SPECIFICITY OF ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODIES FOR PROTEINASE 3

To the Editor:

Niles et al[1] recently reported that patients with Wegener's granulomatosis have anti-neutrophil cytoplasmic autoantibodies (ANCA) that are specific for a 29-Kd neutrophil serine proteinase. They noted that ANCA with this specificity produce cytoplasmic indirect immunofluorescence microscopy staining of alcohol-fixed neutrophils (ie, are C-ANCA using the nomenclature adopted at the Second International ANCA Workshop held in The Netherlands, May 1989). We have shown that a second major category of ANCA (ie, P-ANCA) produces perinuclear staining of alcohol-fixed neutrophils, and most often have specificity for myeloperoxidase (MPO).[2,3]

Recently, Ludemann et al[4] reported data indicating that C-ANCA are specific for a serine proteinase that has biochemical properties like those of proteinase 3 (PR3), which is a neutrophil constituent that has been characterized by Kao et al.[5] Niles et al[1] also raised the possibility that their 29-Kd protein is identical with PR3, as did Goldschmeding et al.[6] We have used purified PR3 and monoclonal antibodies specific for PR3 (both produced by Dr John Hoidal) to show that the neutrophil serine proteinase with which C-ANCA react is in fact PR3.

As reported by Niles et al,[1] Goldschmeding et al,[6] and Ludemann et al,[4] using Western blot analysis, we observed the reactivity of some C-ANCA sera with an approximately 29-Kd fraction of neutrophil cytoplasm (29-Kd ANCA). MPO-specific P-ANCA (MPO-ANCA) did not react with the 29-Kd band, but did react with bands corresponding to the position of MPO in Western blot electrophoretograms.

We have previously used an enzyme-linked immunosorbent assay (ELISA) with MPO as antigen to demonstrate the specificity of

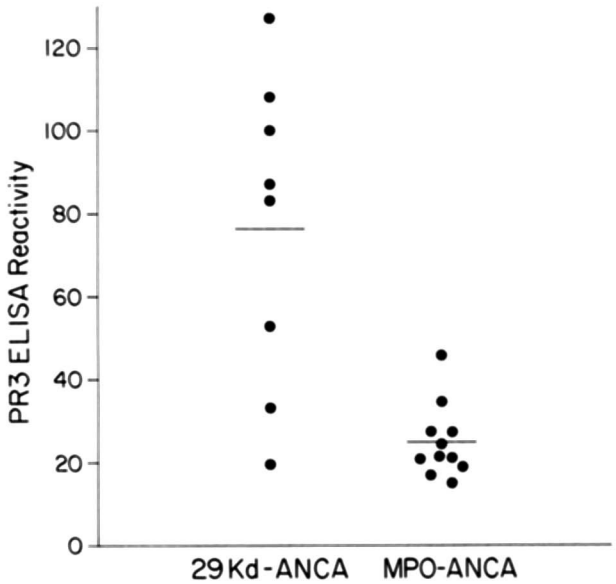

Fig 1. Graph comparing the PR3-reactivity of eight 29-Kd ANCA-positive sera to that of 11 MPO-ANCA-positive sera. The results are expressed as a percentage of a positive control serum. The horizontal lines are the group means.

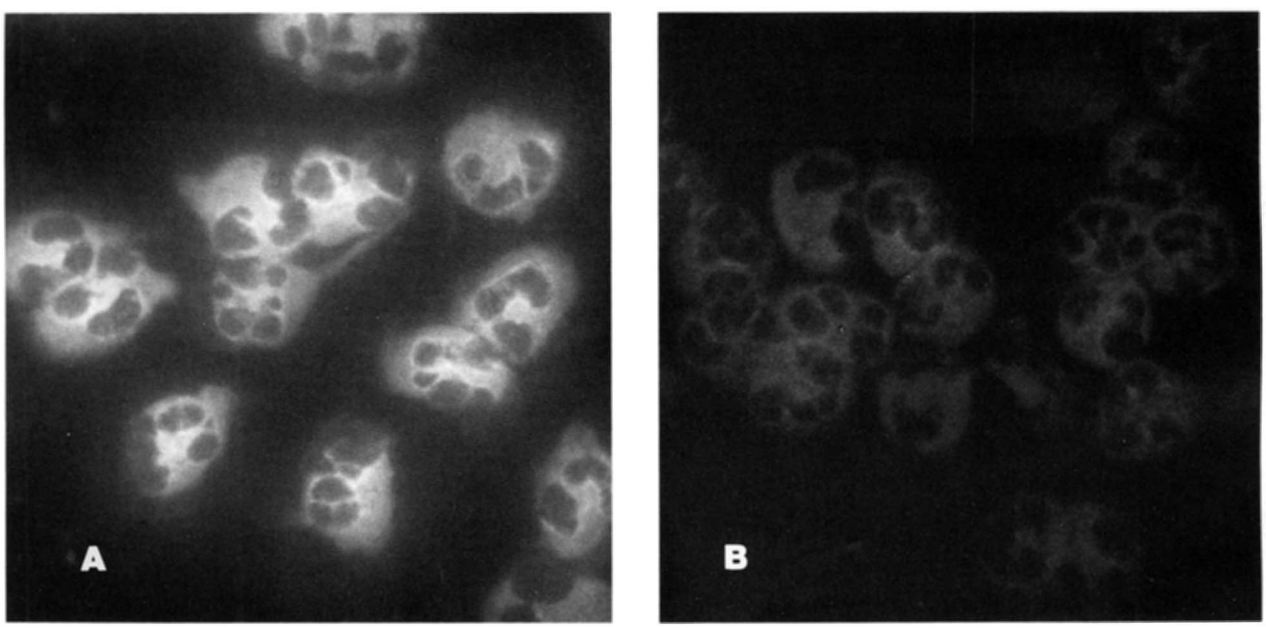

Fig 2. Blocking of 29-Kd ANCA staining with anti-PR3 but not with anti-MPO. (A) Alcohol-fixed neutrophils reacted sequentially with monoclonal anti-MPO, 1:800 29-Kd ANCA serum, and fluoresceinated anti-human IgG (20-second photographic exposure). (B) Alcohol-fixed neutrophils reacted sequentially with monoclonal anti-PR3, 1:800 29-Kd ANCA serum, and fluoresceinated anti-human IgG (60-second photographic exposure).

P-ANCA for MPO.[2,3] In an analogous fashion, we used an ELISA with purified PR3 as antigen to test the specificity of 29-Kd ANCA for PR3. Eight 29-Kd ANCA sera produced statistically significant reactivity with PR3 ($P = .001$ by the nonparametric Wilcoxon's rank sum test). In the PR3-ELISA, 8 29-Kd ANCA gave a mean value of 76.4 ± 37.7 compared with 24.8 ± 8.9 for 11 MPO-ANCA (Fig 1). Fifty normal control sera gave a mean of 9.5 ± 7.5. The MPO-ANCA and 29-Kd ANCA groups did not differ in ELISA reactivity with neutrophil cytoplasm nitrogen bomb cavitate,[2] with means of 101.1 ± 15.3 and 89.4 ± 31.9, respectively. The 29-Kd ANCA with the lowest PR3-ELISA value (ie, 19.5) also had the lowest nitrogen cavitate-ELISA value (ie, 41.6).

The specificity of 29-Kd ANCA for PR3 was further substantiated by indirect immunofluorescence microscopy blocking studies. The C-ANCA staining pattern produced by 29-Kd ANCA could be completely blocked by PR3-specific monoclonal antibodies, but was not blocked by monoclonal anti-MPO or monoclonal anti-DNA antibodies (Fig 2). Conversely, the P-ANCA staining pattern produced by MPO-ANCA could be completely blocked by anti-MPO monoclonal antibodies, but not by the anti-PR3 antibodies.

In summary, we agree with Niles,[1] Ludemann,[4] and Goldschmeding[6] that the approximately 29-Kd autoantigen with which C-ANCA react is a neutrophil serine proteinase; and further conclude that this protein is in fact PR3.

J. CHARLES JENNETTE
Department of Pathology
University of North Carolina
JOHN R. HOIDAL
Department of Medicine
University of Utah
Salt Lake City
RONALD J. FALK
Department of Medicine
University of North Carolina
Chapel Hill

REFERENCES

1. Niles JL, McCluskey T, Ahmad MF, Arnaout MA: Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. Blood 74:1888, 1989

2. 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 318:1651, 1988

3. Jennette JC, Wilkman AS, Falk RJ: Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. Am J Pathol 135:921, 1989

4. Ludemann J, Utecht B, Gross WL: Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. J Exp Med 171:357, 1990

5. Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR: Proteinase 3. A distinct human polymorphonuclear leukocyte proteinase that produces emphysema in hamsters. J Clin Invest 82:1963, 1988

6. Goldschmeding R, van der Schoot CE, ten Bokkel Huinink D, Hack CE, van den Ende ME, Kallenberg CGM, von dem Borne AEGK: Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. J Clin Invest 84:1577, 1989

RESPONSE

Drs Jennette, Hoidal, and Falk have convincingly shown that circulating autoantibodies from some patients with Wegener's granulomatosis are directed against proteinase 3, an elastinolytic serine protease of 29-Kd originally described by Dewald et al[1] and later characterized by Kao et al.[2] These data, in addition to the similarities in the physicochemical and functional properties of p29 and proteinase 3,[2,3] suggest that they represent the same protein. However, comparison of the N-terminus of p29 with that of proteinase 3[4] (Fig 1) shows that although the first 10 residues are identical or conserved, significant differences are present at positions 11 and 14.

These differences, if confirmed, could reflect polymorphic variants of the same protein. Alternatively, they may indicate the presence of two or three highly homologous proteins reactive with Wegener's granulomatosis autoantibodies in different patients. More extensive amino acid sequence data would be needed to distinguish between these possibilities.

```
                  1         10        20
p29:              I V G G H E A Q P H S X P - Y M A S L Q M
                  : : : : : : : : : : : : : : : : : : : : :
p29b:             I V G G H E A Q P H S R P - Y M A S L E M
                  : : : : : : : : : : : : : : : : : : : : :
ACPA antigen:     I V G G H E A Q P H I R P I Y M A
                  : : : : : : : : : : : : : : :
PROTEINASE 3:     I V G G H E A E P R W X P G
```

Fig 1. N-terminal sequence of p29[3]; p29b, purified independently by Gabay et al[5]; ACPA antigen, purified using an autoantibody from a patient with Wegener's Granulomatosis[4]; and proteinase 3.[4] The single letter amino acid code is used (X = undetermined). Identical residues are indicated by (:) and conserved residues by (.). A gap (−) was introduced to maximize homology.

JOHN L. NILES
MIR F. AHMAD
ROBERT T. McCLUSKEY
M. AMIN ARNAOUT
Renal Unit
Department of Medicine
Massachusetts General Hospital, East Charlestown

REFERENCES

1. Dewald B, Rindler-Ludwig R, Bretz U, Baggiolini M: Subcellular localization and heterogeneity of neutral proteases in neutrophilic polymorphonuclear leukocytes. J Exp Med 141:709, 1975

2. Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR: Proteinase 3: A distinct polymorphonuclear leukocyte proteinase that produces emphysema in hamsters. J Clin Invest 82:1963, 1988

3. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA: Wegener's granulomatosis autoantigen is a novel serine proteinase. Blood 74:1888, 1989

4. Ludemann J, Utecht B, Gross WL: Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. J Exp Med 171:357, 1990

5. Gabay JE, Scott RW, Campanelli D, Griffith J, Wilde C, Marra MN, Seeger M, Nathan CF. Antibiotic proteins of human polymorphonuclear leukocytes. Proc Natl Acad Sci (USA) 86:5610, 1989

1990 75: 2263-2264

Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3 [letter; comment]

JC Jennette, JR Hoidal and RJ Falk

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.