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The Role of Neutrophils in Rheumatoid Arthritis – Experiments *In Vitro*: A Change of Conception?

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1. Introduction

Essential cells of innate immunity, neutrophils are often considered to be a homogenous population of terminally differentiated cells (Chakravarti et al., 2009). These cells represent the body's primary line of defence against invading pathogens such as bacteria, and constitute 40-60% of the white blood cell population. Neutrophils are short-lived polymorphonuclear phagocytes. They are known as first-responding inflammatory cells migrating towards the site of inflammation (Chakravarti et al., 2009**;** Edwards et al., 1997).
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 tissues. Neutrophils become activated by agents that include bacterial products and cytokines or chemokines, such as TNF-α, IL-8 or IFN-γ. The primed neutrophils are then mobilized to the site of infection or inflammation and encounter activating signals to trigger bacterial killing. (Wright et al., 2010; Cascao et al., 2009)
It must be noted, that the functions of resting blood neutrophils and primed neutrophils may be very different. Thus, many of the regulatory functions of macrophages are shared by primed (but not resting) neutrophils (Wright et al., 2010). For this reason, *in vitro* experiments using freshly isolated blood neutrophils often fail to recognize the full functional repertoire and capacity of neutrophils.

2. Oxidative metabolism of neutrophils

In a chronic inflammatory process, such as rheumatoid arthritis (RA), large numbers of neutrophils are attracted across the synovial membrane, and become activated. The number of neutrophils in synovial fluid (SF) of patients with RA can reach $5x10^{-9}$ (Edwards et al., 1997). Neutrophils possess a range of potent proteinases and hydrolases, and have the ability to generate a series of reactive oxygen intermediates (ROI) via the combined activities of NADPH (reduced form) oxidase and myeloperoxidase (MPO) (Robinson et al., 1992; Duluray et al., 1990; Nurcombe et al., 1991a). If the neutrophils are not efficiently depleted, their production of inflammatory mediators, such as ROI, could prolong the inflammatory reaction. In fact, inappropriate release of ROI from activated neutrophils is responsible for joint damage observed in RA (Edwards et al., 1997). Beside ROI and inflammatory

mediators, neutrophils are also an important source of proteolytic enzymes which play a role in degradation of articular structures. Wojtecka-Lukasik et al. were the first to isolate collagenase from peripheral blood neutrophils to a degree which allowed determination of its physicochemical properties and assessment of its effects on the biological activity of some drugs used in treatment of rheumatic diseases (Wojtecka-Lukasik et al., 1974). In addition to the active enzyme, its latent form and activator were discovered and described in the rheumatoid joint fluids (Dancewicz et al., 1978).

Neutrophils are able to form extracellular structures, named neutrophils extracellular traps (NETs). NETs are composed by nuclear components, such as chromatin DNA (i.e. histones anchored to this molecular backbone), and cytoplasmic components, such as granular peptides and enzymes. Upon activation, the induction of activation of NADPH oxidase was reported, suggesting that formation of NETs is ROI-dependent (Cascao et al., 2009). Neutrophils die upon release of these structures. However, this is a form of cell death different from apoptosis and necrosis, named "NETosis" (Steinberg et al., 2007). NETs represent an unconventional form of immune response, because these structures remain active even after the neutrophil's death. The presence of nucleic acid can contribute to the development of autoimmune diseases, such as systemic lupus erythematosus (SLE) in which there is an exacerbated reaction against the host DNA (Wartha et al., 2007).

As was shown by Wenthworth (Wenthworth et al., 2002), antibodies catalyze the generation of hydrogen peroxide from singlet molecular oxygen and water. This process can lead to efficient killing of bacteria, regardless of the antigen specificity of the antibody. Hydrogen peroxide production by antibodies alone was found to be not sufficient for bacterial killing. Wenthworth et al. suggested that the antibody-catalyzed water-oxidation pathway produced an additional molecular species with a chemical signature similar to that of ozone. This species is also generated during the oxidative burst of activated human neutrophils and during inflammation. These observations suggest that alternative pathways may exist for biological killing of bacteria that are mediated by potent oxidants previously unknown to biology (Wentworth et al., 2002).

More recently (Yamashita et al., 2008), it was discovered that four amino acids themselves (tryptophan, methionine, cysteine and histidine) are able to catalyze the production of an oxidant with the chemical signature of ozone from singlet oxygen in the water-oxidation pathway. The resultant oxidant with the chemical signature of ozone exhibited significant bactericidal activity in human neutrophils. These results also suggest that an oxidant with the chemical signature of ozone produced by neutrophils might potentiate a host defence system, when the host is challenged by high doses of infectious agents. These findings provide biological insights into the killing of bacteria by neutrophils (Yamashita et al., 2008).

From a different point of view, it is believed that ROI function as second messengers (Hitchon & El-Gabalawy, 2004). Typically, second messengers are short-lived molecules that at the time of activation of a receptor, act specifically on effectors to alter their activity transiently. Indeed, ROI can be generated at the time of receptor activation and they are short-lived, as the other second-messengers (Fillipin et al., 2008). ROI produced by phagocytes are critical for protection against invading microorganisms but also seem to have important physiological roles in priming the immune system. It has been demonstrated that exposure to ROI down-regulate the activity of T lymphocytes: ROI produced by phagocytes also seem to have essential physiological roles in priming the

immune system as second messengers (Jones, 2006). Hitchon and El-Gabalawy propose that the physiological production of ROI by phagocytes in response to an antigen affects T-cell antigen interactions and possibly induces apoptosis in autoreactive arthritogenic T cells, thereby preventing autoimmune responses (Hitchon & El-Gabalawy, 2004).

3. Oxidative metabolism and apoptosis of neutrophils cultured in physiological concentrations of SF, oxygen and cyclic loaded pressure

Although the presence of activated neutrophils in SF is well documented, it is still unknown how the neutrophils are activated, how they interact with other cells, and how long they persist at the site of inflammatory joint.

It was identified that addition of SF to neutrophils results in activation of neutrophils measured by a rapid chemiluminescence (CL) response (luminol- and lucigenin-dependent). Luminol-dependent CL is capable of monitoring both intracellular and extracellular ROI generation (NADPH oxidase and MPO), as luminol freely penetrates the neutrophil's cell membrane. Lucigenin-dependent CL, on the other hand, measures only the rate of extracellular ROI secretion (NADPH oxidase dependent) because lucigenin does not penetrate neutrophils, and light emission detected is independent of the activity of MPO. Pre-incubation of normal blood neutrophils in 10% SF enhanced the luminol- and lucigenin-CL, suggesting that both MPO and NADPH oxidase activity were activated in parallel during exposure to 10% SF from RA patients (Bender et al., 1986). Synovial fluid (20% concentration) isolated from RA patients activated blood neutrophils, leading to increase of luminol dependent CL over a 50 range (Nurcombe et al., 1991b). In contrast, the same fluid activated to a much lower range (two or three fold) of maximal rates of lucigenin dependent CL. All the mentioned reports were performed with SF in concentrations that did not exceed 20%. Other studies, confirmed that SF (used in concentration of 25%) produced rapid and parallel responses (luminol- and lucigenin-CL) in neutrophils.

Our recent results (Gajewski et al., 2009), in contrast to earlier studies, indicate that higher concentrations of SF (up to 80%) have a quite different effect on lucigenin-dependent and luminol-dependent CL response. Increased concentrations of SF resulted in a reduction of luminol-dependent CL response and a very significant increase of lucigenin-dependent CL, reflecting extracellular ROI generation. This effect was observed irrespective of the stimulator used and whether neutrophils were isolated from SF or blood from either RA patients or healthy subjects. This indicates that increasing SF concentration results in higher extracellular ROI secretion and lower MPO-dependent ROI production. The promotion of extracellular release of ROI observed in this experiment is likely to be associated with the high concentrations of SF used, and raises the possibility that extracellular activity of neutrophils may be a general characteristic which prolongs the inflammatory process (Gajewski et al., 2009).

Similar studies were performed by Bell et al. (1995). In these studies they examined the hypothesis that persistent inflammatory responses in RA may result from inhibition of neutrophils apoptosis by factors in SF. The effects of aging in culture and addition of SF on apoptosis was investigated using SF in a concentration range 0-75%. A significant effect of SF on promotion of apoptosis of synovial fluid neutrophils was observed at concentrations of 50% and above (Bell et al., 1995).

It has been proposed that the process of hypoxic-reperfusion injury contributes to the persistence of synovitis in the inflamed joint. The generation of pathological, exercise

induced intra-articular pressure leading to occlusion of the microcirculation is central to this mechanism (Hitchon & El-Gabalawy, 2004). Several observations show that neutrophils-mediated lysis of surrounding cells during ischemia-reperfusion is largely mediated by ROI (Smith et al., 1989). Superoxide dismutase (SOD) can significantly reduce cellular lysis and damage. Treatment with SOD prior the reperfusion reduces the concentration of the potentially injurious ROI**.** Similarly, administration of catalase decreases hydrogen peroxide concentration and reduces tissue damage during reperfusion (Smith et al., 1989). In the investigation of Nguyen (Nguyen et al., 2005) it was concluded that neutrophils play a significant role in injuring cell membranes reloading following periods of unloading, and that this membrane damage was mediated by MPO. A substantial, synergistic effect on the level of muscle lysis when both mechanical loading and neutrophils were applied to muscle cells *in vitro* was found. Loading alone caused only a 1.7% lysis of muscle cells, while co-culturing with neutrophils in the absence of loading resulted in only 3.5% lysis. However, loading in the presence of neutrophils resulted in 12.6% lysis of muscle cells under otherwise identical culture conditions in the presence of SOD (Nguyen et al., 2005). These observations provide a direct link between changes in mechanical loads applied to tissue, and an increase in damage that is induced by inflammatory cells. According to these results, pressure could be also considered as an important factor in *in vitro* experiments.

Another factor that is not often considered and influences neutrophils metabolism and survival is the concentration of molecular oxygen used to incubate the cell suspensions. Most *in vitro* experiments are performed in air or air mixtures of 21% O_2. Rarely, if ever, will such high concentration of oxygenation occur *in vivo*. For example, the O_2 tension within SF has been reported to be low as 1-2%. It was recently showed that local O_2 concentration affects neutrophils apoptosis. Only under hypoxic conditions, such as those typically found in RA joints, anti-apoptotic pathways are trigged. Neutrophils normally have a short life in the circulation (8-12h), whereas within SF with physiological oxygen concentration, neutrophils lifetime can be extended, increasing their potential to cause damage and promote inflammation (Cross et al., 2006).

The arguments that the prolonged lifespan of activated neutrophils in patients with RA may contribute to the progression of the inflammatory process to chronicity were supported by experiments in which neutrophils were exposured to SF (50%). In these conditions, in whole blood, neutrophils were stimulated with equal volume of SF and trans-differentiation of neutrophils into dendritic like cells was observed (Iking-Konert et al., 2005). It may be suggested that neutrophils from SF undergo major alterations, including trans-differentiation to cells with dendritic-like characteristics, probably induced by T cell derived cytokines. Exposure of SF, which contained considerable amounts of cytokines, induced a similar receptor pattern on blood derived neutrophils of healthy donors. This effect was also achieved by T cell supernatant alone (Iking-Konert et al., 2005).

Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. The mutual activation of neutrophils and T cells might contribute to perpetuation of the local inflammatory process, and eventually to the destructive process in RA (Angellini et al., 2002).

Actual findings confirm that extreme conditions within the joint have influence on neutrophils metabolism. Neutrophils in synovial tissues have different features than blood neutrophils. The proposal to use conditions, as close as possible to physiological conditions, is strongly recommended for *in vitro* experiments on neutrophils role in RA.

4. Immune cells – a new, diffusely expressed adrenergic and cholinergic organ? The neuromodulatory aspects of inflamed joint cavity: the importance of cholinergic system

One of the truly remarkable discoveries in modern biology is the finding that the nervous system and the immune system use a common language for intra- and inter-system communication (Blalock, 2005). This biochemical information circuit between neurons and immune cells allows the immune system to function as a sensory organ, and completes our ability to be cognizant not only of the world we can seen hear, taste, touch and smell, but also of bacteria, viruses, antigens or tumor cells. Recognition of such "non-cognitive stimuli" by the immune system results in transmission of information to the central nervous system to cause a physiological response that is ultimately beneficial to the host and detrimental to the infectious agent (Blalock, 2002, 2005).

The idea that immune cells were a source of neuropeptides was viewed by many as heretical; as is often the case when fundamental and unexpected discoveries are made (Blalock, 2005). Concurrent with early observation on production of neuropeptides by immune cells emerging studies reported that neuropeptide/neurotransmitter receptor were present on the same cells (Wybran et al., 1979).

It has been proposed that activity of neutrophils in RA may be influenced by neurotransmitters, including agonists of adrenergic and cholinergic receptors. This conclusion was based on the results of pharmacological studies that suggested the existence of several subtypes of functional adrenergic and cholinergic receptors of human neutrophils. An important modulatory mechanism in neutrophils is the interplay between stimulatory and inhibitory receptors. For example, activation of neutrophils is antagonized by agents that stimulate adenylate cyclase (AC). These agents include B-adrenergic agonists, prostaglandins of the E series, adenosine and histamine. These effects appear to be mediated through adrenergic receptors, activating AC, which leads to an increase in cyclic AMP. Based on the hypothesis of the opposing actions of cAMP and cGMP, it was suggested that the agonists of cholinergic receptors might stimulate leukocyte activation (Gajewski et al., 1995).

The effect of the adrenergic receptors isoproterenol (ISO) and the cholinergic receptor carbachol on ROI production was measured by CL. Activation of neutrophils by OZ (stimulating phagocytosis), PMA (activation of NADPH oxidase) and fMLP (stimulation of chemotactic receptors) leads to elevation of the CL reponse (Gajewski et al., 1997).

The adrenergic receptor antagonist ISO has inhibitory effects on the CL of both peripheral blood neutrophils and SF neutrophils, preactivated by all three stimulators. The inhibitory effect was unchanged after addition of carbachol. Carbachol itself does not influence the CL of neutrophils isolated from either blood or SF, and preactivated by OZ, but PMA modulates the response of fMLP stimulated cells. It causes a significant increase of both luminol-and lucigenin CL expressed by peripheral blood neutrophils, whereas it reduces the response of SF neutrophils. For the first time, to our knowledge, we showed that the cholinergic agonist, carbachol, has been able to increase CL of peripheral human blood neutrophils. In contrast, the same treatment resulted in a decreased CL of neutrophils isolated from SF of patients with RA. These results support the hypothesis that neutrophils, upon cholinergic stimulation may affect the parasympathetic system – both its pro-inflammatory, and its anti-inflammatory activities. With the milieu of the cells determining the nature of the stimulation (Gajewski et al., 1997).

The observed opposite effects may suggest that different subtypes of muscarinic receptors are expressed on neutrophils presented in blood and synovial fluid, respectively. Because there are five distinct genes encoding muscarinic cholinergic receptors subtypes, we investigated the expression of mRNA encoding these receptors subtypes (i.e. m1-m5) in neutrophils isolated from healthy blood donors and patients with RA. Our results demonstrated for the first time the presence of m3, m4 and m5 muscarinic receptor subtypes in human blood neutrophils (Bany et al., 1999). The lack of mRNA for m4 muscarinic receptor subtype in neutrophils isolated from SF, may contribute to the opposite responses to cholinergic stimuli observed in neutrophils from blood and SF (Gajewski et al., 1997, Bany et al., 1999).

These findings were partially confirmed by Tracey (Tracey, 2002). The molecular dovetail between the cholinergic nervous system and the innate immune system is a nicotinic α-bungarotoxin-sensitive macrophage acetylcholine receptor. Exposure of human macrophages, but not peripheral blood monocytes, to nicotine or acetylcholine inhibits the release of TNF-α, IL-1 and IL-8 in response to endotoxin. Tissue macrophages, but not circulating monocytes, produce most of the TNF-α which appears systemically during an excessive inflammatory response. Interaction between the macrophage cholinergic receptor and its ligand inhibits the synthesis of pro-inflammatory cytokines (TNF-α, IL-1 and IL-18) but not anti-inflammatory cytokines (such as IL-10). Acetylcholine inhibits the expression of TNF-α protein in macrophages, but not the induction of TNF-α mRNA levels, indicating that activation of the cholinergic receptor transduces intracellular signals that inhibit cytokine synthesis at a post-transcriptional stage. As compared with macrophages, monocytes are refractory to the cytokine-inhibiting effects of acetylcholine: only supra-physiological concentrations of cholinergic agonist inhibit cytokine synthesis in monocytes (Tracey, 2002).

Further studies on the "reprogramming" of blood neutrophils (contradictory effects of adrenergic and cholinergic systems) into other cell types, SF neutrophils, (both systems inhibiting CL response) are expected to yield new insight into event related to RA therapy.

5. Long-lived dedifferentiated neutrophils

In the clinical setting it was observed, that neutrophils are present in high number in the synovial tissues during the initial stages of RA, and are described to persist in the SF during the course of this disease (Cascao et al., 2009). Patients in an active disease state may have in the SF cellular infiltrate up to 90% of neutrophils (Edwards et al., 1997).

Neutrophils that enter a joint are exposed to multiple factors such as cytokines and extreme physical conditions (low oxygen concentration, high pressure conditions). Recent achievements in neutrophil research indicate that certain inflammatory conditions induce a phenotyphic switch in circulating neutrophils toward a resident neutrophil with different functions (Chakravarti et al., 2009).

Neutrophils isolated from RA patients contain high levels of class II MHC RNA (Cross et al., 2003). It was identified that neutrophils isolated from SF express different types and numbers of surface receptors like CD49, CD80, CD83, CD86, HLA-DR, have increased cell surface ICAM-1 (Iking-Konert et al., 2005).

There are many differences in protein expression between SF and blood-derived neutrophils in RA patients. Neutrophils from RA SF have mobilized pre-formed molecules from intracellular stores to the cell surface and activated genes expression resulting from

enhanced transcription and translation (Quayle et al., 1997). Consequently, several gene products, such as IL-8 and MMP-9, are up-regulated, allowing not only the up-regulation of cell function but also development of new cellular responses, such as antigen-presentation to T cell via activated MHCII expression (Cascao et al., 2009). Neutrophils isolated from the SF of RA patients expressing MHCII, CD80 and CD86 are able to stimulate T-cell proliferation (Wright et al., 2010). 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 (Sandilands et al., 2005). 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 than other antigen-presenting cells. Thus, their function within SF could be different from that of other antigen-presenting cells (Wright et al., 2010).

Inflammatory reprogramming may increase neutrophil viability. As was shown, 8-17% neutrophils of the global neutrophil population have the potential to persist for more than 72 h under inflammatory conditions (Chakravarti et al., 2008). This is in contrast to the circulating neutrophils whose survival is measured in hours. The mechanism of this persistence remains unknown, but it seems that the protein kinases are largely implicated in survival of these long-lived neutrophils (Cronstein et al., 1992). The phospholipids metabolic pathway leading to leukotriene B4 (LTB4) synthesis also illustrates differences between these long-lived neutrophils and circulating neutrophils. As was shown, a significant amount of the 5-lipoxygenase (5-LO) is localized to the nuclear membrane in long-lived neutrophils, in basal conditions, a phenomenon absent in circulating neutrophils (Chakravarti et al., 2008).

As was mentioned, neutrophils exposure to SF (50%) induces transdifferentiation of neutrophils into dendritic like cells (Iking-Konert et al., 2005). When the neutrophils were cultured with TNF-α, IFN-γ and IL-4, the resultant cells had morphologic, cytochemical, and phenotypic features of macrophages. In contrast to the starting population, they were negative for myeloperoxidase, specific esterase and lactoferrin. It appears that, in response to the cytokines present in SF, postmitotic neutrophils can become macrophages (Araki et al., 2004).

Neutrophils are known to phagocytose invading pathogens and harmful particles. However, in the study of Rydel-Tormanen et al. (Rydel-Tormanen et al., 2006) it was demonstrated that neutrophils are also able to engulf apoptotic neutrophils or cell debris resulting from secondary necrosis of neutrophils. Previously, neutrophils phagocytosing apoptotic cells and nuclei have been described in blood smears from patients with systemic lupus erythematosus (SLE), a feature called LE cells (Bohm, 2004). Moreover, in inflammatory foci, apparently viable neutrophils with phagosomes enclosing were found with what appeared to be whole apoptotic neutrophils and apoptotic nuclei. Neutrophils may thereby contribute to clearance and resolution of inflammation, thus acting as a back up system in situations when the macrophages clearance system is insufficient and/or overwhelmed. It is apparent that neutrophils have the abilities needed to mimic macrophage behavior and express most, if not all, surface receptors used by macrophages in the process of phagocytosis, suggesting the mechanisms to be similar in the two types of cells (Rydell-Tormanen et al., 2006).

6. Long-density granulocytes (LDG) and endothelial progenitor cells (EPC)

Two studies (Denny et al., 2010, Hacbarth & Kajdacsy-Bella, 1986) have reported the presence of an abnormal subset of neutrophils in the peripheral circulation of SLE patients.

Low density granulocytes (LDG) are present in PBMC preparations derived from lupus patients. LDGs display an activated phenotype, induce significant endothelial cytotoxicity and synthesize sufficient levels of type I IFNs to disrupt the capacity of endothelial progenitor cells (EPC) to differentiate into mature endothelial cells.
EPCs have been shown to play a role in the neovascularization that occurs in diseased tissues. Given the extensive neovascularization that occurs in RA, it was suggested by Denny (Denny et al., 2010) that EPCs are recruited to the arthritic synovium, where they might contribute to expansion of the synovial microcirculation. The VCAM-1/very late activation antigen 4 adhesive system critically mediated EPC adhesion to cultured RA fibroblasts. As was shown, in 3 diverse animal models used to investigate cell homing in arthritis, EPCs preferentially localized to inflamed synovium compared to normal synovium (Silverman et al., 2007). This correlates well with earlier observations that the number of EPCs per mm^2 identified immunihistochemically in postsurgical human RA synovial tissue (ST) was elevated ~25-fold over the number of EPCs localized in normal ST (Ruger et al., 2004).
The findings provide evidence of a possible role of EPC in the synovial neovascularization that is critical to RA pathogenesis; and it may be suggested that neutrophils play a crucial role in this phenomenon. As was showed by Schruefer (Schruefer et al., 2004), neutrophils are critically involved in angiogenesis. Growing evidences indicates that angiogenesis can be initiated by inflammatory cytokines, including IL-8. Human neutrophils release a variety of proinflamatory cytokines, including IL-8, which was originally identified as a potent activator of human neutrophils (Baggiolini et al., 1989). Subsequently, IL-8 was shown to stimulate angiogenesis by promoting proliferation of endothelial cells, moreover IL-8 inhibits endothelial cell apoptosis and induces the upregulation of endothelial matrix metalloproteinase-2 and -9, which also play an important role in angiogenesis.
Neovascularization is a hallmark of diverse pathological conditions, including RA. Microcirculatory expansion occurs either through angiogenesis (the proliferation and branching off of pre-existing microvessels, by IL-8 from neutrophils), or by vasculogenesis (the de novo formation of blood vessels from circulating EPCs). This phenomenon may be restored by LDG depletion (Denny et al., 2010).
Inhibition of neovascularization and thus inhibition of the expansion of invasive tissue in RA is the desired effect. A clearer understanding of the role of neutrophils in biologic processes that guide EPCs to the angiogenic tissue and exert contradictory effects on mirocirculatory expansion may lead to the development of the novel tools to modulate these activities (Silverman et a., 2007).

7. Neutrophil-mediated monocyte recruitment. View on neutrophil-monocyte axis

The sequence of phagocyte recruitment to the site of inflammation comprises initial extravasation of neutrophils followed by a subsequent emigration of monocytes. The experiments of Gallin (Gallin et al., 1982) pointed to the importance of ready made neutrophil granule proteins in the recruitment of monocytes. Granule proteins are stored in 4 distinct sets of granules. Primary and secondary granules discharged from emigrated neutrophils contain mainly antimicrobial polypeptides. Rapidly mobilized secretory vesicles contain mainly receptors important for adhesion and recognition of foreign particles. Tertiary granules released during transendothelial migration contain mainly proteases (Soehlein et al., 2009).

It has been shown that neutrophils that have migrated to the site of inflammation can up-regulate their production of chemokines, supporting the notion that, in this way, neutrophils participate in the regulation of leukocyte accumulation (Soehlein et al., 2009). In terms of production, the principal chemokine produced by neutrophils is IL-8, which activates neutrophils in an autocrine loop. IL-8 binds to CXCR2 expressed not just on neutrophils, but also monocytes. IL-8 also mediates adhesion both human neutrophils and monocytes to the endothelium (Soehlein et al., 2009).

Adhesion of neutrophils to the endothelial cells results in rapid release of secretory vesicles: proteinase-3 and azurocidin (also known as cationic antimicrobial protein). Both azurocidin and proteinase-3 are strongly positively charged and may therefore act with negatively charged endothelial proteoglycans. Azuricidin was recently shown (Soehnlein et al., 2009) to induce monocyte extravasation, it has been demonstrated that depletion of neutrophils reduces the recruitment of inflammatory monocytes. Interestingly, this deficiency in recruitment can be almost completely rescued by the local application of the supernatant from activated human neutrophils (Soehlein et al., 2009).

Activated neutrophils are short-lived cells. Their apoptosis is a tightly regulated process involving ROI and pathogens. Once neutrophils migrate toward the site of inflammation, their life span increases because of the presence of survival signals in the inflammatory milieu. In response to pro-inflammatory signals, neutrophils not only extend their life span, but also release a web of DNA in which granule proteins are enweaved (Brinkmann et al., 2004). Exposure of granule proteins and entrapment within a net of DNA may contribute to creating a gradient of chemotactic stimuli relevant to monocyte recruitment. Apart from release of granule proteins, apoptotic neutrophils may release attraction signals leading to influx monocytes. In recent years, several apoptotic cell-derived "find-me" signals were identified. Among them is lysophosphatidylcholine (LPC), of which the latter has received much attention. LPC was identified (Kim et al., 2002) as an "eat-me" signal on the apoptotic cell surface. More recently, it has been shown that changes in membrane composition of apoptotic cells (negative surface charges) in initiate attractive signals for phagocytes including monocyte. It is feasible that apoptotic neutrophils generate such electric signals resulting in electrotaxis of monocytes (Zhao et al., 2006).

In addition, neutrophil granule proteins enhance the production of ROI by monocytes (Soehnlein et a., 2008). Thus, various experimental setups provide evidence that the axis of neutrophils and inflammatory monocytes promotes and sustained inflammation. Taken together, the multifaceted action of neutrophils in recruiting and activating monocytes may offer a powerful target for interfering with the sustained inflammatory reponse in RA (Soehnlein et al., 2009).

8. Summary

The research conditions of most neutrophil experiments differ considerably from the physiological environment of the joint, i.e. the presence and concentration of cytokines such as TNF-α, IL-1β, IL-6, IL-8, IL-12, IL-17, IL-18, IL-23 and IFN-γ (McInnes et al., 2007), and physical factors such as viscosity, oxygen concentration, and pressure. Each of these differences can influence neutrophil metabolism and activation (Gajewski et al., 2006, 2010a; Cross et al., 2006). Reasoning that conditions imitating physiological environment are required for proper conclusions, we developed and used culture systems which more accurately simulated conditions in the joint (Gajewski, et al., 2010b). The proposal to

conduct studies in more physiological conditions, is strongly recommended for *in vitro* experiments on neutrophil's role in RA.

Findings confirm that neutrophils in synovial tissues have different features than blood neutrophils. The old view of neutrophils as a terminally differentiated cell completely focused on destroying pathogens and tissues is no longer held (Cascao et al., 2009). Our understanding of the role of neutrophils 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, the functions of macrophages (Cascao et al., 2009).

Neutrophils are key cells in the immune response due to their dual anti-infection and pro-inflammatory roles, being critical effectors in both innate and humoral immunity. Neutrophils generate chemotactic signals and cytokines that recruit, differentiate and activate B and T lymphocytes and antigen presentating cells (APCs), thus establishing a "bridge" between the innate and adaptive immune system. Neutrophils seem to be important "decision-shapers" (Cascao et al., 2009) in this complex system and further understanding of the specific roles of these cells may help to answer one of the main questions in the immune system domain; "What triggers an immune response?" (Cascao et al., 2009).

The knowledge about neutrophil complex biology and their role in immune-mediated inflammatory diseases is expected to reveal promising new therapeutic targets.

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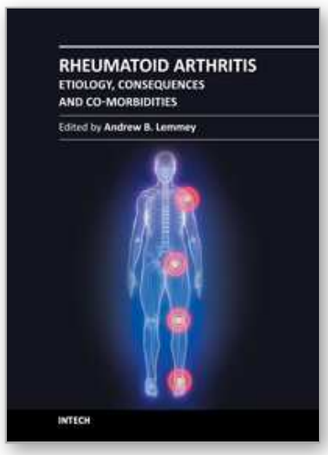

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The purpose of this book is to provide up-to-date, interesting, and thought-provoking perspectives on various aspects of research into current and potential treatments for rheumatoid arthritis (RA). This book features 16 chapters, with contributions from numerous countries (e.g. UK, USA, Japan, Sweden, Spain, Ireland, Poland, Norway), including chapters from internationally recognized leaders in rheumatology research. It is anticipated that Rheumatoid Arthritis - Etiology, Consequences and Co-Morbidities will provide both a useful reference and source of potential areas of investigation for research scientists working in the field of RA and other inflammatory arthropathies.

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