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Review

Neutrophil function in inflammation and inflammatory diseases

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Abstract

In inflammatory conditions such as RA, the neutrophil has tended to be dismissed as a short-lived, terminally differentiated, irrelevant bystander cell. However, this is clearly not the case. A better understanding of the complex heterogeneous pathways and processes that constitute RA, in parallel with a more sophisticated knowledge of neutrophil biology has identified many potential roles for these cells in the persistence of inflammation and progression of joint damage, which should not be underestimated. Not only are neutrophils found in high numbers within the rheumatoid joint, both in synovial tissue and in joint fluid, they have a huge potential to directly inflict damage to tissue, bone and cartilage via the secretion of proteases and toxic oxygen metabolites, as well as driving inflammation through antigen presentation and secretion of cytokines, chemokines, prostaglandins and leucotrienes. Drugs already used to treat RA down-regulate many neutrophil functions, including migration to the joint, degranulation and production of inflammatory mediators, and these cells should be considered as important targets for the development of new therapies in the future.

Key words: Neutrophils, Inflammation, Rheumatoid arthritis, Cytokines, Chemokines, MHC Class II, Apoptosis, TNF, IL-6, Biologics.

REVIEW

Introduction

Our understanding of the role of the neutrophil in inflammation has changed fundamentally over recent years. The initial perception of the neutrophil playing a passive role and merely responding to external signals has now been replaced by an appreciation that activated neutrophils can perform most (if not all) of the functions of macrophages. It is now recognized that appropriately activated neutrophils secrete a variety of pro-inflammatory cytokines and express MHC Class II (MHCII) in a manner that allows presentation of antigen to, and activation of, T cells. It is also recognized that neutrophils contribute to the pathogenesis of a number of human diseases such as chronic obstructive pulmonary disease, Behçet's disease and inflammatory arthritis. In some of these conditions, neutrophils appear to have been inappropriately activated to release tissue-damaging molecules (such as proteases) or, alternatively, molecules that can promote inflammation such as chemoattractants (eicosanoids and chemokines) or cytokines. Evidence is also accumulating from animal models that neutrophils can contribute to both the initiation and the progression of models of arthritis. Indeed, their presence in diseased joints of patients with RA (both in SF and in synovial membranes), and the identification of neutrophil-derived products in these tissues, strongly implicates them in the molecular pathology of this disease.

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Overview of neutrophil function

Neutrophils represent the body's primary line of defence against invading pathogens such as bacteria, and constitute ~40–60% of the white blood cell population. In the circulation of healthy adults, neutrophils exist in a resting state, which ensures that their toxic intracellular contents are not accidentally released to damage host tissue. Neutrophils become activated via a two-stage process. Resting neutrophils can become primed by agents that include bacterial products and cytokines or chemokines, e.g. TNF-α, GM-CSF, IL-8 and IFN-γ [1] and primed neutrophils are then mobilized to the site of infection or inflammation, where they encounter activating signals to trigger bacterial killing.

Priming occurs via two separate mechanisms. Rapid priming (within minutes of the cell receiving a signal) results from the mobilization of intracellular granules that possess pre-formed receptors (Fig. 1) to the plasma membrane. This process increases the number (and sometimes the affinity) of surface-expressed plasma membrane receptors by mechanisms that do not involve protein biosynthesis. Often, however, the priming agent will also result in activation of transcription factors that trigger the *de novo* expression of molecules (e.g. receptors and cytokines), which enhance neutrophil function or lifespan. Thus, the molecular properties and hence functions of resting blood neutrophils and primed neutrophils are very different. For this reason, *in vitro* experiments using freshly isolated blood neutrophils often fail to recognize the full functional repertoire and capability of neutrophils. Many of the regulatory functions of macrophages are shared by primed (but not resting) neutrophils.

The migration of neutrophils from the circulation to the site of inflammation is controlled by interactions with the vascular endothelium. L-selectin expressed on the surface of neutrophils allows loose tethering to ligands expressed on the surface of endothelial cells [such as E- and P-selectin, and P-selectin glycoprotein ligand-1 (PSGL-1)] as it rolls along the endothelium (Fig. 2). This induces conformational changes in integrin adhesion molecules including very late antigen-4 (VLA-4; $\alpha_4\beta_1$-integrin, CD49d/CD29b), lymphocyte function-associated antigen-1 (LFA-1; $\alpha_L\beta_2$-integrin, CD11a/CD18) and macrophage antigen-1 (MAC-1; $\alpha_M\beta_2$-integrin, CD11b/CD18),

Fig. 1 Properties of neutrophils. (**A**) Neutrophil functions and products that can actively drive inflammatory processes. (**B**) Summary of neutrophil granules and their contents. Neutrophil granules are mobilized upon priming of the cell: secretory vesicles are mobilized first, followed by gelatinase granules, specific granules and finally azurophilic granules.

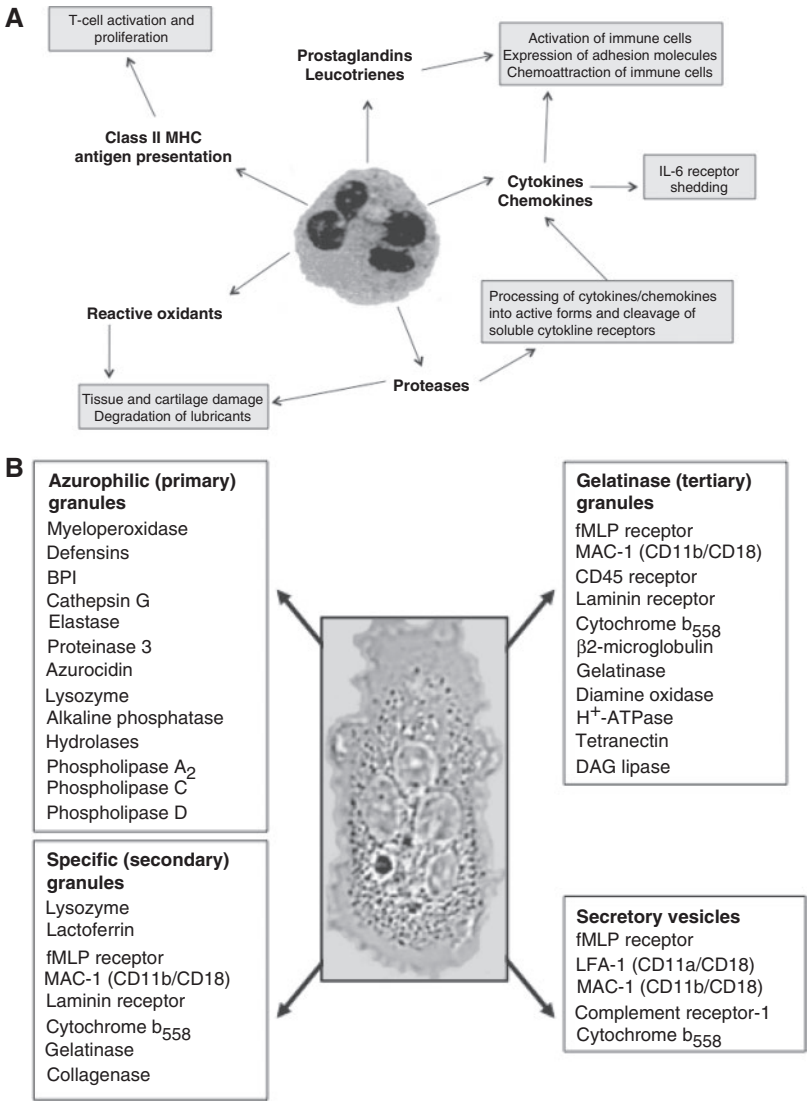
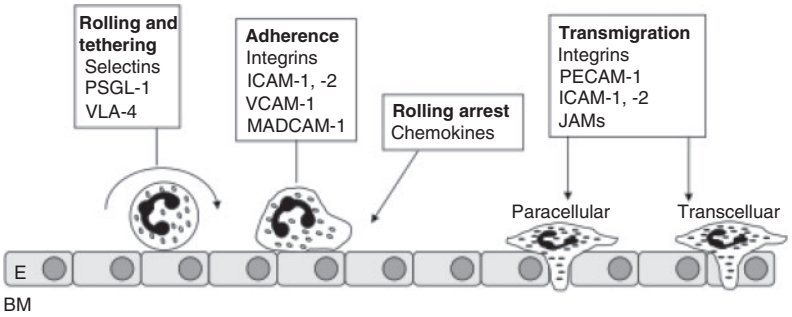


FIG. 2 Neutrophil diapedesis. To pass from the peripheral blood to the site of inflammation, the neutrophil adheres to the endothelial wall using selectins, integrins and adhesion molecules. Rolling arrest precedes transmigration through the endothelial lining of the blood vessel, and chemotaxis to sites of inflammation. E: endothelial cell; BM: basement membrane. (Figure adapted from Ley *et al.* [2].)



which engage with adhesion molecules on the surface of endothelial cells, such as intercellular adhesion molecule (ICAM)-1 and -2, vascular cell-adhesion molecule-1 (VCAM-1) and mucosal vascular cell-adhesion molecule-1 (MADCAM-1), leading to high-affinity ligand binding and strong adherence. Rolling arrest is mediated by binding of chemoattractants such as IL-8 to neutrophil receptors following high-affinity adherence to the endothelium [2]. Neutrophils then migrate into tissue through the junctions between neighbouring endothelial cells (paracellular migration) using surface ligands including ICAM-2, PECAM-1 (platelet endothelial-cell adhesion molecule-1) and proteins of the junctional adhesion molecule (JAM) family [3]. Transcellular transmigration may also occur under conditions of high ICAM-1 expression and density, with a small minority of neutrophils penetrating and passing through pores in the cytoplasm of endothelial cells [4].

Once neutrophils have left the circulation and passed through the endothelium, they migrate towards inflamed tissue along a chemotactic gradient. Exposure of neutrophils to chemoattractants such as *N*-formylmethionyl-leucyl-phenylalanine (fMLP) and complement component 5a (C5a) induces cellular polarization of chemoreceptors and formation of actin-rich pseudopodia at the leading edge of the cell [5]. At the site of infection, membrane receptors for complement proteins and immunoglobulins recognize and bind opsonized bacteria leading to the formation of pseudopodia, phagocytosis of the pathogen and destruction within the intracellular phagosome. Neutrophils possess an arsenal of proteases (Fig. 1) and can generate reactive oxygen species (ROS) in order to rapidly kill phagocytosed pathogens, but these toxic molecules can also damage host tissue following their release from inappropriately activated neutrophils in autoimmune diseases.

Comparison of the function of primed/activated neutrophils with macrophages

Macrophages have long been considered as central to the pathogenesis of RA due to their ability to activate T cells by antigen presentation, and to secrete many of the pro-inflammatory mediators found within SF and tissue such as cytokines, chemokines and MMPs [6]. However, macrophages are often found in relatively low numbers at inflammatory sites compared with neutrophils. While resting peripheral blood neutrophils are relatively short lived, undergoing apoptosis within 12–18 h, primed and activated neutrophils within tissues have undergone molecular changes that extend their life span and alter their molecular properties, thereby allowing them to carry out many functions that have historically been attributed to macrophages. Delayed apoptosis, together with stimulated functions such as the synthesis of pro-inflammatory mediators (cytokines and chemokines) and ability to present antigen to T cells, equips tissue neutrophils with the capability to actively drive inflammatory processes. Indeed, many of the cytokines and chemokines implicated in RA are potent regulators of neutrophil activity (Table 1).

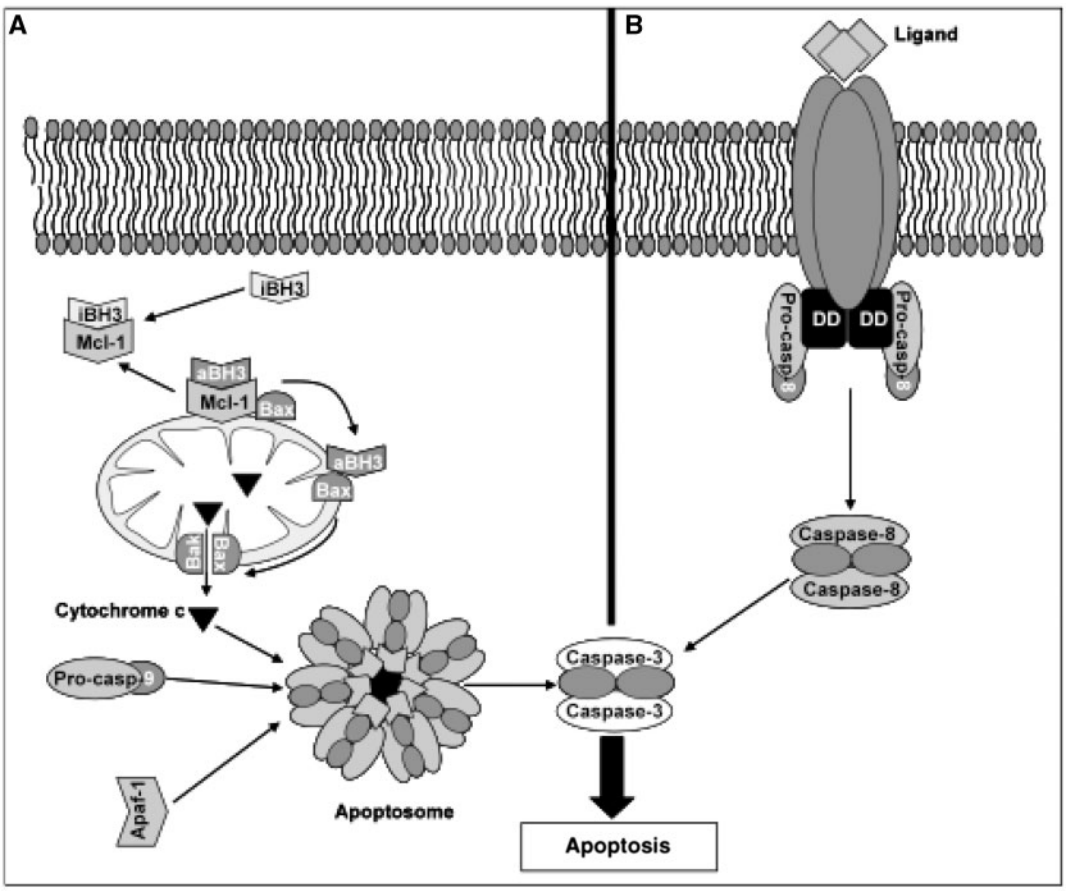
Apoptosis

Apoptosis is controlled by two distinct signalling pathways—intrinsic and extrinsic—both of which culminate in activation of caspases (cysteine–aspartic acid proteases) that cleave intracellular proteins culminating in the death of the cell (Fig. 3). Apoptosis is required for the resolution of inflammation, and defective or delayed apoptosis is implicated in the pathogenesis of inflammatory disease. Neutrophils that have migrated into RA joints display molecular changes associated with a delay in apoptosis, and thus have an enhanced potential to mediate host tissue damage because of their extended life span [18, 19]. The intrinsic apoptotic pathway regulates constitutive neutrophil apoptosis, and the key protein in this pathway is the anti-apoptotic protein myeloid cell leukaemia-1 (Mcl-1), which is expressed in high levels in freshly isolated cells, but has a short half-life of ~2–3 h. Mcl-1 is highly regulated by post-translational modifications, such as phosphorylation and ubiquitination, and cellular levels of the protein correlate closely with apoptosis [14, 20]. The extrinsic pathway to apoptosis is regulated by death receptors such as Fas, TNF-related apoptosis-inducing ligand (TRAIL) receptors-1 and -2

Table 1 Neutrophil-activating factors found within SF [7–17]

	Priming	Adhesion and chemotaxis	ROS and granule enzyme release	Apoptosis delay	MHCII	Production of inflammatory mediators
C5a		Yes				
G-CSF	Yes	Yes	Yes	Yes		Yes
GM-CSF	Yes		Yes	Yes	Yes	Yes
IFNγ	Yes			Yes	Yes	Yes
IgG/IgM			Yes			
IL-1β	Yes	Yes	Yes	Yes		
IL-6		Yes				
IL-8	Yes	Yes	Yes			
IL-15				Yes		
TNF-α	Yes	Yes	Yes	Yes		Yes

Fig. 3 The intrinsic and extrinsic pathways to apoptosis. (**A**) The intrinsic pathway is regulated by proteins of the Bcl-2 family. Anti-apoptotic proteins such as Bcl-2 and/or Mcl-1 sequester activator BH3 (aBH3) proteins and Bax/Bak. Under conditions of stress, inactivator (iBH3) BH3 proteins antagonize Bcl-2/Mcl-1 releasing the aBH3 proteins leading to oligomerization of Bax/Bak and mitochondrial outer membrane permeabilization. Cytochrome c leakage from the mitochondria combines with caspase-9 and Apaf-1 and initiates formation of the apoptosome, resulting in caspase-3 activation and apoptosis. (**B**) The extrinsic pathway to apoptosis is triggered by the engagement of a ligand with a death receptor, recruitment of intracellular death and death engagement domains (DD), and activation of caspases-8 and -3. Cross-talk between the extrinsic and intrinsic pathways is mediated by caspase cleavage of Bcl-2 family proteins such as Mcl-1 and Bid.



and TNF receptors-1 and -2. Engagement of a death receptor with its ligand (e.g. FasL, TRAIL and TNF-α) induces apoptosis via activation of caspase-8 [21]. Some cross-talk exists between the intrinsic and extrinsic apoptotic pathways, as Mcl-1 is a target of caspase-8 cleavage [15, 22].

As well as being implicated in many cancers [23, 24], Mcl-1 plays a key role in the pathophysiology of inflammatory disorders, and has been shown to be elevated in synovial fibroblasts [25], macrophages [26] and lymphocytes [27], as well as neutrophils [28] from inflammatory arthritis patients. Dysregulation of the intrinsic apoptotic pathway in RA has also been reported to occur via increased expression of Bax and Bcl-x_L in synoviocytes [29] and B cells [30], and Bcl-2 in $CD4^+CD28^-$ T-cell clones [31] and RA synovial tissue [32]. Death receptor-mediated apoptosis is very rarely observed within RA synovial cells despite high levels of both Fas and FasL [33]. This may be explained by high expression of Fas-associated death domain-like IL-1β-converting enzyme (cFLIP) in synovial tissue [34]. cFLIP inhibits caspase-8 activation by blocking its engagement with intracellular domains on death receptors, and expression of cFLIP is under the control of Nuclear Factor (NF)-κB, a transcription factor that is activated by TNF-α [35]. TNF-α signalling, via NF-κB, results in the up-regulation of proteins such as Bfl-1, TNFR-associated factor (TRAF)-1 and -2 and X-linked inhibitor of apoptosis protein (XIAP), all of which inhibit apoptosis [64]. cFLIP can also associate with TRAF-1 and -2, as well as with kinases such as RIP and Raf-1, activating NF-κB and ERK signalling pathways [36]. TNF-α has a dynamic effect on neutrophils: at low concentrations its effect is biphasic, promoting early apoptosis in a sub-population of cells, but delaying apoptosis in the remaining cells [37]. It is thought that this mechanism is controlled through stimulation of different signalling pathways via each TNF receptor. While both TNF receptors promote early cell death, only TNFR1 can delay apoptosis via NF-κB-controlled expression of pro-survival genes such as Bfl-1 and TRAF-1 [15, 38, 39]. At high concentrations, TNF-α induces neutrophil apoptosis via death receptor signalling through both TNF receptors, leading to caspase-8 activation and the loss of anti-apoptotic proteins such as Mcl-1 via caspase cleavage [15]. Thus, local concentrations of TNF can have opposing effects on neutrophil function in inflammation.

MHCII

It is now widely accepted that activated neutrophils are capable of presenting antigens via MHCII, thereby stimulating T-cell activation and proliferation. Expression of MHCII molecules in neutrophils from healthy donors can be induced by culture in the presence of GM-CSF, IL-3 and/or IFN-γ and is highly donor dependent [10, 11]. Activation of neutrophils *in vitro* by fMLP, lipopolysaccharide (LPS) or phorbol myristate acetate (PMA), or by cross-linking of MAC-1, has also been shown to induce rapid expression of MHCII, together with T-cell co-stimulatory molecules (CD80 and CD86) [40]. Appropriately cultured neutrophils are able to process and present tetanus toxoid (TT) antigen via MHCII, stimulating proliferation of TT-specific T cells [41, 42], and cross-linking of neutrophil MHCII by super-antigens has also been shown to increase IL-8 production by neutrophils [43]. Neutrophils isolated from the SF of RA patients have been shown to express MHCII, CD80 and CD86, to transcribe and express MHCII molecules in culture, and are able to stimulate T-cell proliferation [11]. Indeed, the levels of expression of MHCII and co-stimulatory molecules on neutrophils from SF have been reported to be equivalent to or greater than the levels of expression on monocytes and B cells [40]. Apart from their ability to stimulate T cells in this way, it is also possible that neutrophils can expose cryptic epitopes, as they possess different proteases from other antigen-presenting cells. Thus, their function within inflamed joints could be quite different from that of other antigen-presenting cells.

Production of inflammatory mediators

In addition to their ability to present antigen, primed neutrophils actively synthesize and secrete cytokines, chemokines, leucotrienes and prostaglandins, and by virtue of their accumulation in large numbers within inflammatory tissue, such as the RA joint, may contribute significantly to local production of inflammatory mediators. In particular, neutrophils have been shown to synthesize and secrete IL-8 in response to a number of stimuli, including TNF-α and GM-CSF [12]. Activated neutrophils have also been reported to synthesize IL-1, -1RA, -6, -12, TGF-β, TNF-α, oncostatin M and BLyS [16, 44, 45], which can subsequently activate both neutrophils and other cells of the immune system. Neutrophils are a significant source of leucotrienes and prostaglandins, especially leucotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2), which are synthesized from arachidonic acid by lipoxygenases and cyclo-oxygenases, respectively. LTB_4 is a neutrophil chemoattractant, and can promote neutrophil adherence and migration by up-regulation of MAC-1 [46]. PGE_2, conversely, has a mainly anti-inflammatory effect on neutrophils, inhibiting phospholipase-D activity and increasing intracellular cyclic-adenosine monophosphate (cAMP) concentrations, which result in decreased calcium influx, loss of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase assembly and lower levels of endothelial adhesion and chemotaxis [47]. PGE_2 has also been reported to delay neutrophil apoptosis [48].

Receptor expression

Neutrophil priming increases the abundance and/or activity of receptors on the plasma membrane that facilitate rapid recognition, phagocytosis and killing of bacteria (e.g. fMLP receptors and complement receptors) or activation by immune complexes (Fcγ receptors). Fcγ receptors bind immunoglobulins such as RF, and may be responsible for neutrophil-mediated damage within the synovial joint. Neutrophils express three types of Fcγ receptor: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). FcγRI is a high-affinity IgG receptor that binds monomeric IgG. It is not expressed on resting blood

neutrophils but can be induced by cytokines such as IFN-γ, and is expressed on neutrophils isolated from the SF of RA patients [49]. FcγRII is constitutively expressed by neutrophils, and binds monomeric IgG with low affinity, but has much higher affinity for dimers or aggregates of IgG. The FcγRIIa isoform is an activating receptor, which is up-regulated by priming agents such as TNF-α and GM-CSF [50]. The FcγRIIb isoform generates an inhibitory signal, and a defect in FcγRIIb function is associated with increased disease severity and joint erosions in RA patients [51]. Treatment with infliximab has been shown to induce a switch between expression of the FcγRIIa and -b isoforms in blood neutrophils from RA patients, resulting in improvements in disease activity [52]. FcγRIII binds complexes of IgG with low affinity, and the FcγRIIIb isoform of the receptor, which is exclusively expressed on neutrophils, is highly abundant with 100 000–200 000 receptors per cell. FcγRIIIb is constantly shed by activated neutrophils, but intracellular stores of the receptor are mobilized during priming to maintain expression [7].

Engagement of IgG immune complexes with either FcγRIIa or FcγRIIIb initiates very different functional responses in neutrophils. Signalling via FcγRIIa initiates chemotaxis, phagocytosis and killing when neutrophils are challenged with serum-opsonized bacteria. However, FcγRIIIb has been shown to play a pivotal role in the secretion of ROS in response to immune complexes, but little or no role in phagocytosis or killing of serum-opsonized bacteria [53]. Indeed, neutrophils from individuals who are genetically deficient in FcγRIIIb show no impairment in bacterial phagocytosis or killing [54]. Activation of neutrophils by immune complexes can be one of the mechanisms of joint damage in RA, and the discovery that blocking the signalling via FcγRIIIb ablates neutrophil activation by immune complexes without compromising host defence identifies this receptor as a potential therapeutic target [53].

Mitochondria

There has long been a belief that neutrophils possess few, if any, functional mitochondria, fitting with the idea that these cells can perform under conditions that are limited in O_2 (e.g. inflamed tissues) and do not require mitochondrial respiration for functions such as the respiratory burst. The idea that neutrophils were devoid of mitochondria, however, made them unique among immune cells and it was difficult to imagine how conventional apoptotic control mechanisms, which rely upon the release of mitochondrial proteins, could operate. It has recently been shown that neutrophils possess a complex network of mitochondria, the function of which is not critical to initiation of a rapid respiratory burst or phagocytosis, but rather is involved in chemotaxis and regulating apoptosis [55]. These mitochondria play little or no role in oxidative metabolism leading to ATP generation. Maintenance of mitochondrial membrane potential appears to be essential in delaying apoptosis in infectious states such as sepsis [56], and indeed loss of mitochondrial membrane potential has been identified as an early marker of apoptosis [55].

Neutrophils in inflammatory disease

Pannus/cartilage

Neutrophils are by far the most abundant immune cell in SF aspirated from the joint of RA patients, but can also be observed at the pannus/cartilage interface in synovial tissue [57, 58], the site of active destruction of cartilage and bone. Upon migration into RA joints, activated neutrophils encounter aggregates of immunoglobulins (e.g. RF), both within the SF and deposited on the surface of the joint. These complexes of immunoglobulins engage Fcγ receptors on the surface of the neutrophil, triggering degranulation and production of ROS either into the SF, or directly onto the surface of the joint in a process termed 'frustrated phagocytosis' [59].

Activation of primed neutrophils, either by phagocytosis of opsonized bacteria or by frustrated phagocytosis, generates the rapid production of ROS via the action of NADPH oxidase. A resting neutrophil has very little capacity to produce ROS, as NADPH oxidase is a multi-component enzyme that is assembled at the plasma membrane during priming [60]. There are at least six components of the oxidase: $p22^{phox}$ and $gp91^{phox}$ (which comprise the membrane-expressed cytochrome b_{558}), and the cytosolic proteins $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and rac-2. Activation of the respiratory burst generates ROS production (O_2^-, $HO^\bullet$, $^1O_2$ and H_2O_2) by NADPH oxidase, and hypochlorous acid (HOCl) via the action of myeloperoxidase. Oxidative stress as a result of inappropriate release of ROS by neutrophils is implicated in the pathology of RA, and neutrophils isolated from RA SF show evidence of having initiated ROS production *in vivo* [61]. Oxygen radicals cause damage to DNA, oxidation of lipids, proteins and lipoproteins, and may be implicated in mutations to immunoglobulins that lead to the formation of RF [62, 63]. HOCl released into the tissue surrounding the neutrophil protects granular proteases from the anti-proteinases found within SF, and neutrophil granule enzymes such as myeloperoxidase and lactoferrin have been found in SF from RA patients [64, 65]. Neutrophil activation also triggers the release of neutrophil granules, several of which are implicated in damage to host tissue, particularly in the destruction of the collagen matrix within cartilage, as well as bacterial killing (Table 2).

Neutrophil-derived proteases are also important mediators of inflammation and have been shown to play a role in the proteolytic activation of cytokines and chemokines. Membrane-associated proteinase-3 can cleave and activate the pro-cytokine forms of TNF-α, IL-1β and IL-8, whereas cathepsin G and elastase may play a role in the degradation of soluble TNF-α and IL-6. In addition, neutrophil serine proteases have been shown to activate cell receptors (such as toll-like receptor 4 and epidermal growth factor receptor) and cleave the adhesion molecules ICAM-1 and VCAM-1 [70].

TABLE 2 Neutrophil granules contain a variety of proteases that are used for bacterial killing, but which may also be implicated in damage to host tissue in RA

Granule	Granule enzyme	Role in tissue damage	Reference
Azurophilic (primary)	Myeloperoxidase	Production of HOCl	[66]
	Elastase	Degradation of lubricin in SF and degradation of cartilage matrix	[67]
Specific (secondary)	Lactoferrin	Up-regulation of neutrophil adhesion molecules and delayed apoptosis	[65, 68]
	Collagenase	Degradation of cartilage matrix	[67]
	Gelatinase	Degradation of cartilage matrix	[69]
Gelatinase (tertiary)	Gelatinase	Degradation of cartilage matrix	[69]

Animal models

Many animal models of inflammatory arthritis show that neutrophils are the first immune cells to enter the arthritic joint, and that early measures of joint inflammation correlate to neutrophil infiltration. The K/BxN mouse model closely resembles human RA, with mice developing progressive joint disease, characterized by rapid onset of symmetrical arthritis in peripheral joints. The pathology of the arthritis that develops in these mice is similar to RA, with pannus formation, synovial hyperplasia, joint swelling and cartilage destruction [71]. Serum transfer from K/BxN mice can induce arthritis in other mouse models, and initiates rapid onset of arthritis that is closely associated with influx of neutrophils into the synovial cavity. Neutrophil-depleted mice are completely resistant to the inflammatory effects of arthritogenic serum from K/BxN mice [71]. In the K/BxN serum-transfer mouse model, neutrophil-derived LTB_4 has been identified as a key mediator of inflammation and cartilage destruction [72], and inhibition of leucotriene synthesis has also been shown to decrease neutrophil migration into knee joints of mice with antigen-induced arthritis (AIA) [73]. LTB_4 receptor (BLT1) knock-out also prevents the development of neutrophil-mediated arthritis in mice injected with K/BxN serum, and BLT1 blockade in wild-type mice has been shown to reverse K/BxN serum-induced disease [74]. In this study, the transfer of wild-type neutrophils to BLT1 knock-out mice not only induced the development of arthritis, but also facilitated the influx of $BLT1^{-/-}$ neutrophils into the joints.

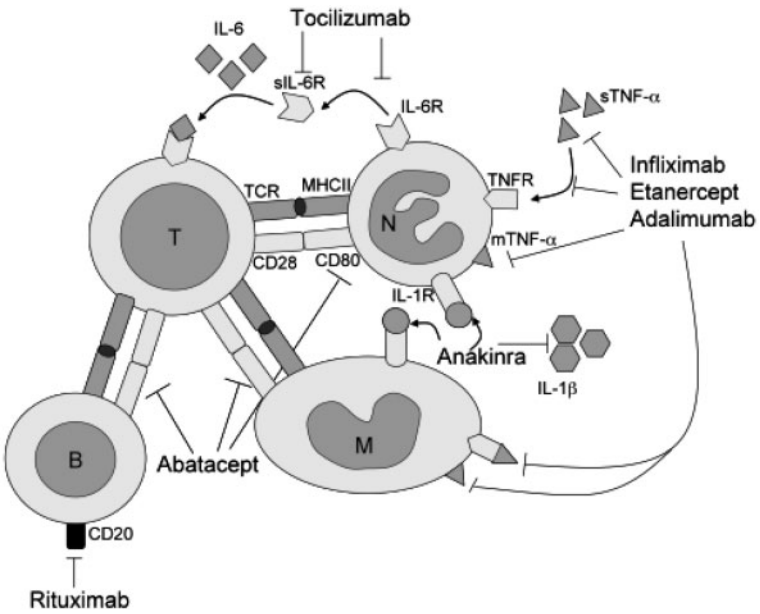
The K/BxN model has also shown that mice deficient in Vav or PLCγ are resistant to the development of arthritis due to defective activation of neutrophil integrins and Fcγ receptors [75]. PI3 kinase has been shown to be integral to the development of arthritis following K/BxN serum transfer, and PI3 kinase knock-down or inhibition significantly diminishes neutrophil infiltration and joint damage [76]. Chemokine receptors have been shown to be essential in neutrophil recruitment and joint damage in AIA, and blockade of CXCR1/2 decreases neutrophil influx, synovial cytokine production and tissue damage [73, 77]. Depletion of the C5a receptor protects mice against the development of antibody-induced arthritis and CIA, completely inhibiting cartilage and bone erosion, and suppressing neutrophil infiltration to the joint [78]. G-CSF has been identified as a key mediator of neutrophil adhesion and joint infiltration, and G-CSF knock-out mice are resistant to CIA [17]. IL-17 has also been shown to induce significant neutrophil joint infiltration and cartilage damage in models of immune-complex-mediated arthritis, which may be a direct effect, or due to the up-regulation of other neutrophil chemoattractants [79].

Biologics and their targets

Drug intervention therapy for RA is directed at many elements of the immune system. While non-steriod anti-inflammatory drugs (NSAIDS), DMARDs and biologics do not specifically target neutrophil function, many of them exert inhibitory effects on neutrophils (Fig. 4). Most NSAIDs inhibit the action of the cyclo-oxygenase-1 and -2 (COX-1 and -2) enzymes, which metabolize arachidonic acid into inflammatory mediators of the prostaglandin family. NSAIDs have been shown to inhibit neutrophil adherence, decrease degranulation and oxidant production, inhibit neutrophil elastase activity and induce neutrophil apoptosis [22, 80, 81]. Corticosteroids provide an anti-inflammatory signal by blocking the activity of phospholipase A_2, thus inhibiting production of pro-inflammatory mediators such as leucotrienes and prostaglandins. Corticosteroids have been shown to inhibit neutrophil degranulation and ROS production, decrease production of inflammatory mediators, and prevent neutrophil adhesion and migration into RA joints [58, 82–84]. Paradoxically, while corticosteroids inhibit many neutrophil functions, they also delay neutrophil apoptosis, although the mechanisms underlying this effect and its relevance *in vivo* are not fully understood [85]. The most commonly prescribed DMARD, MTX, is a potent down-regulator of the immune response in RA [86] and has been reported to abrogate delayed neutrophil apoptosis, and decrease neutrophil chemotaxis, LTB_4 synthesis and ROS production [87–89]. Other commonly prescribed DMARDs have been reported to decrease neutrophil chemotaxis, degranulation and ROS production, induce neutrophil apoptosis and prevent neutrophil-mediated collagen destruction [90–92].

The introduction of biologic therapies, typified by TNF inhibitors (TNFi), has revolutionized the treatment of RA, providing a mechanism to treat those patients most severely affected by the disease. TNF is a cytokine that influences many elements of immune dysregulation in

FIG. 4 Summary of the immune targets of biologic therapies. Anti-TNF drugs infliximab, etanercept and adalimumab target soluble (sTNF-α) and membrane-expressed TNF-α (mTNF-α), facilitating blockade of signalling and removal of mTNF-α-expressing cells. Anakinra is an IL-1 receptor (IL-1R) antagonist, which competitively binds the IL-1R and inhibits IL-1 signalling. Abatacept prevents the co-stimulation of T cells by blocking engagement of CD28 with CD80/86, preventing signalling from MHCII on antigen-presenting cells to the TCR. Rituximab targets and removes $CD20^+$ B cells. Tocilizumab inhibits IL-6 signalling by blockade of the soluble (sIL-6R) and transmembrane (IL-6R) IL-6 receptors. M: macrophage; B: B cell; T: T cell; N: neutrophil.



disease, such as bone resorption, auto-antibody production, cytokine synthesis and cellular infiltration into diseased joints. TNF primes the neutrophil respiratory burst; up-regulates the expression of adhesion molecules, cytokines and chemokines; and at high local concentrations can stimulate ROS production in adherent neutrophils [8, 12, 15, 93]. Three different TNFi are now available for RA patients who fail to respond adequately to standard DMARD therapy. Infliximab and adalimumab are monoclonal antibodies against TNF, while etanercept is a TNFRII fusion protein. All three drugs sequester soluble TNF, and can also bind TNF expressed on the surface of immune cells, facilitating removal of such cells by antibody-dependent cellular cytotoxicity (ADCC) or by initiating apoptosis [94]. The many reported physiological effects of anti-TNF therapy include: blockade of cytokine and chemokine synthesis, e.g. IL-1α, -1β, -6, -8 and TNF-α, by synovial tissue and peripheral blood cells [95, 96]; lowering of serum protease levels [97]; increased apoptosis of monocytes and macrophages [98, 99]; and restoration of regulatory T-cell function [100]. Few reports of the direct effect of anti-TNF agents on neutrophils have been published, but these drugs have been shown to decrease mobilization of neutrophils from peripheral blood to inflamed joints [101], and decrease *ex vivo* neutrophil ROS production [102]. While other biologics such as rituximab and abatacept do not directly target neutrophils, the decrease in auto-antibody titres induced by rituximab [103] removes the neutrophil-activating immune complexes from serum and SF. Blockade of T-cell stimulation by abatacept [104] will also prevent activation of T cells by MHCII-presenting neutrophils.

Tocilizumab, a monoclonal antibody that blocks the soluble and tissue-expressed IL-6 receptor, is also proving to be a highly effective biologic agent in RA [105]. Like TNF-α, IL-6 is a pleiotropic cytokine that is implicated in many aspects of inflammation associated with RA, including the acute-phase response, B-cell differentiation, antibody production, T-cell proliferation, endothelial cell activation, neutrophil recruitment, RANKL expression and osteoclast activation [106, 107]. Cells of the immune system that do not constitutively express the IL-6 receptor can engage with IL-6 bound to soluble IL-6 receptors (sIL-6Rs) via dimerization with the ubiquitously expressed GP130 co-receptor [108], a process termed trans-signalling. Neutrophils are a major source of sIL-6Rs, which they shed in large quantities when activated, and their accumulation in high numbers within the synovial joint could contribute significantly to IL-6 signalling within the synovium via trans-signalling [109].

The success of anti-cytokine therapies has led the search for potential new targets of biologic therapies. These targets include other cytokines and/or their receptors, protein kinase signalling and signalling networks controlling apoptosis. Many of these new therapies have

Table 3 Emerging biologic therapies for the treatment of RA

Target	Name	Stage/model	Reported effects	Reference
A_3 adenosine receptor	CF101	CIA	Decreased joint swelling, cartilage damage and cytokine production	[110]
		Phase II	Improvements in disease activity: ACR20 (55.6%), ACR50 (33.3%), ACR70 (11.5%)	[111]
BLyS	Belimumab	Phase II	Improvements in disease activity: ACR20 (35%), ACR50 (14%), decrease in RF titre	[112]
CXCR1/2 (IL-8 receptor)	DF2162	CIA	Decreased joint swelling and neutrophil influx	[113]
		AIA	Decreased joint swelling, neutrophil influx and local cytokine and chemokine production	[114]
GM-CSF	mAb 22E9	SCW	Decreased joint swelling, cytokine production, cellular influx and cartilage damage	[115]
	CAM-30001	*In vitro*	Decreased cytokine production by monocytes	[116]
IL-15	HuMax-IL15	Phase I/II	Improvements in disease activity: ACR20 (63%), ACR50 (38%) and ACR70 (25%)	[117]
IL-17		CIA	Decreased inflammation and bone erosion through suppression of IL-1β, IL-6, TNF-α and RANKL synthesis	[118]
JAKs	CP-690550	CIA and AIA	Decreased inflammatory cell infiltration and joint damage	[119]
		Phase II	Improvements in disease activity: ACR20 (81%), ACR50 (54%) and ACR70 (28%)	[120]
NAMPT; visfatin	APO866	CIA	Decreased cytokine production, synovial hyperplasia and cellular influx	[121]
p38 MAP kinase	SC-409	SCW	Decreased joint swelling, bone destruction and cytokine production	[122]
	ARY-797	Phase I/II	Dose-dependent inhibition of cytokine production and improvement in disease activity	[123]
PI3 kinase	AS-605240	CIA	Suppressed development of severe arthritis, decreased neutrophil infiltration and joint swelling	[124]
RANKL	Denosumab	Phase II	Decrease in erosions and suppression of bone turnover	[125]
SYK	R406/R788	AIA	Decreased joint inflammation	[126]
		CIA	Decreased erosions, pannus formation and synovitis	[126]
		Double-blind, placebo-controlled	Improvements in disease activity: ACR20 (72%), ACR50 (57%), ACR70 (40%) and decrease in serum IL-6 and MMP3	[127]

SCW: staphylococcus wall-induced arthritis.

shown success in animal models of arthritis and are now in clinical trials. One of the key observations in effective drug therapy in animal models of arthritis is the decrease in neutrophil influx that correlates with improvements in disease activity. Many of the therapies under development target key activators of neutrophils, such as GM-CSF, IL-15 and IL-17, the IL-8 and adenosine receptors, and intracellular signalling molecules such as spleen tyrosine kinase (SYK), Janus kinase (JAK) and p38 MAP kinase (Table 3). A number of other pharmacological agents under development directly target apoptosis, although these are currently being developed as potential anti-cancer therapies. However, they may also be of value in treating autoimmune conditions such as RA. Although still mainly in the pre-clinical stages, these agents include TRAIL death receptor agonists, activators of caspases and small molecule inhibitors of the Bcl-2 family of proteins [128].

Rheumatology key messages

- Activated (but not bloodstream) neutrophils possess many of the molecular properties of macrophages.
- Recent experimental evidence points to neutrophils as drivers of inflammatory processes.
- Many drugs used to treat RA can either directly or indirectly target neutrophil function.

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