L18. Granuloma formation in granulomatosis with polyangiitis

Extravascular granuloma formation is one of the key features in granulomatosis with polyangiitis (GPA). In most patients, GPA begins in the upper respiratory tract as active, eventually necrotizing extravascular granulomatous disease, with or without local vasculitis. Typically, neutrophilic microabscesses with micronecrosis occur, later bordered by ill-defined granulomas that may proceed to geographic necrosis. In contrast to other types of granulomatous diseases, e.g. sarcoidosis or tuberculosis, the granulomatous inflammation in GPA typically shows very poorly delineated granulomas, or only scattered giant cells, within a dense heterogeneous population of inflammatory bystander cells, mainly lymphocytes, plasma cells, dendritic cells, neutrophils, eosinophils and later fibroblasts (figures 1 and 2). The lymphocytic infiltrate may be organized to form primary and secondary follicles corresponding to ectopic lymphoid tissue. Inflammatory cells, necrosis and fibroblast proliferation deeply infiltrate the submucous tissues of the upper respiratory tract and may destructively encroach on cartilage and bone with active resorption (figure 2).

Morphological studies of granulomatous lesions in GPA must always take the following into account:

- several different inflammatory cell types constituting “the” granuloma;
- possible differences in activation profiles and mediator expression (e.g. cytokines) between circulating and lesional inflammatory cells;
- comparison of seemingly specific phenomena in GPA with other types of purulent and/or granulomatous inflammation.

Four important components of the extravascular granulomatous inflammation in GPA will be discussed:

- neutrophils and micronecrosis;
- macrophages and giant cells – the granulomatous reaction;
- ectopic lymphoid tissue: potential source of auto-antigen production;
- fibroblast mediated destruction of cartilage and bone.

Neutrophils and micronecrosis

Several studies have shown that in respiratory tract lesions, a purulent neutrophil reaction is an essential feature of GPA [1–3]: small neutrophilic aggregates (microabscesses) around small necrotic areas that contain neutrophilic debris. It has been suggested that micronecrosis with neutrophilic microabscesses constitutes the early phase in the development of the pathognomonic macrophage granuloma in GPA [1]. However, it is not clear how necrotic lesions are induced. Are they solely ANCA-induced and/or do they result from exposure to another initiating injurious agent? Animal models demonstrating PR3-ANCA induced acute pulmonary and renal vascular injury...
lack extravascular necrotizing granulomatous inflammation of the respiratory tract [4].

Could NETosis play a key role in GPA granuloma? Several recent studies have investigated NETosis as possible pathogen defence mechanism in GPA. ANCA-stimulated neutrophils release neutrophil extracellular traps (NETs), consisting of extracellular fibrous structures of chromatin and various proteins, including PR3 and MPO [5,6]. Kessenbrock et al. [5] have demonstrated NETs in glomerulonephritic lesions in small vessel vasculitis, suggesting a role for PR3-ANCA induced NETosis in the pathogenesis of GPA. Myeloid dendritic cells uploaded with and activated by NETs induced ANCA in susceptible mice [7]. However, no study has investigated NETs in granulomatous extravascular disease in GPA.

In contrast to circulating cells that show an increase of IL-17-positive T cells in GPA, lesional IL-17 expression in acute ANCA-associated glomerulonephritis has been demonstrated mainly in neutrophils [8]. This is also true in granulomatous lesions of the respiratory tract in GPA. In several other inflammatory diseases, neutrophils have been detected as a source of IL-17. It remains to be examined whether the effects of neutrophil-derived IL-17 in GPA on inflammatory response differ from those in other diseases.

**Macrophages and giant cells – the granulomatous reaction**

In GPA, macrophage accumulations justifying the name of “granulomatosis” constitute only a small minority of cells among a multitude of other inflammatory cells, as has been pointed out very clearly by Jennette [3]. Histologically, the granulomatous nature of the inflammation of GPA is therefore often difficult to perceive. The development of granuloma, i.e. the recruitment of nodularly arranged macrophages and fusion of macrophages to giant cells has been explained as a secondary reactive response to the acute necrotic lesions [1,3,9]. Macrophages and giant cells with phagocytosed apoptotic debris, and apoptotic neutrophils surrounding necrotic areas have been shown in necrotizing granulomatous lesions of the respiratory tract in GPA [3,9]. It has been speculated that local antigen-presentation is maintained by such macrophages [3]. The B cell-activating cytokine APRIL (A Proliferation-Inducing Ligand), a survival factor for long-lived plasma cells, has been...
found in its secreted form within macrophages and giant cells [10]. Therefore in GPA, granuloma could support the generation of an APRIL-rich niche for sustained autoimmune reaction. However, it is not known whether macrophage granuloma is a prerequisite for activity and/or induction of autoimmune processes in affected organs. Apoptotic debris of neutrophils occurs in the course of multiple purulent inflammations at various body sites, among them the respiratory tract. The nature of the antigen remains unknown, as well as the alterations of the immune system in GPA that eventually lead to granuloma formation in GPA.

**Ectopic lymphoid structures: potential source of auto-antibody production**

The inflammatory infiltrate in GPA displays features of ectopic lymphoid-like tissue (ELT) neoformation, which may help to maintain an immunopathological response against an auto-antigen, which is otherwise tolerated. PR3-expressing neutrophils, dendritic cells and cells of the adaptive immune response (T- and B-cells, plasma-cells) are clustered in the infiltrate, with lymphocytes frequently organized in follicles [11,12,13]. Local follicle formation and B-cell survival appear to be sustained by cytokines as IL-17, lymphoid chemokines and B-cell survival factors (e.g. APRIL) [10,14]. However, ELT can also occur as reactive lesions in many non-autoimmune chronic inflammatory disorders such as chronic obstructive pulmonary disease [15], and is frequently seen in chronic unspecified rhinosinusitis. Although the mere detection of ELT in granulomatous inflammation of GPA therefore alone cannot prove its significance for auto-antibody production within the granuloma, it has been shown that nasal mucosa can be a place of B lymphocyte maturation, selection and auto-antibody production [11,13,16]. Genetic studies of the overall mutation pattern of CD20+ B-cells and plasma cells from granulomatous GPA tissues show an ongoing selection in Ig V genes resembling that of other autoimmune diseases [17,18].

**Fibroblast-mediated destruction of cartilage and bone**

Only recently have fibroblasts received attention as activated effector cells of tissue destruction in GPA, although a prominent and deep submucous fibroblast reaction is a typical feature of chronic upper respiratory tract granulomatous disease (figure 2). Indeed, deep biopsies with cartilage resp. bone destruction typically show a direct contact of the inflammatory infiltrate to nasal skeletal tissue, with fibroblast rich peristomal/ perichondral tissue and active destruction of skeletal tissue (figure 2). Necrosis of bone or cartilage as a part of widespread geographical necrosis is seen more rarely. Kesel et al. [19] demonstrated in in vivo experiments that xenografted nasal mucosa of GPA patients showed massive destruction of co-implanted human cartilage, mediated by human fibroblasts. An up-regulated production of metalloproteinases 1, 3 and 13 as mediators of extracellular matrix breakdown at sites of invasion and pro-inflammatory cytokines IL-6/8 was found in fibroblasts in vivo and in vitro, as well as delayed apoptosis of fibroblasts of GPA patients in vitro [19]. Further studies will be needed to better understand the cellular interactions of “the granuloma” and its mechanisms giving rise to tissue autodestruction and possibly to autoimmunity in GPA.

**Disclosure of interest**: the authors declare that they have no conflicts of interest concerning this article.

**References**

In contrast to secondary lymphoid organs (SLOs) that arise during development at predetermined locations, the formation of tertiary lymphoid organs (TLOs) can occur in adults at ectopic sites in any tissue in the context of persistent inflammatory disorders, such as autoimmune diseases, cancer, and organ transplantation [1–3]. The molecular mechanisms underlying the organization of chronic inflammatory infiltrates into ectopic lymphoid tissue recapitulate some of those involved in lymphoid organogenesis during development [4,5] and hence this process has been referred to as lymphoid neogenesis. Beyond anatomical similarities with SLOs, ectopic lymphoid tissues are fully functional and support the development of local adaptive immune responses, including the priming of naïve lymphocytes [6], generation of memory subsets, and germinal center reactions (clonal expansions, somatic hypermutations, immunoglobulin class switching and antibody production), which are suspected to contribute to the exacerbation of chronic inflammatory diseases [7,8].

**Tertiary lymphoid organs (TLOs) in rejected organs**

We have undertaken several studies in the context of alloimmunity and have demonstrated 1/that the graft is not only the target of the alloimmune response but also a site where this response actually develops, so as to optimize the communication between the targeted tissue and the immune effectors [9], 2/that TLOs provide survival signal to B cells, allowing them to escape rituximab-induced apoptosis, thereby thwarting therapeutic efficiency [10], 3/that anti-MHC humoral response is more intense and more diverse in TLOs [the abnormal activation of CD4+ T cells promotes the development of an exaggerated pathogenic immune humoral response in TLOs due to a defective immune regulation] [11] and the intragraft microenvironment interferes with peripheral deletion of autoreactive immature B cells that, in turn, produce antibodies against intracellular autoantigens [12], 4/that intragraft humoral immune response appears uncoupled from the systemic response and that TLO formation recapitulate organogenesis of SLOs [13].

While these data demonstrate that chronic rejection is associated with the development of lymphoid nodular infiltrates within rejected organs, evidence for the involvement of these lymphoid structures in the rejection process came from a model of rat aortic interposition model where lymphoid nodular infiltrates was evidenced in the adventitia of the chronically rejected aortic grafts [3]. We could demonstrate that nodular lymphoid structures were functional ectopic germinal centers that participate in the rejection process of the grafted organ. In addition, this showed that the vascular stroma was sufficient to promote and support lymphoid neogenesis.

**Tertiary lymphoid organs (TLOs) in atherothrombosis**

Interestingly, TLOs are also present in the context of atherothrombosis. Indeed, TLOs were detected in the adventitia of human atherosclerotic arteries as early as the 1950s [14], a process that has been recently revisited [15–18]. We could also characterize adventitial lymphoid aggregates distributed all along the aortic segment in atherosclerosis-prone ApoE KO mice. These structures were defined as TLOs because they are composed of B cell follicles surrounded by 1 T cells, a prototypic organization reported for ectopic germinal centers [4]. IgM and IgD staining revealed the presence of two subsets of B cells with different maturation states. Moreover, these TLOs included blood vessels, lymphatic networks, and FRC-like cells, suggesting that these aggregates are proper structures to induce and maintain local immune responses. These adventitial blood and lymphatic networks are essential for the recruitment and drainage of immune effectors [19–22]. Interestingly, the TLOs were polarized towards the media, suggesting that effectors inside the aortic wall may be involved in the lymphangio genesis and angiogenesis associated with the formation of adventitial TLOs. In this respect, it is important to note that...