Activation, apoptosis and clearance of neutrophils in Wegener's granulomatosis
Rossum, Aart Pieter van

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 14-01-2020
Chapter 4

Standardized assessment of proteinase 3 expression on the neutrophil membrane requires priming.
Analysis in ANCA-associated vasculitis and controls

André P. van Rossum, Minke G. Huitema, Coen Stegeman, Marc Bijl, Karina de Leeuw, Miek A. van Leeuwen, Pieter C. Limburg, Cees G.M. Kallenberg

Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, The Netherlands

Submitted
ABSTRACT

Background. In PR3-ANCA associated vasculitis, PR3-ANCA are supposed to bind and activate neutrophils that express proteinase 3 on the membrane (mPR3). mPR3 expression can be present on the total population or on a subpopulation of neutrophils, ranging from 0% to 100%. This percentage is stable within an individual, and increased percentages have been observed in ANCA-associated vasculitis (AAV). Conditions for assessment of mPR3 expression have not been analyzed. In this study, we analyzed mPR3 expression on neutrophils before and after priming with TNF-α in order to assess whether standardized assessment of mPR3 expression requires priming. Furthermore, we compared differential mPR3 expression with membrane expression of (other) activation markers. Using neutrophils before and after priming with TNF-α, we assessed whether percentages of mPR3⁺ neutrophils are increased in patients with ANCA- associated vasculitis compared to disease- and healthy controls.

Patients and Methods. Neutrophils from patients with PR3- and MPO-ANCA-associated vasculitis, disease controls as patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), and healthy controls were analyzed before and after priming with TNF-α for mPR3 expression by flow cytometry. Furthermore, neutrophils were analyzed for expression of CD35, elastase and TNF-Receptor I and II before and after priming.

Results. 31% of all individuals (n=46) analysed showed minimal expression for mPR3 on all neutrophils before priming, whereas after priming a clear mPR3⁺ subset was observed next to mPR3⁻ neutrophils corresponding with bimodal mPR3 expression. Within individuals bimodal for mPR3 expression, uniform expression was seen for CD35, elastase and TNF-Receptor I and II after priming in all neutrophils. In patients with PR3- or MPO-ANCA- associated vasculitis, percentages of mPR3⁺ neutrophils were significantly increased (68 ± 6% and 71 ± 5% versus 46.4 ± 5.6%; p < 0.01 and p < 0.05, respectively) compared to healthy controls after priming. Percentages of mPR3⁺ PMN were increased in patients with SLE (70 ± 4%; p <0.01) as well, but not increased in RA (46.1 ± 6.6%).

Conclusion. In conclusion, standardized assessment of proteinase 3 on the membrane of neutrophils requires priming. Differential expression of mPR3 on neutrophils is independent from activation status, differential mobilization of vesicles or responsiveness to TNF-α. Percentages of mPR3⁺ neutrophils are increased in AAV and SLE, but not in RA.

Key words. proteinase 3, Wegener's granulomatosis, systemic lupus erythematosus, vasculitis, chronic inflammation
INTRODUCTION

Proteinase 3 (PR3) is a proteolytic enzyme, mainly stored in the azurophilic granules and, to a lesser extent, in the specific granules and secretory vesicles of neutrophils. Upon priming with low dose pro-inflammatory cytokines as TNF-α or IL-8, proteinase 3 is translocated to the cell membrane. Translocation of PR3 from the different pools to the cell membrane is sequentially regulated, starting off with exocytosis of readily mobilizable secretory vesicles followed by specific granules, and, finally by limited exocytosis of the azurophilic granules. Based on membrane bound proteinase 3 (mPR3) expression, two subsets of neutrophils can be defined: neutrophils that hardly express proteinase 3 (mPR3− neutrophils) and neutrophils that substantially express proteinase 3 (mPR3+ neutrophils). Individuals in whom both subsets are simultaneously present are defined as being bimodal in their mPR3 expression. Individuals in whom only one subset (mPR3− or mPR3+ neutrophils) is present are defined as monomodal in their mPR3 expression. The percentage of mPR3+ neutrophils has been reported to range from 0% to 100% of the total population of neutrophils within individuals. Furthermore, the percentage of mPR3+ neutrophils is stable within an individual and is not affected by neutrophil activation, disease activity or therapy, as such implying genetic control. In Wegener’s granulomatosis (WG), the percentage of mPR3+ neutrophils is increased. WG is a systemic auto-immune disease, characterized by the presence of anti-neutrophil cytoplasm autoantibodies (ANCA) which are in most cases directed against PR3. PR3-ANCA can activate neutrophils in vitro, resulting in degranulation and oxidative burst. Membrane proteinase 3 expression is associated with neutrophil activation after stimulation with PR3-ANCA. Also in rheumatoid arthritis the percentage of mPR3+ neutrophils has been reported to be increased. Other studies reporting mPR3 expression on neutrophils in patients with PR3-ANCA associated vasculitis, did not find increased percentages of mPR3+ neutrophils in chronic inflammatory disease controls such as patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) as compared to healthy controls. In these studies, neutrophils were analysed for their mPR3 expression without priming with TNF-α. However, isolation procedures alone can prime neutrophils, possibly due to mechanical stress during centrifugation procedures resulting in translocation of PR3 to the plasma membrane. mPR3 expression is normally not seen in whole blood experimental procedures, implying that neutrophils need priming to express mPR3. Induction of mPR3 expression during the isolation procedure is not controllable and may vary between experiments. Hence, percentages of mPR3 expressing neutrophils may not be accurately assessed when neutrophils are not additionally primed with TNF-α.

Therefore, in the present study we first analyzed mPR3 expression on neutrophils before and after priming with TNF-α in order to assess whether assessment of mPR3 expression requires priming. Furthermore, we compared differential mPR3 expression with membrane expression of (other) activation markers. Using neutrophils before and after priming with TNF-α, we assessed whether percentages of mPR3+ neutrophils are increased in patients with ANCA-associated vasculitis. In order to assess the specificity of the suggested increase in percentages of mPR3+ neutrophils for ANCA-associated vasculitis, we included disease controls from patients with chronic inflammatory diseases, namely RA and SLE.
MATERIALS AND METHODS

Patients and controls

As the percentage of mPR3+ neutrophils is supposed to be genetically determined, it is not affected by age, disease activity or therapy. Therefore, we have included all patients with ANCA-associated vasculitis, SLE and RA who where consecutively followed at the out-patient clinic:

- **ANCA-associated vasculitis:** A diagnosis of Wegener’s granulomatosis (WG), Churg Strauss Syndrome (CSS) or Microscopic Polyangiitis (MPA) was established according to the Chapel Hill criteria. PR3- or MPO-ANCA were determined by indirect immunofluorescence (IIF) assay on ethanol-fixed neutrophils and by capture ELISA with specificity for PR3 or MPO, as described earlier. The PR3-ANCA associated vasculitis group consisted of 25 patients with WG. The MPO-ANCA associated vasculitis group consisted of 5 patients with WG, 5 patients with MPA, 2 patients with CSS, and 1 patient with unclassified MPO-associated vasculitis.

- **Rheumatoid arthritis (RA):** Patients fulfilled the criteria of the American College of Rheumatology criteria for definite RA.

- **Systemic lupus erythematosus (SLE):** Diagnosis was established according to the American College of Rheumatology criteria for SLE.

- **Healthy controls:** Healthy laboratory personnel were included as controls.

Additional information of patients and controls is given in Table 1.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>PR3-ANCA</th>
<th>MPO-ANCA</th>
<th>RA</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>25</td>
<td>25</td>
<td>13</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>20</td>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>5</td>
<td>8</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>42 ± 9</td>
<td>59 ± 16</td>
<td>53 ± 18</td>
<td>50 ± 13</td>
<td>49 ± 14</td>
</tr>
<tr>
<td>Inactive disease</td>
<td>-</td>
<td>24 (96%)</td>
<td>12 (93%)</td>
<td>15 (60%)</td>
<td>24 (96%)</td>
</tr>
<tr>
<td>Treatment:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP, AZA, MTX, MMF</td>
<td>-</td>
<td>12 (48%)</td>
<td>5 (38%)</td>
<td>18 (72%)</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>-</td>
<td>7 (28%)</td>
<td>5 (38%)</td>
<td>1 (4%)</td>
<td>10 (40%)</td>
</tr>
</tbody>
</table>

**HC:** Healthy Control; **PR-ANCA:** PR3-ANCA-associated vasculitis; **MPO-ANCA:** MPO-ANCA-associated vasculitis; **RA:** Rheumatoid Arthritis; **SLE:** Systemic Lupus Erythematosus; **CP:** cyclophosphamide; **AZA:** azathioprine; **MTX:** methotrexate; **MMF:** mycophenolate mofetil

Isolation and priming of neutrophils

Neutrophils of healthy individuals were isolated from EDTA-anticoagulated blood by centrifugation on Polymorphprep™ (Nycomed, Oslo, Norway) and hypotonic lysis of contaminating erythrocytes with ice-cold ammonium chloride buffer. Cells were washed with cold Hanks’ balanced salt solution (HBSS) without Ca²⁺ / Mg²⁺ (GIBCO / Life Technologies, Breda, The Netherlands) and resuspended in Hanks’ balanced salt solution (HBSS) with Ca²⁺ / Mg²⁺ (GIBCO / Life Technologies) to obtain 1 x 10⁷ cells /ml. Part of the samples were primed with 2 ng/ml of recombinant TNF-α (Boehringer Mannheim, Germany) for 15 min at 37°C prior to analysis of membrane expression. Non-primed neutrophils were analyzed for membrane expression immediately after isolation.
Membrane expression on neutrophils

Membrane expression of proteinase 3, elastase, TNF-α-Receptor I, TNF-α-Receptor II and CD35 (or Complement Receptor 1, CR1) were measured using flow cytometry as previously described. All steps were performed on ice. Shortly, samples containing 10^6 neutrophils were fixed with 0.5% paraformaldehyde for 10 minutes, washed with PBS / 1% BSA by centrifugation at 1200 x g, 4°C for 3 min, and incubated with 0.5 mg/ml heat-aggregated goat immunoglobulins (IgG; Sigma, Zwijndrecht, The Netherlands) for 15 min to saturate Fcγ receptors. Next, cells were stained with a saturating dose of mouse monoclonal IgG1 antibody (MAb) directed against human PR3 (PR3G-3), human elastase (NP57, Dako Cytomation, Glostrup, Denmark), TNFRI (clone: 16830, R&D Systems, Minneapolis, USA), TNFRII (clone: 22235, R&D Systems), human CD35 (Ber-Mac-DRC, Dako Cytomation) or with an irrelevant IgG1 control antibody (MCG1; IQProducts, Groningen, The Netherlands) for 30 min. Next, non-bound antibodies were washed off with PBS / 1% BSA. This step was followed by 30 min incubation with phycoerythrin (PE)-conjugated goat anti-mouse antibody (Southern Biotechnology Associates, Inc., Birmingham, USA) in the presence of 0.5 mg/ml heat-aggregated goat IgG and subsequent washing step. Fluorescence intensity was analyzed on an ELITE flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA), calibrated using the Flow-Set® fluorospheres (Beckman Coulter, Hialeah, FL, USA). Bimodal mPR3 expression was defined as the presence of 10% - 90% mPR3+ cells. The percentage of mPR3+ cells within primed neutrophils was calculated by integration of the peak representing the mPR3+ cells compared to the peak of mPR3− cells, irrespective of the isotype control. The level of PR3 or CD35 was calculated as mean fluorescence intensity (MFI PR3 or CD35) of specific binding corrected for the non-specific binding of the isotype control antibody (MFI NSB).

STATISTICS

Statistical analysis was performed using Mann-Whitney and GraphPad Prism, version 3.0 (GraphPad Software, San Diego, CA).

RESULTS

Patterns of mPR3 expression before and after priming. We analyzed mPR3 expression on neutrophils of patients (n=124) and healthy controls (n=25) before and after priming with TNF-α (2 ng/ml). Based on the percentage of mPR3 expressing neutrophils before and after priming we could distinguish the following mPR3 expression patterns: 1) Individuals (n=46) who did not demonstrate a separate population of mPR3 expressing neutrophils before priming (monomodal low mPR3 expression), whereas after priming a separate population of mPR3 expressing neutrophils (10 - 90% mPR3+ cells) could be observed (bimodal mPR3 expression) (Figure 1A and E); 2) Individuals (n=36) in whom before and after priming bimodal mPR3 expression could be observed (Figure 1B and F);
Figure 1. Patterns of mPR3 expression before and after priming with TNF-α. Neutrophils were isolated and stained for mPR3 and CD35 expression before (A, B, C, D) and after priming with TNF-α (E, F, G, H). A and E): Representative overlay of an individual showing monomodal expression of mPR3 on neutrophils before priming (A), whereas after priming (E) a bimodal expression of mPR3 from the same individual is observed. B and F): Individual in whom before (B) and after priming (F) bimodal mPR3 expression is observed. C and G): Individual in whom all neutrophils before (C) and after priming (G) show apparent mPR3 expression (> 90% mPR3<sup>+</sup> cells). D and H): Individual in whom all neutrophils show a lack of mPR3 expression before (D) and after (H) priming (> 90% mPR3<sup>-</sup> cells). Grey peak represents isotype control, black line represents mPR3 expression, and CD35 expression is represented by the dashed line.
3) Individuals (n=16) in whom all neutrophils before and after priming showed apparent mPR3 expression (> 90% mPR3+ cells) (Figure 1D and G); and 4) Individuals (n=15) in whom all neutrophils showed a lack of mPR3 expression before and after priming (< 10% mPR3+ cells) (Figure 1D and H). Thus, neutrophils often require the additional step of priming with TNF-α in order to express proteinase 3 on the membrane (Figure 1A vs 1B). This indicates that priming with TNF-α is required for standardized assessment of proteinase 3 on the membrane of neutrophils.

Figure 2. CD35 expression on non-primed neutrophils from individuals with different patterns of mPR3 expression before and after priming with TNF-α. Analysis of CD35 expression showed a significantly (p < 0.05) lower level of CD35 expression on neutrophils of individuals who were monomodal before priming but bimodal in the mPR3 expression after priming compared with individuals already bimodal in the mPR3 expression before priming. Horizontal line denotes the median. Asterisk: p < 0.05

Next, we compared levels of the activation marker CD35 on neutrophils of individual with monomodal low mPR3 expression before priming but bimodal in mPR3 expression after priming with individuals already bimodal in mPR3 expression before priming. A significantly higher level of CD35 expression was present on neutrophils of the latter individuals compared to neutrophils of the former individuals already before priming (395 ± 40 MFI, n=36, and 290 ± 23 MFI, n=46; p < 0.05) (Figure 2). This suggests that neutrophils expressing mPR3 without additional priming with TNF-α are already in a primed state.

Differential mPR3 expression is independent of (other) activation markers
To investigate whether differential mPR3 expression in bimodal individuals results from differences in mobilization of the secretory vesicles that transport PR3 to the membrane, we analyzed CD35 levels on both mPR3 subsets in bimodal individuals. mPR3+ neutrophils in bimodal individuals did not have higher levels of CD35 compared to mPR3− neutrophils in these bimodal individuals, indicating no differences in mobilization of secretory vesicles between both subsets (data not shown). In addition, analysis of TNF-α-Receptor I and TNF-α-Receptor II expression on neutrophils in bimodal individuals demonstrated uniform expression of these receptorson the two mPR3 subsets, suggesting that differential mPR3 expression is not
due to differences in responsiveness to priming with TNF-α between mPR3− and mPR3+ subsets (Figure 3A).

Figure 3. Monomodal expression of TNFRI, TNFRII, elastase, and CD35 expression in bimodal mPR3 donors. (A) Neutrophils of a bimodal mPR3 individual were isolated and stained for TNFRI (black line) and TNFRII (dashed line). Isotype control is represented by the grey peak. (B and C) Neutrophils of a bimodal mPR3 individual showing monomodal expression of elastase and CD35 before (B) and after (C) priming with TNF-α.

Percentages of mPR3 expressing neutrophils were stable before and after priming with TNF-α (data not shown). Membrane expression of elastase on non-primed and primed neutrophils was weakly present and always uniform (Figure 3B and 3C). Occasionally, we observed a slight upregulation of mPR3 after priming with TNF-α on the mPR3− subset in bimodal individuals, comparable with elastase expression. (Figure 3B and 3C).

**Percentage of mPR3 expressing neutrophils in inflammatory diseases**

As priming with TNF-α is required for standardized assessment of mPR3 expression on neutrophils, we analyzed the percentage of neutrophils expressing mPR3 in patients with PR3-ANCA-associated vasculitis (n=25), MPO-ANCA associated vasculitis (n=13), systemic lupus erythematosus (n=25), rheumatoid arthritis (n=25), and healthy controls (n=25) after priming with TNF-α. In this analysis, also individuals monomodal in mPR3 expression were included. To define monomodal mPR3− or mPR3+ individuals, we analyzed mPR3 expression on the mPR3− subset and the mPR3+ subset in a random sample of 25 bimodal individuals (Figure 4A and B).

Figure 4. mPR3 expression on mPR3 subsets in bimodal individuals and monomodal individuals. In A, a random sample of bimodal individuals (patients and healthy controls, n=25) were analyzed for expression of mPR3 after priming of neutrophils with TNF-α. In B, minimal expression of mPR3 on the mPR3- subset is presented. Based upon these values, an mPR3 expression ≤ 60 MFI was defined as mPR3− neutrophils, and > 60 MFI as mPR3+ neutrophils. In C, mPR3 expression of all individuals monomodal in their mPr3 expression are depicted. Results are expressed as MFI of mPR3 from with irrelevant binding by isotype control was substracted.
We found that mPR3 expression on neutrophils of the mPR3⁻ subset never reached expression levels higher than 60 MFI. As such, an MFI of 60 was defined as the cut-off value for mPR3 expression. Therefore, monomodal mPR3⁺ individuals were defined as individuals in whom > 90% of neutrophils had mPR3 expression exceeding 60 MFI, and monomodal mPR3⁻ individuals as individuals in whom > 90% of neutrophils had mPR3 expression below 60 MFI. Patients and controls who had a monomodal expression of mPR3 are depicted in Figure 4C with their respective mPR3 expression and subsequent division into monomodal mPR3⁻ and mPR3⁺ individuals. Including both monomodal and bimodal mPR3 individuals, we found that patients with PR3-ANCA associated vasculitis and MPO-associated vasculitis had a significantly higher percentage of mPR3⁺ PMN (68.1 ± 5.5%, n=25, and 71.1 ± 4.5%, n=14, respectively) than healthy controls (46.4 ± 5.6%, n=25; p < 0.01 and p < 0.05, respectively) (Figure 5 and 6). Furthermore, also patients with systemic lupus erythematosus showed significantly higher percentages (70.1 ± 4.1%, n=25; p < 0.01) of mPR3⁺ neutrophils compared to controls (Figure 5 and 6). However, percentages of mPR3⁺ PMN were not increased in patients with rheumatoid arthritis (46.1 ± 6.6%, n=25) (Figure 5 and 6).

**Figure 5.** Percentages of mPR3⁺ neutrophils are increased in ANCA- associated vasculitis and systemic lupus erythematosus after priming with TNF-α. Patients with PR3-ANCA associated vasculitis (PR3-ANCA, n=25), MPO-ANCA- associated vasculitis (MPO-ANCA, n=13), systemic lupus erythematosus (SLE, n=25), rheumatoid arthritis (RA, n=25), and healthy controls (HC, n=25) were analyzed for their percentage of mPR3⁺ neutrophils after priming with TNF-α. **: p < 0.01. Horizontal line denotes the median.

**DISCUSSION**

In the present study, we first analyzed whether priming of neutrophils is required for standardized assessment of PR3 expression on the membrane. We found that in almost half of all individuals with bimodal mPR3 expression analyzed, substantial mPR3 expression was only seen after priming with TNF-α. In these individuals, distinct separation of the mPR3⁻ and the mPR3⁺ subset could only accurately made after priming with TNF-α. Neutrophils of these individuals had a lower expression of the activation marker CD35 than individuals with bimodal mPR3 expression already showing mPR3 expression without the additional priming step with TNF-α. This suggests that neutrophils expressing mPR3 without additional priming with TNF-α are
already in a primed state. Furthermore, we demonstrate that differential expression of mPR3 on both subsets within individuals with bimodal mPR3 expression did not originate from differential mobilization of secretory vesicles or activation status between both subsets after priming with TNF-\(\alpha\), as both subsets expressed similar levels of CD35 on both mPR3\(^{-}\) neutrophils and on mPR3\(^{+}\) neutrophils. Furthermore, also another proteinase, i.e. elastase showed uniform expression on all neutrophils.

**Figure 6.** Distribution of the percentages of mPR3\(^{+}\) neutrophils in various inflammatory diseases after priming with TNF-\(\alpha\). Histogram of the distribution of mPR3\(^{+}\) neutrophils in patients with PR3-ANCA associated vasculitis (PR3-ANCA, n=25), MPO-ANCA-associated vasculitis (MPO-ANCA, n=13), systemic lupus erythematosus (SLE, n=25) and rheumatoid arthritis (RA, n=25); all versus healthy controls (HC, n=25).

Analysis of TNF-\(\alpha\)-Receptor I and TNF-\(\alpha\)-Receptor II expression on neutrophils in individuals with bimodal mPR3 expression demonstrated uniform expression of these receptors on the two mPR3 subsets, suggesting that differential mPR3 expression is not due to differences in responsiveness to priming with TNF-\(\alpha\). These observations are in line with other studies\(^{7;10;19}\), showing that differential expression of mPR3 is not the result of differences in activation, mobilization of vesicles or responsiveness to priming with TNF-\(\alpha\). On the other hand, the present study suggests that neutrophils have to reach a certain level of activation in order to express proteinase 3 on the membrane. So, a subpopulation of neutrophils translocates proteinase 3 to the membrane when activated, and the percentage of this subpopulation is stable within an individual which is in agreement with other studies\(^{7;8;10;11}\).

In the present study, we standardized the assessment of proteinase 3 expression on the membrane of neutrophils in patients with ANCA-associated vasculitis and disease controls by priming isolated neutrophils with TNF-\(\alpha\). We found that percentages of mPR3\(^{+}\) neutrophils were increased in patients with PR3- and MPO-ANCA-associated vasculitis, and systemic lupus erythematosus compared to healthy controls. Percentages of mPR3\(^{+}\) neutrophils in rheumatoid arthritis were not increased. This contrasts with a study by Witko-Sarsat et al who showed increased percentages of mPR3\(^{+}\) neutrophils in rheumatoid arthritis\(^{10}\). However, the respective neutrophils in that study were not primed in vitro with TNF-\(\alpha\). As such, clear distinction between the
two subsets is less accurate and more dependent on priming by isolation procedures in vitro or during in vivo as a result of active disease in the patient analyzed. In 1998, Muller-Kobold et al. already showed that neutrophils from Wegener’s patients with active disease have an increased expression of mPR3 compared to Wegener’s patients with quiescent disease.

In PR3-ANCA associated vasculitis, autoantibodies directed against PR3 (PR3-ANCA) activate neutrophils by binding to PR3 on the membrane of neutrophils. As such, high numbers of neutrophils that are able to express PR3 on the membrane after priming are an obvious risk factor in this disease. However, increased numbers of mPR3+ neutrophils were found in MPO-ANCA associated vasculitis and SLE as well. The clinical significance of this increased mPR3 expression on neutrophils in these disorders is not yet clear. Increased mPR3 expression might, possibly, play a role in the pathophysiology of these autoimmune inflammatory diseases. Membrane-bound proteinase 3 (mPR3) on neutrophils is catalytically active against extracellular matrix proteins such as fibronectin and, surprisingly, resistant to inhibition by physiological inhibitors. The functions of proteinase 3 (PR3) are quite diverse. For instance, PR3 is able to cleave important pro-inflammatory cytokines as TNF-α, IL-1 and IL-18 into a bioactive form, whereas other serine proteases like human neutrophil elastase can not. Furthermore, PR3 has caspase-like activities since it cleaves the cell cycle inhibitor p21, and, as such, induces apoptosis in endothelial cells. Due to these functions, PR3 can be regarded as an important regulator of inflammation.

In conclusion, standardized assessment of proteinase 3 on the membrane of neutrophils requires priming. Furthermore, differential expression of mPR3 on neutrophils is independent of activation status, mobilization of vesicles or responsiveness of these neutrophils to TNF-α. Percentages of neutrophils that express proteinase 3 after priming with TNF-α are increased in PR3- and MPO-ANCA- associated vasculitis and SLE, but not in RA. Whereas a pathophysiological role for (increased) mPR3 expression has been strongly suggested in PR3-ANCA associated vasculitis; its role in other ANCA- associated vasculitides and SLE requires further studies.

REFERENCES


