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The Role of Neutrophils in the Induction of Glomerulonephritis by Anti-Myeloperoxidase Antibodies

Hong Xiao,* Peter Heeringa,[†] Zhi Liu,[‡]
Dennis Huugen,[†] Peiqi Hu,[‡] Nobuyo Maeda,*
Ronald J. Falk,[§] and J. Charles Jennette*

From the Departments of Pathology and Laboratory Medicine,
Dermatology,[†] and Medicine,[§] University of North Carolina at
Chapel Hill, Chapel Hill, North Carolina; and the Department of
Clinical and Experimental Immunology,[‡] Cardiovascular
Research Institute Maastricht, University Maastricht,
The Netherlands*

In humans, circulating anti-neutrophil cytoplasm autoantibodies (ANCAs) with specificity for myeloperoxidase (MPO) are strongly associated with the development of pauci-immune necrotizing and crescentic glomerulonephritis (NCGN). In mice, we have demonstrated that intravenous injection of mouse antibodies specific for mouse MPO induces NCGN that closely mimics the human disease. We now report that the development of NCGN in this experimental model is accompanied by glomerular accumulation of neutrophils and macrophages. Neutrophil infiltration was most conspicuous at sites of glomerular necrosis and crescent formation, with macrophages also most numerous in crescents. Lymphocytes, however, were sparse in acute lesions. Importantly, mice that were depleted of circulating neutrophils with NIMP-R14 rat monoclonal antibodies were completely protected from anti-MPO IgG-induced NCGN. These findings provide direct evidence that neutrophils play a major role in the pathogenesis of anti-MPO-induced NCGN in this animal model and implicate neutrophils in the induction of human ANCA disease. This raises the possibility that therapeutic strategies to reduce circulating neutrophils could be beneficial to patients with ANCA-induced NCGN. (*Am J Pathol* 2005, 167:39–45)

Anti-neutrophil cytoplasm autoantibodies (ANCAs) are specific for constituents of the primary granules of neutrophils and the peroxidase-positive lysosomes of

monocytes.¹ The two major antigen specificities are for myeloperoxidase (MPO-ANCA) and proteinase 3 (PR3-ANCA).^{1–3} ANCAs are found in 80 to 90% of patients with necrotizing and crescentic glomerulonephritis (NCGN) that is characterized immunohistologically by the absence or paucity of immunoglobulin in vessel walls (ie, pauci-immune NCGN).^{1,4,5} ANCA NCGN is the most common form of aggressive glomerulonephritis and often is accompanied by a pauci-immune systemic necrotizing small vessel vasculitis, such as microscopic polyangiitis or Wegener's granulomatosis.^{4–6}

Numerous observations suggest that neutrophils are important effector cells in the pathogenesis of human ANCA NCGN. In renal biopsies from patients with ANCA NCGN, activated neutrophils are present in affected glomeruli and in the renal interstitium.⁷ The number of activated intraglomerular neutrophils correlates with the severity of renal injury as reflected in serum creatinine levels.⁷ *In vitro*, ANCAs can activate cytokine-primed neutrophils, causing an oxidative burst, degranulation, release of inflammatory cytokines, and damage to endothelial cells.^{8,9} Recently, our laboratory developed an experimental animal model of NCGN that involves the adoptive transfer of mouse anti-MPO lymphocytes into immune-deficient mice or the passive infusion of mouse anti-MPO IgG into either immune-deficient or immune-competent mice.¹⁰ The resulting NCGN has remarkable pathological similarity to human ANCA glomerulonephritis.

In the current study, we investigated the hypothesis that neutrophils are key effector cells in the pathogenesis of MPO-ANCA-mediated NCGN in this experimental model. The results show that anti-MPO-induced NCGN is associated with the accumulation of neutrophils and macrophages at sites of glomerular injury, and that neu-

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Address reprint requests to J. Charles Jennette, Department of Pathology and Laboratory Medicine, 303 Brinkhous-Bullitt Building, University of North Carolina, Chapel Hill, NC 27599-7525. E-mail: jcj@med.unc.edu.

trophil-depleted mice are protected from induction of NCGN by anti-MPO IgG. Taken together, these results indicate that neutrophils play a major role in anti-MPO-induced NCGN in this model.

Materials and Methods

Mice

Breeding pairs of 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 lacking MPO (MPO^{-/-} mice) were the sixth-generation progeny of a backcross into B6 mice originally generated by Aratani and colleagues.¹¹ MPO^{-/-} mice (8 to 10 weeks old) were used for immunization and as donors of anti-MPO antibodies. Wild-type (WT) B6 mice (9 to 10 weeks old) were used as recipients for passive transfer experiments. The University of North Carolina Institutional Animal Care and Use Committee approved all animal experiments.

Preparation of Pathogenic Mouse Anti-Murine MPO IgG and Control Mouse Anti-Bovine Serum Albumin (BSA) IgG

The purification of mouse MPO and the immunization of MPO^{-/-} mice were performed as previously described.¹⁰ Briefly, mouse MPO was purified from WEHI-3 cells by Dounce homogenization, Concanavalin A affinity chromatography, ion exchange, and gel filtration chromatography. MPO^{-/-} mice were immunized intraperitoneally with 10 μ g of purified murine MPO or BSA in complete Freund's adjuvant. Development of antibodies was monitored by anti-MPO enzyme-linked immunosorbent assay. The presence of circulating anti-MPO antibodies was confirmed in selected animals by indirect immunofluorescence microscopy assay on murine neutrophils as described.¹⁰ The IgG fraction was isolated from the serum of MPO^{-/-} mice immunized with murine MPO by 50% ammonium sulfate precipitation and protein G affinity chromatography as previously described.¹⁰ The IgG fractions were concentrated, sterilized by ultrafiltration, and the protein concentrations were determined by Coomassie protein assay reagent kit (Pierce, Rockford, IL). The purity of the isolated antibodies was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Induction of Anti-MPO Disease

Two different regimens were used to produce anti-MPO-induced glomerulonephritis. Studies of the effects of neutrophil depletion on induction of glomerulonephritis used one intravenous injection of 50 μ g/g body weight of anti-MPO IgG in phosphate-buffered saline (PBS) on day 0 with sacrifice on day 6. Control mice received the same dose of anti-BSA or no IgG. For more detailed evaluation of glomerular infiltration by neutrophils, a more severe

expression of disease was induced by a double dose of anti-MPO IgG. The first intravenous 50 μ g/g body weight dose of anti-MPO IgG was administered on day 0, the second dose was administered on day 3, and the mice were sacrificed on day 6. The control group of B6 mice for this study received the same amount of anti-BSA IgG on days 0 and 3. Induction of circulating anti-MPO was monitored in all mice by anti-MPO enzyme-linked immunosorbent assay.¹⁰

Laboratory and Pathological Evaluation of Disease Induction

Five days after injection of anti-MPO and 1 day before sacrifice, mice were placed in metabolic cages for 12 hours to collect urine for analysis. Urine was tested by dipstick for hematuria, proteinuria, and leukocyturia (Roche Diagnostics Corp., Indianapolis, IN). A reference range was established from the urine analysis of 305 normal B6 mice, which had hematuria 0.1 ± 0.4 , proteinuria 1.0 ± 0.1 , and leukocyturia 0.0 ± 0.1 . Thus, using the mean plus 2 SDs for the reference range, abnormal hematuria was set at >0.9 , proteinuria >1.2 , and leukocyturia >0.2 . Mice were euthanized on day 6 with methoxyflurane. At the time of postmortem examination, samples of kidney were fixed in 10% formalin and processed for light microscopy, or snap-frozen and processed for immunofluorescence microscopy. For light microscopy, specimens were stained with hematoxylin and eosin, periodic acid-Schiff, and Masson trichrome stains. For immunofluorescence microscopy to detect glomerular localization of immune determinants, frozen sections were stained with fluoresceinated antibodies specific for mouse IgG, IgM, IgA, C3, and MPO (ICN/Cappel, Aurora, OH). For detection of leukocytes in frozen tissue, sections were stained with rat antibodies to neutrophils (anti-Gr-1, clone RB6-8C5; BD Pharmingen, Franklin Lakes, NJ), monocytes/macrophages (anti-F4/80 clone A3-1, Research Diagnostics, Flanders, NJ; and anti-CD68, clone FA11, Serotec, Raleigh, NC), and lymphocytes (anti-CD3, clone KT3; Beckman Coulter, Fullerton, CA). Rat antibody binding was detected using peroxidase-labeled secondary rabbit anti-rat IgG and tertiary goat anti-rabbit IgG antibodies (DAKO, Carpinteria, CA). Staining was generated with 3-amino-9-ethylcarbazole and hydrogen peroxide. Sections were counterstained with hematoxylin. Leukocyte localization was expressed as the average leukocytes per cross-section of glomeruli based on evaluating an average of 48 glomeruli per specimen (range, 30 to 80 glomeruli). For more detailed evaluation of neutrophil infiltration in the mice that received two doses of anti-MPO, immunostaining for neutrophils was performed on 5- μ m paraffin sections of kidney using a biotin-labeled rat monoclonal anti-mouse neutrophil antibody NIMP-R14 (Cedarlane Laboratories Ltd., Ontario, Canada) with detection achieved with peroxidase-conjugated streptavidin (BioGenex, San Ramon, CA) and a DAKO Liquid DAB+ substrate-chromogen system (DAKO, Carpinteria, CA). A kidney from each of six mice that received two doses of anti-MPO was evaluated at two levels of section

Figure 1. Neutrophil depletion by NIMP-R14. B6 mice were injected either with 1 mg of NIMP-R14 rat anti-murine neutrophil monoclonal antibody ($n = 7$) (open circles) or control rat IgG ($n = 6$) (filled diamonds). Circulating neutrophils were quantified at different time points by cell counting of blood smears stained with Diff-Quik. Data are expressed as percentage of neutrophils in the blood. A single dose of the NIMP-R14 caused severe neutropenia in mice for more than 5 days.

at least 50 μm apart for a total of 12 levels. Similarly, one kidney from six mice that received two doses of anti-BSA was evaluated at two levels of section for a total of 12 levels. Neutrophil accumulation was quantified by direct counting of stained cells in glomeruli at each of the levels of section. Results were expressed as the number of glomeruli with positive staining for neutrophils, the average number of neutrophils per positive glomerular cross-section, and the average number of neutrophils per all glomerular cross-sections.

In Vivo Kinetics of Circulating Neutrophils after Injected Anti-Neutrophil Antibodies

To evaluate the kinetics of neutrophil depletion, B6 mice ($n = 7$) were injected intraperitoneally with 1 mg of the monoclonal rat anti-murine neutrophil antibody, NIMP-

R14, in 0.5 ml of PBS. NIMP-R14 selectively depletes mouse neutrophils *in vivo*.^{12–14} The control groups ($n = 6$) received rat IgG (1 mg of IgG in 0.5 ml of PBS). Neutrophil depletion was assessed before injection and on day 1, 2, 3, 4, 5, and 6 after antibody injection by direct cell counting of peripheral blood smears stained with Diff-Quik Giemsa Stain Set (Dade Behring Inc., Newark, DE).

Effect of Neutrophil Depletion on the Induction of Glomerulonephritis by Anti-MPO IgG

B6 mice ($n = 6$) were injected intraperitoneally with 1 mg of NIMP-R14 monoclonal antibody in 0.5 ml of PBS. The control groups ($n = 6$) received the same amount of control rat IgG. Both experimental and control mice received 50 $\mu\text{g/g}$ body weight of anti-mouse MPO IgG by intravenous injection 16 hours after receiving the anti-neutrophil antibodies. The effect of NIMP-R14 on peripheral blood leukocytes was determined by differential cell counting of neutrophils, monocytes, and lymphocytes in Diff-Quik Giemsa-stained peripheral blood smears. Introduction of circulating anti-MPO was monitored by anti-MPO enzyme-linked immunosorbent assay. The mice were sacrificed on day 6 and kidney tissue processed for light and immunofluorescence microscopy.

Statistical Analysis

Ranked analysis of variance and Kruskal-Wallis tests were used to evaluate differences across groups, with differences between specific groups evaluated within the ranked analysis of variance test.

Results

Depletion of Circulating Neutrophils after Injection of NIMP-R14 Monoclonal Antibodies

Within 1 day after a single injection of 1 mg of NIMP-R14 monoclonal antibody in 0.5 ml of PBS into B6 mice ($n = 7$), the number of circulating neutrophils was dramatically reduced from 14% of white blood cells to 1%, and re-

Figure 2. Neutrophil depletion prevents anti-MPO antibody-induced necrotizing and crescentic glomerulonephritis. B6 mice were pretreated with the neutrophil-depleting antibody NIMP-R14 ($n = 6$) or control rat IgG ($n = 6$). Sixteen hours later, the mice received the pathogenic anti-MPO IgG (50 $\mu\text{g/g}$ body weight). Mice were sacrificed for pathological examination 6 days after receiving anti-MPO IgG. All mice that received neutrophil-depleting antibodies and anti-MPO IgG had normal glomeruli by light microscopy (left). In contrast, all mice that received control rat IgG and anti-MPO IgG developed segmental fibrinoid necrosis (long arrow, middle, and arrow, right) and cellular crescents (short arrow, middle). Periodic acid-Schiff stain.

Table 1. Pathologic Findings in Mice that Received One Injection of Anti-MPO IgG, Anti-MPO IgG after Depletion of Neutrophils, Anti-BSA IgG, or No Injection of IgG

Group	Cres (%)	Nec (%)	IgG (0 to 4+)	IgM (0 to 4+)	IgA (0 to 4+)	C3 (0 to 4+)	MPO (0 to 4+)	PMN (Gr-1)	Mac (F4/80)	Mac (CD68)	Lym (CD3)
Anti-MPO	11.5 ±2.8	5.8 ±1.1	0.5 ±0.0	0.5 ±0.0	0.2 ±0.3	0.3 ±0.3	0.0 ±0.0	0.41* ±0.11	0.86* ±0.05	0.80* ±0.22	0.10 ±0.03
Anti-MPO and PMN depletion	0	0	0.5 ±0.0	0.6 ±0.2	0.1 ±0.2	0.4 ±0.2	0.0 ±0.0	0.14 ±0.06	0.07 ±0.03	0.08 ±0.02	0.06 ±0.04
Anti-BSA	0	0	0.5 ±0.0	0.50 ±0.0	0.2 ±0.3	0.5 ±0.0	0.0 ±0.0	0.11 ±0.04	0.08 ±0.05	0.09 ±0.04	0.07 ±0.03
B6 control	0	0	0.7 ±0.3	0.6 ±0.2	0.2 ±0.3	0.5 ±0.0	0.0 ±0.0	0.08 ±0.04	0.07 ±0.03	0.09 ±0.04	0.08 ±0.02

* $P < 0.002$.

Pathologic findings in mice that received one dose of anti-MPO IgG (anti-MPO), anti-BSA IgG (anti-BSA), or no injection (B6 control). One set of mice had depletion of neutrophils with NIMP-R14 before receipt of anti-MPO IgG (anti-MPO and PMN depletion). Crescent formation (Cres) and necrosis are expressed as the percentage of glomeruli affected. Immunofluorescence microscopy staining of glomeruli is expressed as the mean intensity of staining for IgG, IgM, IgA, C3, and MPO on a scale of 0 to 4+. Immunoenzyme microscopy to detect glomerular neutrophils (PMNs), monocytes, and macrophages (Mac) and lymphocytes (Lym) is expressed as average number of leukocytes per glomerular cross section.

maintained at this low level for up to 5 days. Thereafter, neutrophils gradually returned toward normal (Figure 1). Control mice ($n = 6$) injected with the same volume of control IgG exhibited normal levels of circulating neutrophils.

Prevention of Anti-MPO IgG-Induced NCGN by Neutrophil Depletion

To directly determine whether neutrophils are required for MPO-ANCA-mediated NCGN, B6 mice ($n = 6$) were pretreated with a single intraperitoneal injection of neutrophil-specific NIMP-R14 monoclonal antibody (1 mg of IgG in 0.5 ml of PBS) before injection of anti-MPO IgG. A differential leukocyte count of Giemsa-stained blood smears 16 hours after the injection of NIMP-R14 antibody revealed $1.1 \pm 0.4\%$ neutrophils, $1.1 \pm 0.4\%$ monocytes, and $97.8 \pm 0.6\%$ lymphocytes. In contrast, control mice had $14.0 \pm 4.4\%$ neutrophils, $1.4 \pm 0.7\%$ monocytes, and $84.6 \pm 4.3\%$ lymphocytes. The difference between the two groups was statistically different for neutrophils ($P < 0.0001$) and lymphocytes ($P < 0.0001$) but not for monocytes ($P > 0.2$).

Sixteen hours after injection of either anti-neutrophil (NIMP-R14) or control IgG, mice received an intravenous injection of anti-MPO IgG. After 5 days, mice injected with anti-MPO IgG without neutrophil depletion developed urine abnormalities consistent with glomerulonephritis, ie, 2.5 ± 0.5 hematuria, 1.9 ± 0.55 proteinuria, and 0.8 ± 0.8 leukocyturia. In contrast, mice that were depleted of circulating neutrophils by pretreatment with NIMP-R14 anti-neutrophil antibodies had urine analysis results that were within the reference ranges, ie, 0.2 ± 0.4 hematuria, 1.0 ± 0 proteinuria, and 0 ± 0 leukocyturia. Levels of circulating anti-MPO IgG in the neutrophil-depleted mice were similar to those found in the control mice (2.02 ± 0.12 in neutrophil-depleted mice with no NCGN versus 1.78 ± 0.72 in anti-MPO mice with NCGN ($P = 0.25$).

All mice that were pretreated with normal rat IgG before receiving anti-MPO rather than with NIMP-R14 developed focal glomerular necrosis (mean, $5.8 \pm$

1.1% of glomeruli with necrosis) and glomerular crescents (mean, $11.5 \pm 2.8\%$ of glomeruli with crescents) whereas none of the mice that were pretreated with NIMP-R14 developed glomerular necrosis or crescents (Figure 2 and Table 1). Glomeruli that did not have necrosis or crescents appeared normal by light microscopy with no hypercellularity and no increase in matrix material.

Glomerular immunoglobulin and complement deposition in mice that received anti-MPO alone was absent or sparse and similar to that in mice that received anti-MPO along with neutrophil depletion; and also was similar to glomerular immunoglobulin and complement localization in mice that received anti-BSA IgG or healthy control mice that received no immunoglobulin

Figure 3. Glomerular leukocytes 6 days after injection of a single dose of anti-MPO IgG. The panels show infiltration of an inflamed glomerulus by neutrophils that are scattered throughout the tuft (top left marked with anti-Gr-1), macrophages that are concentrated in the crescent (top right marked with anti-CD68 and bottom right marked with anti-F4/80), and a few lymphocytes (bottom left).

Table 2. Pathologic Findings in Mice that Received Two Injections of Anti-MPO IgG or Anti-BSA IgG

Group	Crescents (%)	Necrosis (%)	% Glomeruli with neutrophils	Neutrophils per positive glomerulus	Neutrophils per all glomeruli
Anti-MPO	12.0 ± 6.1	17.8 ± 7.8	30.27 ± 6.98*	3.08 ± 1.02*	0.97 ± 0.48*
Anti-BSA	0.0	0.0	5.97 ± 2.49	1.06 ± 0.08	0.07 ± 0.03

Mice received two doses of anti-MPO IgG (anti-MPO) or anti-BSA IgG (anti-BSA) on day 0 and day 3 with sacrifice on day 6. Crescent formation and necrosis are expressed as the percentage of glomeruli affected. Immunoenzyme microscopy to detect glomerular neutrophils is expressed as percentage of glomeruli with any neutrophils, average number of neutrophils per cross section of glomeruli with neutrophils, and average number of neutrophils per cross section of all glomeruli.

*Neutrophil infiltration is significantly higher ($P < 0.001$) in the anti-MPO group by all three methods of measuring glomerular infiltration.

(Table 1). Glomerular leukocyte phenotyping demonstrated that mice that received anti-MPO without neutrophil depletion had statistically significant increased glomerular infiltration by neutrophils and monocytes/macrophages but not lymphocytes when compared to control mice that received no immunoglobulin injection.

(Table 1). Neutrophils were most numerous in glomeruli with inflammation and necrosis, and macrophages tended to cluster within crescents (Figure 3). Mice that had neutrophil depletion before receiving anti-MPO IgG and mice that received anti-BSA IgG had no significant increase in neutrophils, monocytes/mac-

Figure 4. Glomerular neutrophil accumulation in B6 mice that received two doses of anti-MPO IgG on day 0 and day 3 followed by sacrifice on day 6. Mice that received anti-BSA IgG had only rare neutrophils in a few glomeruli (not shown). Immunoenzyme microscopy using an antibody specific for mouse neutrophils demonstrated that mice that received anti-MPO IgG had a marked increase in glomerular neutrophils especially at sites of necrosis or crescent formation. **a:** The glomerulus has segmental fibrinoid necrosis (arrow) with brown-staining neutrophils most numerous at the margins of the necrosis. **b:** The glomerular tuft on the left has scattered clusters of neutrophils and the hilar arteriole on the right (arrow) contains aggregated neutrophils. **c:** At the plane of section examined, the glomerular tuft has a few scattered neutrophils and Bowman's space contains numerous neutrophils (arrow). **d:** The glomerulus has extensive fibrinoid necrosis (between arrows) with some residual neutrophils at the periphery in a crescent. Within the necrosis, there are scattered brown granules that probably are neutrophil fragments.

rophages, or lymphocytes when compared to control mice (Table 1).

Glomerular Influx of Neutrophils after Induction of Glomerulonephritis with Two Doses of Anti-MPO IgG

To induce more robust glomerular injury, two doses of anti-MPO were given at day 0 and day 3. These mice developed hematuria (2.6+), proteinuria (2.8+), leukocyturia (1.4+), and elevated blood urea nitrogen (45.2 mg/dl) in contrast to the mice that received two doses of anti-BSA IgG and had no significant elevation in hematuria (0+), proteinuria (0.9+), leukocyturia (0+), or blood urea nitrogen (25.5 mg/dl). The blood urea nitrogen in mice that received anti-MPO was significantly elevated over the blood urea nitrogen in the control mice ($P < 0.04$). By light microscopy, all mice that received anti-MPO IgG had glomerular necrosis (average $17.8 \pm 7.8\%$ of glomeruli involved in each mouse) and crescents (average $12.0 \pm 6.1\%$ of glomeruli involved) whereas mice that received anti-BSA IgG had no renal histological abnormalities. Thus, these mice with two doses of anti-MPO at days 0 and 3 had more severe glomerular injury with a greater degree of necrotizing injury than mice that received only one dose of anti-MPO IgG at day 0. However, even in the mice that received two doses of anti-MPO, most glomeruli had no abnormalities by light microscopy. This is similar to acute focal human ANCA NCGN in which glomeruli that do not have necrosis or crescents often appear histologically normal.

Immunohistological staining of paraffin sections with NIMP-R14 for neutrophils demonstrated that induction of glomerulonephritis by anti-MPO IgG was accompanied by glomerular influx of neutrophils (Table 2 and Figure 4). Although increased neutrophils occurred in some glomeruli that had not yet developed histological lesions, neutrophils were concentrated at sites of segmental necrotizing glomerular injury, and occasionally were identified in Bowman's space in glomeruli with necrosis or crescents, and in a few afferent arterioles (Figure 4). Positively staining fragments of neutrophils were present in some necrotic areas (Figure 4d).

Discussion

In this mouse model of ANCA NCGN, neutrophils and macrophages are present at the sites of acute glomerular injury, and neutrophil depletion completely protects mice from induction of NCGN by anti-MPO IgG even in the presence of levels of circulating anti-MPO IgG that cause NCGN in 100% of mice with normal levels of neutrophils. These observations support the concept that neutrophils are the key effector cells in anti-MPO antibody-induced NCGN.

Numerous *in vitro* studies have documented the ability of human ANCA IgG (ie, both anti-MPO and anti-PR3 IgG) to activate neutrophils with the resultant release of toxic oxygen metabolites, lytic and toxic proteases, nitric

oxide, and inflammatory cytokines.^{15–19} This activation involves increased display of ANCA antigens at the surface of neutrophils facilitating both the attachment of the antigen-binding portion of the autoantibodies as well as the engagement of Fc receptors on the surface of neutrophils.^{20–22} Neutrophils that have been activated by ANCA IgG adhere to and kill endothelial cells *in vivo*^{23–25} and are induced to migrate through endothelial monolayers.²⁶ If these events that have been observed repeatedly *in vitro* are reproduced *in vivo* by ANCA IgG-induced stimulation on neutrophils, this would provide a clear pathogenic mechanism for the mediation of acute inflammatory vascular injury, including glomerulonephritis and vasculitis, by activation of neutrophils by ANCA IgG.⁸

The experiments reported here provide evidence for an important role for neutrophils in this experimental model, but they do not rule out the participation of monocytes in disease induction. Monocytes express both MPO and PR3 and can be activated by ANCA IgG to release many of the same mediators that are released by neutrophils after activation by ANCA.^{27–30} Under the conditions tested in the current model, any activation of monocytes that may be occurring is not sufficient to produce detectable injury in the absence of neutrophils. However, the immunophenotyping of leukocytes demonstrated the clear participation of macrophages in the necrotizing and especially the crescentic lesions. One possibility is that the initial induction of acute necrotizing injury is primarily mediated by neutrophils, whereas the macrophage infiltration is a component of the innate inflammatory response to the injury and plays a major role in the initiation of crescent formation. The leukocyte phenotyping revealed only a few lymphocytes at the sites of acute injury. This lack of substantial participation of lymphocytes in the acute injury is consistent with the observation that this model of glomerulonephritis can be induced by injecting anti-MPO IgG into immune-deficient mice that lack functional T cells.¹⁰

In summary, our results demonstrate that neutrophils are crucial in the induction of NCGN by anti-MPO antibodies in this experimental model. This supports the possibility that neutrophils are similarly important in human ANCA NCGN. If this is the case, therapeutic strategies that target ANCA-induced neutrophil recruitment and activation could be beneficial in the treatment of ANCA diseases.

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