

Immune phenomena in localized and generalized Wegener's granulomatosis

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Received on May 7, 2003; accepted in revised form on August 11, 2003.

Clin Exp Rheumatol 2003; 21 (Suppl. 32): S49-S54.

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EXPERIMENTAL RHEUMATOLOGY 2003.

Key words: Wegener's granulomatosis, granuloma, necrosis, vasculitis, ANCA.

ABSTRACT

Wegener's granulomatosis (WG) is characterized by granulomatous inflammation and systemic vasculitis with a predilection for the lungs and kidneys. In most patients WG begins with a localized organ involvement of the upper respiratory tract that progresses to systemic disease (generalized WG) (1). Because of the life-threatening nature of systemic vasculitis, much effort has concentrated on elucidating the pathogenesis of the vasculitis. However, based upon a renewed interest in (innate) immune defenses against microbes, a better understanding of the chronic granulomatous inflammation may contribute to a more precise insight into the early genesis of WG. Thus, this review focuses on summarizing and discussing data for a potential pattern of disease, i.e. from localized to generalized WG with a special emphasis on granulomatous lesions of the upper respiratory tract and their alterations during the disease course.

Definitions of limited, localized and generalized WG

In their original reports on a previously unrecognized new disease entity Heinz Klinger and Friedrich Wegener described a granulomatous inflammation accompanied by generalized vasculitis (2-4). In the fifties of the last century this disease became known as Wegener's granulomatosis. Later, Carrington and Liebow introduced the term "limited" WG to characterize predominant involvement of the lungs in the absence of kidney involvement (5). Recently the European Vasculitis Study Group (EUVAS) refined the term "limited" WG by determining two subgroups previously subsumed under the category of limited forms. This determination of subgroups was based on clinical and

pathological considerations in order to define disease stages. "Localized" WG was defined as WG restricted to the upper and/or lower respiratory tract. "Early systemic" WG included any organ involvement except for renal and imminent vital organ failure. Finally, "generalized" WG included renal involvement and/or imminent organ failure. Two other subgroups, namely "severe renal" and "refractory" disease, were defined to cover the full spectrum of the disease (6). Some patients with localized WG may progress to generalized disease whereas others do not, for as yet unknown reasons. Clinical and experimental evidence suggests that there are further differences between proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) and myeloperoxidase (MPO)-ANCA positive WG, ANCA-negative WG and patients displaying features of two granulomatous diseases, like WG and Crohn's disease (7; reviewed in 8). Previous studies detected ANCA by immunofluorescence and anti-proteinase 3 antibodies by ELISA less frequently in patients with limited disease as compared to generalized WG (9). This may reflect true differences in disease stage, but methodical improvements, i.e. the use of capture-ELISA or the detection of ANCA directed against the pro-form of PR3 might be more sensitive in initial disease stages and to changes in disease activity (10).

Pattern and mechanisms in the upper respiratory tract: Granulomas, necrosis, vasculitis

The current understanding of a classic histopathology of WG comprises a granulomatous inflammation resulting in a geographic pattern of tissue necrosis with a variable number of multinucleated giant cells and a necrotizing vasculitis (1,11, 12). The presence of

all three morphologic features is not always a given and may differ with respect to their appearance in involved organs as well as the time course of the disease. However, we and others have observed a predominance of granulomatous inflammation compared to signs of vasculitis in the upper respiratory tract, especially in localized WG (1,13,14). Granulomas of the upper respiratory tract can affect or extend towards other regions of the head, such as the retro-orbital tissues or the meninges. Renal granulomas are rare (15). Immunohistologic studies by Fienberg have suggested that WG may start as granulomatous disease in the upper respiratory tract and systemic vasculitis may subsequently develop (16,17). WG granulomas of the respiratory tract display different morphologies, ranging from palisading micro-

granulomas and necrotizing granulomas to neutrophilic microabscesses (11,12). They can be found in close vicinity to inflamed vessels or at extravascular sites (11,12,16,17). Physiologically, granulomas are formed by the immune system to deal with intracellular microbes and antigens that are difficult to digest (18). Therefore, as early work by F. Wegener himself already proposed, the presence of granulomas in the upper respiratory tract might suggest an infectious or other environmental cause as the initial triggering step in the pathogenesis of WG. The chronicity of the inflammatory response may indicate a failed attempt of the granulomas to fulfill their task, *i.e.* an infectious or other trigger is not resolved but instead may persist and lead to geographic necrosis of the tissue and vascular inflammation.

Cellular compositions

To elucidate tissue injury in the upper respiratory tract there have been studies on granulomas, necrosis and vascular inflammation in the upper respiratory tract of both localized and generalized WG (14,19,20). Figures 1 a-c depict the corresponding morphologic patterns (a: ill-defined epithelioid cell granulomas; b: geographic necrosis; and c: necrotizing and inflamed venules, *i.e.* vasculitis) that can be found in nasal lesions of both localized and generalized WG. On the cellular level, the granulomatous inflammation of the upper respiratory tract of both localized and generalized WG is characterized by CD4⁺ T cells, CD8⁺ T cells as well as monocytes, CD20⁺ B lymphocytes and CD68⁺ macrophages and CD68⁺ multinucleated giant cells (19). A strong expression of CD26 (optional

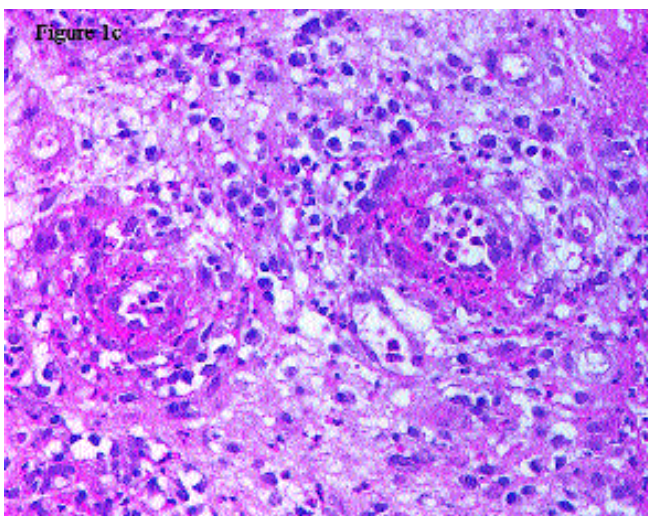
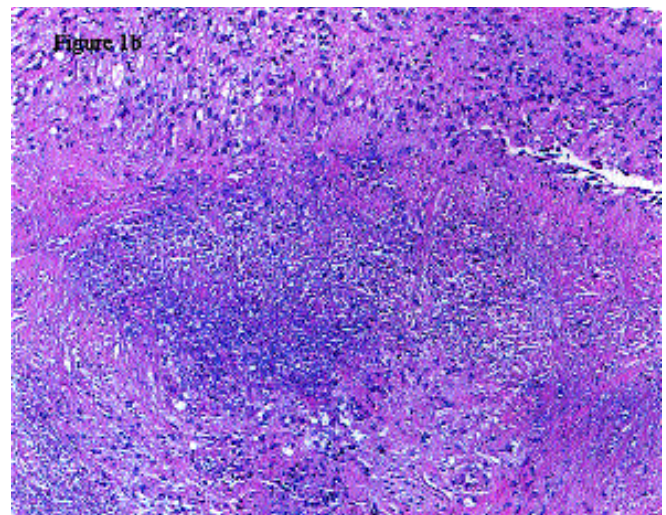
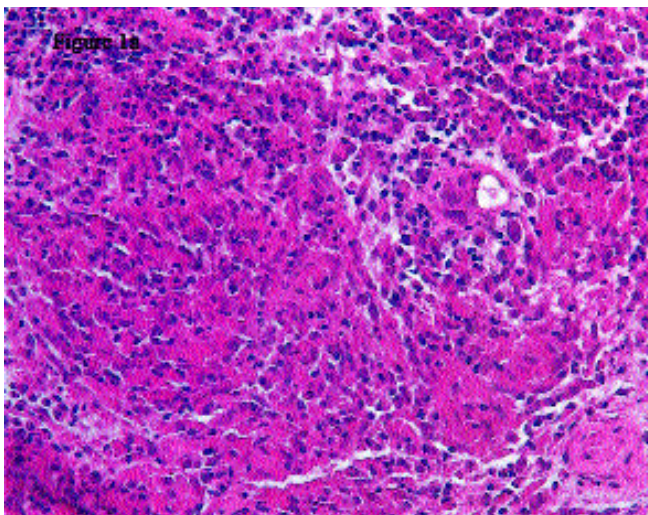


Fig. 1. (a) H (hematoxylin) & E (eosin) staining showing ill-defined epithelioid cell granulomas in a nasal biopsy from a patient with localized WG (original magnification x 400). (b) H&E staining showing geographic necrosis bordered by histiocytes in a nasal biopsy from a patient with localized WG (original magnification x 400). (c) H&E staining showing venules with fibrinoid necrosis and a mixed inflammatory infiltration of vessel walls, *i.e.* vasculitis in a nasal biopsy from a patient with localized WG (original magnification x 630).

Th1 marker) on CD4⁺ T cells in granulomatous lesions of the upper respiratory tract is predominantly seen in localized WG, whereas this is less evident in generalized WG (19). These observations were extended by Balding and colleagues (20), who described a predominant Th2 type immune response in nasal lesions of generalized WG, although the presence and extent of inflammatory lesions within the nasal tissue were not described in their study and FACS antibodies used for histologic interferon (IFN) and CCR5 detection (20). Furthermore, CD28⁻ T cells are present or even enriched in granulomatous lesions of the upper and lower respiratory tract of generalized WG (21, 22).

Cytokines

TNF (tumor necrosis factor) as well as IFN have been shown to be important for the formation and necrosis of granulomas, especially in animal models of tuberculosis (23,24). Recently, CD68⁺ alveolar macrophages have been described as one major source of TNF in sarcoidosis granulomas (25). In WG, peripheral blood and granuloma (CD4⁺ CD28⁻) T cells as well as monocytes/macrophages are major sources of TNF production (22, 26, 27, 28). In addition, low numbers of TNF⁺ multinucleated giant cells have been found in granulomatous lesions of nasal WG tissues (Fig. 2). Animal models led to the interesting hypothesis that the less organized and at times poorly defined structure of granulomatous lesions in WG may be due to a slightly impaired production of tissue TNF in relation to the amount of TNF actually needed to meet the antigenic challenge (23, 29). Nonetheless, a beneficial effect of anti-TNF therapy in generalized WG has been demonstrated (30), suggesting that balancing or down-regulating TNF levels targets an important disease mechanism in WG. Further, increased levels of soluble TNF receptor have been shown in WG (31). IFN expression was also observed in granulomatous lesions of the upper respiratory tract in localized (19, 32), and to a lesser extent in generalized WG (19, 20). Based on these observations it may

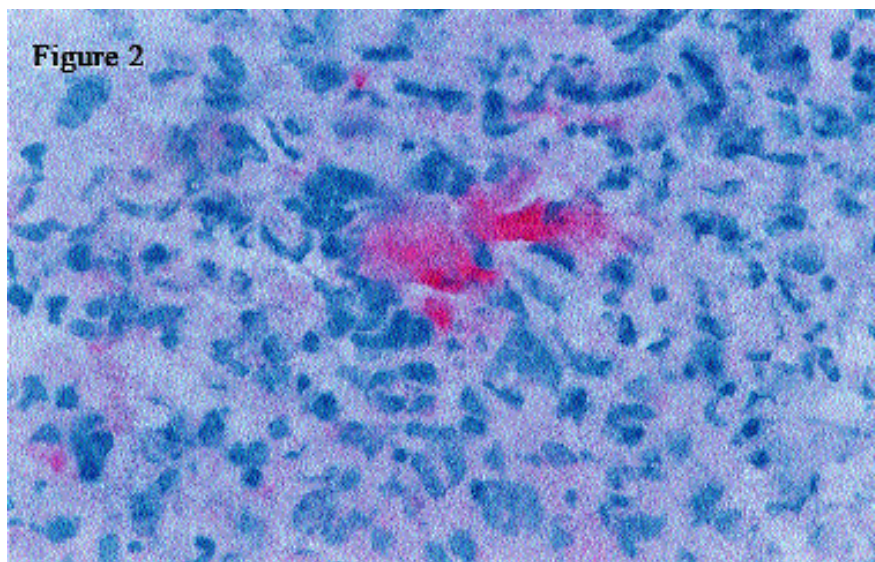


Fig. 2. Immunohistochemistry showing a multinucleated giant cell with a positive perinuclear staining for TNF (22) in a nasal biopsy from a patient with localized WG. Briefly, nasal biopsies of WG patients were submerged in tissue freezing medium, snap-frozen in liquid nitrogen and stored until use at -80°C. Six µm serial cryostat sections were fixed in acetone (30 min), followed by fixation in chloroform (30 min). Incubation with the primary monoclonal antibody (clone TNF-E; 58) was performed for 30 min and immunostaining was undertaken according to the alkaline phosphatase – anti alkaline phosphatase method with New Fuchsin development (59). Finally, slides were counterstained with hematoxylin and mounted. Immunostainings were controlled by implementing the secondary reagents alone to confirm specificity or enzyme development by itself. The stainings were evaluated by AM and KH-U. (Original magnification x 630)

be hypothesized that changes in the microenvironment, for instance from Th1 (IFN) to Th2 (IL-4) dominated cytokine profiles at granulomatous lesions of the upper respiratory tract, may accompany or even promote disease progression (19, 20). Phenotypically and functionally restricted cellular subsets such as IFN- and TNF-producing peripheral blood- and granuloma CD4⁺CD28⁻ T cells may play an important role in early granuloma formation (22).

Migration patterns

With respect to an accumulation of inflammatory cells in tissues, i.e. granulomas, in response to chemotactic gradients the expression of chemokine receptors can indicate migratory capabilities and patterns. It has been shown that RANTES (regulated on activation normal T cell expressed and secreted/CCL5) is expressed in WG granulomas of the respiratory tract (33). RANTES production by endothelial cells can be synergistically induced by IFN + TNF (34), which may represent a mechanism of leucocyte migration into

granulomatous areas. In localized WG, a higher expression of CCR5 (receptor for RANTES, associated with type 1 immune responses) on memory T cells may favor recruitment of type 1 effector memory T cells in granulomatous lesions of the upper respiratory tract, whereas in generalized WG CCR3 (receptor for RANTES, associated with type 2 immune response)-mediated recruitment of type 2 effector memory T cells may play an additional role (20, 35, 36). Moreover, early expansion of a subset of Th1-like CD4⁺CD28⁻CCR5⁺ effector memory T cells might contribute to granuloma formation in the beginning of the disease (35, 36).

Pattern and mechanisms of localized and generalized WG: Infection(?)-induced inflammation

It has been suggested that WG evolves on the basis of a genetic predisposition to an exaggerated Th1-like reaction with granuloma formation in response to environmental triggers and/or the autoantigen, proteinase 3 (37). So far there is no cause and effect relationship for any microorganism implicated in

Table I. Predominant phenotypic/cell surface and functional markers mainly of different T cell subsets occurring during the WG disease course at different locations according to the ELK classification by DeRemee *et al.* (57), (numbers in parentheses refer to the literature).

WG disease phase/location	Phenotypic and functional characteristics
Localized / inflammatory lesions (histology)	CD4 ⁺ CD26 ⁺⁺ (19) CD4 ⁺ IFN ⁺⁺ (19) CD3 ⁺ CCR5 ⁺⁺ (35) TNF ⁺ (upper respiratory tract)
Localized/peripheral blood	Mononuclear leucocytes: IFN ⁺ (19) CD4 ⁺ CD28 ⁺ CCR5 ⁺⁺ (35) CD4 ⁺ CD45RO ⁺ CCR5 ⁺ (36)
Generalized / inflammatory lesions (histology)	CD4 ⁺ CD26 ⁺ (19) CD4 ⁺ CD28 ⁺ : IFN ⁺ ; TNF ⁺ (19, 22) CD3 ⁺ CCR5 ⁺ (35) (upper respiratory tract) CD3 ⁺ : IL-4 ⁺ ; CCR3 ⁺ Eosinophils (20) (upper respiratory tract) CD3 ⁺ CD28 ⁺ (21) Macrophages, CD4 ⁺ CD45RO ⁺ : RANTES ⁺ ; IFN mRNA ⁺ (33) (lower respiratory tract) CD3 ⁺ : IL-2 ⁺ ; IL-4 ⁺ ; CCR5 ⁺ (20) (kidney)
Generalized/peripheral blood	HLA-DR ⁺ CD4 ⁺ : IFN ⁺ ; TNF ⁺ (27) CD4 ⁺ CD28 ⁺ : IFN ⁺ ; TNF ⁺ (21, 22) CD4 ⁺ CD28 ⁺ CCR5 ⁺ (35) CD4 ⁺ CD45RO ⁺ CCR3 ⁺ (36)

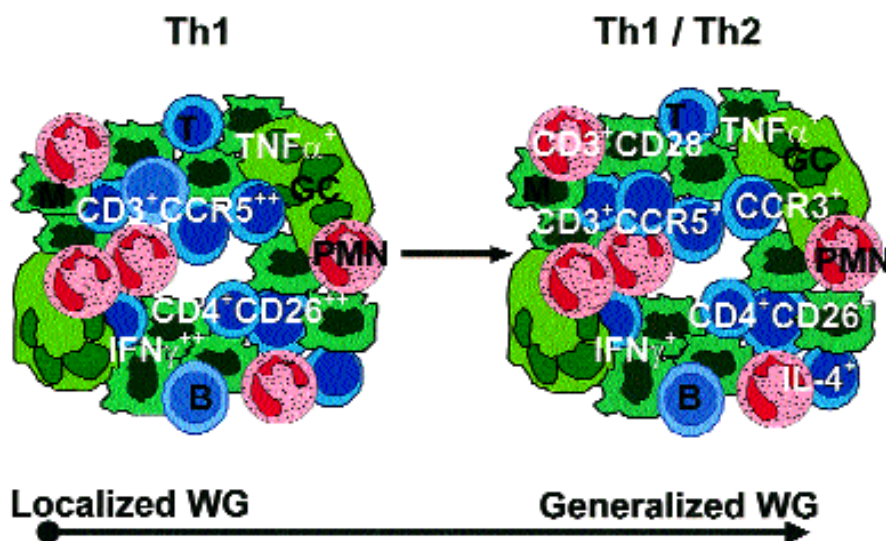


Fig. 3. Summary of the principal structure of nasal granulomatous lesions in localized and generalized WG (see also Table I). WG nasal granulomatous lesions consist of neutrophilic granulocytes (PMN), monocyte-derived tissue macrophages (M), multinucleated giant cells of the Langhans and foreign body type (GC), T cells (T), B cells (B). CD4⁺CD26⁺⁺ cells produce IFN⁺⁺ in granulomatous lesions of localized WG (19). IL-4 positive cells have been shown in granulomatous lesions of generalized WG (20). CD3⁺ T cells are mainly CD28⁺ (21). CD4⁺CD28⁺ T cells are IFN⁺ and TNF⁺ in granulomatous lesions of WG (22). CCR5⁺⁺ T cells are predominantly found in granulomatous lesions of localized WG, whereas CCR3⁺ T cells are detected in generalized WG (20, 35). Changes in the cytokine milieu of a potential disease starting point (nasal granuloma) may promote and/or accompany the course of the disease from localized to generalized WG. (original art by AM, WLG, PL).

(early) WG pathogenesis, but a significant association between nasal carriage of *S. aureus* and WG relapse rates has been found (38,39). A break of tolerance, molecular mimicry or superantigen driven processes may eventually result in B cell expansion and ANCA production. CD4⁺CD28⁺CCR5⁺ effector memory T cells might contribute to granuloma formation and could potentially play a role in the pathology of autoreactivity, either directly by maintaining the inflammatory response or as a result of bystander activation (35, 36).

Pattern and mechanisms of generalized WG: ANCA-induced small vessel inflammation and destruction

PR3-ANCA, B and T cells

Numerous experimental *in vitro* and *in vivo* studies have demonstrated that interaction of ANCA with cytokine-primed neutrophils and monocytes and/or their enzymatic constituents leads to leukocyte recruitment, endothelial damage and subsequent destruction of blood vessels, representing major mechanisms of vasculitis (40-46, reviewed in 47). Recently, Xiao and co-workers reported that MPO-ANCA cause glomerulonephritis and vasculitis in mice (48). They provide convincing *in vivo* evidence for the induction of glomerulonephritis and vasculitis by ANCA. With this study compelling proof for a direct pathogenicity of the autoantibody has been given. It has been demonstrated that T cell help is required for the production of circulating ANCA autoantibody by plasma cells (49). Further, induction of proliferation and IL-10 production by CD4⁺ T cells as detected by ELISA following stimulation with PR3 for 10 days was shown (50).

However, ELISA detects the composite cytokine response of a bulk population of specifically and unspecifically activated cells. In acute myelogenous leukemia PR3-specific T cells have also been found. In this clinical condition PR3-specific T cells produce Th1-type cytokines in response to PR3 as detected by single cell analysis using cytokine flow cytometry (51). A TNF⁺ response has also been determined in re-

sponse to another potential autoantigen, the G1 domain of the proteoglycan aggrecan in ankylosing spondylitis recently (52). Since plasticity, *i.e.* the capacity to activate alternative cytokine programs, of effector memory T cells is limited (53), further studies are necessary to determine the exact pattern and time course of cytokine production in response to PR3 in WG. Besides PR3-specific T cells, a regulatory T cell subset in WG, as defined by CTLA4 expression, may also play a role for disease progression (54). A microarray gene chip analysis combined with real-time, quantitative PCR yielded significant responses of DIF-2, COX-2 and IL-8 genes in ANCA-activated leucocytes (55). The IEX-1/DIF-2 molecule seems to be involved in regulating the apoptosis of activated T and B cells (56), which may offer another clue for a better understanding of the role of T and B cells in ANCA vasculitis.

Conclusion

Granulomatous lesions represent an important feature of Wegener's granulomatosis. Table I and Figure 3 summarize our current understanding of the patterns of granulomatous lesions, especially in the upper respiratory tract, and provide a hypothesis of the transition from localized to generalized WG. Changes of the granulomatous microenvironment in the upper respiratory tract need to be more closely evaluated, to elucidate mechanisms important during early, localized WG. Understanding the connection between the granulomatous lesions and the subsequently evolving generalized vasculitis remains one of the challenges in explaining the pathogenesis of Wegener's granulomatosis.

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