18 Bach JF. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocrine Reviews 1994; 15: 516-541.
19 Shehadeh N, Calcinaro F, Bradley BJ, Bruchlim I, Vardi P, Lafferty KJ. Effect of adjuvant therapy on development of diabetes in mouse and man. Lancet 1994; 343: 706-707.
20 Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzei G, Calvin D, Annunziata N, Doetschman T. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. Nature 1992; 359: 693-699.
21 Kühn R, Löhler J, Rennick D, Tajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993; 75: 263-274.
22 Florquin S, Goldman M. T cell subsets in glomerular diseases. Springer Semin Immunopathol 1994; 16: 71-80.
23 Pelletier L, Pasquier R, Rossert J, Vial M-C, Mandet C, Druet P. Autoreactive T cells in mercury-induced autoimmunity. Ability to induce the autoimmune disease. J Immunol 1988; 140: 750-754.
24 Saoudi A, Kuhn J, Huygen K, de Kozak Y, Velu T, Goldman M, Druet P, Bellon B. Th2 activated cells prevent experimental autoimmune uveoretinitis, a Th1-dependent autoimmune disease. Eur J Immunol 1993; 23: 3096-3103.
25 Ishida H, Muchamuel T, Sakaguchi S, Menon S, Howard M. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB-W mice. J Exp Med 1994; 179: 305-310.
26 Fossati L, Takahashi S, Merino R, Iwamoto M, Aubry J-P, Nose C, Spach R, Motta R, Izui S. An MRL-MpJ-lpr/lpr substrain with a limited expansion of lpr double-negative T cells and a reduced autoimmune syndrome. Int Immunol 1993; 5: 525-532.
27 Llorente L, Richaud-Patin Y, Flor R, Alcocer-Varela J, Wijdenes J, Morel-Fourrier B, Galanaud P, Emilie D. In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjögren's syndrome and systemic lupus erythematosus: a potential mechanism of B lymphocyte hyperactivity and autoimmunity. Arthritis Rheum 1994; 37: 1647-1655.
28 Llorente L, Zou W, Levy Y, Richaud-Patin Y, Wijdenes J, Alcocer-Varela J, Morel-Fourrier B, Brouet J-C, Alarcon-Segovia D, Galanaud P, Emilie D. Role of interleukin-10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med 1995; in press.
29 Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner H, Hafler DA. Increased frequency of interleukin 2-responsible T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994; 179: 973-984.
30 Panitch HS, Hirsch RL, Schindler J, Johson KP. Treatment of multiple sclerosis with γ interferon. Exacerbations associated with activation of the immune system. Neurology 1987; 37: 1097-1102.
31 Katzidis PD, Chu C-Q, Brennan FM, Maini RN, Feldmann M. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J Exp Med 1994; 179 : 1517-1527.
32 Elliott MJ, Maini RN, Feldmann M, Fox-Long A, Charles P, Katsidis P, Brennan M, Malder J, Bijl H, Ghrayeb J, Woody J. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to TNFα, safety, clinical efficacy and regulation of acute phase response. Arthritis Rheum 1993; 36: 1681-1686.
33 Noël LH, Aucouturier P, Monteiro RC, Preud'homme J-L, Lesavre P. Glomerular and serum immunglobulin G subclasses in membranous nephropathy and anti-glomerular basement membrane nephritis. Clin Immunol Immunopath 1988; 46: 186-194.
34 Oliveira DBG, Lachmann P. Autoimmunity. In Lachmann, J, Peters D, Rosen FS, Walport MJ eds. Clinical aspects of immunology, Oxford, 1993, Blackwell Scientific Publications, pp 717-743.
35 Kallenberg CGM, Mulder AHL, Stegeman CA. Activation of granulocytes by anti-neutrophil cyoplasmic antibodies [ANCA] in Wegener's granulomatosis: a predominant role for the IgG3-subclass. JASN 1994; 5: 832 [Abstract].

Animal models in ANCA-vasculitis

R. KETTRITZ, JIA JIN YANG, K. KINJOH, J. C. JENNETTE & R. J. FALK
Department of Medicine and Pathology, University of North Carolina at Chapel Hill, North Carolina, USA

Anti-neutrophil cytoplasmic autoantibodies (ANCA) have been found in sera of patients with systemic necrotizing small-vessel vasculitis and necrotizing crescentic glomerulonephritis [1,2,5]. A strong association between ANCA and these diseases has been established over the last years [3–5]. Furthermore, results of *in vitro* experiments have shown that ANCA may participate in the pathogenesis of these diseases [6–11]. An animal model of ANCA-induced vasculitis is an essential step in the proof that ANCA are indeed pathogenic.

Koch's postulates for establishing causal relationship were described in the context of anthrax and tuberculosis [12]. Molecular modifications [13,14] of these postulates have been developed for non-infectious diseases. Application of Koch's postulates to ANCA would request that: *1)* ANCA must be isolated from affected subjects, *2)* ANCA must produce disease when transmitted to or induced in healthy animals and the resultant disease should mirror the features of the human disease, *3)* ANCA must be isolated from animals with such an experimentally induced disease.

Koch's postulates have been met by Goodpasture's syndrome, which is an example of a disease caused by antibodies developed against the non-collagenous domain of type IV collagen. Antibodies have been isolated from the circulation and from the affected vessels of patients. Transfer of autoantibody-containing plasma of patients into monkeys induces the characteristic disease [15].

The pathogenic role of ANCA is not established because no animal model exists. What can we learn from existing animal models?

Spontaneous vasculitis in MRL and SCG mice

The first models of spontaneous lupus erythematosus in mice were the New Zealand Black mice (NZB) and the (NZB x W)F1 mice developed by mating NZB with New Zealand White mice (NZW). Since then, different strains of lupus mice have been produced. One of them, the MRL/Mp-lpr/lpr mouse developed by Murphy and Roths [16], serves as an experimental model of human systemic lupus erythematosus [SLE] and rheumatoid arthritis [17,18]. This mouse model provides an example for spontaneous autoimmunity with necrotizing small- and medium-sized vasculitis that is not necessarily caused by immune complexes and is rather comparable with the pauci-immune vasculitis in ANCA-associated diseases. Mice homozygous for the autosomal recessive lpr gene develop excessive lymphoproliferation (lpr), proliferative glomerulonephritis and, in con-

trast to other lupus mice strains, necrotizing vasculitis affecting small- and medium-sized arteries and arterioles. Sequential studies on 170 mice showed fibrinoid necrosis and neutrophil influx in the acute stage of the disease followed by occurrence of lymphocytes and fibroblasts and a chronic reparative stage with infiltration of mononuclear cells and fibroblasts [23]. Kidney (34.6%) and urine bladder (29.8%) were the most frequently affected organs. Immunofluorescence studies revealed the presence of immune complex components in only 6 out of 36 kidneys with necrotizing vasculitis.

What causes the vasculitis in MRL/Mp-lpr/lpr mice? The most characteristic immunological finding is a massive accumulation of T-cells with polyclonal B-cell stimulation resulting in the occurrence of a variety of autoantibodies including antibodies against ds-DNA, ss-DNA, Sm and histones and rheumatoid factors [rev. in 24]. The coexistence of many different autoantibodies makes it almost impossible to reveal a causal relationship.

The murine lpr gene is located on chromosome 19. The product of this gene is identified as a cell surface protein (Fas antigen) belonging to the TNF (tumour necrosis factor)/ NGF (nerve growth factor) receptor family [19,20] and is directly involved in the pathway of apoptosis [20,21,22]. The assumption is that lpr mice have a genomic deletion of parts of the Fas gene leading to a deficiency in apoptosis with accumulation of lymphocytes, some of which are autoreactive, that are normally deleted.

Harper reported that 20% of female MRL/Mp-lpr/lpr mice are positive for ANCA [25]. In this study, murine monoclonal IgG antibodies were produced and tested against different antigens using ELISA and Western blot. All these monoclonal antibodies were polyreactive and bound to myeloperoxidase (MPO), lactoferrin and DNA.

Recently Kinjoh *et al.* established a recombinant inbred mouse strain (spontaneous crescentic glomerulonephritis forming mouse [SCG/Kj]) [27]. These mice were derived from BXSB and MRL/Mp-lpr/lpr strains and have been selected for the highest frequency of crescent formation in the kidneys. After 9 weeks of age, 97% of the animals develop crescent formation; 58% of the females and 34% of the males have crescentic glomerulonephritis with only slight fine granular deposits of immunoglobulins along the glomerular basement membrane. This finding is similar to the pauci-immune necrotizing crescentic glomerulonephritis in human ANCA diseases in that the apparent amount of immune complex deposition is less than would be expected to cause such severe inflammation and necrosis. The SCG/Kj strain is also characterized by a high incidence of systemic vasculitis of small arteries and arterioles. The organs most frequently affected with vasculitis are spleen (37%) followed by ovary, uterus, heart and stomach. In contrast to MRL/Mp-lpr/lpr mice, no vasculitis of the kidneys was found. These mice have P-ANCA with reactivity for MPO. Furthermore monoclonal antibodies developed from these mice show specificity for human MPO. When these hybridomas are transferred into the peritoneum of nude mice, proteinuria occurs; however no histological evidence for glomerulonephritis or vasculitis develops during the few weeks that the animals survive with the hybridomas.

Vasculitis induced by mercuric chloride ($HgCl_2$)

In susceptible strains of rats such as Brown Norway (BN) rats, mercuric chloride induces a T-cell dependent polyclonal B-cell activation [28]. This leads to generation of anti-glomerular basement membrane antibodies with linear [29] and, later, granular pattern [30] of immunofluorescence staining of the glomerular basement membrane. These changes are associated with proteinuria but not with mesangial proliferation by light microscopy. The $HgCl_2$-induced disease is characterized by development of antibodies, including autoantibody production to DNA, collagen and thyroglobulin [30]. An increased IgE level [31] has been reported and is thought to be an indirect marker for the Th2 subpopulation of CD4+ cells. More recently, it was reported that BN rats given $HgCl_2$ develop autoantibodies to MPO as well [32]. Mathieson described histological injuries including a necrotizing vasculitis [33,34]. $HgCl_2$ was given over 10 days and autopsy studies were performed on days 10, 12, 16, and 18 [33]. The histological examination showed changes in multiple organs. The most remarkable finding was a necrotizing vasculitis with a mononuclear infiltrate in submucosal vessels of the gut which was primary and not secondary to mucosal necrosis. It has been shown by the same investigators that the parasite flora of the gut is at least partly responsible for the predominant manifestation in this location. Pretreatment of the animals with antimicrobial drugs diminished the severity of the tissue injury. Anti-MPO antibodies were detectable from day 10, reached a peak on day 12, and later resolved. The authors emphasize the similarities with human systemic vasculitis.

Conflicting results exist as to whether the autoimmune syndrome can be transferred into healthy animals. Pelletier *et al.* [35] have shown that the transfer of autoreactive CD4+ T-cells from rats exposed to $HgCl_2$ can transfer the disease, whereas Quasim *et al.* [36] reported that injecting normal BN rats with serum from rats with $HgCl_2$-associated vasculitis failed to induce disease. The occurrence of many autoantibodies in these animals makes an evaluation of causal significance of any particular antibody difficult.

Anti-MPO associated pauci-immune glomerulonephritis

In 1993, Brouwer *et al.* reported a model for pauci-immune necrotizing crescentic glomerulonephritis in the BN rat [37]. The disease is induced in human MPO-immunized rats by unilateral kidney perfusion with the lysosomal enzyme extract and H_2O_2 5 weeks after immunization. The extract contains MPO with trace amounts of proteinase 3 (PR3) and elastase. MPO was present in a granular pattern along the glomerular basement membrane (GBM) at 4 hours but was no longer present at 24 hours, 4 days and 10 days after perfusion; immunoglobulin (Ig) G and complement were present along the GBM in a granular pattern at 4 and 24 hours, but were scanty or had disappeared completely at 4 and 10 days after perfusion. In contrast, deposits of IgG and C3 were still present in MPO-immunized rats perfused with the lysosomal extract without H_2O_2, MPO and H_2O_2, and MPO alone at 4 and 10 days after perfusion. MPO-immunized rats perfused with the lysosomal enzyme extract and H_2O_2, in contrast to control-immunized and/or control-perfused rats, developed a proliferative glomerulonephritis characterized by intra- and extracapillary cell proliferation, ruptured Bowman's capsule, periglomerular granulomatous inflammation, and formation of giant cells. Monocytes, polymorphonuclear leukocytes (PMN), and to a far lesser extent, T cells were found in the glomeruli. Interstitial infiltrates consisted of monocytes, PMN, and T cells. Granulomatous vasculitis of small vessels was found at 10 days after perfusion. Brouwer *et al.* reported this rat model closely resembled human anti-MPO associated pauci-immune necrotizing crescentic glomerulonephritis.

In 1994, Brouwer reported that after renal perfusion with lysosomal enzymes with H_2O_2, MPO-immunized Lewis rats showed persistence of IgG and C3 deposition along the GBM. At 10 days after perfusion, BN rats showed complete disappearance of IgG and C3 deposits if perfusion was performed at 10 days after immunization, in contrast to IgG and C3 deposits if perfusion was performed at 10 weeks after immunization [38]. Subsequently, Brouwer *et al.* noted that the

perfusion of H_2O_2 could be replaced by ischaemia/reperfusion injury [39].

Yang *et al.* repeated Brouwer's study in spontaneously-hypertensive (SH) rats and BN rats [40]. Rats were immunized with human MPO. When circulating anti-MPO antibodies were detectable by indirect immunofluorescence microscopy and ELISA, blood pressure was measured, then perfusion of the left kidney of each rat was done with either MPO+H_2O_2, MPO, H_2O_2 alone or MPO+H_2O_2+neutral protease. Rats were killed on day 4 or day 10 after perfusion, and specimens were examined by light and immunofluorescence microscopy. Pathological lesions and numerous deposits of IgG, C3 and MPO were found in immunized rats perfused with MPO+H_2O_2 with or without neutral protease, or MPO alone, in both rat strains and on both day 4 and day 10. In the kidneys, a proliferative glomerulonephritis that ranged in severity from mild mesangial hypercellularity to severe inflammation with focal necrosis and occasional cresent formation was seen. Several kidneys also had segmental necrotizing vasculitis affecting arterioles and small arteries. There was focal tubulointerstial injury commensurate with the glomerular injury. Deposits of IgG were predominantly in a diffuse global granular-to-linear pattern, with granular C3 deposits predominantly in capillary walls. There were weak linear basement membrane deposits of MPO in these kidneys. In immunized rats, 93% of the glomeruli that developed proliferative glomerulonephritis had IgG depositions, and 88% of them had C3 depositions. The degree of histological injury was proportional in intensity to the amount of IgG immune deposits. SH rats sustained more damage and higher blood pressure than BN rats. No lesion was observed in immunized rats perfused with H_2O_2 or in the non-perfused right kidneys. Yang *et al.* concluded that this experimental model was of immune complex vasculitis, and did not resemble human ANCA-associated pauci-immune disease.

Aggravation of Masugi nephritis by MPO-ANCA in a rat model for anti-GBM antibody-induced glomerulonephitis

Kobayashi and colleagues reported exacerbation of anti-GBM disease with anti-rat MPO antibodies [41]. Histological studies performed after 3 and 15 hours focused on the number of polymorphnuclear leukocytes per glomerulus, fibrin accumulation and the distribution of rat MPO in the affected kidneys. The authors showed that the administration of MPO-ANCA followed by anti-GBM antibodies increases neutrophil influx and fibrin accumulation. Furthermore they revealed by direct immunofluorescence a deposition of rat MPO along the glomerular basement membrane exclusively in the group that received both autoantibodies. Only the kidney eluate of this group contained anti-MPO antibodies. The findings suggest aggravation of Masugi nephritis by anti-MPO ANCA. This could be explained by the in vitro observation that ANCA-induced neutrophil activation requires a synergistic neutrophil priming process, which in the Kobayashi model could be the anti-GBM-induced inflammation.

Summary

The animal models described to date suggest pathogenicity of ANCA. Unfortunately, none of the models unequivocally proves that ANCA are pathogenic. Further search for a suitable animal model to document or exclude causal significance of ANCA in vasculitis is needed.

References

1 Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotizing glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? Br Med J 1982; 285:606.

2 Jennette JC, Falk RJ. Antineutrophil cytoplasmic autoantibodies and associated diseases: a review. Am J Kidney Dis 1990; 15: 517-529.

3 van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA, van der Giessen M, van der Hem GK, The TH. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985; i: 425-429.

4 Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N Engl J Med 1988; 318: 1651-1657.

5 Kallenberg CGM, Brouwer E, Weening JJ, Cohen Tervaert JW. Anti-neutrophil cytoplasmic antibodies: Current diagnostic and pathophysiological potential. Kidney Int 1994; 46: 1-15.

6 Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. Proc Natl Acad Sci USA 1990; 87: 4115-4119.

7 Lai KN, Lockwood CM. The effect of anti-neutrophil cytoplasm autoantibodies on the signal transduction in human neutrophils. Clin Exp Immunol 1991; 85: 396-401.

8 Porges AJ, Redecha PB, Kimberly WT, Csernok E, Gross WL, Kimberly RP. Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via FcgammaRIIa. J Immunol 1994; 153: 1271-1280.

9 Mulder AHL, Heeringa P, Brouwer E, Limburg PC, Kallenberg CGM. Activation of granulocytes by anti-neutrophil cytoplasmic antibodies[ANCA]: a FcgammaRII-dependent process. Clin Exp Immunol 1994;

10 Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. Kidney Int 1992; 41: 375-383.

11 Savage CO, Pottinger BE, Gaskin G, Pusey CD, Pearson JD: Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity towards cultured endothelial cells. Am J Pathol 1992; 141: 335-342.

12 Carter KC. Essays of Robert Koch, Greenwood Press, Westport, Conneticut,1987.

13 Falkow S. Molecular Koch's postulates applied to microbial pathogenicity. Rev Infect Dis 1988; 10 (Suppl.2): S274-S276.

14 Hall PA, Lemoine NR. Koch's postulates revisited. J Pathol 1991; 164: 283-284.

15 Lerner RA, Glassock RJ, Dixon FJ. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. J Exp Med 1967; 126: 989-1004.

16 Murphy E, Roths J. A single gene model for massive lymphoproliferation with immune complex disease in the new mouse strain MRL. Proceedings of the 16th International Congress on Hematology, Kyoto, Japan 1976, pp. 69-72.

17 Hang L, Theofilopoulos AN, Dixon FJ. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. J Exp Med 1982; 155: 1690-1701.

18 Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, McConahey PJ, Murphy ED, Roths JB, Dixon FJ. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. J Exp Med 1978; 148: 1198-1215.

19 Watanabe-Fukunaba R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice by defects in Fas antigen that mediates apoptosis. Nature 31992; 56: 314-317.

20 Itoh N, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991; 66: 233-243.
21 Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasuagai T, Kitamura Y. Lethal effect of the anti-Fas antibody in mice. Nature 1993; 364: 806-809.
22 Yoshino T, Kondo E, Cao L, Takahashi K, Hayashi K, Nomura S. Inverse expression of bcl-2 protein and Fas-antigen in lymphoblasts in peripheral lymph nodes and activated peripheral blood T and B lymphocytes. Blood 1994; 83: 1856-1861.
23 Hewicker M, Trautwein G. Sequential study of vasculitis in MRL mice. Lab Anim 1987; 21: 335-341.
24 Cohen PL, Eisenberg RA. Lpr and gld: Single gene models of systemic autoimmunity and lymphoproliferative disease. Ann Rev Immunol 1991; 9: 243-269.
25 Harper JM, Lockwood CM, Cooke A. Anti-neutrophil cytoplasm antibodies in MRL-lpr/lpr mice. Clin Exp Immunol 1993; 93 (Suppl.1): 22.
26 Moyer CF, Reinisch CL. The role of vascular smooth muscle cells in experimental autoimmune vasculitis. I. The initiation of delayed type hypersensitivity angiitis. Am J Pathol 1984; 117: 380-390.
27 Kinjoh K, Kyoguko M, Good R. Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. Proc Natl Acad Sci USA 1993; 90: 3413-3417.
28 Hirsch F, Coudere J, Sapin C, Fournier G, Druet P. Polyclonal effect of HgCl2 in the rat, its possible role in an experimental autoimmune disease. Eur J Immunol 1982; 12: 620-625.
29 Sapin C, Druet E, Druet P. Induction of anti-glomerular basement membrane antibodies in the Brown-Norway rat by mercuric chloride. Cin Exp Immunol 1977; 28: 173-179.
30 Pusey CD, Browman C, Morgan A, Weetman AP, Hartley B, Lockwood CM. Kinetics and pathogenicity of autoantibodies induced by mercuric chloride in the brown Norway rat. Clin Exp Immunol 1990; 81: 76-82.
31 Prouvost-Danon A, Abadie A, Sapin C, Bazin H, Druet P. Induction of IgE synthesis and potentiation of anti-albumin IgE antibody response by HgCl2 in the rat. J Immunol 1981; 126: 699.
32 Esnault VLM, Maathieson PW, Thiru S, Oliveira DB, Lockwood CM. Autoantibodies to myeloperoxidase in brown Norway rats treated with mercuric chloride. Lab Invest 1992; 67: 114-120.
33 Mathieson PW, Thiru S, Oliveira DBG. Mercuric chloride-treated brown Norway rats develop widespread tissue injury including necrotizing vasculitis. Lab Invest 1992; 67: 121-129.
34 Quasim FJ, Mathieson PW, Thiru S, Oliveira DBG. Cyclosporin A exacerbates mercuric chloride-induced vasculitis in the brown Norway rat. Lab Invest 1995; 72: 183-190.
35 Pelletier L, Pasquier R, Rossert J, Vial M-C, Mandet C, Druet P. Autoreactive T cells in mercury-induced autoimmunity. J Immunol 1988; 140: 750-754.
36 Quasim FJ, Mathieson PW, Thiru S, Oliveira DBG, Lockwood CM. Further characterization of an animal model of systemic vasculitis. In: Groos WL ed. ANCA-Associated Vasculitides: Immunological and Clinical Aspects. New York, Plenum Press, 1993, p. 133.
37 Brouwer E, Huitema MG, Klok PA, Weerd HD, Tervaert JWC, Weening JJ, Kallenberg CGM. Anti-myeloperoxidase-associated proliferative glomerulonephritis: an animal model. J Exp Med 1993; 177: 905-914.
38 Brouwer E, Klok PA, Huitema MG, Limburg PC, Weening JJ, Kallenberg CGM. Strain differences in immune responsiveness between Brown Norway and Lewis rats; implications for the animal model of anti-myeloperoxidase associated glomerulonephritis. Submitted.
39 Brouwer E, Klok PA, Huitema MG, Weening JJ, Kallenberg CGM. Renal ischemia/reperfusion injury contributes to renal damage in rat model of anti-myeloperoxidase associated proliferative glomerulonephritis. Submitted.
40 Yang JJ, Jennette JC, Falk RJ. Immune complex glomerulonephritis is induced in rats immunized with heterologous myeloperoxidase. Clin Exp Immunol 1994; 97:466-473.
41 Kobyashi K, Shibata T, Sugisaki T. Aggravation of rat Masugi nephritis by heterologous ant-rat myeloperoxidase [MPO] antibody. Clin Exp Immunol 1993; 93 (Suppl 1): 20.

The diversity of perinuclear antineutrophil cytoplasmic antibodies (pANCA) antigens

A. WIIK[1], L. STUMMANN[1], L. KJELDSEN[2], N. BORREGAARD[2], S. ULLMAN[3], S. JACOBSEN[4] & P. HALBERG[4]
[1]Department of Autoimmunology, Statens Seruminstitut, Artillerivej 5, [2]Department of Haematology and [3]Dermatology, Rigshospitalet, Blegdamsvej 9, [4]Department of Rheumatology, Hvidovre Hospital, Copenhagen, Denmark

Autoantibodies to human polymorphonuclear granulocytes (PMNs) giving rise to a perinuclear/nuclear fluorescence pattern using indirect immunofluorescence (IIF) technique and smears of leucocytes as substrate have been known to exist since 1959 [reviewed in 1]. These autoantibodies were first described in patients with leucopenia, often as part of Felty's syndrome, and later in the majority of patients with active rheumatoid arthritis (RA), some patients with systemic lupus erythematosus (SLE), and most patients with drug-induced lupus (DIL) caused by treatment with hydralazine and procainamide [1]. Further studies also disclosed such autoantibodies in ulcerative colitis (UC) [2].

Because of the perinuclear/nuclear reactivity of these autoantibodies they were initially termed granulocyte-specific anti-nuclear antibodies (GS-ANA) [1]. In the First International Workshop on ANCA in Copenhagen, Falk and Jennette drew the attention to the fact that certain cationic proteins from PMN lysosomes would redistribute to the perinuclear area upon dissolution of granule membranes by ethanol fixation causing an artefactual antigen distribution [3]. This pattern was thus commonly seen with anti-cytoplasmic antibodies (ANCA) recognizing myeloperoxidase (MPO) [3,4,5]. Since the great interest arose from the detection of ANCA in Wegener's granulomatosis (WG) [6,7,8], and not from anti-neutrophil antibodies in rheumatologic patients [1], a discussion on nomenclature for these antibodies led to the proposal to use the term pANCA for antibodies giving rise to a perinuclear/nuclear IIF pattern and cANCA for antibodies giving rise to a cytoplasmic IIF pattern [9]. This review will use the term pANCA even if some anti-neutrophil antibodies may be targeting true nuclear antigens in RA (Wiik, Gross, Kallenberg, personal communication). The vasculitis nomenclature used here is the one proposed by the Chapel Hill Consensus Conference [10].