antigen-presenting functions or transmit signals to naive T-cells remains still puzzling. Neutrophils have also been shown to modulate the maturation, activation and functions of NK cells, either by themselves or in cooperation with other cell types [9]. In this context, it is worth mentioning that neutrophils, by interacting with specific subsets of peripheral blood myeloid DC (e.g., 6-sulpho LacNAc+ DC, also known as slanDC) can strongly potentiate IFNγ release by NK cells [10]. Importantly, the potential pathophysiological relevance of a cell network among neutrophils, slanDC, and NK cells has been suggested by immunohistochemical studies that have revealed their colocalization in several chronic inflammatory pathologies, such as Crohn’s disease and psoriasis [10]. Finally, it has also been proposed that mature postmitotic neutrophils can also “transdifferentiate” into much-longer-lived cells with macrophage- or DC-like characteristics, which might constitute a further manner for neutrophils to act as regulatory cells of the adaptive immune response [1,3].

In view of the continuously emerging findings in the field, it is predictable that in the next years there will be the discovery of additional, unsuspected biologic features that neutrophils possess.

Disclosure of interest: the author declares that he has no conflicts of interest concerning this article.

Acknowledgements: This work was supported to MAC by grants from Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR-PRIN 2009MFXE7L), and Associazione Italiana per la Ricerca sul Cancro (AIRC-IG 11782).

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Available online 26 February 2013

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http://dx.doi.org/10.1016/j.lpm.2013.01.033

L34. Neutrophils in ANCA-associated vasculitis: Still under investigation

Introduction

The pathogenesis of ANCA-associated vasculitis (AAV) is unknown but is consistent with a primary role for neutrophils in the acute injury. Neutrophils are cardinal cells in the pathophysiological process in AAV since they are both effector cells responsible for endothelial damage and targets of autoimmunity. The neutrophil granule proteinase 3 (PR3) and myeloperoxidase (MPO) are the only two main target antigens for ANCA [1]. This selectivity is surprising regarding the diversity of the proteins stored within cytosolic granules. It has been proposed that the formation of Neutrophil Extracellular Traps (NETs) that are composed of DNA extruded from dying neutrophils, which bear all the cationic granule proteins including PR3 and MPO, could be involved in the pathophysiology of AAV [2]. However, NET formation does not explain this selectivity towards PR3 and MPO. Anti-PR3 ANCA are found in sera from patients with granulomatosis with polyangiitis (GPA), which is characterized by granulomatous inflammation of the upper and/or lower respiratory tract whereas anti-MPO ANCA are present in sera from patients with microscopic polyangiitis (MPA), idiopathic necrotizing crescentic glomerulonephritis, and less frequently in Churg-Strauss syndrome, which associates late onset asthma, hypereosinophilia and small vessel vasculitis.

Neutrophil activation during ANCA-associated vasculitis: role of ANCA

During the pathological course of AAV, accumulation of neutrophils at the inflammatory site is a key event initiated by the
so-called “priming” of these cells. Priming can be induced by inflammatory cytokines, adhesion, bacterial products (lipopolysaccharide), or lipid mediators and is characterized as a “ready to go” state, which results in a faster and higher response upon exposure to a second stimulus. Priming is necessary to induce membrane expression of PR3 and MPO that can be accessible to ANCA to trigger neutrophil activation. Primed neutrophils incubated in vitro with IgG purified from sera containing anti-PR3 ANCA or anti-MPO ANCA are able to produce superoxide anion and release lytic granular proteins [3]. ANCA-induced neutrophil activation requires both antigen binding, Fc receptor (FcγRIIA or FcγRIIb) as well as β2-integrin engagement. Several recent articles have reviewed our current knowledge about the signaling pathways involved in ANCA-induced neutrophil activation [4]. The parallel development of animal models allowed to study the in vivo role of ANCA in vasculitis and it is now admitted that anti-MPO ANCA are pathogenic (see “The animal models” section). However, in the presence or absence of ANCA, there are no controversies about the crucial role of activated neutrophils in endothelial damages [5]. Further investigations are needed to identify the molecular mechanisms regulating the complex neutrophil/endothelium interactions [6] and whether they are dysregulated in AAV.

**The ANCA antigens: keys in the pathophysiology of ANCA-associated vasculitis**

MPO and PR3 are two granule-stored microbicidal proteins expressed by neutrophils but also by monocytes [2]. MPO is highly abundant (up to 5% of dry weight) and is exclusively found in azurophilic granules. MPO is a key component of the phagocyte oxygen-dependent intracellular microbicidal system [7]. Nonetheless, it progressively became clear that MPO was not solely a bacterial protein but a key player in the balance between innate and adaptive immunity through its pro and anti-inflammatory functions. It has been demonstrated that MPO can oxidize low density lipoproteins and extracellular matrix proteins within the blood vessel wall, implicating MPO in the physiopathology of atherosclerosis [8] that is now considered as an “immune” disease [9]. Further studies will be required to understand the immunomodulatory role of MPO in AAV.

PR3 also called myeloblastin, specifically expressed by neutrophils and monocytes, belongs to the neutrophil serine protease family and is classically localized in azurophilic granules with its homologs: elastase, cathepsin G and azurocidin. After phagocytosis of pathogens, PR3 is secreted in the phagolysosome to play its microbicidal function. Although PR3 shares more than 60% sequence homology with neutrophil elastase, PR3 has some structural and functional peculiarities [10]. One specific feature of PR3 is its bimodal membrane expression at the resting neutrophil surface, meaning that some neutrophils lack membrane PR3 (mPR3−) whereas others express (mPR3 +). Interestingly, patients suffering from AAV have an increased proportion of mPR3 + cells [11,12]. Interestingly, CD177 (also called human neutrophil antigen B1, NB1), a glycosylphosphatidylinositol (GPI)-linked membrane receptor is co-expressed on the same neutrophil subset that expressed membrane PR3 [13,14]. It has been suggested that NB1 could bind PR3 thus acting as a receptor for PR3. PR3 association with membrane involved a hydrophobic patch responsible for its interaction with lipids and its membrane anchorage [15,16]. PR3 could be externalized during apoptosis in association with specific partner proteins including the phospholipids scramblase1 [17] and calreticulin, a chaperone protein involved in the recognition of apoptotic cells by macrophages [18]. Accordingly, it can be proposed a new role of PR3 in counteracting anti-inflammatory mechanisms induced by apoptotic cell clearance [19,20] thus impairing the resolution of inflammation and promoting autoimmunity.

**Dysregulated granular proteins synthesis and survival in neutrophils in ANCA-associated vasculitis**

Neutrophils from AAV patients re-express genes coding for PR3 and MPO, despite the fact that these genes are normally restricted to the promyelocytic stage during granulocytic differentiation [21]. Gene expression profile studies performed on blood leukocytes has shown a signature consisting of more than 200 genes expressed in neutrophils from AAV patients whereas a lymphocyte signature was observed in SLE patients [22]. Neutrophils are not simple terminal effectors cells but are also capable of immunomodulation with the secretion of a great variety of cytokines, chemokines [23] that can instruct all immune cells (monocytes, dendritic cells, T cells and B cells) through an active cross-talk. For instance, neutrophils are a major source of B-cell-Activating Factor (BAFF) and A Proliferating-Inducing Ligand (APRIL), which are both members of the TNF superfamily and implicated in fundamental processes of B lymphocytes homeostasis [24]. Notably, serum levels of BAFF are elevated in GPA compared to controls [25]. Neutrophils from patients with AAV have shown an enhanced rate of apoptosis measured in vitro as compared to controls when activated by ANCA [26]. In contrast, spontaneous apoptosis of neutrophils was delayed in patients with AAV [27]. In keeping with this notion, neutrophils from GPA patients have an increased expression of the cytosolic proliferating cell nuclear antigen [28] that is associated with increased neutrophil survival [29]. Whether these disturbances in the balance survival/apoptosis might again impact the clearance of apoptotic neutrophils that is a key step in the resolution of inflammation [30] is not known but should require attention.

**Conclusion**

New perspectives in the research on neutrophils in AAV should include studies to determine whether some intrinsic dysregulation could favor a failure in the resolution of inflammation.
specific investigation of the functions and the immunomodulatory roles of the target antigens, either MPO or PR3 should be carried out in order to confirm their specific and presumably differential involvement in MPA and GPA, respectively as suggested by the recent genomic study comparing GPA and MPA [31].

Disclosure of interest: the author declares that he has no conflicts of interest concerning this article.

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