-MPO IgG induced NCGN is influenced by genetic background**

C57BL/6J (B6), 129S6/SvEv (129S6), 129S1/SvImJ (129S1), LP/J (LP), WSB/EiJ (WSB), NZO/H1ijJ (NZO), PWK/PhJ (PWK), NOD/LtJ (NOD), DBA1, DBA2, Aj, C3H and CAST/Ei (CAST) mice were injected with the same dose of anti-MPO IgG. As shown in table 1 and figure 1, severity of NCGN as measured by percentage of glomeruli with crescents ranged from greater than 60% in 129S6 and CAST mice to no disease induction in NOD, DBA1 and DBA2 mice. The observed differences in pathogenicity could be determined either by protective alleles in mice with less severe NCGN or disease promoting alleles in mice with more severe NCGN.

The nephritogenicity of anti-MPO IgG was tested in F1 mice generated by B6 backcross with 129S6 mice, and F2 mice generated by (B6x129S6) F1 intercross [30]. The severity of NCGN in 129S6 × B6 F2 spanned the extremes between B6 and
129S6 mice, although there was a clear trend toward the less severe end of this spectrum (Figure 1).

**The genetic influence is mediated by neutrophils**

Chimeric mice were created by transplantation of $1.5 \times 10^7$ 129/S6 bone marrow cells (BM) into irradiated Rag2⁻/⁻/B6 recipients, and B6 BM into irradiated Rag2⁻/⁻/129S6 recipients. Four weeks after transplantation greater than 95% of circulating leukocytes were derived from the donor. Chimeric mice were injected with nephritogenic doses of anti-MPO IgG to determine the relative importance of bone marrow-derived cell in pathogenesis of NCGN [30]. B6 mice with circulating 129S6 leukocytes developed 79% crescents (69–85%), which is similar to 129S6 mice, whereas 129S6 mice that with circulating B6 leukocytes had 17% crescents (9–22%), which similar to but slightly more severe than B6 mice. Thus, the genetic origin of the circulating leukocytes determined the severity of NCGN.

To test if this effect was mediated through genetically determined differences in neutrophil function, the ability of anti-MPO IgG to activate 129S6, 129S1 and B6 neutrophils respectively was tested in vitro. Neutrophils were primed with TNFα, incubated with anti-MPO IgG, and activation measured by superoxide generation. Anti-MPO IgG caused more activation of neutrophils from 129S6 compared to neutrophils from B6 or

<table>
<thead>
<tr>
<th>Strains</th>
<th>Mouse numbers (n)</th>
<th>n with crescents</th>
<th>Mean % crescents when present</th>
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<tbody>
<tr>
<td>B6</td>
<td>27</td>
<td>27</td>
<td>9.5</td>
</tr>
<tr>
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<td>24</td>
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<tr>
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<tr>
<td>CAST</td>
<td>5</td>
<td>5</td>
<td>61.2</td>
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Data from [30].

NA: not applicable because no crescents were observed.

**Table I**

Crescent formation induced by the same does of anti-MPO IgG in different strains of mice

**Figure 1**

Comparison of the severity of anti-MPO induced glomerulonephritis in B6, 129S6 and 129S1 strains of mice, and in B6 × 129S6 F1 and F2 mice, represented as the percentage of glomeruli with crescents

Data from [30].
129S1 mice, suggesting that genetic differences in neutrophil activation by anti-MPO IgG is the basis for the difference in disease severity among strains. Thus, in this model, differences in disease severity are caused by genetic influences on the innate immune response rather than the adaptive immune response.

**Comparative genomic analysis**

To search for genes that influence the pathogenicity of anti-MPO, genetic variation among mouse strains was evaluated with high-density genotyping at 542,190 single nucleotide polymorphisms (SNPs) using a Mouse Diversity Array [31,32]. Regions that are identical by descent (IBD) were identified [33]. Although 129S6 and 129S1 have very different NCGN severity (figure 1, table I), they are very closely related genetically (table II) with most genetic differences clustered. B6 differed from the other three strains at over 20% of SNPs, LP from 129 at 7% to 8%, and the 129 S1 and S6 from each other at less than 1% (table II).

B6 mice are identical by descent (IBD) with 129 in more than 27% of the genome. The remaining 73% that is not IBD is a likely location of alleles influencing severity of NCGN, however, genes in an IBD region could influence genes in the non-IBD regions and have an effect on disease phenotype. Ninety percent of mismatches between 129S1 and 129S6 accounting for the 3.3% non-IBD genome is confined to 15 regions over 90.8 Mb on nine chromosomes contain 761 known genes and are candidates for loci for causing the differences in NCGN severity between 129S1 and 129S6. Several of the known genes in the non-IBD regions are involved in pathways that have been incriminated in the pathogenesis of AAV, including genes involved in complement activation, Fc receptor engagement, antibody-cell surface interaction, epigenetic gene regulation, and cell signaling.

To identify quantitative trait loci (QTL) for disease severity based on percentage crescents, 100 female B6 × 129S6 F2 mice were genotyped at 76 SNPs using Sequenom iPEx MassARRAY [34,35]. Similar studies are currently underway using 129S51 × 129S6 F2 mice. No QTL peaks with sufficient genome-wide significance were detected, although suggestive QTL peaks were observed on chromosomes 4, 5, 7, 9, and 10 [30]. The absence of a well-defined QTL suggests that the marked differences in disease severity between 129S6 and B6 are the result of multiple gene interactions. This conclusion also is supported by the observation that NCGN severity in 129S6 × B6 F2 mice has a wide range of severity spanning the extremes between 129S6 and B6 mice.

**Conclusion**

Genetic background has a substantial effect on the pathogenicity of anti-MPO IgG in a mouse model of human MPO-ANCA disease. This genetic influence appears to be the result of interactions between multiple genes, and acts through genetically determined modulation of the receptivity of neutrophils to activation by MPO-ANCA.

**Disclosure of interest:** the author has not supplied his declaration of conflict of interest.

**References**

Lectures


L10. Animal models of ANCA-associated vasculitis: Effector mechanisms and experimental therapies

ANCA and their interaction with neutrophils and monocyes

Four distinct diseases are characterized by the appearance of anti-neutrophil cytoplasmic autoantibodies (ANCA), namely granulomatosis with polyangiitis (GPA, formerly called Wegener’s granulomatosis), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA, formerly

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