Neutrophil, Basophil, and Eosinophil Granulocyte Functions in the Elderly

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Contents

Abstract

The immune response changes within aging. Aged persons >65 years old display a predisposition to inflammation and infection combined with an increase in morbidity and mortality than younger individuals. Extensive studies about consequences of aging on adaptive immunity exist, but the effects of aging on the innate immune system remain only partly understood. Neutrophil granulocytes (polymorphonuclear leukocytes, PMN) as the largest leukocyte population in blood build the first line of defense against pathogenic microorganisms and play an important role in regulation of the immune response. In vitro studies demonstrate that PMN functions such as phagocytosis, reactive oxygen species

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(ROS) production, intracellular killing, neutrophil extracellular trap (NET) formation, and chemotaxis are changed in elderly persons, whereas the number of circulating neutrophils is unaltered compared to young persons. However, data from different studies regarding PMN functions are still inconsistent due to different isolation and analysis techniques, contaminating cells, and preactivation. Interestingly, most of the adhesion surface molecules/receptors of PMN are not impaired in function and expression with age. But age-related alterations in receptor-dependent signal transduction, membrane content, and fluidity may result in a decline of PMN function and rescue from apoptosis. Studies investigating age-associated defects of basophil and eosinophil granulocytes are limited, mainly due to their low number in periphery. These cells mediate protection against infections with parasites and are involved in antigen presentation, T-helper 2 cell differentiation, but promote inflammatory responses in allergy and autoimmune diseases. This chapter gives an overview about the age-related modulations in human granulocytes.

Keywords

Adhesion · Polymorphonuclear leukocytes · Basophils · Eosinophils · Neutrophils · Phagocytosis · Chemotaxis · Degranulation · Intracellular killing · Inflammation · Apoptosis · G-CSF · GM-CSF · fMLP · IL-1RA · IL-1ß · IL-3 · IL-8 · TNF-α · Toll-like receptor · MAPK · MyD88 · NETs · IRAK · p38 · ERK1/2 · Membrane fluidity · CD62L

Introduction

Elderly persons are more susceptible to microbial infections with an increase in morbidity and mortality due to impaired innate and adaptive immunity, termed immunosenescence (Panda et al. 2009; Wessels et al. 2010). In general, age-related changes include a decreased response to vaccination and increased incidence of infections, inflammatory and autoimmune diseases, and cancer. There are many approaches to uncover the molecular and cellular changes surrounding immune system dysfunctions. However, additional factors such as nutrition, fitness, social components, and diseases influence immunity of elderly persons making the detection of stand-alone, age-dependent changes more difficult. To exclude those factors, the SENIEUR protocol was created to clearly separate age-related from non-age-related alterations of the immune system (Ligthart et al. 1994). This protocol sets the criteria in order for a healthy elderly person to participate in immunogerontological studies. The effects of aging are well-documented for the adaptive immune system, e.g., the alterations in T cell count, phenotype, and function as well as reduced ability of B cells to synthesize high-affinity antibodies (► Chap. 34, "Older Human B Cells and Antibodies"). But in the meantime, the importance of the innate immune system in fighting invading microorganisms and the cooperation with the adaptive immune system to ensure optimal immune response has become more widely accepted. Neutrophil granulocytes display alterations of function, surface molecule

expression, apoptosis, and signal transduction with aging. Analyses of number and functions of human basophils and eosinophils in aging are rare objectives in age research but, like neutrophils, might have a future potential to modify age-related immune deficiencies. The age-associated changes and their effect on the attenuation of granulocyte functions will be summarized and discussed by reviewing the literature.

Neutrophils

Neutrophils are key effector cells of the innate immune system. They are the first cells migrating rapidly to sites of infection and recognize and engulf microorganisms by phagocytosis. Neutrophils destroy and degrade invaded pathogenic bacteria and fungi via the release of reactive oxygen species and antimicrobial and proteolytic granule proteins, which are delivered to the phagosomes and to the extracellular environment (Panda et al. 2009; Wessels et al. 2010; Mantovani et al. 2011). Another function of neutrophils is NETosis, the release of a matrix composed of DNA, chromatin, and granule proteins to capture extracellular bacteria within so-called neutrophil extracellular traps (NETs), a zinc-dependent process (Sørensen and Borregaard 2016, Hasan et al. 2013).

Additionally, neutrophils produce chemokines and cytokines that recruit and regulate the inflammatory response of macrophages, T cells, and neutrophils themselves. Finally, they initiate an apoptotic program where they are digested by macrophages without causing tissue damage and necrosis and, therefore, support the resolution of the inflammatory response (Panda et al. 2009; Wessels et al. 2010).

Human neutrophils have a life span of 5.4 days, and their half-life circulation time in blood is 6–12 h (Schroeder et al. 2006; Panda et al. 2009; Pillay et al. 2010; Summers et al. 2010). The adult bone marrow has to produce $1–2 \times 10^{11}$ neutrophils per day to sustain a sufficient cell number to efficiently fight infections (Uciechowski and Rink 2014). This continuous production is controlled by granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-3.

Maintenance of neutrophil numbers is again controlled by phagocytosis of apoptotic neutrophils by macrophages, termed "efferocytosis." Efferocytosis decreases the synthesis of IL-23 and IL-17 and dampens G-CSF production (Stark et al. 2005; Kruger et al. 2015).

To maintain the function of neutrophils summoned to infected tissue, "survival" factors such as lipopolysaccharide (LPS), hypoxic environment, complement factors, and pro-inflammatory cytokines counteract apoptotic programs in neutrophils (Fig. 1) (Lee et al. 1993; Hannah et al. 1995; Panda et al. 2009). To fulfill their tasks in the defense against bacterial and fungal infections, specific functions are regulated by specific receptors. These receptors are for *N*-formyl-methionyl-leucyl-phenylalanine peptide (fMLP), GM-CSF, complement, CD16 (FcγRIII), and IL-8 (CXCL8) (Butcher et al. 2001; Uciechowski and Rink 2014). Neutrophils also express CD66b, CD11a, CD11b, CD11c, CD18, CD14, and CD15, constitutively, and MHC class II,

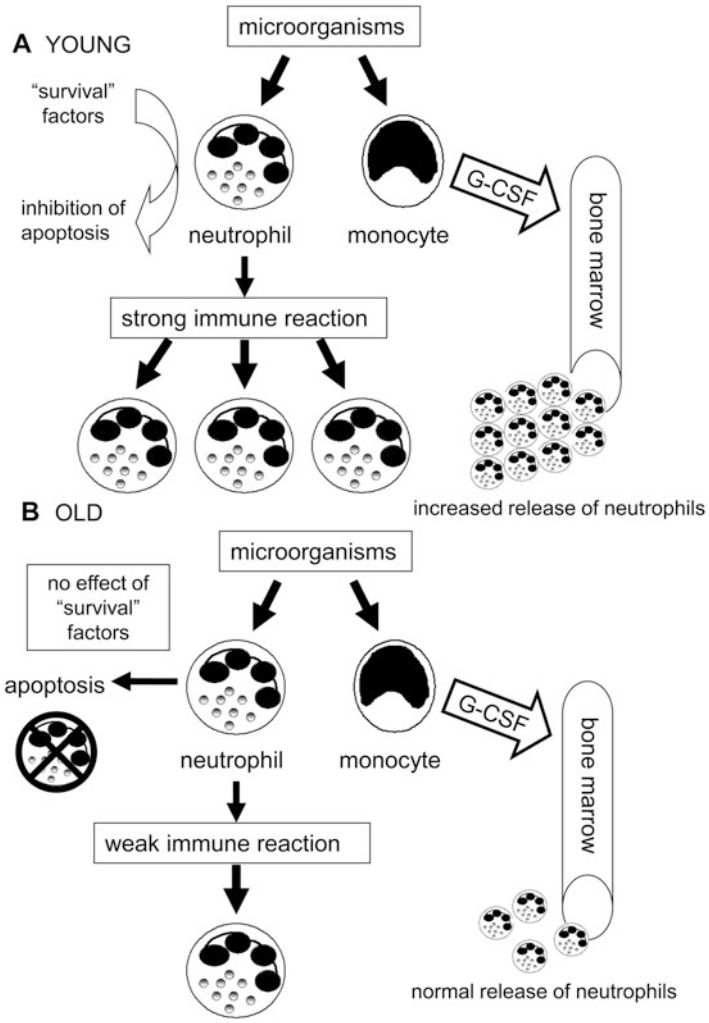

Fig. 1 Recruitment and apoptosis of neutrophils during infection. (**a**) After phagocytosis of invading microorganisms, the apoptosis of neutrophils in young individuals is blocked via the release of survival factors. Additionally, G-CSF induces the release of a large number of neutrophils from the bone marrow leading to physiological neutrophil leukocytosis. (**b**) In elderly persons, the inhibition of apoptosis of neutrophils is impaired, and the recruitment of neutrophils from the bone marrow is not enhanced. This might result in an exhaustion of neutrophils and consequently lead to a reduced immune response with age

CD64 (FcγRI), upon stimulation (Schroeder et al. 2006; Wessels et al. 2010). Additionally, pattern recognition receptors, e.g., toll-like receptors (TLRs), binding conserved molecular structures of most microorganisms, participate in the inflammatory response of PMN and other cells of the innate and adaptive immune system (O'Neill 2006). Neutrophils express all TLRs with the exception of TLR3

(Mantovani et al. 2011). In addition, GM-CSF, IL-15, and IL-18 activate neutrophils (Fortin et al. 2007, 2009; Panda et al. 2009; Wessels et al. 2010).

The role of neutrophils in the immune response has been underestimated for a long time, and their function has been reduced to being only end-differentiated phagocytes with limited transcriptional activity. This view has now changed since neutrophils possess a more active transcriptional program (Ericson et al. 2014) than expected, an extended life (Pillay et al. 2010), as well as the ability of to bridge and regulate innate and adaptive immune responses (Panda et al. 2009, Wessels et al. 2010). In addition, the bandwidth of neutrophil functions and cell interactions with dendritic cells, natural killer (NK) cells, macrophages, and nonimmune cells has also emerged in the last years (Kruger et al. 2015). However, resting highly purified human neutrophils synthesize only a limited pattern of cytokines and chemokines after stimulation (Schroeder et al. 2006). Interestingly, there are some reports that neutrophils are also able to synthesize pro-inflammatory cytokines such as IL-1ß, tumor necrosis factor-α (TNFα), IL-6, and IL-17 described by others (Ferretti et al. 2003; Wessels et al. 2010; Mantovani et al. 2011). These observations are mostly results of monocyte contaminations in the neutrophil isolates or have only been seen using PCR methods. Neutrophils produce large amounts of the anti-inflammatory interleukin-1 receptor antagonist (IL-1RA) after stimulation or after high accumulation of neutrophils (Kolling et al. 2001; Schroeder et al. 2005). Thus, neutrophils are not only able to recruit other immune cells to sites of infection but can also create an anti-inflammatory environment that supports the clearance of inflammation.

Neutrophils and Aging

It is well known that aging results in a predisposition to inflammation as well as to infections, which is associated with higher rates of mortality and morbidity (Franceschi et al. 2000; Wessels et al. 2010).

One might assume that impaired defense against invading pathogens such as fungi and bacteria is accompanied by a reduced amount of neutrophils as seen within the T and B cell system of elderly persons. But there are no alterations in the number of precursor cells in the bone marrow or of circulating neutrophils (Chatta et al. 1993; Born et al. 1995). Moreover, neutrophils have been described to be significantly increased in the aged (Cakman et al. 1997). Therefore, one can assume that functional defects of neutrophils such as recruitment or killing are probably responsible for age-associated susceptibility to infection. Studies in mice supported this thesis since microenvironmental factors associated with advanced age resulted in impaired neutrophil function (Geiger and Rudolph 2009). In addition, the mixture of these factors may be crucial since reduced killing of *Candida albicans* (Murciano et al. 2008), but not that of other microbes such as *Streptococcus pneumoniae*, could be shown (Kovacs et al. 2009).

Neutrophil precursor cells show a reduced proliferative response to G-CSF only (Fig. 1), whereas responses to GM-CSF and IL-3 are not affected (Chatta et al. 1993). Elderly persons also display a normal neutrophilia during infection (Lord

et al. 2001), indicating that GM-CSF and IL-3 mediate sufficient neutrophil production. However, loss of apoptotic rescue and a normal recruitment of neutrophils by G-CSF during infection might promote an impaired immune response with age (Fig. 1). In the case of severe chronic infection, neutropenia can be observed in the elderly, suggesting that persistent infection in the elderly impairs neutrophil recruitment (Born et al. 1995).

Function

Although neutrophil count can be elevated and adherence to endothelia appears to be unchanged in elderly persons, impaired PMN functions can be seen including a decline in phagocytic capacity in healthy elderly individuals accompanied by reduced intracellular killing (Lord et al. 2001, Butcher et al. 2000). This decline in function may contribute to increased susceptibility to bacterial infections in the elderly population. In contrast, aged persons fulfilling the SENIEUR criteria who also exhibit elevated numbers of granulocytes are functionally normal (Ginaldi et al. 1999; Plackett et al. 2004).

Studies that analyze phagocytosis of *Escherichia coli (E. coli)* and opsonized bacteria or yeast and opsonized zymosan by neutrophils have all demonstrated a significant impairment in phagocytic function in the elderly (Esparza et al. 1996, Wenisch et al. 2000; Alonso-Fernandez et al. 2008; Simell et al. 2011; Sauce et al. 2016). Additionally, changes in actin polymerization and decrease in the antibody-dependent phagocytosis mediated by Fc receptors are reported (Rao et al. 1992). Interestingly, the functions of these receptors are not changed, immunoglobulin and complement levels are normal, and serum from elderly donors opsonizes bacteria normally so that phagocytosis itself is impaired (Wenisch et al. 2000; Butcher et al. 2001). Butcher et al. (2001) have shown that one of the receptors involved in recognizing antibodies on the surface of bacteria, CD16, is significantly reduced with age and may contribute to the observed decline in neutrophil phagocytic function with age (Butcher et al. 2001).

After phagocytosis of pathogenic microorganisms, the phagosomes fuse with lysosomes containing bactericidal substances and build the phagolysosome. Therein the pathogen will be intracellularly killed. Besides other destructive components contained within the phagolysosome, intracellular killing is dependent on the generation of reactive oxygen species (ROS), termed respiratory or oxidative burst. This respiratory burst induces production of superoxide, hydrogen peroxide, and hypochloric acid, which are all toxic to microbes. Contradictory findings describing the respiratory burst after fMLP stimulation in neutrophils of the elderly have been reported. Some groups determined decreased respiratory burst activity after either fMLP (Biasi et al. 1996; Braga et al. 1998; Tortorella et al. 2000), GM-CSF, or LPS stimulation (Tortorella et al. 1996). Wenisch et al. (2000) showed a significant reduction in generation of ROS after stimulation with *Staphylococcus aureus* (*S. aureus*) but normal ROS production after stimulation with *E. coli*. These results are in concordance with reported reduced ability of elderly to fight infections caused

by gram-positive bacteria (Whitelaw et al. 1992), since *S. aureus* frequently causes postoperative sepsis in the elderly. Other studies using SENIEUR selected persons could not detect a difference in respiratory burst compared to younger persons even after stimulation with fMLP (Butcher et al. 2000; Lord et al. 2001). The application of different stimuli led to various results, based on the assumption that distinct pathways of neutrophilic activation are involved. An early report by Tortorella et al. (2004) indicated that signaling pathways may be impaired. Neutrophils obtained from elderly humans and stimulated with GM-CSF displayed a significant reduction in phosphorylated ERK1/2 levels and an even larger decrease in ERK1/2 activation. No changes in GM-CSF-induced p38 MAPK phosphorylation were observed (Tortorella et al.; 2004). This coincides with Larbi and colleagues reporting that p38 signaling is not involved in GM-CSF delayed apoptosis in any age groups (Larbi et al. 2004). Decreased antioxidant production, e.g., glutathione (Alonso-Fernandez et al. 2008), is also impaired in the elderly.

A recent report by Sauce et al. (2016) described that resting blood neutrophils have a normal ROS production but a decreased oxidative burst in response to fMLP. The reduced phagocytosis and ROS production seem to be associated with the upregulation of an immunosuppressive CD16bright/CD62Ldim neutrophil subpopulation (Sauce et al. 2016). In contrast, another group found spontaneous higher levels of intracellular ROS in neutrophils from individuals 60–89 years old, whereas zymosan-induced extracellular ROS levels were lower in this group compared to young and nonagenarian (>90 years old) groups. Since there was also a positive correlation with heat shock protein (HSP)70 levels and zymosan-induced extracellular ROS levels, the authors suggest that older people less than 90 years old have a higher risk of mortality (Kovalenko et al. 2014). Both spontaneous higher ROS production and circulating levels of inflammatory markers in the elderly had been described before; these authors found a negative correlation with HSP70 levels and higher spontaneous ROS levels (Ogawa et al. 2008).

There are only few reports about intracellular killing of fungi and bacteria in elderly people. In these reports the capability of stimulated and unstimulated neutrophils to destroy *Candida albicans* was reduced by 10–50% in the elderly, and *E. coli* killing was 44% lower than that of young persons (Plackett et al. 2004; Uciechowski and Rink 2014). Murciano et al. (2008) also reported an impaired destruction of *Candida albicans* in the elderly (Murciano et al. 2008).

The reason for impaired intracellular killing in neutrophils of the elderly is not clear yet. Although Piazzolla postulated that cytoskeleton-affecting compounds are responsible for the alteration of fMLP-stimulated superoxide generation (Piazzolla et al. 1998), this does not illuminate the selective discrimination of one stimulant against the other. It is possible that triggering various signal transduction pathways after recognition of the pathogen and consequent activation of the neutrophil are responsible for an impaired defense toward one pathogen, whereas the response to another remains unaltered in the elderly.

Interestingly, it has been described that non-stimulated neutrophil production of ROS and nitric oxide (NO) was, respectively, 38 and 29% higher in the elderly compared to younger subjects (Nogueira-Neto et al. 2016). This might indicate that

because of higher levels of ROS in the elderly under resting conditions, the production cannot be triggered to reach levels of younger ones after stimulation.

Neutrophils respond to various chemotactic products such as complement factor C5a, chemokine IL-8 (=CXCL8), or bacterial fMLP, released either by the host or by the invading organism (Uciechowski and Rink 2014). The migration to the infected tissue is mediated through binding of CD15 (Lewis X) on neutrophils to P- and L-selectin on endothelial cells (rolling) and, secondly, via interaction of CD11a/CD18 and CD11b/CD18 on neutrophils and CD54 on endothelial cells (adhesion) followed by transmigration through the endothelium (diapedesis) following a chemotactic gradient. It has been reported that chemotaxis remains largely unaltered in the elderly (Biasi et al. 1996) or at least display a normal reaction after stimulation with fMLP (Butcher et al. 2000; Lord et al. 2001). Many in vitro studies described a decreased chemotaxis of neutrophils from healthy aged donors stimulated with GM-CSF and fMLP or complement (Wenisch et al. 2000; Niwa et al. 1989; Alonso-Fernandez et al. 2008; Fortin et al. 2006; Fulop et al. 2004). A lower actin polymerization of elderly neutrophils after fMLP stimulation or changes in signaling pathways are discussed as underlying mechanisms (Rao et al. 1992).

Corberand et al. (1981) reported significantly decreased chemotaxis in people over 80 years old but no significant difference in 60- to 70-year-old compared with young persons. In contrast, Niwa et al. (1989) presented opposite results finding a correlation between 60- and 70-year-old people with decreased chemotaxis and respiratory burst and mortality 7 years after the initial study. No difference between people older than 80 years old and the young could be seen. The authors suggested that individuals with the most suitable neutrophils survived into the oldest age group, and therefore, no difference between the over-80-year-old persons and the younger ones could be observed. Similar data have been obtained for degranulation and superoxide production in response to stimulants such as fMLP (Esparza et al. 1996; Tortorella et al. 2000).

Several new reports described how and by which mechanisms recruitment is decreased with aging. The authors showed a diminished response to IL-8 of human neutrophils from elderly individuals and to chemokine neutrophil-activating protein 3 (NAP-3, CXCL1) of neutrophils in aged mice (Dalboni et al. 2013; Brubaker et al. 2013; Sapey et al. 2014). The consequence of chemotactic defects in neutrophils from the elderly might be an impaired migration into infected or injured tissues leading to delayed wound healing, occurrence of severe wound infections, and reduced pathogen clearance. Alonso-Fernandez et al. (2008) found no differences in chemotaxis of centenarians compared to younger persons suggesting that a defect in chemotaxis contributes to a defective immune reaction in aged persons.

As aforementioned, NETs are a new recently discovered function of neutrophils, helping in the immune response against rapidly dividing bacteria. Neutrophils from aged mice synthesize fewer NETs after infection with *S. aureus* or PMA stimulation (Tseng et al. 2012). LPS- and IL-8-induced NET formation showed a significant age-related decline suggesting a defect in proximal signaling with regard to age-associated reduction in NET and ROS generation (Hazeldine et al. 2014).

Apoptosis

Differentiation, development of tissue, and homeostasis but also neurodegenerative and immune diseases and cancer are dependent on apoptotic processes. Neutrophils exhibit a fast apoptotic rate in vitro as well as in vivo. It is important that apoptosis is well-balanced to ensure PMN survival and production; in case of imbalance, an enhanced risk to develop chronic inflammatory diseases is arising.

The regulation of apoptosis of neutrophils is important to maintain longer survival in inflamed tissue or the resolution of inflammation. Without stimulation, the susceptibility of neutrophils to apoptosis is either slightly increased in the elderly (by GM-CSF) or unaffected by aging (Fulop et al. 1997; Panda et al. 2009; Wessels et al. 2010).

It has been shown that the rescue from apoptosis by survival factors G-CSF, GM-CSF, IL-2, TNFα, IL-6, steroids, and LPS of neutrophils is disturbed with aging. Increased apoptotic rates of neutrophils at the site of infection might cause decreased bactericidal function (Fig. 1). DiLorenzo and coworkers reported a significant age-related decrease of formation of O^{2-} and chemotaxis, whereas no significant correlation between age and the expression of the death receptor CD95 (APO1, Fas) on the granulocyte membrane could be detected. The authors suggest that an increase of CD95-mediated apoptosis of neutrophils might play a minor role in the impairment of PMN function (Di Lorenzo et al. 1999).

Consequently, an imbalance of pro- and antiapoptotic molecules of the B cell leukemia (Bcl)-2 family, such as Bax, Bad, myeloid cell leukemia (Mcl)-1, or BclXL, can be assumed.

Fulop et al. (2002, 2004) investigated the role of antiapoptotic Mcl1 and proapoptotic Bax in decreased apoptosis inhibition in PMN of the elderly. The authors found that the expression of Bax was unchanged in old and young persons; also treatment with GM-CSF could not modulate Bax expression. In contrast, Mcl1 was found to be upregulated after GM-CSF stimulation in young persons, whereas there was no difference between stimulation and spontaneous apoptosis in the elderly. By comparing the Bax/Mcl1 ratio after GM-CSF stimulation, only a slight difference in the Bax/Mcl1 ratio in aged persons was detected, but Mcl1 expression was increased relative to Bax in neutrophils from younger individuals. These findings indicate an important role of Bax and Mcl1 in the survival of neutrophils mediated by GM-CSF.

The Janus tyrosine kinase (Jak)2 signal transducer and activator of transcription (Stat)5 signal transduction pathway is also modulated in elderly persons (Plackett et al. 2004; Fulop et al. 2004). Since Jak2 is related to the expression of antiapoptotic Bcl-2, there might be a possible link between Jak2 and Mcl1 being involved in the decreased rescue of neutrophils from apoptosis (Fig. 2) (Fulop et al. 2004). Larbi et al. (2005) presented evidence that a modulation in the p42/p44 (ERK1/2) mitogen-activated protein kinase (MAPK) activation occurs in PMN of elderly subjects under GM-CSF stimulation and is in part responsible for the decreased apoptotic decline of PMN in the elderly. This might be the reason why GM-CSF was not able to downregulate caspase-3 activation in neutrophils of elderly persons. Interestingly, the authors observed that GM-CSF changed the proapoptotic phenotype to an antiapoptotic phenotype by alteration of the Bcl-2 family members Bax and Bcl-xL in young neutrophils in an MAPK-independent way, whereas this could not be seen in aged neutrophils.

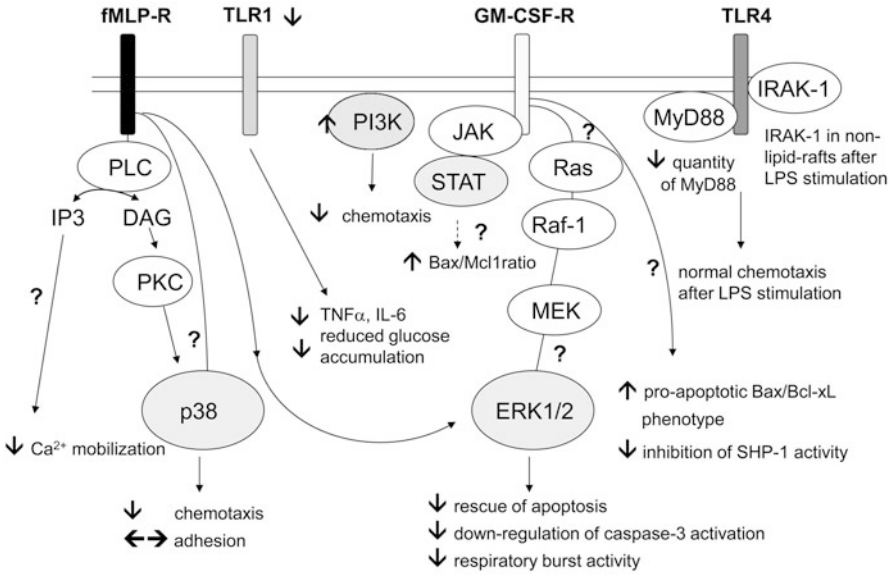

Fig. 2 Signaling in neutrophils of the elderly. Age-related impairment in intracellular signaling after binding of the appropriate ligands to their receptors leading to altered functions of neutrophils. Question marks display defects in different signaling pathways associated with age (Modified and adapted from Fulop et al. 2004). *PLC* phospholipase C, *DAG* diacylglycerol, *IP3* inositol trisphosphate, *MEK* MAPK (mitogen-activated protein kinase)/ERK kinase, *ERK* extracellular signal-regulated kinase, *PKC* protein kinase C, *PI-3K* phosphoinositide-3 kinase, *TLR* toll-like receptor

Age-associated loss of GM-CSF ability to induce neutrophil functions and impede apoptosis via phosphoinositide-3 kinase/Akt (PI3K/Akt), extracellular signal-regulated protein kinase (ERK), and Stat signaling pathway inhibition is caused by an amplified activity of SHP-1, an inhibitor of Src family of tyrosine kinases, and suppressors of cytokine signaling (SOCS) (Tortorella et al. 2004, 2006, 2007; Fortin et al. 2006, 2007).

Taken together, these modulations might be responsible for the creation of a proapoptotic environment and could explain the increased incidence of infections in the elderly (Figs. 1 and 2).

Signal Transduction

Activation of the fMLP receptor via phospholipase C (PLC) leads to the production of diacylglycerol (DAG) and inositol trisphosphate (IP3); the latter initiates the enhancement of intracellular Ca^{2+}. DAG induces the membrane translocation of protein kinase C (PKC) and phosphorylation of MAPK family members.

Intracellular Ca^{2+} is decreased in stimulated neutrophils from elderly persons (Fig. 2), suggesting that there is an impairment in Ca^{2+} flux during cell signaling (Fulop et al. 1997, 2004; Lipschitz et al. 1991). Interestingly, resting neutrophils of elderly subjects show an enhanced level of intracellular Ca^{2+} (Wenisch et al. 2000). Preactivation, modulation of the aged plasma membrane followed by altered receptor and adapter protein linkage, and defects in the early phase of signal transduction might lead to the impairment of Ca^{2+} mobilization of aged neutrophils after fMLP stimulation. By investigating the impaired Ca^{2+} mobilization in aged neutrophils, Klut et al. (2002) found heterogeneity of the examined neutrophils concerning time and magnitude of the response. A reduced number of neutrophils in the elderly were able to generate an effective reaction, hinting at a possible subpopulation (▶ Chap. 38, "Signal Transduction Changes in Human Neutrophils with Age").

After fMLP stimulation, PKC might also activate the p38 signal pathway, which is involved in regulating gene transcription, chemotaxis, and adhesion. The ERK1/2 signaling pathway is also triggered after fMLP stimulation playing a role in adhesion and respiratory burst activity. Defects in the signal cascades of both pathways and the decrease in activation and phosphorylation levels of p38 and ERK1/2 MAPKs are suggested to affect impaired neutrophilic functions in the elderly (Fig. 2) (Fulop et al. 2004).

GM-CSF is able to activate the Jak/Stat pathway, the Ras-Raf-1-MEK-ERK1/2 pathway, and phosphatidylinositol 3 kinase (PI-3K)-triggered signaling (Fulop et al. 2004). Investigating the role of protein tyrosine phosphatases (PTP), especially Src homology domain-containing protein tyrosine phosphatase-1 (SHP-1), Fortin et al. (2006) suggested a differential effect of GM-CSF on phosphatase activity in modulating neutrophil functions with aging. SHP-1 is a negative regulator of signal transduction and can negatively regulate Src kinases, such as the Jak or Lyn kinase, elicited by GM-CSF in PMN. When recruited to the plasma membrane and activated, SHP-1 dephosphorylates proteins activated by receptors and inhibits cell activation. SHP-1 phosphatase activity could not be downregulated after short stimulation with GM-CSF in the neutrophils of the elderly persons in contrast to neutrophils of young. In lipid rafts from neutrophils of elderly, SHP-1 is continuously present, whereas in the neutrophils of young donors, SHP-1 is rapidly dissociated after stimulation by GM-CSF and is recruited back during a longer period of stimulation. SHP-1 is also constantly recruited to Lyn, which cannot be relieved by GM-CSF. Consequences of the dysregulated GM-CSF signaling in neutrophils of aged donors are impaired protection from apoptosis, delayed respiratory burst, and degranulation functions of neutrophils. These modulations together with the abovementioned changes in the Jak2-Stat5 and ERK1/2 signaling pathways might contribute to the decreased GM-CSF effects on neutrophils (Fortin et al. 2006, 2007). Sapey and colleagues described decreased neutrophil migration to different inflammatory stimuli in elderly people but no lack in chemokinesis (Sapey et al. 2014). It could be shown that constitutive activation of PI-3K was responsible for the decreased migration. Blocking of PI-3Kγ or δ restored accuracy of the directional chemotaxis. Figure 2 summarizes the effects of aging in signal transduction.

Adhesion Surface Molecules and Receptors

After receiving a chemotactic signal, the rolling neutrophil adheres via integrin molecules to endothelial cells and migrates through the endothelium (diapedesis) toward the site of infection. Adhesion appears not to be impaired in the elderly. After stimulation with fMLP, zymosan, phorbol myristate acetate (PMA), or calcium ionophores, human neutrophils from young and elderly persons displayed no difference in adhesion to plastic, gelatin, and bovine aortic endothelium (Biasi et al. 1996; Plackett et al. 2004).

In a very recent paper, it could be observed that in non-stimulated neutrophils, obtained from young (18–30 years old) and elderly (65–80 years old) human volunteers, CD11b expression was only elevated in neutrophils from the aged group. Additionally, a 69% higher non-stimulated in vitro neutrophil/endothelial cell adhesion was observed for neutrophils isolated from elderly donors [Nogueira-Neto et al. 2016].

Furthermore, a normal or enhanced adherence of neutrophils to endothelia or thrombocytes has been described, but it is not clear whether this has an effect on increased tissue migration in vivo. One might argue that increased adherence is caused by slightly enhanced expression of CD15 (Lewis X) and CD11b (Mac-1, complement receptor 3) on neutrophils (Esparza et al. 1996). In contrary, no increase of CD11b and CD15 but a decrease of CD62L (L-selectin) was observed by others (Butcher et al. 2001). Interestingly, the expression of the other two integrins, CD11a (leukocyte function antigen, LFA-1) and CD11c (p150, 95) involved in cell adhesion, is not affected (Lord et al. 2001, Butcher et al. 2000, 2001; Esparza et al. 1996).

De Martinis et al. (2004) compared the expression of CD50 (ICAM-3; a ligand for CD11a/CD18) and CD62L adhesion molecules in peripheral blood granulocytes and monocytes between healthy elderly and young persons. They found a decrease in the percentage of granulocytes and monocytes expressing CD62L in the elderly but no alteration in the density expression on both cell types suggesting a pre-activation which might contribute to the pro-inflammatory status in aging. The authors described a downregulation of the density expression of CD50 at a per cell level on granulocytes and a decrease of CD50 density expression on monocytes but an expansion of CD50-positive cells in elderly persons. Changes in cell adhesion and decreased chemotactic functions may be associated with the loss of CD62L which may contribute to the enhanced susceptibility to acute infections in elderly persons. The shedding of CD62L from the cell surface of neutrophils and constitutive ROS production are also signs of preactivation/low-grade inflammation as postulated by other groups (Franceschi et al. 2000; Biasi et al. 1996; De Martinis et al.; 2004) and confirm well with the observation of enhanced Ca^{2+} flux in elderly persons. Yet, one has to be cautious with regard to the purification process of neutrophils since some substances may cause a decrease in CD62L expression (Schroeder et al. 2006; Wessels et al. 2010).

Noble et al. (1999) observed a significantly lower recruitment of early activation marker CD69 from the vesicles to the plasma membrane after stimulation with PMA in elderly people (fulfilling the SENIEUR criteria) than in younger persons. fMLP in

contrast had no influence in different expression of CD69 in young and elderly persons, suggesting again the impairment of distinct pathways within aging.

There is growing evidence that aging is accompanied by changes in receptor signaling pathways and membrane fluidity (Larbi et al. 2004; Fulop et al. 2004, Fortin et al. 2006). In contrast to other cells, the fluidity of the PMN membrane increases with age, caused by alterations in the cholesterol/phospholipid content of the membrane (Alvarez et al. 2001). These modulations result in changed function of lipid rafts, which directly influence TLRs and GM-CSF signaling. Additionally, actin, which may play a role in cell surface receptor movement and expression, has been indicated to contribute to the changed ROS production (Rao et al. 1992). In summary, these alterations in signaling may impair the effector functions of neutrophils in aging.

After stimulation, the fMLP receptor which is coupled to a pertussis toxin-sensitive G protein induces the production of superoxide anion, hydrogen peroxide, NO, and an increase in intracellular free calcium. The influence of aging on the release of free radicals has been investigated by different laboratories for a long period of time (reviewed by Schroeder and Rink 2003). Some investigators reported a decreased synthesis of free radicals by neutrophils of elderly persons but found no change in the expression of fMLP receptor number (Biasi et al. 1996; Tortorella et al. 2000; Fulop et al. 2004), whereas others could not confirm those data (Lord et al. 2001). A recent study by Fulop et al. (2004), examining neutrophils isolated from young and aged persons who met criteria defined by the SENIEUR protocol, showed a significantly lower production of superoxide anion under fMLP stimulation and/or GM-CSF priming in PMN from elderly persons compared with younger ones. Fulop et al. (2004) postulate the existence of a subpopulation of neutrophils in aged persons, which seems to be responsible for a significantly higher superoxide anion production after 48 h when compared with younger PMN, although they found a reduced superoxide anion production after 24-h stimulation with fMLP and GM-CSF in elderly persons. The authors suggest that PMN from elderly persons might act heterogeneously to downregulate responses to stimulation than PMN from younger persons, which react more efficiently.

Toll-like receptors are pattern recognition receptors with the specificity to recognize substances consisting of conserved motifs of bacteria, fungi, and virus. Ten different human TLRs have been identified, including three intracellularly located types. After ligand binding, the central adapter molecule, myeloid differentiation primary response protein 88 (MyD88), transduces signals into the cell by recruiting a cascade of serine-threonine kinases and IL-1 receptor-associated protein kinases (IRAKs), leading to nuclear factor kappa B (NF-kB)-dependent transcription of pro-inflammatory genes. Although the existence of a MyD88-independent way, stimulation via TLR leads to the release of pro-inflammatory cytokines such as IL-1, IL-6, or TNF-α. The additional production of chemokines and upregulation of surface molecules through TLR signaling build a bridge between innate and adaptive immune responses. Some reports describe an influence of age on TLR. Renshaw et al. (2002) observed that LPS (ligand for TLR4, gram-negative bacteria)-stimulated macrophages from aged mice synthesize less IL-6 and TNF- α than younger

ones. This study was confirmed by Boehmer et al. (2004). Additionally, a lower TLR4 mRNA level compared with those of younger macrophages was found in aged macrophages by Renshaw et al. (2002), whereas others did not observe a variation in TLR4 surface expression with age. These results are difficult to transfer to human beings since increased levels of circulating pro-inflammatory cytokines are generally observed in elderly individuals, especially because aged monocytes after LPS stimulation produce significantly higher amounts of IL-6 and TNF-α (Fagiolo et al. 1993; Gabriel et al. 2002).

By studying the expression of TLR2 (ligand: components of gram+ bacteria) and TLR4, Fulop et al. (2004) did not observe any changes in the proportion of neutrophils expressing TLR2/TLR4 nor in the expression of both receptors on the surface of neutrophils. However, an increase of TLR4 expression in unstimulated raft and non-raft fractions and no redistribution after LPS stimulation in elderly persons in contrast to younger individuals could be detected (Fig. 2). The authors did also not find any differences in fMLP and GM-CSF receptor expression with aging (Fulop et al. 2004).

Although TLR2 and TLR4 expression remains unchanged, an association of IRAK-1 with lipid rafts was not detected after stimulation with LPS. Additionally, the main adapter protein of the TLR signaling pathway, MyD88, was significantly reduced in the plasma membrane of elderly persons (Fig. 2). These observations show once again that age-related alterations influence receptor-driven signal transduction but do not explain normal LPS-mediated chemotaxis of neutrophils from elderly persons. It is conceivable that other signaling pathways could be involved or that a non-receptor-driven function of LPS might exist (Gabriel et al. 2002; Fulop et al. 2004).

After stimulation with a specific TLR1/TLR2 ligand, old individuals showed an age-associated reduction in TNF-α and IL-6 production in human monocytes (van Duin and Shaw 2007). In addition, a strong correlation of decreased cytokine production with lower surface expression of TLR1 but not TLR2 on monocytes was found. Additionally, a decreased TLR4 expression on monocytes from aged people was detected. This had also been reported but, in contrast to a reduced TLR4 expression on monocytes, TLR4 surface expression on neutrophils from aged donors was unaltered (Fulop et al. 2004). In this context, Qian et al. (2014) found reduced expression and function of TLR1 in neutrophils and an underlying age-dependent deficiency in PMN bioenergetics (reduced glucose accumulation). Carrying the TLR1 genotypes 743AA/1805GG associated with a negative TLR1 phenotype may contribute to lower surface expression in elderly people as well as higher TNF-α, CXCL8, and CCL2 plasma levels (Uciechowski et al. 2013) (► Chap. 48, “Role of TLR Polymorphisms in Aging and Age-Related Diseases”).

Finally, enhanced chemokine receptor (CXCR2; receptor for CXCL8) expression on circulating neutrophils did not improve recruitment of elderly neutrophils (Brubaker et al. 2013). The authors suggest that a defect in CXCR2 signaling may be responsible for the reduced recruitment.

However, age-dependent alterations in cytokine production of neutrophils have not been observed, but reported low-grade inflammation of resting neutrophils may

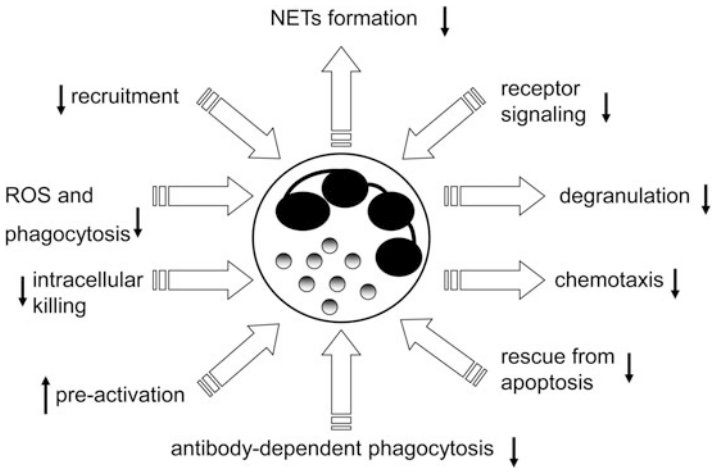

Fig. 3 Overview of impaired PMN functions with aging

have been initiated by cytokines. By investigating cytokine production of neutrophils in the elderly, one must also take into account that aged monocytes produce significantly more pro-inflammatory cytokines after stimulation than those of younger persons (Fagiolo et al. 1993; Gabriel et al. 2002).

Taken together, neutrophilic functions are also affected through aging. Receptors such as TLR1, CD62L, CD50, and CD16 are modified in expression, whereas other surface molecules such as CD11a and CD11c plus CD18 were not found to be altered with age. The decline in different signal transduction pathways being responsible for receptor-mediated responses and apoptotic rescue mechanisms is indicated. Additionally, altered plasma membrane content and fluidity of neutrophils in the elderly appear to influence signal transduction. In addition, important functions of neutrophils such as phagocytosis, degranulation, intracellular killing, chemotaxis, ROS production, and NETS formation are impaired with age (Fig. 3). It should be pointed out that different PMN isolation techniques, monocyte contaminations, purity grade and preactivation of neutrophils during isolation, and different selection criteria of aged persons cannot be excluded as a possible explanation for the controversial results published by distinct groups of investigators (Wessels et al. 2010; Uciechowski and Rink 2014).

Basophils

The circulating blood contains only a small cell number of basophils (0.5%). They have a short life span (1–2 days), but under inflammatory conditions, they rapidly proliferate in the bone marrow and migrate to the lung, blood, liver, and spleen (Min et al. 2012). The main function of basophils is mediating protection against

ectoparasite infection and expulsion from the intestine (Karasuyama and Yamanishi 2014; Min et al. 2012).

Their name originates from blue staining of their cytoplasmic granules after exposure to a basic dye. Studying basophils, especially their role in host immunity, is complicated since there are low amounts of them in the periphery and they are difficult to separate from mast cells. Basophils and mast cells derive from the granulocyte-monocyte progenitor and can release a similar pattern of mediators after activation. Specific markers for basophils are the high-affinity receptor for IgE (FcεRI) and CD11b and no expression of the mast/stem cell growth factor receptor ckit/CD117. IL-3, immune complexes, IL-18, IL-25, IL-33, LPS, and complement factor C5a are the main factors regulating basophil generation and functions.

It has been shown that basophils can act as antigen-presenting cells by picking up antigen-IgE complexes resulting in antigen-specific CD4+ T-helper type 2 (Th2) polarization (Yoshimoto et al. 2009). Activated parasite-specific CD4+ T cells release IL-3 which in turn induces IL-4 expression in basophils. By producing IL-4, basophils are able to mediate the differentiation from naïve CD4+ T cells into IL-4-producing Th2 cell effector cells (summarized in Min et al. 2012).

How exactly basophils contribute to allergic and helminth immunity is not fully understood. They are the main producers of IL-4 during primary helminth infection (van Panhuys et al. 2011).

Basophils have been reported to play a critical role in allergic inflammation by secreting IL-4 in response to IL-3 or FcεRI cross-linking (Denzel et al. 2008). In experiments where basophils were specifically depleted, it has been shown that basophils did not mediate Th2 cell priming in vivo and only interact with T cells in lung tissues. Surprisingly, deletion of IL-4 and IL-13 in T cells or basophils shows that these basophil cytokines may not be required in primary helminth immunity (Sullivan et al. 2011).

Basophils and Aging

The number of studies investigating the role of basophils in aging is limited (Panda et al. 2009). In older publications, contradictory findings were reported. Schwarzenbach et al. (1982) described a delayed degranulation of basophils in the elderly, whereas Marone et al. (1986) could show that basophils from aged individuals had higher histamine release after anti-IgE stimulation and higher sensitivity to a standard concentration of anti-IgE than young individuals (Marone et al. 1986). Analyses of total IgE, CD23, and Th2 cytokines IL-4, IL-10, and IL-13 did not show significant differences in serum samples from 37 young and 62 old individuals (Di Lorenzo et al. (2003). However, significantly lower percentage and absolute numbers of basophils had been found in healthy aged individuals than in younger ones (Song et al. 1999). There were also significant and negative correlations between age and basophil numbers in healthy volunteers (Song et al. 1999).

Smith et al. (2001) observed impairment in type 2 responsiveness by using two different in vivo models. The aged mice had reduced pulmonary granulomas and delayed rejection of intestinal worms. In addition, no eosinophilia and decreased production of antigen-specific IgE could be detected in aged mice. These results may indicate an increased incidence of diseases induced by type 1 cytokines and a reduced adequate antiparasitic response of eosinophils and basophils with age.

Juvenile mice with a defect in basophil-mediated type 2 response (IL-4) after helminth infection appear to be a crucial factor to develop allergic conditions contrary to older mice (Nel et al. 2011). This may support the idea that the aged possess a delayed onset of allergic symptoms and fewer allergies.

In aging, there is a switch from Th1 to Th2 response; because basophils are involved in Th2 cell differentiation (Min et al. 2012) and play a role in increasing humoral immune memory responses (Denzel et al. 2008), the impact of basophils in aging is worth further examination.

Eosinophils

The main function of eosinophils is to fight parasitic helminths which are eliminated by degranulation of granule proteins and generation of superoxide radicals. Eosinophils were discovered by Paul Ehrlich in 1879, and their name originates from red staining of the cytoplasmic granules by the acid dye eosin. They are terminally differentiated cells, they have a half-life in blood of about 18 h, and their cytoplasmic crystalloid (also termed secretory, specific, or secondary) granules store different, preformed cationic proteins (Muniz et al. 2012). In blood, only 1–6% of leukocytes are eosinophils; they are mainly distributed in the skin and mucosa of the gut and respiratory and reproductive systems. How long eosinophils do survive in healthy tissue is not known; several days or even weeks are supposed (Behm and Ovington, 2000).

By producing transforming growth factor (TGF)-β, basic fibroblast growth factors (bFGF), platelet-derived growth factor (PDGF), matrix metalloproteases (MMPs), and vascular endothelial growth factors (VEGF), they are involved in tissue remodeling and repair.

Eosinophils are able to modulate the immune response by the release of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8; the Th1 cytokines IL-12 and IFN-γ; the Th2 cytokines IL-4, IL-5, IL-9, IL-13, and IL-25; as well as suppressing cytokines TGF-β and IL-10 (Hogan et al. 2008).

Degranulation of secondary granule proteins, eicosanoids, leukotrienes, and reactive oxygen species can also influence remodeling of healthy and disease conditions (Kita 2011).

Like mast cells and basophils, eosinophils also express IgE receptors and are activated by antigen-IgE complexes. Since eosinophils express CD80/CD86 and MHC II molecules, they may act as antigen-presenting cells as well (Behm and Ovington 2000).

Eosinophils are mainly involved in hypereosinophilia, defined as peripheral blood eosinophil counts >1,500/μL, causing organ damage and mortality, and in immune hypersensitivity syndromes, including asthma, dermatitis, and rhinitis, tissue injury, tumor immunity, and allergic diseases. Therefore, their role as benign effector cells became indistinct (Hogan et al. 2008).

Eosinophils and Aging

There are only few studies about the influence of aging regarding to alterations in eosinophil numbers and functionality. Using a rat model of bronchial asthma, the impact of aging on the allergic airway response was examined (Yagi et al. 1997). In contrast to aged animals, significantly higher levels of specific IgE antibody and enhanced amounts of eosinophils and neutrophils were detected in bronchoalveolar lavage fluid from young animals. Similar results were reported in mice (Smith et al. 2001). When measuring chemotactic activity of eosinophils in the supernatant of cultured lymph node cells, aged rats failed to show activity contrary to young rats. From these data, the authors indicated that aged rats are defective in eosinophil accumulation in sites exposed to antigen, which might be also due to T cells also altered with age. In two studies investigating women from the Women's Health and Aging Studies, mortality, frailty, and age were not found to be associated with changes in eosinophils and basophils (Leng et al. 2007, 2009).

Di Lorenzo and colleagues investigated rhinitis symptoms in a follow-up study by reanalyzing 108 rhinitis patients after 15 years. A tendency to milder rhinitis symptoms and a decrease in allergic parameters (e.g., skin prick test, serum total, and specific IgE) could be observed in the older group. Interestingly, the alterations in rhinitis symptoms seemed to be related to changes in the nasal eosinophils (Di Lorenzo et al. 2013).

Another group observed increased blood neutrophil and platelet counts but significantly decreased eosinophil and lymphocyte numbers in aged individuals (79–87 years of age) (Starr and Deary 2011).

Eosinophils are hardly present in dermis and did not show any dependence on age; therefore, their impact on skin aging is very low (Petrov et al. 2013).

When comparing the functions of blood eosinophils isolated from young and old subjects with asthma, eosinophils did not differ in adhesive and chemotactic capabilities as well as their number in sputum. However, eosinophils from the elderly (55–88 years of age) displayed decreased superoxide anion production and a significantly delayed IL-5-induced degranulation of eosinophil-derived neurotoxin (Mathur et al. 2008). These observations concerning altered degranulation are in concert with a study in which basophils had been investigated (Schwarzenbach et al. 1982). The authors suggest that alterations in eosinophilic function may affect the response to medication and the manifestation of asthma.

The further investigation of eosinophilic properties will be important to elucidate their role in enhanced morbidity and mortality associated with allergy, asthma, autoimmune diseases, and atherosclerosis during aging.

Conclusions and Future Directions

Although PMN counts are normal or slightly increased in aged persons compared to young individuals, aging influences the functional properties of neutrophils. The observed changes are significantly reduced phagocytosis along with decreased antibody-dependent phagocytosis of neutrophils from elderly subjects. In addition, the other toxic mechanisms to destroy pathogenic microorganisms such as reactive oxygen species generation, degranulation, NETs formation, and intracellular killing are impaired by age. New studies of chemotaxis have shown that migratory responses of neutrophils from elderly persons are in fact altered. The decline in functionality, impaired Ca^{2+} mobilization, and delayed rescue from apoptosis during aging appear to arise from defects of several signaling pathways, altered plasma membrane components, and modulated protein tyrosine phosphatase activity. The molecular mechanisms responsible for those alterations in signal transduction and why distinctive stimuli cause different effects have still not been fully resolved. However, by analyzing the role of microRNAs of human neutrophils in senescence, six microRNAs involved in chemokine and cytokine signaling, Ras pathway, and regulation of the actin cytoskeleton have been identified (Ward et al. 2011). In addition, Zhang et al. (2015) reported that heterogeneity in pro-inflammatory activity of murine neutrophils is associated with their aging and showed that neutrophils aged in the circulation lost CD62L expression and upregulated CXCR4 and CD11b. They also suggest that microbiota-derived molecules through TLRs and MyD88 may contribute to neutrophil aging under steady-state conditions. This might be an interesting approach for research of human neutrophils in the elderly since shedding of CD62L is one feature of neutrophils found in aged individual as well as different neutrophil subsets exist in mice and humans (Tseng and Liu 2014; Sauce et al. 2016). Figure 3 and Table 1 summarize the age-related changes in neutrophils. The few studies in which basophils and eosinophils had been investigated indicate that decreased ROS production and degranulation appear to be associated with age

Table 1 Age-related changes of neutrophils

Parameter/function	Stimulants/targets	Reported effect
Number of circulating neutrophils		←→ Chatta et al. 1993; Born et al. 1995; ↑ Cakman et al. 1997
Number of precursors in bone marrow		←→ Chatta et al. 1993
Proliferation of neutrophilic precursors in response to	G-CSF	↓ Chatta et al. 1993
	IL-3	←→ Chatta et al. 1993
	GM-CSF	←→ Chatta et al. 1993
Phagocytosis	Opsonized bacteria, yeast	↓ Esparza et al. 1996; Wenisch et al. 2000; Butcher et al. 2001; Simell et al. 2011
	Antibody-dependent CD16-mediated	↓ Butcher et al. 2001; Simell et al. 2011

(*continued*)

Table 1 (continued)

Parameter/function	Stimulants/targets	Reported effect
Respiratory burst	fMLP	↓ Biasi et al. 1996; Braga et al. 1998; Tortorella et al. 2000; Sauce et al. 2016; ←→ Lord et al. 2001; Butcher et al. 2000
	GM-CSF, LPS	↓ Tortorella et al. 1996
	Gram-positive bacteria	↓ Wenisch et al. 2000
	Gram-negative bacteria	←→ Wenisch et al. 2000
	Non-stimulated	↑ Ogawa et al. 2008; Kovalenko et al. 2014; Nogueira-Neto et al. 2016; Sauce et al. 2016
Degranulation	fMLP	↓ Esparza et al. 1996; Tortorella et al. 2000
Chemotaxis	fMLP, GM-CSF, LPS; CXCL8	↓ Corberand et al. 1981; Niwa 1989; Wenisch et al. 2000; Dalboni et al. 2013; Brubaker et al. 2013; Sapey et al. 2014; ←→ Lord et al. 2001; Butcher et al. 2000
Intracellular killing	Gram-negative bacteria, fungi	↓ Plackett et al. 2004; Murciano et al. 2008; Simell et al. 2011
Adhesion	Endothelia, thrombocytes	←→ Biasi et al. 1996; ↗ Esparza et al. 1996
	CD11a-c/CD18	←→ Noble et al. 1999; Butcher et al. 2001; De Martinis et al. 2004; ↗ Esparza et al. 1996; Nogueira-Neto et al. 2016 (CD11b)
	CD15	←→ De Martinis et al. 2004; ↗ Esparza et al. 1996
	CD50	↘De Martinis et al. 2004
	CD62L	↓ De Martinis et al. 2004
NET formation		↓ Hazeldine et al. 2014; Tseng and Liu 2014
Apoptosis		←→ Tortorella et al. 1999; ↗ Fulop et al. 1997
Rescue by	IL-2, LPS, G-CSF, GM-CSF	↓ Fulop et al. 1997
CD95-induced apoptosis; expression of CD95		←→ Fulop et al. 1997
Downregulation of caspase-3 activity	GM-CSF	↓ Larbi et al. 2004
Bax/Mcl1	GM-CSF	↑ Fulop et al. 2002, Fulop et al. 2004
Antiapoptotic phenotype	GM-CSF	↓ Larbi et al. 2004
Inhibition SHP-1 activity	GM-CSF	↓ Fortin et al. 2006
Jak2-Stat5 pathway	GM-CSF	↓ Fulop et al. 2004
Signal transduction defects		↗ Tortorella et al. 2004; Fulop et al. 2004

(*continued*)

Table 1 (continued)

Parameter/function	Stimulants/targets	Reported effect
Intracellular Ca^{2+} level		↑ Wenisch et al. 2000
Intracellular Ca^{2+} mobilization	fMLP	↓ Lipschitz et al. 1991
ERK1/2 MAPK pathway	GM-CSF	↓ Tortorella et al. 2004
p38 MAPK pathway	GM-CSF	←→ Tortorella et al. 2004; Larbi et al. 2004
Plasma membrane fluidity	Receptor signaling in relation with lipid rafts	↓ Fulop et al. 2004; Yuli 1982; Alvarez et al. 2001
Expression of surface molecules		
	CD69	↓ Rao et al. 1992
	fMLP-R	←→ Biasi et al. 1996; Fulop et al. 1997; Tortorella et al. 2000; ↘ Lord et al. 2001
	GM-CSF-R	←→ Fulop et al. 2004
	TLR2/TLR4, TREM-1	←→ Fulop et al. 2004
	TLR1	↓ Qian et al. 2014

↓ decreased; ↘ slightly decreased; ↑ increased; ↗ slightly increased; ←→ unchanged

Table 2 Age-related changes of basophils

Parameter/function	Stimulants/ targets	Reported effect
Absolute number of basophils		↓ Song et al. 1999
Number of IL-4-producing basophils (mice)		←→ Nel et al. 2011
IL-4, IL-13 production	Allergic condition	←→ Di Lorenzo et al. 2003
Response to parasites (mice)	Intestinal worms	↓ Smith et al. 2001
Degranulation		↓ Schwarzenbach et al. 1982; ↑ Marone et al. 1986

↓ decreased; ↘ slightly decreased; ↑ increased; ↗ slightly increased; ←→ unchanged

contributing to reduced antiparasitic and lower allergic responses. In Tables 2 and 3, the age-related changes of basophils and eosinophils are compiled.

Further research is required since neutrophils, basophils, and eosinophils display more features than formerly assumed; it would shed light on the deficiencies that occur during the aging process and could be beneficial to the elderly in the future.

Table 3 Age-related changes of eosinophils

Parameter/function	Stimulants/ targets	Reported effect
Number of eosinophils		←→ Leng et al. 2007, 2009; ↓ Starr and Deary 2011
Airway response (rat)	Asthma model	↓ Yagi et al. 1997
Response to parasites (mice)	Intestinal worms	↓ Smith et al. 2001
Chemotaxis (rat)		↓ Yagi et al. 1997
Chemotaxis (human)		←→ Mathur et al. 2008
Degranulation	IL-5	↓ Mathur et al. 2008
Adhesion		←→ Mathur et al. 2008
Production of ROS		↓ Mathur et al. 2008

↓ decreased; ↘ slightly decreased; ↑ increased; ↗ slightly increased; ←→ unchanged

Cross-References

- ► Effects of Aging on Human Toll-Like Receptor Function
- ► Older Human B Cells and Antibodies
- ► Signal Transduction Changes in Human Neutrophils with Age

References

Alonso-Fernandez P, Puerto M, Mate I, Ribera JM, de la Fuente M (2008) Neutrophils of centenarians show function levels similar to those of young adults. J Am Geriatr Soc 56:2244–2251

Alvarez E, Ruiz-Guttierrez V, Sobrino F et al (2001) Age-related changes in membrane lipid composition, fluidity and respiratory burst in rat peripheral neutrophils. Clin Exp Immunol 124:95–102

Behm CA, Ovington KS (2000) The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. Parasitol Today 16:202–209

Biasi D, Carletto A, Dell'Agnola C et al (1996) Neutrophil migration, oxidative metabolism, and adhesion in elderly and young subjects. Inflammation 20:673–681

Boehmer ED, Goral J, Faunce DE et al (2004) Age dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokines by production and mitogen-activated protein kinase expression. J Leukoc Biol 75:342–349

Born J, Uthgenannt D, Dodt C et al (1995) Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. Mech Ageing Dev 84: 113–126

Braga PC, Sala MT, Dal Sasso M et al (1998) Influence of age on oxidative bursts (chemiluminescence) of polymorphonuclear neutrophil leukocytes. Gerontology 44:192–197

Brubaker AL, Rendon JL, Ramirez L, Choudhry MA, Kovacs EJ (2013) Reduced neutrophil chemotaxis and infiltration contributes to delayed resolution of cutaneous wound infection with advanced age. J Immunol 190(4):1746–1757

Butcher S, Chahel H, Lord JM (2000) Ageing and the neutrophil: no appetite for killing? Immunology 100:411–416

Butcher SK, Chahel H, Nayak L et al (2001) Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. J Leukoc Biol 70:881–886

Cakman I, Kirchner H, Rink L (1997) Zinc supplementation reconstitutes the production of interferon-alpha by leukocytes from elderly persons. J Interf Cytokine Res 17:469–472

Chatta GS, Andrews RG, Rodger E et al (1993) Hematopoietic progenitors and aging: alterations in granulocytic precursors and responsiveness to recombinant human G-CSF, GM-CSF, and IL-3. J Gerontol 48:M207–M212

Corberand J, Ngyen F, Laharrague P et al (1981) Polymorphonuclear functions and aging in humans. J Am Geriatr Soc 29:391–397

Dalboni TM, Abe AE, de Oliveira CE, Lara VS, Campanelli AP, Gasparoto CT, Gasparoto TH (2013) Activation profile of CXCL8-stimulated neutrophils and aging. Cytokine 61(3):716–719

De Martinis M, Modesti M, Ginaldi L (2004) Phenotypic and functional changes of circulating monocytes and polymorphonuclear leucocytes from elderly persons. Immunol Cell Biol 82:415–420

Denzel A, Maus UA, Gomez MR, Moll C, Niedermeier M, Winter C, Maus R, Hollingshead S, Briles DE, Kunz-Schughart LA, Talke Y, Mack M (2008) Basophils enhance immunological memory responses. Nat Immunol 9:733–742

Di Lorenzo G, Balistreri CR, Candore G et al (1999) Granulocyte and natural killer activity in the elderly. Mech Ageing Dev 108:25–38

Di Lorenzo G, Pacor ML, Pellitteri ME, Listi F, Colombo A, Candore G, Mansueto P, Lo Bianco C, Ditta V, Rini GB, Caruso CA (2003) study of age-related IgE pathophysiological changes. Mech Ageing Dev 124:445–448

Di Lorenzo G, Leto-Barone MS, La Piana S, Ditta V, Di Fede G, Rini GB (2013) Clinical course of rhinitis and changes in vivo and in vitro of allergic parameters in elderly patients: a long-term follow-up study. Clin Exp Med 13(1):67–73

Ericson JA et al (2014) Gene expression during the generation and activation of mouse neutrophils: implication of novel functional and regulatory pathways. PLoS ONE 9:e108553

Esparza B, Sanchez H, Ruiz M et al (1996) Neutrophil function in elderly persons assessed by flow cytometry. Immunol Investig 25:185–190

Fagiolo U, Cossarizza A, Scala E et al (1993) Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol 23:2375–2378

Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J Immunol 170:2106–2112

Fortin CF, Larbi A, Lesur O et al (2006) Impairment of SHP-1 down-regulation in the lipid rafts of human neutrophils under GM-CSF stimulation contributes to their age-related, altered functions. J Leukoc Biol 79:1061–1072

Fortin CF, Larbi A, Dupuis G, Lesur O, Fulop T (2007) GM-CSF activates the Jak/STAT pathway to rescue polymorphonuclear neutrophils from spontaneous apoptosis in young but not elderly individuals. Biogerontology 8:173–187

Fortin CF, Ear T, McDonald PP (2009) Autocrine role of endogenous interleukin-18 on inflammatory cytokine generation by human neutrophils. FASEB J 23:194–203

Franceschi C, Bonafe M, Valensin S et al (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254

Fulop T Jr, Fouquet C, Allaire P et al (1997) Changes in apoptosis of human polymorphonuclear granulocytes with aging. Mech Ageing Dev 96:15–34

Fulop T Jr, Larbi A, Linteau A et al (2002) Role of Mcl-1 and Bax expression alterations in the decreased rescue of human neutrophils from apoptosis by GM-CSF with aging. Ann N Y Acad Sci 973:305–308

Fulop T, Larbi A, Douziech N et al (2004) Signal transduction and functional changes in neutrophils with aging. Aging Cell 3:217–226

Gabriel P, Cakman I, Rink L (2002) Overproduction of monokines by leukocytes after stimulation with lipopolysaccharide in the elderly. Exp Gerontol 37:235–247
Geiger H, Rudolph KL (2009) Aging in the lympho-hematopoietic stem cell compartment. Trends Immunol 30:360–365
Ginaldi L, De Martinis M, D'Ostilio A et al (1999) The immune system in the elderly: III. Innate immunity. Immunol Res 20:117–126
Hannah S, Mecklenburgh K, Rahman I et al (1995) Hypoxia prolongs neutrophil survival in vitro. FEBS Lett 372:233–237
Hasan R, Rink L, Haase H (2013) Zinc signals in neutrophil granulocytes are required for the formation of neutrophil extracellular traps. Innate Immun 19:253–264
Hazeldine J, Harris P, Chapple IL et al (2014) Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals. Aging Cell 4:690–698
Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, Kay AB, Rothenberg ME (2008) Eosinophils: biological properties and role in health and disease. Clin Exp Allergy 38:709–750
Karasuyama H, Yamanishi Y (2014) Basophils have emerged as a key player in immunity. Curr Opin Immunol 31:1–7
Kita H (2011) Eosinophils: multifaceted biological properties and roles in health and disease. Immunol Rev 242:161–177
Klut ME, Ruehlmann DO, Li L et al (2002) Age-related changes in the calcium homeostasis of adherent neutrophils. Exp Gerontol 37:533–541
Kolling UK, Hansen F, Braun J, Rink L, Katus HA, Dahlhoff K (2001) Leucocyte response and anti-inflammatory cytokines in community acquired pneumonia. Thorax 56:121–125
Kovacs EJ, Palmer JL, Fortin CF, Fulop T, Goldstein DR, Linton PJ (2009) Aging and innate immunity in the mouse: impact of intrinsic and extrinsic factors. Trends Immunol 30:319–324
Kovalenko EI, Boyko AA, Semenkov VF, Lutsenko GV, Grechikhina MV, Kanevskiy LM, Azhikina TL, Telford WG, Sapozhnikov AM (2014) ROS production, intracellular HSP70 levels and their relationship in human neutrophils: effects of age. Oncotarget 5 (23):11800–11812
Kruger P, Saffarzadeh M, Weber AN, Rieber N, Radsak M, von Bernuth H, Benarafa C, Roos D, Skokowa J, Hartl D (2015) Neutrophils: between host defence, immune modulation, and tissue injury. PLoS Pathog 11:e1004651
Larbi A, Dupuis G, Douziech N et al (2004) Low-grade inflammation with aging has consequences for T-lymphocyte signaling. Ann N Y Acad Sci 1030:125–133
Larbi A, Douziech N, Fortin C, Linteau A, Dupuis G, Fulop T Jr. (2005) The role of the MAPK pathway alterations in GM-CSF modulated human neutrophil apoptosis with aging. Immun Ageing 2(1):6
Lee A, Whyte MK, Haslett C (1993) Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. J Leukoc Biol 54:283–288
Leng SX, Xue QL, Tian J, Walston JD, Fried LP (2007) Inflammation and frailty in older women. J Am Geriatr Soc 55:864–871
Leng SX, Xue QL, Tian J, Huang Y, Yeh SH, Fried LP (2009) Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: results from the Women's Health and Aging Studies I. Exp Gerontol 44:511–516
Ligthart GJ, Corberand JX, Geertzen HGM et al (1994) Necessity of the assessment of health status in human immunogerontological studies: evaluation of the SENIEUR protocol. Mech Ageing Dev 55:89–105
Lipschitz DA, Udupa KB, Indelicato SR et al (1991) Effect of age on second messenger generation in neutrophils. Blood 78:1347–1354
Lord JM, Butcher S, Killampali V et al (2001) Neutrophil ageing and immunesenescence. Mech Ageing Dev 122:1521–1535
Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11:519–531
Marone G, Poto S, Dimartino L, Condorelli M (1986) Human basophil releasability.1. Age-related-changes in basophil releasability. J Allergy Clin Immunol 77:377–383

Mathur SK, Schwantes EA, Jarjour NN, Busse WW (2008) Age-related changes in eosinophil function in human subjects. Chest 133:412–419

Min B, Brown MA, LeGros G (2012) Understanding the roles of basophils: breaking dawn. Immunology 135:192–197

Muniz VS, Weller PF, Neves JS (2012) Eosinophil crystalloid granules: structure, function, and beyond. J Leukoc Biol 92:281–288

Murciano C, Yanez A, O'Connor JE, Gozalbo D, Gil ML (2008) Influence of aging on murine neutrophil and macrophage function against Candida albicans. FEMS Immunol Med Microbiol 53:214–221

Nel HJ, Hams E, Saunders SP, Mangan NE, Smith P, Atzberger A, Flavell RA, Akira S, McKenzie AN, Fallon PG (2011) Impaired basophil induction leads to an age-dependent innate defect in type 2 immunity during helminth infection in mice. J Immunol 186:4631–4639

Niwa Y, Kasama T, Miyachi Y et al (1989) Neutrophil chemotaxis, phagocytosis and parameters of reactive oxygen species in human aging: cross-sectional and longitudinal studies. Life Sci 44:1655–1664

Noble JM, Ford GA, Thomas TH (1999) Effect of aging on CD11b and CD69 surface expression by vesicular insertion in human polymorphonuclear leukocytes. Clin Sci 97:323–329

Nogueira-Neto J, Cardoso AS, Monteiro HP, Fonseca FL, Ramos LR et al (2016) Basal neutrophil function in human aging: implications in endothelial cell adhesion. Cell Biol Int. https://doi.org/10.1002/cbin.10618. [Epub ahead of print]

O'Neill L (2006) How Toll-like receptors signal: what we know and what we don't know. Curr Opin Immunol 18:3–9

Ogawa K, Suzuki K, Okutsu M, Yamazaki K, Shinkai S (2008) The association of elevated reactive oxygen species levels from neutrophils with low-grade inflammation in the elderly. Immun Ageing 5:13

Panda A, Arjona A, Sapey E, Bai FW, Fikrig E, Montgomery RR, Lord JM, Shaw AC (2009) Human innate immunosenescence: causes and consequences for immunity in old age. Trends Immunol 30:325–333

Petrov VV, Vasil'eva OV, Kornilova NK, Gunin AG (2013) Age-related changes in mast cells and eosinophils of human dermis. Ontogenez 44(3):179–185

Piazzolla G, Tortorella C, Serrone M et al (1998) Modulation of cytoskeleton assembly capacity and oxidative response in aged neutrophils. Immunopharmacol Immunotoxicol 20:251–266

Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L (2010) In vivo labeling with $2H_2O$ reveals a human neutrophil lifespan of 5.4 days. Blood 116:625–627

Plackett TP, Boehmer ED, Faunce DE et al (2004) Aging and innate immune cells. J Leukoc Biol 76:291–299

Qian F, Guo X, Wang X, Yua X, Chen S, Malawista SF, Bockenstedt LK, Allore HG, Montgomery RR (2014) Reduced bioenergetics and toll-like receptor 1 function in human polymorphonuclear leukocytes. Aging 6:131–139

Rao KMK, Currie MS, Padmadabhan J et al (1992) Age-related alterations in actin cytoskeleton and receptor expression in human leukocytes. J Gerontol 47:B37–B44

Renshaw M, Rockwell J, Engleman C et al (2002) Cutting edge: impaired toll-like receptor expression and function in aging. J Immunol 169:4697–4701

Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, Insall RH, Stockley RA, Lord JM (2014) Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. Blood 123(2):239–248

Sauce D, Dong Y, Campillo-Gimenez L, Casulli S, Bayard C, Autran B, Boddaert J, Appay V, Elbim C. Reduced oxidative burst by primed neutrophils in the elderly individuals is associated with increased levels of the CD16bright/CD62Ldim immunosuppressive subset. J Gerontol A Biol Sci Med Sci. 2016. pii:glw.062. [Epub ahead of print]

Schroeder AK and Rink L (2003) Neutrophil immunity of the elderly. Mechanisms of Ageing and Development 124:419–425

Schroeder AK, von der Ohe M, Fleischer D et al (2005) Differential synthesis of two interleukin-1 receptor antagonist variants and interleukin-8 by peripheral blood neutrophils. Cytokine 32:246–253

Schroeder AK, von der Ohe M, Kolling U et al (2006) Polymorphonuclear leucocytes selectively produce anti-inflammatory interleukin-1 receptor antagonist and chemokines, but fail to produce pro-inflammatory mediators. Immunology 119:317–327

Schwarzenbach HR, Nakagawa T, Conroy MC, Deweck AL (1982) Skin reactivity, basophil de-granulation and ige levels in aging. Clin Allergy 12:465–473

Simell B, Vuorela A, Ekstrom N, Palmu A, Reunanen A, Meri S, Kayhty H, Vakevainen M (2011) Aging reduces the functionality of anti-pneumococcal antibodies and the killing of *Streptococcus pneumoniae* by neutrophil phagocytosis. Vaccine 29:1929–1934

Smith P, Dunne DW, Fallon PG (2001) Defective in vivo induction of functional type 2 cytokine responses in aged mice. Eur J Immunol 31:1495–1502

Song C, Vandewoude M, Stevens W, De Clerck L, Van der Planken M, Whelan A, Anisman H, Dossche A, Maes M (1999) Alterations in immune functions during normal aging and Alzheimer's disease. Psychiatry Res 85:71–80

Sørensen OE, Borregaard N (2016) Neutrophil extracellular traps – the dark side of neutrophils. J Clin Invest 126:1612–1620

Stark MA, Huo Y, Burcin TL, Morris MA, Olson TS et al (2005) Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity 22:285–294

Starr JM, Deary IJ (2011) Sex differences in blood cell counts in the Lothian Birth Cohort 1921 between 79 and 87 years. Maturitas 69:373–376

Sullivan BM, Liang HE, Bando JK, Wu D, Cheng LE, McKerrow JK, Allen CDC, Locksley RM (2011) Genetic analysis of basophil function in vivo. Nat Immunol 12:527–U243

Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM et al (2010) Neutrophil kinetics in healthand disease. Trends Immunol 31:318–324

Tortorella C, Polignano A, Piazzolla G et al (1996) Lipopolysaccharide-, granulocyte-monocyte colony stimulating factor- and pentoxifylline-mediated effects on formyl-methionyl- leucine-phenylalanine-stimulated neutrophil respiratory burst in the elderly. Microbios 85:189–198

Tortorella C, Piazzolla G, Spaccavento F et al (1999) Age related effects of oxidative metabolism and cyclic AMP signaling on neutrophil apoptosis. Mech Ageing Dev 110:195–205

Tortorella C, Piazzolla G, Spaccavento F et al (2000) Regulatory role of extracellular matrix proteins in neutrophil respiratory burst during aging. Mech Ageing Dev 119:69–82

Tortorella C, Stella I, Piazzolla G et al (2004) Role of defective ERK phosphorylation in the impaired GM-CSF-induced oxidative response of neutrophils in elderly humans. Mech Ageing Dev 8:539–546

Tortorella C, Simone O, Piazzolla G, Stella I, Cappiello V, Antonaci S (2006) Role of phosphoinositide 3-kinase and extracellular signal-regulated kinase pathways in granulocyte macrophage-colony-stimulating factor failure to delay Fas-induced neutrophil apoptosis in elderly humans. J Gerontol A Biol Sci Med Sci 61:1111–1118

Tortorella C, Simone O, Piazzolla G, Stella I, Antonaci S (2007) Age-related impairment of GM-CSF-induced signalling in neutrophils: role of SHP-1 and SOCS proteins. Ageing Res Rev 6:81–93

Tseng WT, Liu GY (2014) Expanding roles of neutrophils in aging hosts. Curr Opin Immunol 29:43–48

Tseng CW, Kyme PA, Arruda A, Ramanujan VK, Tawackoli W, Liu GY (2012) Innate immune dysfunctions in aged mice facilitate the systemic dissemination of methicillin-resistant S. aureus. PLoS One 7(7):e41454

Uciechowski P, Rink L (2014) Basophil, eosinophil, and neutrophil functions in the elderly. In: Massoud A, Rezaei N (eds) Immunology of aging. Springer, Berlin/Heidelberg, pp 47–63

Uciechowski P, Oellig EM, Mariani E, Malavolta M, Mocchegiani E, Rink L (2013) Effects of human Toll-like receptor 1 polymorphisms on ageing. Immun Ageing 10:4

van Duin D, Shaw AC (2007) Toll-like receptors in older adults. J Am Geriatr Soc 55:1438–1444

van Panhuys N, Prout M, Forbes E, Min B, Paul WE, Le Gros G (2011) Basophils are the major producers of IL-4 during primary helminth infection. J Immunol 186:2719–2728

Ward JR, Heath PR, Catto JW, Whyte MKB, Milo M, Renshaw SA (2011) Regulation of neutrophil senescence by microRNAs. PLoS One 6:e15810

Wenisch C, Patruta S, Daxbock F et al (2000) Effect of age on human neutrophil function. J Leukoc Biol 67:40–45

Wessels I, Jansen J, Rink L, Uciechowski P (2010) Immunosenescence of polymorphonuclear neutrophils. Sci World J 10:145–160

Whitelaw DA, Rayner BL, Willcox PA (1992) Community-acquired bacteremia in the elderly: a prospective study of 121 cases. J Am Geriatr Soc 40:996–1000

Yagi T, Sato A, Hayakawa H, Ide K (1997) Failure of aged rats to accumulate eosinophils in allergic inflammation of the airway. J Allergy Clin Immunol 99:38–47

Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, Nakanishi K (2009) Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4(+) T cells. Nat Immunol 10:706–754

Yuli I, Tamonga A, Snyderman R (1982) Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. Proc Natl Acad Sci USA 79: 5906–5910.

Zhang D, Chen G, Manwani D, Mortha A, Xu C, Faith JJ, Burk RD, Kunisaki Y, Jang JE, Scheiermann C, Merad M, Frenette PS (2015) Neutrophil ageing is regulated by the microbiome. Nature 525(7570):528–532