Current knowledge on cellular interactions in the WG-granuloma

P. Lamprecht, W.L. Gross

Department of Rheumatology, University Hospital of Schleswig-Holstein, Campus Lübeck, and Rheumaklinik Bad Bramstedt, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Peter Lamprecht, Wolfgang L. Gross.

Supported by a grant from the Wegener's Granulomatosis Association, Kansas City USA (PL, WLG), grant La 1339 (PL, WLG) from the German Research Society (Deutsche Forschungsgemeinschaft/DFG), Innovationsfonds Schleswig-Holstein (PL), and grant No. 02.2 from the Verein zur Förderung der Erforschung und Bekämpfung rheumatischer Erkrankungen Bad Bramstedt e.V., Germany (PL).

Please address correspondence to: Peter Lamprecht, MD, Department of Rheumatology, University Hospital of Schleswig-Holstein, Campus Lübeck, and Rheumaklinik Bad Bramstedt, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: lamprecht@rheuma-zentrum.de

Key words: Wegener's granulomatosis, granuloma, proteinase 3, ANCA, lymphiod neoformation, autoimmunity.

ABSTRACT

*Wegener's granulomatosis (WG) usually starts as granulomatous disease of the respiratory tract (so-called localized WG) before it converts to systemic disease (generalized WG) with the emergence of proteinase 3-specific antineutrophil cytoplasmic autoantibodies (PR3-ANCA) and PR3-ANCA associated autoimmune vasculitis. So far, it remains unresolved how tolerance to "Wegener's autoantigen" PR3 is broken and the immune response to PR3 sustained. Further, the relationship between granulomatous lesions and systemic vasculitis is poorly understood. None of the ANCA-animal-models has reproduced granulomata typical of WG so far. A number of endogenous and exogenous factors (HLA-DPB1*0401 / PTPN22*620W, respiratory epithelial barrier dysfunction?* S. aureus, *cPR3?) could favour initial formation of granulomata in the respiratory tract and break of tolerance. PR3 induces dendritic cell maturation via the protease activated receptor (PAR)-2 and evokes a strong Th1-type T-cell response in WG. Clusters of PR3$^+$ cells (neutrophils/monoctyes) surrounded by antigen-presenting cells, Th1-type CD4$^+$CD28- effector memory T-cells, maturing B- and plasmacells are found in WG-granulomata of the upper respiratory tract. Thus, WG-granulomata might provide the necessary "proinflammatory environment" for the break of tolerance and display features of lymphoid-like tissue neoformation, in which autoimmunity to PR3 could be sustained. Subsequent PR3-ANCA associated systemic vasculitis gives rise to new inflammatory lesions in many other organs, thereby promoting a self-perpetuating pathology characterized by inflammation and autoimmunity to PR3.*

Introduction

Wegener's granulomatosis (WG) is a potentially organ and/or life-threatening, chronic inflammatory and autoimmune disease of as yet unknown etiology, characterized by necrotizing granulomatous inflammation, glomerulonephritis and an autoimmune vasculitis associated with the highly WG-specific proteinase 3 (PR3)-directed antineutrophil cytoplasmic autoantibodies (PR3-ANCA). WG usually starts as granulomatous disease of the respiratory tract (so-called localized WG*) before it converts to systemic disease (generalized WG*) with the emergence of PR3-ANCA and PR3-ANCA associated autoimmune vasculitis. In spite of immunosuppressive treatment, relapse rates are high and seem to be related to the persistence of granulomata (1).

So far, the relationship between granulomatous lesions and systemic vasculitis in WG is poorly understood. An animal model displaying both pathological features of WG is still missing. While glomerulonephritis and vasculitis have been induced by myeloperoxidase (MPO)-ANCA in mice and rat models mimicking human microscopic polyangiitis recently, the significance of PR3-ANCA induced exacerbation of paniculitis (an uncommon manifestation of WG) in another animal model is unclear. Stronger pulmonary and renal inflammation is seen in LPS primed mice in this model (2). *In vitro* studies support the concept that PR3-ANCA interact with neutrophil granulocytes causing premature intravascular neutrophil activation and degranulation with subsequent endothelial damage and further leukocyte recruitment to the site of vasculitis (3). None of the aforementioned animal models has reproduced granulomata typical of WG so far. The disease course of WG with apparent primary involvement of the respiratory tract suggests that WG may be initiated by an aberrant cell-mediated immune response to an exogenous or endogenous antigen in the respiratory tract with a subsequent break of tolerance to PR3 (4). In this review, we will discuss current knowledge of the pathology and pathophysiological mechanisms in the formation of WG-granulomata.

Morphological spectrum and formation of the WG-granuloma

WG-granulomata show a wide morpho-

*According to the EUVAS definitions of WG-subgroups.

logical spectrum. The lesions display different morphologies such as neutrophilic microabcesses, necrotizing palisading granulomas, and sometimes epitheloid cell granulomas. The American pathologist R. Fienberg reviewed open lung biopsies of WG patients and described the formation of granulomatous lesions in WG. In his study, the earliest lesions in the lung are foci of swollen collagen fibers representing apparent tissue injury and/or necrosis. Next, mononuclear histiocytes migrate to the vicinity of the necrosis. Neutrophil granulocytes, lymphocytes, epitheloid cells and multinucleated giant cells appear subsequently. Sometimes, the histiocytes become orientated in a pallisading manner around the central necrosis area. The central necrosis may be confluent or may show an irregular serpiginous pattern known as "geographic" necrosis. Granulomatous lesions in the lung can be found in vicinity to inflamed vessels, but also at distant extravascular sites (5, 6). Thus, WG-granulomata are not solely a consequence of a vascular injury, but rather could represent an inherent trait of inflammation occurring at different sites of tissue damage in many – but not all – organs once the aberrant cellular immune response has been established in WG.

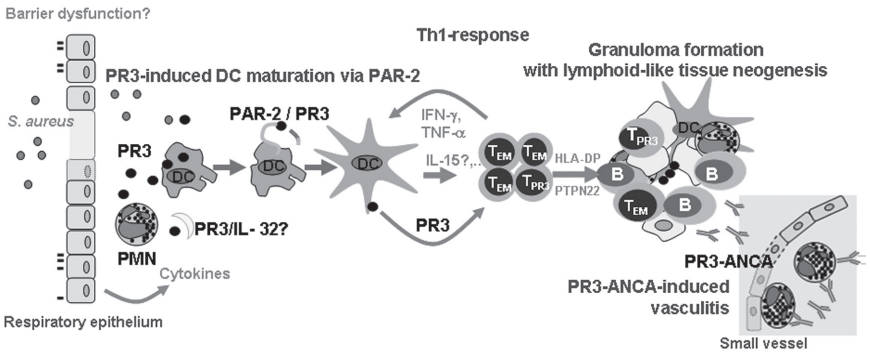

Fig. 1. *Hypothesis on lymphoid neoformation in WG-granulomata as key to self-perpetuating inflammation and autoimmunity in WG:* Following hypothetical barrier dysfunction and potentially triggered by exogenous factors (*e.g. S. aureus*), PR3 is released from neutrophil granulocytes. PR3 induces DC maturation via the protease activated receptor (PAR)-2. PR3 also enhances proinflammatory IL-32 action (15). Dendritic cells (DC) and other antigen-presenting cells (APC) induce a Th1-type effector memory T-cell (T_{EM}) response via cytokines such as IL-15 (?) and PR3 specific T-cells. Subsequent granuloma formation might provide the necessary "proinflammatory environment" for the break of tolerance. Lymphoid-like tissue neoformation in WG-granulomata could sustain autoimmunity to PR3. PR3-ANCA formation results in PR3-ANCA associated vasculitis giving rise to further inflammatory lesions. Genetic factors predispose to granulomatous inflammation (HLA-DPB1*0401) and PR3-ANCA seropositivity (PTPN22*620W), thereby determining disease progression (16).

Lymphoid neoformation in WG-granulomata: key to self-perpetuating inflammation and autoimmunity

So far, it remains unresolved how the immune response to PR3 is induced and sustained. Many questions are unanswered: How is tolerance to PR3 broken? How are PR3-ANCA induced? Why and where does PR3 become the target of autoimmunity in WG (and why not one of the many other neutrophil constituents)? And what is the relation between granulomatous inflammation and autoimmunity in WG?

Animal models give an idea how autoimmunity and immunopathology could be induced and sustained in autoimmune diseases. In many animal models, solely the presence of activated autoreactive T- and B-cells is insufficient to cause autoimmune disease. However, break of tolerance and organ specific autoimmunity is induced in the presence of a "proinflammatory environment" and sustained by neoformation of lymphoid-like structures in inflamed target organs (7). In human autoimmune diseases such as rheumatoid arthritis or Sjögren's syndrome, initiation and maintenance of autoimmune responses in such structures is suggested (8). More recently, we have shown that WG-granulomata contain clusters of $PR3^+$ cells (neutrophils/monoctyes) surrounded by antigen-presenting cells, Th1-type $CD4^+CD28^-$ effector memory T-cells, maturing B- and plasmacells suggestive of neoformation of lymphoid-like tissue (9, 10). Antigen-presenting cells such as mature dendritic cells produce proinflammatory cytokines, which render responder T-cells refractory to the suppressive effect of regulatory cells. Intriguingly, "Wegener's autoantigen" PR3 induces DC maturation via the protease activated receptor (PAR)-2 and evokes a stronger Th1-type T-cell response in WG as compared to healthy and disease controls (11). Thus, WG-granulomata of the upper respiratory tract display features of lymphoid-like tissue neoformation, in which autoimmunity to PR3 could be initiated and sustained by an exaggerated Th1-type response (Fig. 1).

Role of endogenous and exogenous factors in the induction of autoimmunity to PR3

The combined influence of different predisposing genetic factors and exogenous triggers might determine disease severity, phenotype, and outcome ranging from rare "formes frustes" with loco-regional granulomatous inflammation restricted to the respiratory tract to full-blown, $PR3^-ANCA^+$ WG with granulomata and systemic vasculitis. More recently, T-cell function associated genetic factors predisposing to granulomatous inflammation (HLA-DPB1*0401) and PR3-ANCA seropositivity (PTPN22*620W) have been determined (12). *S. aureus* colonization of the respiratory tract is an exogenous factor triggering WG relapses (13). Data from another study suggest that a complimentary peptide (cPR3) coded by an (endogenous or exogenous) antisense DNA strand of PR3 elicits its cognate antibody, which in turn evokes an anti-idiotype of this antibody with specificity for the autoantigen, *i.e.* PR3-ANCA. However, only 7 out of 34 patients with PR3-ANCA also had cPR3 (105-201)-specific antibodies in that study (14). These data raise the question why the primary antibody response (cPR3-antibodies) was weaker and detectable in only few individuals (and also one SLE control) compared to

the secondary response (PR3-ANCA). Taken together, endogenous and exogenous factors favouring autoimmunity to PR3 are still poorly defined and the underlying mechanisms remain to be further elucidated.

Conclusion

A number of endogenous (HLA-DPB1*0401/PTPN22*620W, respiratory epithelial barrier dysfunction?) and exogenous (*S. aureus*, cPR3?) factors could favour initial formation of granulomata in the respiratory tract in WG. WG-granulomata might provide the necessary "proinflammatory environment" for the break of tolerance and neoformation of lymphoid-like structures, which sustain autoimmunity to PR3 in WG. As a result, PR3-ANCA associated systemic vasculitis gives rise to inflammatory lesions in many organs, in which neoformation of lymphoid-like structures may eventually also be seen, thereby promoting a self-perpetuating pathology characterized by inflammation and autoimmunity to PR3.

References

1. BACON PA: The spectrum of Wegener's granulomatosis and disease relapse. *New Engl J Med* 2005; 352: 330-2.
2. HEERINGA P, HUUGEN D, COHEN TERVAERT JW: Anti-neutrophil cytoplasmic autoantibodies and leukocyte-endothelial interactions: a sticky connection? *Trends Immunol* 2005; 26: 561-4.
3. LU X, GARFIELD A, RAINGER GE, SAVAGE CO, NASH GB: Mediation of endothelial cell damage by serine proteases, but not superoxide, released from antineutrophil cytoplasmic antibody-stimulated neutrophils. *Arthritis Rheum* 2006; 54: 1619-28.
4. SARRAF P, SNELLER M: Pathogenesis of Wegener's granulomatosis: Current concepts. *Expert Rev Mol Med* 2005; 7: 1-19.
5. FIENBERG R: A morphologic and immunohistologic study of the evolution of the necrotizing palisading granuloma of pathergic (Wegener's) Granulomatosis. *Semin Resp Med* 1989; 10: 126-32.
6. KRADIN RL, MARK EJ: Case records of the Massachusetts General Hospital: Weekly clnicopathological exercises. Case 18-2002, A 48-year-old man with a cough and bloody sputum. *New Engl J Med* 2002; 346: 1892-9.
7. LANG KS, RECHER M, JUNT T *et al*.: Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat Med* 2005; 11: 138-45.
8. ZINKERNAGEL RM: Antiinfection immunity and autoimmunity. *Ann N Y Acad Sci* 2002; 958: 3-6.
9. KOMOCSI A, LAMPRECHT P, CSERNOK E *et al*.: Peripheral blood and granuloma $CD4^+CD28^-$ T-cells are a major source of IFN-γ and TNF-α in Wegerner's granulomatosis. *Am J Pathol* 2002; 160: 1717-24.
10. VOSWINKEL J, MUELLER A, KRAEMER JA *et al*.: B lymphocyte maturation in Wegener's granulomatosis: a comparative analysis of VH genes from endonasal lesions. *Ann Rheum Dis* 2006; 65: 859-64.
11. CSERNOK E, AI M, GROSS WL *et al*.: Wegener's autoantigen induces maturation of dendritic cells and licences them for Th1 priming via the protease-activated receptor-2 pathway. *Blood* 2006; 107: 4440-8.
12. JAGIELLO P, GROSS WL, EPPLEN J: Complex genetics of Wegener's granulomatosis. *Autoimmun Rev* 2005; 4: 42-7.
13. POPA ER, STEGEMAN CA, KALLENBERG CGM, COHEN TERVAERT JW: Staphylococcus aureus and Wegener`s granulomatosis. *Arthritis Res* 2002; 4: 77-9.
14. PENDERGRAFT III WF, PRESTON GA, SHAH RS *et al*.: Autoimmunity is triggered by cPR-3 (105-201), a protein complementary to human autoantigen proteinase 3. *Nat Med* 2004; 10: 72-79.
15. NOVICK D, RUBINSTEIN M, AZAM T, RABINKOV A, DINARELLO CA, KIM SH: Proteinase 3 is an IL-32 binding protein. *Proc Natl Acad Sci USA* 2006; 103: 3316-21.
16. LAMPRECHT P, CSERNOK E, GROSS WL: Effector memory T-cells as driving force of granuloma formation and autoimmunity in Wegener's granulomatosis. *J Intern Med* 2006; 260: 187-91.