Bone Marrow–Derived Cells Are Sufficient and Necessary Targets to Mediate Glomerulonephritis and Vasculitis Induced by Anti-Myeloperoxidase Antibodies

Adrian Schreiber, Hong Xiao, Ronald J. Falk, and J. Charles Jennette

University of North Carolina at Chapel Hill, Department of Pathology and Laboratory Medicine, Chapel Hill, North Carolina

Clinical and experimental evidence indicate that ANCA cause pauci-immune necrotizing and crescentic glomerulonephritis (NCGN) and systemic small vessel vasculitis in humans. One of the major target antigens for ANCA is myeloperoxidase (MPO). An animal model that closely resembles the human disease is induced by intravenous injection of anti-MPO IgG into mice. The likely primary pathogenic targets for the anti-MPO IgG are circulating neutrophils and monocytes, although other cells have been implicated, including endothelial cells and epithelial cells. Herein is reported a new model for anti-MPO–mediated glomerulonephritis and vasculitis that further documents the pathogenic potential of ANCA and demonstrates that bone marrow (BM)-derived cells are sufficient targets to cause anti-MPO disease in the absence of MPO in other cell type. MPO knockout (Mpo−/−) mice that were immunized with mouse MPO were exposed to irradiation and received a transplant of Mpo+/+ or Mpo−/− BM. Engraftment in mice with circulating anti-MPO resulted in development of pauci-immune NCGN in all mice and pulmonary capillaritis and splenic necrotizing arteritis in some. Anti-MPO IgG also was introduced intravenously into chimeric mice by transplantation of Mpo+/+ BM into irradiated Mpo−/− mice or Mpo−/− BM into irradiated Mpo+/+ mice. Chimeric Mpo−/− mice with circulating MPO-positive neutrophils developed NCGN, whereas chimeric Mpo+/+ mice with circulating MPO-negative neutrophils did not, thereby indicating that BM-derived cells are not only sufficient but also necessary for induction of anti-MPO disease. This novel animal model further documents ANCA IgG interactions with neutrophils as a cause of ANCA-associated glomerulonephritis and vasculitis.

examination after 13 d shows severe NCGN as well vasculitis in various organs. However, the glomerulonephritis is not pauci-immune because there is a moderate amount of predominantly mesangial glomerular deposition of immune complexes. Therefore, this is not a good model of pauci-immune human disease, which often has a small amount of glomerular Ig deposition but usually does not have the extent of deposition that is seen in mice after splenocyte transfer.

A rat model of ANCA disease has been induced by immunizing rats with human MPO, which results in the production of anti-MPO antibodies that cross-react with human and rat MPO (20). This causes focal segmental pauci-immune glomerulonephritis and focal pulmonary capillaritis. However, not all rats developed glomerulonephritis, and, when present, 10% of glomeruli had crescents or necrosis. As in the mouse model that was induced by injection of anti-MPO IgG, the glomerulonephritis was not severe.

Here we report a novel approach to the induction of ANCA glomerulonephritis and vasculitis that provides additional support for the pathogenicity of ANCA, sheds light on the pathogenic mechanisms that are involved, and provides a model that may be better suited than current models for long-term studies of ANCA disease. We report the use of transplantation of MPO-positive bone marrow (BM) into MPO-deficient mice to induce pauci-immune NCGN and SVV.

### Materials and Methods

#### Mice

Wild-type (WT) C57BL/6J (B6) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained by the University of North Carolina Division of Laboratory Animal Medicine. Mice that lacked MPO (Mpo−/− mice) originally were generated by Aratani et al. (21). Table 1 details the 10 experimental groups of mice. Nine- to

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**Table 1. Experimental animal groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Recipient Mouse</th>
<th>BM Donor Strain</th>
<th>Radiation Dosage (rad)</th>
<th>% MPO + Blood Neutrophils</th>
<th>Intravenous Anti-MPO</th>
<th>% with NCGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>900</td>
<td>91.3 ± 6.1</td>
<td>No</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>750</td>
<td>86.2 ± 11.7</td>
<td>No</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>600</td>
<td>49.6 ± 29.9</td>
<td>No</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>450</td>
<td>16.4 ± 4.5</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>900</td>
<td>89.8 ± 6.6</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>Mpo−/−</td>
<td>Mpo−/−</td>
<td>900</td>
<td>0</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>WT</td>
<td>WT</td>
<td>900</td>
<td>100</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>WT</td>
<td>No</td>
<td>900</td>
<td>8.5 ± 3.5</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>Mpo−/−</td>
<td>WT</td>
<td>900</td>
<td>90.2 ± 6.1</td>
<td>Yes</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>WT</td>
<td>None</td>
<td>None</td>
<td>100</td>
<td>Yes</td>
<td>100</td>
</tr>
</tbody>
</table>

*Recipient mice in groups 1 through 9 were either myeloperoxidase (MPO) knockout (Mpo−/−) or wild-type (WT) mice. Groups 1 through 4 and 6 were immunized with mouse MPO before exposure to various dosages of irradiation. After irradiation, mice received bone marrow (BM) transplants from either WT or Mpo−/− mice. The percentage of MPO-positive neutrophils in the blood was an index of BM engraftment. The chimeric mice in groups 8 and 9 received an intravenous injection of anti-MPO IgG after engraftment. Group 10 WT mice received no radiation and no BM transplant before intravenous injection with anti-MPO IgG and thus served as positive controls for groups 8 and 9. The last column indicates whether mice developed necrotizing and crescentic glomerulonephritis (NCGN).*
10-wk-old mice were used as donors of BM cells. Immunization of mice with MPO was begun at 8 to 10 wk of age. All animal experiments were in accordance with Nation Institutes of Health guidelines and approved by the University of North Carolina Institutional Animal Care and Use Committee.

Immunization of Mice

Purification of mouse MPO from WEHI-3 cells and immunization of Mpo−/− mice were performed as described previously (17). Eight- to 10-wk-old Mpo−/− mice were immunized intraperitoneally with 10 μg of purified murine MPO (groups 1 through 4 and 6) or BSA (group 5) in complete (first immunization) or incomplete (booster immunizations) Freund’s adjuvant (Table 1). Mice were immunized at day 0, receive a booster on days 28 and day 35, and were irradiated on days 40 to 45. Development of antibodies was monitored by anti-MPO ELISA (17). Mice that were used for BM transplantation developed anti-MPO titers that were 80% or higher compared with a positive control serum pool that was derived from MPO−/− mice that were immunized by the regimen of Xiao et al. (17). Approximately 85% of immunized mice met this criterion.

BM Transplantation in Mice

Mice were kept in sterile housing conditions with food and water ad libitum (sterile water with neomycin 2 g/L [pH 2.0]). Mice in groups 1 to 9 were γ-irradiated with different whole-body dosages that ranged from 450 to 900 rad (Table 1). BM cells were harvested from femurs and
tibia, erythrocytes were lysed, and 1.5 × 10^7 BM cells were injected intravenously and retro-orbitally. Four different combinations of BM donors and recipients were used: WT into Mpo−/− (groups 1 through 5 and 9), Mpo−/− into WT (group 8), WT into WT (group 7), and Mpo−/− into Mpo−/− (group 6). Recovery of peripheral blood leukocytes and peripheral blood neutrophils was monitored after BM transplantation using the HESKA Veterinary Hematology System. Engraftment was measured as percentage of peripheral blood neutrophils that were positive for MPO by enzyme histochemistry. Extent of engraftment stabilized after 4 to 5 wk. Mice were killed for pathologic examination 8 wk after transplantation.

**Induction of Glomerulonephritis by Intravenous Injection of Anti-MPO IgG**

The IgG fraction from immunized Mpo−/− mice was isolated from serum by 50% ammonium sulfate precipitation and protein G affinity chromatography as described previously (17). Chimeric mice in groups 8 and 9 and control WT mice in group 10 were received an intravenous injection of 50 μg/g body wt anti-MPO IgG in PBS and killed on day 6. Just before the mice were killed, serum and urine samples were collected to evaluate renal function.

**Functional Evaluation of Renal Injury**

Mice were placed in metabolic cages 1 d before being killed, and urine was collected for 12 h. Urine was tested by dipstick (Roche Diagnostics Corp., Indianapolis, IN) for hematuria and leukocyturia, and the extent of hematuria and leukocyturia is expressed as the mean on a scale of 0 (none) to 4 (severe). The albuminuria was determined by Mouse Albumin ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, TX). Using the mean ± 2 SD for the reference range established previously by our laboratory using 305 healthy B6 mice, abnormal

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**Table 2. Extent of glomerular formation and necrosis in various experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>BM Strain</th>
<th>BM Donor Strain</th>
<th>% Glomeruli with Crescents per Mouse</th>
<th>% Glomeruli with Necrosis per Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mpo−/−</td>
<td>Yes</td>
<td>31.6 ± 24.8 (5.9 to 70.2)</td>
<td>15.5 ± 12.7 (2.0 to 37.1)</td>
</tr>
<tr>
<td>2</td>
<td>Mpo−/−</td>
<td>Yes</td>
<td>30.7 ± 26.3 (7.3 to 80.4)</td>
<td>17.1 ± 13.3 (4.2 to 40.2)</td>
</tr>
<tr>
<td>3</td>
<td>Mpo−/−</td>
<td>Yes</td>
<td>5.2 ± 6.1 (0.0 to 14.3)</td>
<td>2.6 ± 3.1 (0.0 to 7.9)</td>
</tr>
<tr>
<td>4</td>
<td>Mpo−/−</td>
<td>Yes</td>
<td>0.0 ± 0.0 (0)</td>
<td>0.0 ± 0.0 (0)</td>
</tr>
<tr>
<td>5</td>
<td>Mpo−/−</td>
<td>No</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Mpo−/−</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>WT</td>
<td>No</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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**Figure 4.** Pathologic findings in Mpo−/− mice transplanted with Mpo−/− or Mpo+/+ BM. None of the MPO-immunized Mpo−/− mice that received 900-rad irradiation followed by transplantation with Mpo−/− BM (group 6) developed glomerular lesions (A; hematoxylin and eosin [H&E]). All of the MPO-immunized Mpo−/− mice that received 900-rad irradiation followed by transplantation with Mpo+/+ BM (group 1) developed glomerular necrosis (B, arrow; H&E) and crescents (C, arrows; periodic acid-Schiff [PAS]); and some of these mice also developed necrotizing arteritis in the spleen with fibrinoid necrosis (D, arrow; PAS), pulmonary alveolar capillaritis with and septal influx of neutrophils (arrow) and intra-alveolar hemorrhage (E; H&E), and, less frequently, pulmonary granulomatous inflammation with multinucleated giant cells (F, arrows; H&E).
hapturic and blood urea nitrogen (BUN) were measured using a Johnson & Johnson Clinical Diagnostics VITROS 250 (Berkshire, UK).

Pathologic Evaluation of Renal Injury

Samples of kidney, lung, spleen, and liver tissue were collected when the mice were killed and fixed in 10% formalin and embedded in paraffin. Four-micrometer sections of specimens were stained with hematoxylin and eosin and periodic acid-Schiff (PAS). The extent of glomerular crescents and necrosis was expressed as the percentage of glomeruli with crescents and necrosis in each mouse. For immunofluorescence microscopy to detect glomerular localization of immune determinants, 4-μm sections of snap-frozen kidney tissue were stained with fluoresceinated goat anti-mouse IgG (Molecular Probes Invitrogen, Carlsbad, CA); goat anti-mouse C3 (ICN/Cappel, Aurora, OH); and antibodies that were specific for mouse IgM, IgA, and MPO (ICN/Cappel, Aurora, OH). Immunofluorescence microscopy staining of glomeruli was expressed as the intensity of staining on a scale of 0 to 4+. For detection of leukocytes, sections of snap-frozen tissue were stained with rat antibodies to neutrophils (anti–Gr-1, clone RB6–8C5; BD Pharmingen, Franklin Lakes, NJ) and monocytes/macrophages (anti-CD68, clone FA11; Serotec, Raleigh, NC). Rat antibody binding was detected using peroxidase-labeled secondary rabbit anti-rat IgG and tertiary goat anti-rabbit IgG antibodies (DAKO, Carpinteria, CA) followed by 3-amino-9-ethylcarbazole and hydrogen peroxide. Sections were counterstained with hematoxylin. Leukocyte localization was expressed as the percentage of positive glomeruli, the average leukocytes per cross-section of all glomeruli, and the average leukocytes per cross-section of positive glomeruli on the basis of evaluation of an average of 48 glomeruli per specimen (range 30 to 80 glomeruli).

Results

Neutrophil Reconstitution after Irradiation and BM Transplantation

MPO-deficient mice were immunized with murine MPO. After developing anti-MPO antibody titers >100% of a positive control standard, mice were irradiated with 450 to 900 rad of whole-body γ irradiation. Various donor and recipient combinations were used as shown in Table 1. The time course of repopulation of the circulating leukocyte pool after irradiation was established (Figure 1). Both MPO-immunized and nonimmunized Mpo−/− mice displayed a similar time course and amount of leukocyte repopulation (Figure 1A). There was a greater increase in the MPO-immunized mice (group 1) after 4 wk compared with the nonimmunized mice (group 5), but this was only marginally significant (P = 0.03). Both group 1 and group 5 had a similar rate of engraftment (Figure 2A, Table 1), thereby ruling out an immune-mediated depletion of repopulating MPO-positive neutrophils. MPO-positive neutrophils were identified by enzyme histochemistry (Figure 2, B and C). The slightly higher level of neutrophils in group 1 might be the result of the inflammatory state that accompanies the glomerulonephritis and vasculitis in these mice but not the group 5 mice. As shown in Figure 2A, there was no significant differ-

Table 3. Glomerular immunofluorescence microscopy findings in various experimental groups expressed on a scale of 0 to 4+

<table>
<thead>
<tr>
<th>Group</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>C3</th>
<th>MPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ± 0.0 (0.5)</td>
<td>0.5 ± 0.0 (0.5)</td>
<td>0.6 ± 0.2 (0.5 to 1.0)</td>
<td>0.5 ± 0.0 (0.5)</td>
<td>0.3 ± 0.3 (0.0 to 0.5)</td>
</tr>
<tr>
<td>2</td>
<td>0.6 ± 0.3 (0.5 to 1.0)</td>
<td>0.5 ± 0.4 (0.0 to 1.0)</td>
<td>0.6 ± 0.3 (0.5 to 1.0)</td>
<td>0.1 ± 0.3 (0.0 to 0.5)</td>
<td>0.4 ± 0.5 (0.0 to 1.0)</td>
</tr>
<tr>
<td>3</td>
<td>0.7 ± 0.3 (0.5 to 1.0)</td>
<td>0.7 ± 0.3 (0.5 to 1.0)</td>
<td>0.7 ± 0.3 (0.5 to 1.0)</td>
<td>0.3 ± 0.3 (0.0 to 0.5)</td>
<td>0.4 ± 0.5 (0.0 to 1.0)</td>
</tr>
<tr>
<td>4</td>
<td>0.6 ± 0.3 (0.5 to 1.0)</td>
<td>0.5 ± 0.4 (0.0 to 1.0)</td>
<td>0.8 ± 0.3 (0.5 to 1.0)</td>
<td>0.1 ± 0.3 (0.0 to 0.5)</td>
<td>0.3 ± 0.3 (0.0 to 0.5)</td>
</tr>
<tr>
<td>5</td>
<td>0.6 ± 0.3 (0.5 to 1.0)</td>
<td>0.5 ± 0.3 (0.0 to 1.0)</td>
<td>1.0 ± 0.0 (1.0)</td>
<td>0.3 ± 0.2 (0.0 to 0.5)</td>
<td>0.0 ± 0.0 (0.0)</td>
</tr>
<tr>
<td>6</td>
<td>0.6 ± 0.3 (0.0 to 1.0)</td>
<td>0.6 ± 0.2 (0.5 to 1.0)</td>
<td>0.8 ± 0.3 (0.5 to 1.0)</td>
<td>0.4 ± 0.4 (0.0 to 1.0)</td>
<td>0.0 ± 0.0 (0.0)</td>
</tr>
<tr>
<td>7</td>
<td>0.8 ± 0.3 (0.5 to 1.0)</td>
<td>0.7 ± 0.3 (0.0 to 1.0)</td>
<td>1.0 ± 0.0 (1.0)</td>
<td>0.3 ± 0.3 (0.0 to 0.5)</td>
<td>0.0 ± 0.0 (0.0)</td>
</tr>
</tbody>
</table>

Figure 5. Immunofluorescence microscopy findings in MPO-immunized Mpo−/− mice that received 900-rad irradiation followed by transplantation with Mpo+/+ BM (group 1). There is low-level staining for IgG, IgM, IgA, and C3 that is similar to staining that typically is seen in human pauci-immune crescentic glomerulonephritis. Scattered MPO staining seemed to correspond to infiltrating neutrophils and sites of neutrophil degranulation. Irregular staining for fibrin corresponded to foci of fibrinoid necrosis and crescent formation.
ence in engraftment with MPO-positive neutrophils between the 900- and 750-rad irradiation groups, but there was a pro-
gressive decline in engraftment with lower irradiation dosages.

**Anti-MPO Antibody Titer after Irradiation and BM Transplantation**

After the irradiation, anti-MPO antibody titers were moni-
tored. Mice that had been immunized with MPO before trans-
plantation (groups 1 through 4) had no significant decrease in anti-MPO antibody titer (Figure 2D). Irradiated nonimmunized
MPO-deficient mice showed no change in their baseline anti-
MPO antibody level after engraftment with MPO-positive cells.
Therefore, no new antibody response to MPO was mounted
after irradiation, and the circulating anti-MPO seems to be the
result of residual circulating antibody or residual committed
plasma cells or both.

**Urinary Abnormalities after BM Transplantation**

Eight weeks after irradiation and reconstitution, MPO-pre-
immunized Mpo−/− mice that were irradiated with 900 and
750 rad before transplantation with Mpo+/+ BM developed
comparable levels of marked hematuria, leukocyturia, and
albuminuria (Figure 3, A and B). This was in marked contrast
to nonimmunized Mpo−/− mice that were irradiated with 900
rad before transplantation with Mpo+/+ BM and developed
no hematuria, leukocyturia, or albuminuria. MPO-preimmu-
nized Mpo−/− mice that were irradiated with 600 or 450 rad
before transplantation with Mpo+/+ BM also failed to develop
hematuria, leukocyturia, or albuminuria.

Renal function was impaired in group 1 mice with a signif-
icant increase of creatinine (0.38 ± 0.16 mg/dl; range 0.2 to 0.7)
and BUN (61.7 ± 56.9 mg/dl; range 18 to 178) compared with
group 5 mice (creatinine 0.21 ± 0.06 mg/dl; BUN 31.6 ± 3.3
mg/dl; P < 0.05). All other irradiation groups (groups 2
through 4) had no significant increase in creatinine (750 rad:
0.34 ± 0.05 mg/dl; 600 rad: 0.33 ± 0.05 mg/dl; 450 rad: 0.24 ±
0.05 mg/dl) or BUN (750 rad: 31.4 ± 13.1 mg/dl; 600 rad: 35.6 ±
6.0 mg/dl; 450 rad: 36.9 ± 8.5 mg/dl).

**Histologic Evidence of NCGN**

Mice were killed 8 wk after irradiation and BM transplanta-
tion. All MPO-preimmunized Mpo−/− mice that had been
irradiated with 900 and 750 rad before transplantation with
Mpo+/+ BM (groups 1 and 2) developed glomerular necrosis
and crescents, 71% of preimmunized mice that received 600 rad
(group 3) developed glomerular necrosis and crescents, and
none of the preimmunized mice that received 450 rad (group 4)
or the nonimmunized mice that received 900 rad (group 5)
developed glomerular lesions (Table 1). No glomerular disease
developed in preimmunized Mpo−/− mice that received 900
rad before Mpo−/− BM transplantation (group 6) or in
Mpo+/+ WT mice that received 900 rad before Mpo+/+ WT
BM transplantation (group 7). Figure 3C and Table 2 show that
groups 1 and 2 mice had similar severity of glomerulonephritis
with crescents in approximately 31 to 32% of glomeruli and
fibrinoid necrosis in approximately 15 to 17% of glomeruli
(Figure 4). Group 3 mice had very mild glomerulonephritis,
and groups 4 through 7 had no glomerular disease. These
pathologic findings correlate extremely well with the urinary
findings and renal function.

**Immunofluorescence Microscopy Observations**

Immunofluorescence microscopy demonstrated a paucity of
staining for IgG, IgA, IgM, and C3 with no significant semi-
quantitative differences among the mice in the experimental
or control groups (groups 1 through 7; Table 3, Figure 5). Groups
1 through 4 had slight scattered glomerular staining for MPO
that probably represented infiltrating neutrophils and sites of
neutrophil degranulation. Groups 1 and 2, which had high
levels of engraftment as evidenced by high percentages of
circulating MPO-positive neutrophils, had no staining of ves-
sels away from sites of injury and no staining of other tissue
elements for MPO. Therefore, there was no evidence by immu-
nohistology that BM-derived stem cells were giving rise to cells
other than circulating myeloid leukocytes. Irregular foci of
staining for fibrin seemed to correspond to areas of fibrinoid
necrosis and crescent formation. Groups 5, 6, and 7 had no
glomerular staining for MPO or fibrin.

**Glomerular Neutrophil and Macrophage/Monocyte Accumulation**

Immunohistology demonstrated glomerular accumulation of
neutrophils (anti-GR1) as well as monocytes/macrophages (an-

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**Table 4. Glomerular influx of neutrophils and macrophages in various experimental groups expressed as the percentage of glomeruli that were positive for the cell type, average cells per positive glomerulus, and overall average cells per glomerulus**

<table>
<thead>
<tr>
<th>Group</th>
<th>% Positive Glomeruli</th>
<th>PMN per Positive Glomerulus</th>
<th>PMN per Glomerulus</th>
<th>% Positive Glomeruli</th>
<th>MP per Positive Glomerulus</th>
<th>MP per Glomerulus</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>44.1 ± 9.8</td>
<td>1.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>75.2 ± 17.6</td>
<td>2.1 ± 0.3</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>13.2 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.16 ± 0.0</td>
<td>20.0 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.14 ± 0.0</td>
</tr>
<tr>
<td>6</td>
<td>11.5 ± 1.9</td>
<td>1.2 ± 0.1</td>
<td>0.14 ± 0.0</td>
<td>19.1 ± 6.0</td>
<td>1.2 ± 0.2</td>
<td>0.23 ± 0.1</td>
</tr>
<tr>
<td>7</td>
<td>10.8 ± 0.9</td>
<td>1.4 ± 0.2</td>
<td>0.15 ± 0.0</td>
<td>17.7 ± 0.6</td>
<td>1.0 ± 0.1</td>
<td>0.15 ± 0.0</td>
</tr>
</tbody>
</table>

*MP, glomerular macrophages; PMN, glomerular neutrophils.*
ti-CD68) in mice in the experimental groups, whereas mice in control groups showed no increase above normal (Table 4).

Systemic Manifestation of Vasculitis

Focal pulmonary alveolar capillaritis was identified in the lungs of mice in groups 1 (five of 13), group 2 (four of seven), and group 3 (two of seven; Figure 4G). One mouse in group 1 and one mouse in group 2 also had focal pulmonary granulomatous inflammation with multinucleated giant cells that resembled lesions of Wegener’s granulomatosis (Figure 4F). Three mice in group 1 had necrotizing arteritis in the spleen (Figure D). No vasculitis was identified in groups 4 through 7.

Disease Induction by Passive Transfer of Anti-MPO IgG into Chimeric Mice

Chimeric mice with either Mpo−/− neutrophils and Mpo+/+ tissue (group 8) or Mpo−/+ neutrophils and Mpo−/− tissue (group 9) were established to evaluate the pathogenic target of passively administered anti-MPO IgG compared with disease induction by the same dosage of anti-MPO IgG in WT Mpo+/+ mice (group 10). All mice in group 9 that expressed MPO in BM-derived cells but were otherwise Mpo−/− developed hematuria, leukocyturia, and albuminuria that were comparable to the urinary findings in the group 10 positive control mice (Figure 6). In contrast, none of the group 8 mice with Mpo−/− BM-derived cells and Mpo+/+ tissue developed urinary abnormalities.

Histologic examination revealed that all mice in group 9 that expressed MPO in BM-derived cells but were otherwise Mpo−/− developed glomerular crescents (Figure 7B, arrows, PAS) and necrosis (Figure 7C, arrow, hematoxylin and eosin), but none of the group 8 mice with Mpo−/− BM-derived cells and Mpo+/+ tissue developed glomerular lesions (Figure 7A, PAS). On average, positive control mice (group 10) had 11.0 ± 2.0% of glomeruli with crescents and 5.0 ± 0.0% with necrosis (Figure 6C). Group 9 mice that expressed MPO in BM-derived cells but were otherwise Mpo−/− had crescents in 14.0 ± 8.1% of glomeruli and necrosis in 7.6 ± 5.0%. In contrast, group 8 mice with Mpo−/− BM-derived cells and Mpo+/+ tissue had 0% crescents and 0% necrosis. Therefore, MPO-positive BM-derived cells and not tissue cells are the pathogenic targets of anti-MPO antibodies. In addition, these data demonstrate that BM-derived cells, most likely neutrophils and monocytes, are necessary and sufficient targets for induction of glomerulonephritis by anti-MPO antibodies.

Discussion

ANCA vasculitis is the most common form of aggressive SVV in adults and is characterized in the acute phase by focal necrotizing vascular inflammation that is rich in neutrophils (22,23). There is compelling clinical and experimental evidence that ANCA antibodies that are specific for MPO or PR3 are involved directly in the pathogenesis of ANCA disease, but the pathogenic details have not been elucidated fully (2,3). *In vitro* experimental studies have shown that ANCA IgG can interact with and activate circulating neutrophils, resulting in the release of injurious factors that could be involved in the pathogenesis of vasculitis and glomerulonephritis (2–12). However, for a better understanding of any human disease, including ANCA vasculitis, good animal models are indispensable. Thus far, there are only a few convincing animal models of ANCA disease. One involves passive transfer of murine anti-MPO antibodies or anti-MPO lymphocytes from MPO-immunized MPO−/− mice into Mpo+/+ recipient mice (17). This approach does not involve breaking tolerance and therefore is not a model of the immunogenesis of the ANCA autoimmune response, but it is a feasible model of ANCA pathogenesis. Another model uses immunization of rats with human MPO,
resulting in the production of anti-MPO antibodies that cross-react with rat MPO (20). A model that is more difficult to interpret uses anti-GBM antibodies to induce glomerulonephritis in mice that have been immunized with human MPO (24). Additional animal models not only would help confirm the pathogenic potential of ANCA but also would shed additional light on details of the pathogenic mechanism and provide additional options for future research, including research into the efficacy of treatment strategies.

In this report, we describe two new approaches for modeling human ANCA disease that use transplantation of Mpo+/+ BM into Mpo−/− mice. One approach involves active induction of circulating anti-MPO antibodies in Mpo−/− mice by preimmunization with MPO before transplantation of MPO-positive BM. The other approach involves passive intravenous injection of anti-MPO antibodies into chimeric mice that have circulating MPO-positive BM-derived cells but are otherwise Mpo−/−.

When recipient mice are preimmunized with MPO, the presence of anti-MPO antibodies did not prevent successful engraftment with MPO-positive BM because preimmunized and non-immunized MPO-deficient mice had similar engraftment with MPO-positive BM (Figures 1 and 2). It is interesting that preimmunized mice developed a slight increase in the peripheral neutrophil count compared with nonimmunized counterparts. This likely was the consequence of the inflammatory events that are involved with the developing ANCA disease process and thus would be analogous to observations in patients who have ANCA disease and develop an elevated peripheral neutrophil count (25).

Engraftment with donor MPO-positive BM depended on the dosage of irradiation. Engraftment was more successful at higher irradiation dosages than at lower dosages (Table 1, Figure 2A). The efficacy of engraftment and the resultant proportion of peripheral blood neutrophils that were MPO positive correlated with the induction of glomerulonephritis and systemic vasculitis (Table 1). All mice that received the highest irradiation dosages (900 or 750 rad) and had approximately 90% circulating MPO-positive neutrophils developed glomerulonephritis, and most had evidence for systemic vasculitis, especially pulmonary capillaritis. A total of 71% of mice that received 600 rad and had approximately 50% circulating MPO-positive neutrophils developed glomerulonephritis; however, when present, the glomerulonephritis was less severe as determined by urinalysis and histopathology (Figure 3). None of the mice that received 450 rad and had only 16.4% circulating MPO-positive neutrophils developed glomerulonephritis, although there was a slight increase in glomerular staining for MPO (Table 3) that probably reflected slight subclinical neutrophil accumulation.

There was no difference in the anti-MPO antibody titer among the preimmunized experimental Mpo−/− mouse groups that received different dosages of radiation (Figure 2D). Thus, the differences in disease induction correlate directly with the availability of MPO-positive neutrophils in the circulation to act as pathogenic targets for anti-MPO antibodies. Mpo−/− mice that were not preimmunized with MPO did not develop circulating anti-MPO antibodies (Figure 2D) and did not develop glomerulonephritis (Figure 3) even though they had excellent engraftment with approximately 90% MPO-positive peripheral blood neutrophils. This documents the requirement for anti-MPO antibodies for disease induction in this model. The basis for this failure to mount an immune response against the MPO in the engrafted BM is not known. A likely possibility is that the irradiation caused so much ablation to the immune system that it did not recover during the experimental interval. Alternatively, sequestration of the MPO antigen within neutrophils and monocytes and their precursors may have reduced immunogenicity, although this is unlikely because of the degranulation that occurred at the sites of inflammation. Another, even less likely possibility is tolerization of the reemerging immune system by exposure to high levels of MPO from the engrafted BM. The relatively stable level of anti-MPO antibodies in the preimmunized mice (Figure 2D) suggests that these mice also did not develop an augmented response to the engrafted MPO-positive cells but rather that the anti-MPO antibodies were derived from residual circulating IgG, persistent plasma cells that survived irradiation, or both. Future studies will investigate events at later time points and will include additional booster immunizations in an attempt to
perpetuate or augment the anti-MPO immune response. This may allow the development of a model of long-term ANCA disease that will be a better mimic for human disease and a better model for testing therapeutic regimens.

The most common hypothesis about anti-MPO antibody–induced vasculitis is that the disease is caused by binding of ANCA to neutrophils, with subsequent activation leading to adherence to and injury of vessel walls (2,3). Activation by ANCA of monocytes, which also contain MPO and PR3, may contribute to disease induction, but neutrophils seem to be the dominant effector cells (18). Some investigators, however, have raised the possibility that other cells, such as endothelial cells, renal epithelial cells, or pulmonary alveolar cells, might produce ANCA antigens and could be targets for pathogenic events (13–16). We used two approaches to document the importance of circulating MPO-positive neutrophils in the pathogenesis of ANCA and to rule out a role for other cell types. One approach generated circulating anti-MPO antibodies by active immunization with MPO, and the other approach introduced circulating anti-MPO by passive intravenous injection. Both approaches caused pauci-immune NCGN in mice that had MPO only in BM-derived cells. This demonstrated that Mpo+/ BM-derived cells, primarily neutrophils, are sufficient pathogenic targets for anti-MPO to cause disease. In contrast, generation of circulating anti-MPO antibodies either by active immunization with MPO or by passive injection of anti-MPO IgG did not cause glomerulonephritis in mice that had active immunization with MPO or by passive injection of anti-MPO antibodies. This demonstrated that Mpo+/ BM-derived cells are required pathogenic targets for anti-MPO to cause disease.

Conclusion

These novel animal models further confirm that anti-MPO antibodies cause pauci-immune NCGN and systemic SVV. Furthermore, the data indicate that MPO-positive neutrophils are sufficient and necessary pathogenic targets for the induction of glomerulonephritis and systemic SVV by anti-MPO antibodies.

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