Age-dependent dysregulation of innate immunity

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Abstract | As we age, the innate immune system becomes dysregulated and is characterized by persistent inflammatory responses that involve multiple immune and non-immune cell types and that vary depending on the cell activation state and tissue context. This ageing-associated basal inflammation, particularly in humans, is thought to be induced by several factors, including the reactivation of latent viral infections and the release of endogenous damage-associated ligands of pattern recognition receptors (PRRs). Innate immune cell functions that are required to respond to pathogens or vaccines, such as cell migration and PRR signalling, are also impaired in aged individuals. This immune dysregulation may affect conditions associated with chronic inflammation, such as atherosclerosis and Alzheimer's disease.

The United Nations forecasts that the global human population over the age of 60 will increase more than threefold (to nearly 2 billion individuals) during the first half of the twenty-first century, and that by 2050 it will exceed the size of the global population of young individuals (those individuals who are less than 15 years of age)[1]. This unprecedented growth in the aged population is observed in both developed and developing nations. In the United States, individuals over the age of 65 currently constitute approximately 12% of the population, but they account for over 35% of visits to general internists, 34% of prescription drug use, 50% of hospital stays and 90% of nursing home residents[2]; this partly reflects increased morbidity and mortality from infectious diseases and poor responses to vaccination[3].

With regard to the adaptive immune system, there is evidence for broad-ranging, age-associated alterations in the development and function of B cells and T cells[4–8]. The effects of ageing on the innate immune system had been less well studied, until recently. The diverse cell lineages that mediate innate immunity show heterogeneous ageing phenotypes that reflect their developmental, tissue and activation context. Overall, studies in aged mice (generally older than 20 months) and in humans over the age of 65 years have shown that activation of the aged innate immune system results in dysregulated inflammation. This dysregulation involves both elevated levels of basal inflammation (especially in humans) and an associated impaired ability to mount efficient innate and adaptive immune responses to newly encountered pathogens or vaccine antigens. Indeed, a body of evidence, mainly from human studies, indicates that older adults have elevated levels of pro-inflammatory cytokines, clotting factors and acute phase reactants in the steady state[9–11] — the term 'inflamm-ageing' was coined to describe this phenomenon[12]. Evaluation of several[13,14] (but not all[15]) human cohorts has shown an association between elevated levels of cytokines, such as interleukin-6 (IL-6), and mortality and disability in older individuals.

The mechanisms that underlie these ageing-associated heightened levels of basal inflammation remain incompletely understood but seem to involve changes in the numbers and the functions of innate immune cells, altered expression of pattern recognition receptors (PRRs), activation of PRRs by endogenous ligands associated with cellular damage, and aberrant signalling events downstream of PRR activation that lead to cytokine secretion. In this Review, we discuss evidence, predominantly from mouse and human studies, of ageing-associated alterations in innate immunity; although there are substantial similarities between aged humans and mice, there are also important differences (TABLE 1). These differences probably reflect various factors, including the characteristics of genetically homogeneous inbred mouse strains and intrinsic species-specific differences between humans and mice[16]. We also discuss the implications of age-associated changes in innate immunity for improving responses to infection or vaccination and for age-associated chronic inflammatory diseases.

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Table 1 | **Overview of age-associated alterations in human and mouse innate immune cells**

Cell type	Decreased in ageing	Increased in ageing
Human		
Haematopoietic stem cells	• Lymphoid differentiation[21]	• DNA damage[25] • Myeloid-biased differentiation[21]
Neutrophils	• Chemotaxis[38,40] • Intracellular bacterial killing[50,52] (decreased[53] or unchanged[60] in mice) • Phagocytosis[49–51] (unchanged in mice[60]) • GM-CSF and TREM1 signalling[55,57]	NR
Monocytes	• TLR1–TLR2-induced pro-inflammatory cytokine production[33,98] • TLR-induced co-stimulatory molecule expression[107] • TLR1 and TLR4 expression[33,98]	• TLR5 expression[101] • TLR4-induced cytokine production (by $CD14^+CD16^+$ monocytes)[32]
Macrophages	• WNV-induced DC-SIGN signalling[110] • TNF production (in the skin)[111]	• WNV-induced STAT1 phosphorylation[110] • WNV-induced TLR3 expression[110]
Monocyte-derived DCs and myeloid DCs	• TLR-induced cytokine production[99,118,121] • WNV-induced type I IFN production[121] • Phagocytosis and migration[36] • PI3K activity[36]	• LPS-, ssRNA- and self-DNA-induced cytokine production[36,122] • Basal cytokine production[99]
Plasmacytoid DCs	• TLR-induced cytokine production[37,99,119–121]	• Basal cytokine production[99]
Mouse		
Haematopoietic stem cells	• Lymphoid differentiation[17–20]	• Cytokine- and chemokine-mediated damage[23,24] • Myeloid-biased differentiation[17–20]
Neutrophils	• Chemotaxis[41–44] • NET formation[53]	• Neutrophil inflammation[39,45,46]
Macrophages	• Cytokine production[86,100,102–105] • TLR expression (strain dependent)[86,100,102,103] • MAPK signalling[102,103]	PGE2 production[83]
BMDCs and myeloid DCs	• Co-stimulatory molecule expression (decreased in the $CD8\alpha^+$ subset; and unchanged or decreased in $CD11c^+$ BMDCs)[88,91,93,112] • Antigen uptake (decreased in the $CD8\alpha^+$ subset; and unchanged or decreased in $CD11c^+$ BMDCs)[88,89,93] • Induced NLRP3 function (BMDCs)[125]	• PGE2 production (by BMDCs)[115] • TLR-induced IL-23 production (by BMDCs)[114] • Basal NLRP3 function[124]
Plasmacytoid DCs	• Type I IFN production[113] • IRF7 nuclear translocation[113]	NR

BMDCs, bone marrow-derived dendritic cells; DCs, dendritic cells; DC-SIGN, DC-specific ICAM3-grabbing non-integrin; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN, interferon; IL-23, interleukin-23; IRF7, IFN-regulatory factor 7; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NET, neutrophil extracellular trap; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NR, none reported; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; ssRNA, single-stranded RNA; STAT1, signal transducer and activator of transcription 1; TLR, Toll-like receptor; TNF, tumour necrosis factor; TREM1, triggering receptor expressed on myeloid cells 1; WNV, West Nile virus.

The ageing innate immune cell compartment

Studies in mice have shown that aged haematopoietic stem cells (HSCs) have reduced regenerative potential and fail to efficiently reconstitute myeloablated recipient mice following transplantation. Moreover, aged mouse HSCs are biased towards myeloid differentiation at the expense of lymphopoiesis[17–20], and there is evidence for a similar skewing in human HSCs from older donors[21]. The underlying mechanisms of these findings probably include both ageing-associated cell-intrinsic alterations and microenvironmental changes[22]; for example, the presence of low levels of lipopolysaccharide (LPS) or chemokines, such as CC-chemokine ligand 5 (CCL5), may compromise HSC regenerative capacity or may promote myeloid skewing[23,24]. In addition, DNA damage in the form of double-stranded DNA breaks seems to be increased in HSCs from older humans[25]. These alterations are associated with a decline in adaptive immunity and may contribute to the increased incidence of myeloid malignancies that is associated with ageing[22,26].

The size of most innate immune cell populations seems to either remain stable or decrease with age; for example, studies using the Senior Europeans (SENIEUR) protocol (which selects for successfully aged adults without co-morbid medical conditions) have reported no change or only a mild decrease in the numbers of neutrophils[27,28]. Given this, it is notable that neutrophilia was found to be a risk factor for death in a multivariate analysis of one cohort of aged adults[29]. Monocyte numbers are mostly unchanged in older adults[30,31], although age-associated increases in $CD14^+CD16^+$ inflammatory monocytes have been reported[31–33]. However, decreased percentages and numbers of plasmacytoid dendritic cells (pDCs) have been reported in older adults[34,35] (although not in all studies[36]). Similarly, the number of myeloid DCs (mDCs; also known as conventional DCs) also seems to be decreased or unchanged with age[35,37]. The existing evidence indicates that basal inflammation in older individuals is not associated with increased numbers of myeloid cells, despite the skewing of aged HSCs to

Plasmacytoid dendritic cells (pDCs). Immature DCs with morphology that resembles that of a plasma cell. Mouse pDCs express markers such as B220, which is usually associated with the B cell lineage. In humans, pDCs express CD123 and blood DC antigen 2 (BDCA2; also known as CLEC4C and CD303), and are $CD11c^-$. These DCs are the main producers of type I interferons in response to viral infection.

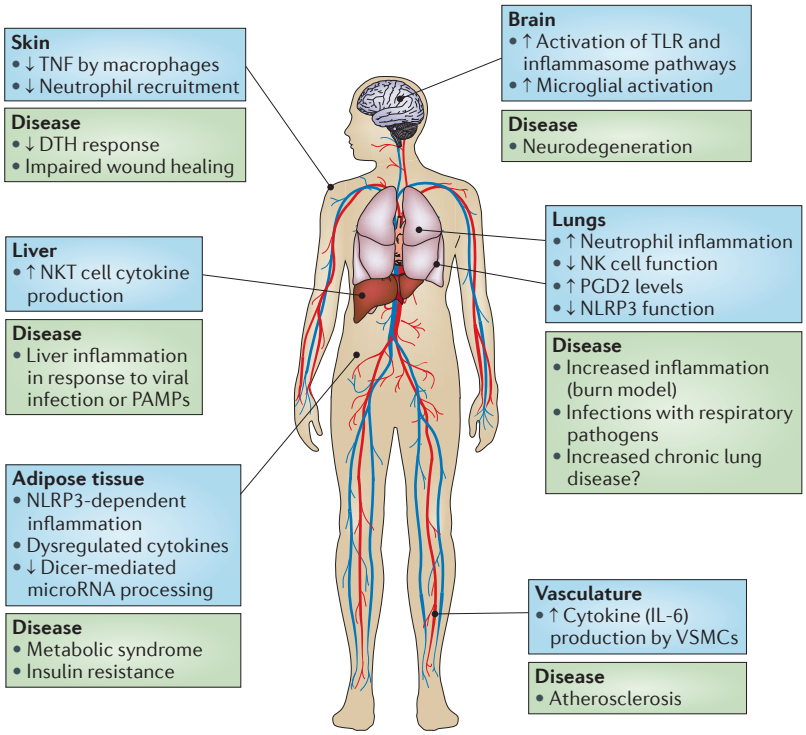

Figure 1 | **Organ-specific changes of the innate immune system associated with ageing and disease.** The effects of ageing in various organ systems are depicted. Diminished innate immune responses are found in some cases, such as in the skin, in which a decrease in tumour necrosis factor (TNF) production by macrophages results in decreased endothelial cell activation and diminished delayed-type hypersensitivity (DTH) responses. In addition, studies in mice have linked diminished neutrophil recruitment to impaired wound healing. Decreased natural killer (NK) cell function and induced NLRP3 (NOD-, LRR- and pyrin domain-containing 3) function in response to influenza virus limit protective responses to infection in the lungs. However, there are also instances of dysregulated inflammatory responses found in ageing; for example, there is increased neutrophil recruitment in the lungs following burn injury in a mouse model. In the liver, there is experimental data showing that NKT cells show exaggerated inflammatory responses that contribute to immune pathology during viral infection or to Toll-like receptor (TLR) activation. In the brain, TLR and inflammasome signalling are increased with ageing and are associated with microglial activation. Signalling via the NLRP3 inflammasome also contributes to increased age-associated inflammation in adipose tissue. Finally, in the vasculature, increased basal production of interleukin-6 (IL-6) by vascular smooth muscle cells (VSMCs) has been found in aged rodents and non-human primates and may provide a potential explanation for the increased prevalence of atherosclerosis with ageing. PAMPs, pathogen-associated molecular patterns; PGD2, prostaglandin D2.

the myeloid cell lineage. This reflects the impaired bone marrow homing, proliferative responses and self-renewal capacity seen in aged HSCs with myeloid skewing[20].

Ageing and innate immune cell migration

Neutrophils are the first cells to migrate to pathogen-infected sites, and their migratory capacity has been extensively studied in the context of ageing. The speed of neutrophil movement, also known as chemokinesis, was found to be unperturbed in older humans[38], but neutrophils from aged mice show impaired chemokinesis in response to IL-8 (REF. 39). By contrast, chemotaxis (that is, directional movement in response to a gradient of a stimulus) of neutrophils from older humans[38,40] and mice[41–43] seems to be impaired. Moreover, impaired neutrophil chemotaxis was found to contribute to delayed wound healing in aged mice — a defect that was associated with lowered expression of intercellular adhesion molecule 1 (ICAM1)[44] (FIG. 1). However, diminished chemotaxis can result in not only reduced neutrophil migration to the sites of inflammation but also defective neutrophil egress from inflamed tissues. Indeed, local neutrophil-mediated inflammation was increased in aged mice, compared with in young mice, following burn-associated lung injury, as well as following bacterial or viral infection[39,45,46]. In the case of burn-associated injury, an age-associated decrease in the expression of CXC-chemokine receptor 2 (CXCR2) by neutrophils and an increase in the expression of ICAM1 by pulmonary endothelial cells contributed to increased neutrophil inflammation in the lungs[39] (FIG. 1). The findings that ICAM1 expression is increased in the burn model but is decreased in the wound-healing model may reflect tissue context and differences between a systemic (burn) and a local response.

Neutrophil migration *in vitro* can also be facilitated or inhibited depending on the presence of pro-inflammatory environmental stimuli, including IL-8 and tumour necrosis factor (TNF)[47], and such age-associated microenvironmental and tissue-related factors probably contribute to the dysregulation of neutrophil activation and migration. Taken together, these results suggest that aged neutrophils have an impaired ability to traffic into and out of sites of infection. Notably, defects in the migration of mouse pulmonary DCs[48] and human monocyte-derived DCs (MDDCs)[36] have also been reported, as discussed below. Such alterations in cell trafficking with ageing could affect the initiation of innate immune responses at sites of infection.

Ageing and innate immune cell effector functions

Neutrophils. Neutrophils from older adults, compared with those from younger adults, showed impaired phagocytosis of opsonized *Escherichia coli* and *Streptococcus pneumoniae*[49–51] as well as a diminished capacity to kill phagocytosed microorganisms[50,52,53]. One potential factor contributing to an age-associated defect in intracellular bacteria killing in human neutrophils could be the diminished expression of dehydroepiandrosterone sulphate, which is a circulating steroid that promotes superoxide generation in neutrophils[54].

Age-associated defects in neutrophil effector function have been associated with impaired signal transduction functions. Examples of this include impaired anti-apoptotic responses to granulocyte/macrophage colony-stimulating factor (GM-CSF) that are mediated through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway[55] and the phosphoinositide 3-kinase (PI3K)–AKT pathway[56]. Neutrophils from older adults, compared with those from younger individuals, show reduced basal expression of suppressor of cytokine signalling 1 (SOCS1) and SOCS3, which negatively regulate JAK–STAT signalling[56], and an impaired response to triggering receptor expressed on myeloid cells 1 (TREM1)[57], which is an activating receptor of the immunoglobulin superfamily that mediates the production of cytokines, chemokines

and reactive oxygen species (ROS). This dysregulated signal transduction may reflect alterations in membrane lipid composition and lipid rafts that result in inappropriate localization or retention in membrane-signalling domains. For example, the negative regulator SH2 domain-containing protein tyrosine phosphatase 1 (SHP1; also known as PTPN6) is excluded from lipid rafts following GM-CSF stimulation of neutrophils from young individuals, but it is retained in rafts in neutrophils from older individuals[58]. Taken together, these findings indicate that ageing impairs several signalling pathways in neutrophils, including the generation of the respiratory burst and apoptotic pathways, which may result in reduced protection from microbial infection.

Although in early studies neutrophil function was reported to be mostly preserved in aged mice[59,60], a recent study in aged mice indicated that neutrophils infected *in vitro* with methicillin-resistant *Staphylococcus aureus* (MRSA) showed decreased production of CXC-chemokine ligand 1 (CXCL1) and CXCL2 (REF. 53). Neutrophils also produce neutrophil extracellular traps (NETs), which are scaffolds of extruded chromatin that contain antimicrobial peptides and proteases, such as elastase and myeloperoxidase, that facilitate the capture and killing of pathogens[61]. NET formation decreased with age in a mouse model of MRSA infection[53], but it remains to be determined whether NETs are altered in aged humans.

NK and NKT cells. Natural killer (NK) cells show decreased cytotoxicity, lung infiltration and interferon-γ (IFNγ) production in a model of influenza virus infection in aged mice, compared with in young mice[62,63] (FIG. 1). In an ectromelia poxvirus model, NK cells from older mice (aged 14–18 months) showed a cell-intrinsic defect in migration to regional lymph nodes[64]. The cytotoxicity of NK cells induced by type I IFNs seems to be decreased in aged mice[65], as does the induced production of IFNγ and granzyme B in response to specific cytokine combinations[66].

In humans, NK cells can be broadly divided into a $CD56^{low}$ population that has cytotoxic activity and a $CD56^{hi}$ population that is responsible for cytokine production[67]. $CD56^{hi}$ NK cells seem to decrease in proportion and in cytokine and chemokine secretion with ageing. In older adults, cytotoxicity is decreased on a per-cell basis, and there is an expansion of the $CD56^{low}$ cytotoxic NK cell compartment[68–73]. Notably, this diminished cytotoxicity is associated with an age-associated defect in the mobilization of perforin to the immunological synapse[74]. Elucidating the mechanisms that underlie age-associated changes in NK cell function may have particular clinical importance, as impaired NK cell function is associated with increased infection rates and mortality in older adult nursing home residents[75].

Invariant NKT (iNKT) cells are characterized by CD1d-restricted recognition of endogenous and bacterial glycolipids[76]. iNKT cell numbers are increased in aged compared with young mice[77]; by contrast, numbers of human iNKT cells in the blood decrease with age and these cells show diminished proliferation in response to the CD1d ligand α-galactosylceramide[78,79]. In mice, NKT cells produce cytokines such as IL-17A, which contributes to immune pathology after viral infection[46]. Consistent with this, studies in non-infectious mouse models indicate that ageing enhances inflammatory responses by NKT cells, and that this leads to liver immunopathology[80,81] (FIG. 1). Thus, NKT cells also contribute to the innate immune dysregulation that is associated with ageing.

Macrophages and DCs. Various macrophage and DC effector functions in aged individuals have been studied. Several reports have shown an age-associated decline in nitric oxide production by mouse and rat macrophages; at the same time, an age-associated increase in PGE2 production and cyclooxygenase 2 (COX2) expression have also been reported[82,84,83]. However, evaluation of the effects of ageing on phagocytosis has produced mixed results. Although several studies report preserved phagocytosis of bacterial targets by aged mouse macrophages[85,86], studies of human monocytes and MDDCs suggest that there is an age-associated impairment in phagocytosis[32,36], and phagocytosis of apoptotic cells by both mouse macrophages and human MDDCs has been reported to be reduced[36,87]. These findings raise the possibility that there are differential age-associated effects on specific phagocytic functions.

Studies of antigen-presenting cells generally show impaired function with ageing[88–90] (with one exception[91]); for example, bone marrow-derived DCs (BMDCs) in aged mice, compared with those in young mice, were shown to be defective in controlling tumour growth in a B16 melanoma model[92]. A recent study of infection with a modified version of *Listeria monocytogenes* showed decreased expression of co-stimulatory proteins and impaired bacterial uptake by $CD8\alpha^+$ conventional DCs in aged mice, particularly at early stages of infection (although the LPS-induced co-stimulatory protein expression was comparable to that in young mice)[93]. After oral infection of mice with the intracellular parasite *Encephalitozoon cuniculi*, $CD11c^+$ DCs from the lymph nodes of adult (but not aged) mice had a decreased ability to prime T cells compared with $CD11c^+$ DCs from young mice[94]. This impaired function was associated with a decrease in IL-12 production by DCs from adult mice that could be compensated for by IL-15 treatment. Microenvironmental alterations, such as disruption of the architecture of the splenic marginal zone, may also contribute to impairments in antigen presentation[95]. Whether these processes are altered in human cells remains unclear; studies using fairly small numbers of people have so far shown preserved antigen presentation function in DCs and monocytes[96,97].

Ageing and PRR signal transduction

Alterations in TLR expression. Ageing is associated with impaired PRR signalling, which may partly be accounted for by the reported alterations in Toll-like receptor (TLR) expression by innate immune cells from older adults, compared with those from younger individuals. Decreased surface expression of TLR1 has been associated

Conventional DCs
Specialized phagocytic antigen-presenting cells that have the classic stellate dendritic cell (DC) morphology. Mouse conventional DCs generally express CD11c, but are highly heterogeneous and are divided into subsets: $CD8\alpha^+$ and $CD4^+$ subsets in secondary lymphoid organs, and $CD8\alpha^+CD103^+$ and $CD4^+CD11b^+$ subsets in the periphery. Human conventional DCs are generally termed myeloid DCs and are lineage-negative MHC class II^+CD11c^+ DCs. Human equivalents to the mouse $CD8\alpha^+$ and $CD4^+$ subsets can be distinguished by expression of blood DC antigen 3 (BDCA3; also known as CD141) and BDCA1 (also known as CD1c), respectively.

Monocyte-derived DCs
(MDDCs). MDDCs can be generated *in vitro* from peripheral blood mononuclear cells in the presence of interleukin-4 and granulocyte/macrophage colony-stimulating factor. MDDCs resemble myeloid dendritic cells (DCs) and may model the differentiation of DCs from monocytes that enter sites of inflammation.

with diminished TLR1–TLR2-induced cytokine production in human monocytes from older individuals[33,98]. Age-associated decreases in human TLR1, TLR3 and TLR8 protein expression by mDCs[99], and in TLR7 and TLR9 expression by pDCs[37], have also been reported. Although substantial age-associated decreases in *Tlr* gene expression have been reported in mice[100], the pattern is less clear in humans. Concordant changes in both protein and gene expression have been reported, such as the age-associated increase in *TLR5* mRNA and protein levels in adherent human monocytes[101], but in other cases changes in gene expression may not be sufficient to account for changes in TLR protein levels[99]. This suggests that post-transcriptional mechanisms contribute to the decreased TLR protein levels. In human monocytes, total TLR1 protein expression was comparable in cells from young and older adults; thus, the reported decrease in cell surface TLR1 levels could result from mechanisms involving TLR1 transport to the plasma membrane[98].

TLR signalling and cytokine production in aged monocytes and macrophages. TLR signalling has a crucial role in linking innate and adaptive immune responses through the induction of expression of co-stimulatory molecules and pro-inflammatory cytokines. One of the first studies to evaluate TLR function in aged C57BL/6 mice showed there to be a generalized decrease in *Tlr* (*Tlr1–Tlr9)* gene expression and TLR-induced TNF and IL-6 production by splenic and peritoneal macrophages, as well as decreased expression of TLR4 protein[100]. By contrast, in aged BALB/c mice, surface expression of TLR2 and TLR4 on peritoneal macrophages was unchanged. Nonetheless, TLR2- and TLR4-induced TNF and IL-6 production were reduced in macrophages from aged BALB/c mice, and they were associated with decreased p38 mitogen-activated protein kinase (MAPK) expression[102,103]. An age-associated impairment in TLR2-dependent cytokine production and signalling was also reported in alveolar macrophages from BALB/c mice in a model of *S. pneumoniae* infection[104], and in macrophages from C57BL/6 mice responding to *Porphyromonas gingivalis*; a decrease was only observed for IL-6 production in response to *P. gingivalis* in aged BALB/c mice[86,105]. These findings provide evidence for an age-associated decline in mouse TLR function and show variation in the underlying mechanisms that may partly reflect genetic background (FIG. 2).

In rhesus macaques, monocyte production of TNF and IL-6 induced by stimulation of TLR2–TLR6 heterodimers, TLR4 and TLR9 was decreased in aged animals, compared with in young animals[106]. In humans, monocytes from older adults produce less TNF and IL-6 in response to TLR1–TLR2 stimulation, and there is an associated decrease in MAPK signalling; decreased cell surface expression of TLR1, but not of TLR2, was associated with this reduced cytokine production[98]. In studies of cell subpopulations, TLR1–TLR2-induced cytokine production was decreased in all monocyte subsets, including classical $CD14^{hi}CD16^{-}$, inflammatory $CD14^{hi}CD16^{+}$ and non-classical $CD14^{low}CD16^{hi}$ monocytes, in aged compared with young adults[33].

TLR-dependent expression of the co-stimulatory molecules CD80 and CD86 following TLR engagement *in vitro* was altered in monocytes from older adults, thereby potentially affecting the efficiency of antigen presentation. Notably, the extent of TLR-induced CD80 and CD86 expression strongly correlated with antibody responses to the trivalent inactivated influenza virus vaccine[107]. These studies of induced TLR function were notable for the use of multivariate statistical modelling to adjust for potential co-variates, such as gender, race, co-morbidities and medication use — such modelling is valuable in human studies, given the considerable heterogeneity in cohorts.

So, monocyte and macrophage function seems to be diminished in aged mice and humans compared with in young individuals. However, these impairments occur in the presence of dysregulated or inappropriately persistent inflammatory responses; for example, basal and LPS-induced TNF production by $CD14^{+}CD16^{+}$ human monocytes increases with age[32]. In addition, human (adherent) monocytes show an age-associated increase in the TLR5-induced production of IL-8 and IL-6 and in the phosphorylation of p38 MAPK and ERK[101]. This increase in TLR5 signalling in monocytes from older adults was not observed in non-adherent cells[98], which suggests that adherence to plastic could result in partial activation that contributes to an age-associated pro-inflammatory bias. The maintenance, or even the enhancement, of TLR5 activity with ageing might also be potentiated by an inflammatory environment in the context of infection. Further studies are needed to clarify the underlying mechanisms, in light of the potential use of TLR5 agonists as vaccine adjuvants[108] (BOX 1) and the association of *TLR5* gene expression with human influenza virus vaccine antibody responses in young adults[109]. Another example of dysregulated TLR responses came from studies of *in vitro* West Nile virus (WNV) infection: in WNV-infected macrophages from older adults, STAT1-dependent downregulation of TLR3 expression was impaired, which resulted in an inappropriate persistence of TLR3 activation. This might contribute to the increased morbidity and mortality that is associated with WNV infection in older adults[110].

Finally, the ageing-associated *in vivo* microenvironment is also likely to contribute to functional defects; for example, in a study of human delayed-type hypersensitivity responses to tuberculin purified protein derivative, impaired T cell migration in the skin of aged adults was linked to decreased TNF production by skin macrophages and a consequent impairment in endothelial cell activation (FIG. 1). Notably, skin macrophages from aged and young humans produced comparable levels of TNF *ex vivo*, which suggests that factors in the microenvironment are responsible for the impaired function *in vivo*[111].

TLR signalling and cytokine production in aged DCs. In mice, there is evidence for preserved DC function with ageing; mouse BMDCs[112] and $CD11c^{+}$ DCs that were isolated from the spleen and lymph nodes showed unperturbed TLR function[91], although lower levels of LPS-induced IL-6 and TNF production by DCs have

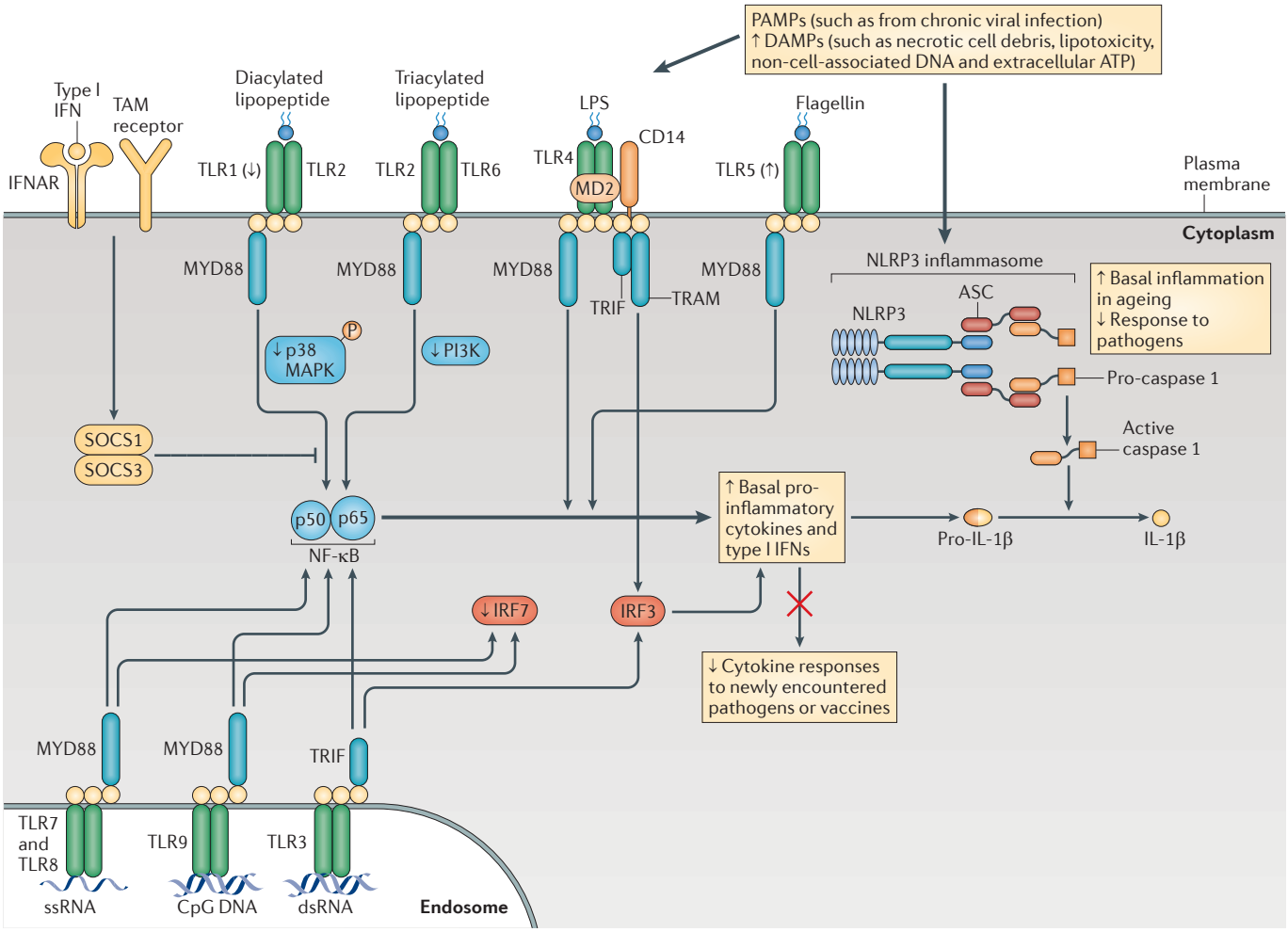

Figure 2 | **Effects of ageing on innate immune PRR signalling.** Toll-like receptor (TLR) and NLRP3 (NOD-, LRR- and pyrin domain-containing 3) signalling pathways are depicted. With ageing, elevated levels of pattern recognition receptor (PRR) ligands (pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)), which could arise from chronic viral infection (such as with cytomegalovirus) or from cell damage, respectively, contribute to an elevated pro-inflammatory state, as manifested by increased basal levels of pro-inflammatory cytokines that may result partly from TLR signalling and partly from NLRP3 signalling. Such basal activation may restrict responsiveness to new pathogens or vaccines, resulting in innate immune failure and impaired adaptive immune responses. Changes in protein expression of TLR1 and TLR5 in humans are indicated; results in mice suggest that there is either a widespread decrease in *Tlr* gene expression or unchanged expression levels, but that there are alterations in intracellular signalling proteins (such as decreased levels of p38 mitogen-activated protein kinase (MAPK)). dsRNA, double-stranded RNA; IFN, interferon; IFNAR, IFNα/β receptor; IL-1β, interleukin-1β; IRF, IFN-regulatory factor; LPS, lipopolysaccharide; MD2, myeloid differentiation factor 2; MYD88, myeloid differentiation primary-response protein 88; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; SOCS, suppressor of cytokine signalling; ssRNA, single-stranded RNA; TAM, TYRO3, AXL and MER; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing IFNβ.

been reported in a T cell receptor-transgenic system[88]. However, an age-associated decrease in type I IFN production by pDCs was found following *in vitro* or *in vivo* challenge with herpes simplex virus type 2 (HSV-2). This ageing-associated dysfunction correlated with decreased nuclear translocation of IFN-regulatory factor 7 (IRF7). In addition, TLR9-dependent type I IFN responses by pDCs were augmented by treatment with antioxidants and in mice subjected to caloric restriction[113].

Another age-associated alteration of TLR function is increased IL-23 expression by mouse BMDCs following combined stimulation with the TLR4 ligand LPS and the TLR7 agonist R848 (also known as resiquimod); this increase is associated with alterations in histone modification at the promoter for the gene encoding IL-23p19 (also known as IL-23α)[114] and similarly resulted in an age-associated increase in the production of prostaglandin E2 (PGE2) by BMDCs. Notably, PGE2 was found to substantially augment IL-23 production by DCs from aged, but not from young, mice[115]. As IL-23 promotes the differentiation of T helper 17 (T_H17) cells, such increases in PGE2 and IL-23 production might underlie the potential increased predisposition to T_H17 cell responses that has been reported

Box 1 | **TLR agonists as vaccine adjuvants for the elderly**

Specific Toll-like receptor (TLR) activators, such as the lipopolysaccharide component lipid A, have recently been used as a strategy to boost immune protection following vaccination in aged individuals. This may be accomplished by the production of pro-inflammatory cytokines, which can enhance the responses of aged $CD4^+$ T cells[190]. Administration of the TLR5 ligand flagellin has been associated with preserved innate immune responses in aged mice and elevated inflammatory responses by specific human cell types from aged individuals[101,191]. Indeed, a vaccine construct that contained flagellin linked to peptides from the influenza virus protein haemagglutinin afforded protection against lung infection with influenza virus in aged mice[191]. However, the protection induced in the aged mice was still inferior to that induced in young mice, which probably reflects defects in the adaptive immune response of aged mice. A clinical study using a similar vaccine construct found that people over 65 years of age showed a robust humoral response without substantial side effects[192].

Several studies have used the TLR9 ligand CpG-containing oligonucleotides in combination with FMS-like tyrosine kinase 3 (FLT3) ligand as an adjuvanted intranasal vaccine against influenza virus and pneumococcus; both vaccines gave similar responses in young and aged mice[193,194]. CpG-containing oligonucleotides added to the pneumococcal conjugate vaccine also restored pneumococcal polysaccharide-specific antibody responses in aged mice[195]. A recent human study found that a TLR4 agonist, glucopyranosyl lipid, enhances the ability of myeloid dendritic cells (mDCs) to respond to influenza virus infection *in vitro*[196], and this was mediated by increased mDC production of granzyme B[196]. Taken together, these studies indicate that TLR engagement has great potential to enhance immune protection in aged individuals, but also that targeted use of TLR agonists — alone or in combination — will be essential, as different TLR activators may have distinct effects in overcoming defects in the adaptive immune systems of aged individuals.

in aged individuals[116,117]. An age-associated increase in PGD2 production has been observed in lung tissue of mice infected with respiratory viruses, and this increase was associated with impaired migration of pulmonary DCs to regional lymph nodes (FIG. 1). Treatment of these mice with PGD2 antagonists resulted in improved DC migration and T cell responses[48].

Primary DCs from aged humans have been reported to have impaired functions; for example, the production of IL-12 downstream of TLR4 stimulation with LPS was reduced in mDCs from older, compared with younger, individuals[118]. Another study revealed a generalized age-associated decrease in TLR-induced cytokine production by mDCs and pDCs, and this strongly correlated with reduced antibody responses to influenza virus immunization[99]. Other studies of human pDCs have shown age-associated decreases in TLR7- and TLR9-induced cytokine production[37], influenza virus-induced type I and type III IFN production[119,120] and WNV-induced type I IFN production[121]. Taken together with other reports indicating that human pDC numbers in peripheral blood decrease with age[34,35], these findings suggest that the antiviral and antitumour functions of pDCs may be particularly affected in older adults. Strikingly, basal levels of intracellular cytokines in primary mDCs and pDCs have been found to increase with ageing[99] — a finding that is consistent with the age-associated pro-inflammatory environment. Such a background of high cytokine production may reflect a degree of basal TLR activation that cannot be further augmented with additional TLR agonist treatment and that could contribute to failed innate immune responses to pathogens or vaccines (FIG. 2).

MDDCs from older adults, compared with those from young adults, showed increased TNF and IL-6 production following TLR4 or TLR8 stimulation; and TNF and IFNα production were also increased following exposure to self DNA *in vitro*[36,122]. By contrast, *in vitro* assays of phagocytosis and a transwell assay of migration showed reduced function of MDDCs from older adults. These phenotypes were associated with impaired PI3K signalling, which has been implicated as both a negative regulator of TLR signalling and a positive regulator of phagocytic function[36]. However, the possibility that the GM-CSF and IL-4 treatment used to generate MDDCs could attenuate potential age-associated differences should also be considered.

Ageing is not always associated with increased TLR signalling in human MDDCs. An age-associated decrease in CD80 and CD86 upregulation and in type I IFN production by MDDCs was observed after infection with WNV. This defect was associated with impaired induction of STAT1 and IRF7 expression[121], and is similar to findings in primary human pDCs[120,121]. Furthermore, WNV-infected MDDCs from older adults, compared with those from young adults, showed decreased nuclear translocation of the positive regulator IRF1 and increased expression of the TLR inhibitory protein AXL (also known as UFO)[121], which is a member of the TYRO3, AXL and MER (TAM) receptor family. Following activation of the IFNα/β receptor (IFNAR)–STAT1 pathway, TAM receptors are expressed and hijack this pathway to produce the inhibitory proteins SOCS1 and SOCS3 (REF. 123); this mechanism provides a link to the STAT1-dependent decrease in type I IFN production that occurs following WNV infection. Taken together, these findings indicate that the effects of ageing on responses by DCs are complex; there is evidence for both inappropriately impaired and augmented responses to PRR engagement that reflect cell type, activation state and tissue context (FIG. 2; TABLE 1).

Signalling downstream of other PRRs. The effects of ageing on cytoplasmic PRRs, such as the NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), remain to be completely elucidated. Notably, a recent study of aged mice deficient in NLRP3 (NOD-, LRR- and pyrin domain-containing 3) implicates the NLRP3 inflammasome in age-associated inflammation in adipose tissue and the brain, with aged knockout animals showing improvement in glucose tolerance as well as in tests of memory and learning[124]. By contrast, aged C57BL/6 mice showed reduced production of IL-1β and IL-18 following NLRP3 activation in the context of influenza virus infection[125]. Taken together, these findings are again consistent with failure in innate immune activation in the context of dysregulated inflammation (FIG. 1).

PRR signalling is known to intersect with macroautophagy, which seems to be defective in various cell types from aged organisms[126]; so, it is probable that autophagic responses to intracellular pathogens are also impaired in aged individuals. At the same time, defects in autophagy may influence PRR signalling via increased generation of ROS from dysfunctional mitochondria that ordinarily would be subject to macroautophagic degradation. As

NOD-like receptors
(NLRs). A family of more than 20 nucleotide-binding oligomerization domain (NOD)-like cytoplasmic pattern recognition receptors that sense pathogens, toxins, endogenous danger signals (such as uric acid) and exogenous crystalline substances (such as alum, silica and asbestos) and induce inflammatory responses.

RIG-I-like receptors
(RLRs). A family of cytoplasmic pattern recognition receptors that are related to the RNA helicase retinoic acid-inducible gene I (RIG-I). They recognize single- and double-stranded viral RNA and mediate antiviral responses, such as type I interferon production.

Inflammasome
A multiprotein complex that consists of a NOD-like receptor, an adaptor protein and pro-caspase 1. On assembly, the complex facilitates the caspase 1-mediated cleavage and production of mature cytokines, such as interleukin-1β and interleukin-18.

signalling downstream of both NLRP3 and RLRs seem to be influenced by impaired autophagy[127–130], and as ROS production also generally increases with age, it is probable that PRR function in aged individuals will be influenced by changes in autophagy[131].

Systemic factors in innate immune ageing

Hormonal changes. Factors that are extrinsic to the immune system also contribute to the heightened pro-inflammatory environment that is associated with ageing. For example, both oestrogen and testosterone decrease IL-6 production *in vitro* and *in vivo*[132–135], and age-associated decreases in oestrogen or androgen production are associated with increases in basal pro-inflammatory cytokine levels[136,137]. Another non-immune contribution to the inflammatory environment is the neuroendocrine axis that links the central nervous system to the periphery via the hypothalamus. Knockdown of expression of the histone deacetylase sirtuin 1 in hypothalamic neurons induced a pro-inflammatory activated state in peripheral $CD4^+$ T cells[138]. Indeed, recent studies indicate that mammalian lifespan itself may be linked to a nuclear factor-κB-dependent inflammatory state in the hypothalamus, which occurs in aged mice and which is linked to decreases in gonadotropin-releasing hormone[139]. Further studies will be required to determine whether an analogous mechanism occurs in ageing humans.

Metabolic changes. Adipose tissue has immunological functions in addition to its well-known functions in energy storage and metabolism. Redistribution of adipose tissue and impairments in metabolic functions, such as insulin sensitivity, in aged individuals are well described[140], and adipose tissue may be a source of the increased systemic levels of pro-inflammatory cytokines that are associated with ageing[141–144]. Increased infiltration of macrophages in adipose tissue of aged mice is reminiscent of that seen in the setting of obesity and supports the idea that obesity is a form of accelerated ageing in adipose tissue[145]. Additional work on the biology of adipose tissue in the context of ageing will be required to obtain mechanistic insights. However, it is notable that saturated fatty acids and ceramides that are generated in the setting of lipotoxicity (for example, in metabolic syndrome) can activate the NLRP3 inflammasome[129,146]. In addition, a recent study identified a decrease in the expression of Dicer, which is a protein that mediates microRNA processing, in adipose tissue from aged mice and in pre-adipocytes from aged humans. Mice with an adipose tissue-specific knockout of Dicer had increased sensitivity to oxidative stress and expression of markers associated with senescence, which implicates microRNA regulation in adipose tissue ageing and suggests it may potentially contribute to the age-associated pro-inflammatory environment[147] (FIG. 1).

DAMPs. Intriguing links between DNA damage and the activation of inflammatory responses constitute an additional potential contributing factor to the age-associated pro-inflammatory environment; for example, DNA damage from ionizing radiation has been associated with IL-6 production by epithelial cells[148]. Indeed, IL-1β, IL-6 and IL-8 are components of the senescence-associated secretory phenotype (SASP), which is the secretome of cytokines, growth factors, extracellular matrix proteins and proteases that are produced by senescent cells in response to DNA damage and that modify the local microenvironment[149]. The SASP is also regulated by signalling via p38 MAPK[150] and by cell-associated IL-1α signalling[151]. Notably, in a cohort of nonagenarians, levels of endogenous non-cell-associated DNA were strongly associated with inflammatory markers, such as C-reactive protein, and were an independent risk factor for mortality[152]. Such DNA, which could be released from senescent or necrotic cells, potentially contains endogenous damage-associated molecular patterns (DAMPs), which activate innate immune responses. Several studies have shown that necrotic cells can activate the NLRP3 inflammasome, probably through the release of cellular ATP[153–155], but it remains possible that additional PRRs, such as those that recognize nucleic acid motifs, could also participate in innate immune activation by endogenous ligands. In light of these findings, chronic infections (such as with cytomegalovirus (CMV), with other herpesviruses or with HIV), ongoing endogenous DNA release by necrotic cells or engagement of innate immune PRRs by other self ligands may all contribute to the increased inflammatory state that is associated with ageing. Although chronic viral infections in humans depend on exposure, it remains unclear whether such pro-inflammatory DNA is intrinsic to ageing per se or instead results from the consequences of co-morbid disease or alterations in nutritional status in aged adults.

Chronic and latent viral infections. Chronic viral infections, particularly with human CMV, have been linked to an age-associated pro-inflammatory environment[156]. CMV is known for cycles of asymptomatic reactivation throughout life, and such reactivation could contribute to an inflammatory microenvironment[157,158]. However, establishing causality, as opposed to associations, in humans has remained elusive. Although CMV reactivation has been shown to be associated with increased IL-6 and TNF levels and with impaired responses to influenza virus vaccine[159,160], a recent longitudinal study that compared CMV-seronegative aged adults, those seroconverting to CMV and those who remained seropositive over a 10-year period failed to show an effect of CMV status on markers of inflammation[161]. This study was the first to longitudinally evaluate individuals who seroconverted to CMV and does not implicate CMV as the dominant driver of an age-associated pro-inflammatory environment. However, the number of new CMV seroconverters in this study was relatively small, and it is also conceivable that multiple rounds of CMV reactivation throughout life are needed to establish a systemic increase in inflammation. Further longitudinal studies of CMV in human cohorts are needed to address these issues.

Macroautophagy
An evolutionarily conserved process in which acidic double-membrane vacuoles sequester intracellular contents (such as damaged organelles and macromolecules) and, through fusion to secondary lysosomes, target them for degradation.

Ageing and chronic inflammatory disease

The evidence discussed for age-associated innate immune dysregulation, in the setting of hormonal and metabolic aetiologies as well as endogenous ligands for PRRs, suggests that the resultant pro-inflammatory state will influence the pathogenesis of not only infectious diseases but also conditions that are associated with chronic inflammation. Here, we illustrate the interface of innate immune activation with two examples of diseases that have a high prevalence in older adults — atherosclerosis and Alzheimer's disease.

Atherosclerosis. Clinical and epidemiological studies have shown that ageing contributes to the development of atherosclerosis[162], which is a chronic inflammatory arterial disease that is characterized by the deposition of lipids within the arterial wall[163]. TLR and NLRP3 signalling have recently been implicated in the development of this disease[164,165]. It is not clear how ageing enhances the development of atherosclerosis, but elevated basal inflammatory responses may contribute to the chronic inflammation in the disease. It is also poorly understood how an atherosclerotic plaque becomes susceptible to rupture, and the exact nature of the complex thrombotic process after rupture, which involves components of the immune system, the coagulation system and stromal cells, remains to be described. Vascular smooth muscle cells (VSMCs) harvested from the aortas of aged mice were found to have pro-atherogenic features, including increased production of pro-inflammatory cytokines, such as IL-6, and chemokines, such as CCL2 (FIG. 1). The expression of TLR4 was also increased in aged VSMCs, and increased production of IL-6 was dependent on TLR4 and myeloid differentiation primary-response protein 88 (REF. 166). VSMCs from aged non-human primates also showed a higher production of pro-inflammatory cytokines, such as IL-1β, TNF and CCL2 (REF. 167). In this study, the increased inflammatory response correlated with increased mitochondrial oxidative stress. Notably, treatment with resveratrol, which is a naturally occurring phenol that has been shown to extend lifespan in various experimental models, reversed the ageing-associated pro-inflammatory phenotype of VSMCs. However, it remains unclear how the inflammatory phenotype of aged VSMCs contributes to the pathogenesis of atherosclerosis.

Alzheimer's disease. Dysregulated innate immune responses in the ageing brain may be relevant to various age-associated neurodegenerative conditions, such as Alzheimer's disease (FIG. 1). PRRs are highly expressed in the brain, with the expression of TLR1–TLR9 reported in both mouse and human microglia. Mouse astrocytes and cortical neurons also show widespread TLR expression, although in humans only TLR3 expression has been reported[168]. Notably, TLR-induced cytokine production by mouse microglia is increased in aged mice compared with in young mice. In humans, a gene expression microarray study of four regions of the brain in young (20–59 years old) and aged (60–99 years old) individuals, and in patients with Alzheimer's disease (74–95 years old) revealed marked upregulation of TLR and caspase 1 expression in older adults. In addition, a gene expression signature of microglial activation was also observed in older adults[169]. Although a limited subset of innate immune genes showed significant differences in expression between older adults and those with Alzheimer's disease, a far greater number of genes were (mostly) upregulated in older, compared with young, adults. These findings suggest a role for chronic inflammation in the brain in precursor stages of Alzheimer's disease.

The effect of TLR activation in the context of neurodegeneration has been investigated mainly in mouse models and seems to be complex. The β-amyloid peptide found in amyloid plaques that are deposited in the brains of individuals with Alzheimer's disease induces a TLR-dependent innate immune response that may facilitate β-amyloid clearance[170–174]. However, systemic inflammation, for example, by administration of LPS, exacerbates β-amyloid plaque formation and cognitive impairment in a transgenic Alzheimer's disease model[175,176], and an association between systemic inflammation and Alzheimer's disease has been reported in humans[177]. Recent studies have also implicated the NLRP3 inflammasome in Alzheimer's disease pathogenesis[178,179]. The beneficial and detrimental effects of innate immune engagement in aged adults with Alzheimer's disease may reflect the timing, location and extent of inflammation, and this remains an area of active investigation.

Conclusions and future perspectives

Considerable progress has been made in elucidating age-associated alterations in innate immunity and their consequences. For some cell types, information on the effects of ageing is only just emerging; one example is myeloid-derived suppressor cells (MDSCs) — a heterogeneous population of immature myeloid cells with potent T cell-suppressive functions[180]. MDSCs are increased in malignancies and other inflammatory states, as well as in aged compared with in young mice[181] and humans[182]. These findings are notable given the substantial age-associated increase in human cancers[183]. A recent report in a mouse model of sterile inflammation described a neutrophil population that constitutively produced IFNγ and that conferred T cell resistance to MDSC suppression — this provides a potential mechanism for innate immune dysregulation that could potentiate chronic inflammation[184]. However, it remains to be seen whether this neutrophil population is present in aged humans or mice.

Understanding the biological basis for altered innate immunity and inflammation in ageing is a challenge that has substantial clinical importance, as restoration or preservation of physiological function is likely to be of equal or greater importance to extension of lifespan. The study of well-characterized human cohorts in the context of co-morbid medical diseases or alterations in functional status, such as the geriatric syndrome of frailty[185], offers the possibility of linking innate immune function and inflammation with biological mechanisms.

Integrative systems biology approaches are also beginning to yield insights into such mechanisms[186]. The use of computational approaches together with model systems will be crucial as our appreciation of the complexity of the immune system and its relationship with the environment increases. For example, the possibility that the gut microbiome may influence vaccine responsiveness[187], probably through engagement of the innate immune system, will require the synthesis of signatures of vaccine responses from gene expression, proteomic and immunological studies with known age-associated alterations in the composition of the human gut microbiome[188,189]. These approaches offer future prospects to a field that has considerable challenge and opportunity.

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