Forum Review Article

Does dysregulation of redox state underpin the decline of innate immunity with ageing?

Helen R Griffiths[1*], Matthew CO Rooney[1], Yvonne Perrie[2]

[1]*Faculty of Health and Medical Sciences, University of Surrey, Stag Hill, Guildford, GU2 7XH, UK*

[2]*Department of Pharmacy, University of Strathclyde,* Scotland

*Corresponding author - h.r.griffiths@surrey.ac.uk

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Abstract

Significance

Anti-bacterial defence invokes the innate immune system as a first responder, with neutrophils phagocytosing and forming NETs around pathogens in a ROS-dependent manner. Increased NOX2 activity and mitochondrial ROS production in phagocytic, antigen-presenting cells affects local cytokine secretion and proteolysis of antigens for presentation to T cells at the immune synapse. Uncontrolled oxidative post-translational modifications to surface and cytoplasmic proteins in antigen presenting cells during ageing can impair innate immunity.

Recent Advances

NOX2 plays a role in the maturation of dendritic cells, but paradoxically, NOX2 activity has also been shown to promote viral pathogenicity. Accumulating evidence suggests that a reducing environment is essential to inhibit pathogen proliferation, facilitate antigenic processing in the endosomal lumen and enable an effective immune synapse between antigen presenting cells and T cells. This suggests that the kinetics and location of ROS production and reducing potential are important for effective innate immunity.

Critical Issues

During ageing, innate immune cells are less well able to phagocytose, kill bacteria/viruses and process proteins into antigenic peptides – three key steps that are necessary for developing a specific targeted response to protect against future exposure. Aberrant control of ROS production and impaired Nrf2-dependent reducing potential may contribute to age-associated immune decline.

Future Directions

Local changes in redox potential may be achieved through adjuvant formulations to improve innate immunity. Further work is needed to understand the timing of delivery for redox modulators to facilitate innate immune cell recruitment, survival, antigen processing and presentation activity without disrupting essential ROS-dependent bacterial killing.

Innovation

Immune memory of a pathogen depends on recognition of a unique antigenic “mark” or epitope on the pathogen. It requires carefully orchestrated interactions between the memory-holding lymphocytes in the adaptive arm of immune system, and the antigen presenting innate immune cells. The uptake and processing of pathogens to produce unique antigenic “marks” by macrophages and dendritic cells is modulated by redox state. With ageing-associated dysregulation of redox state,

impaired immune memory may ensue. Approaches to manipulate redox state in specific subcellular endosomal compartments after antigen uptake should be explored to understand whether vaccination responses may be improved during ageing.

Introduction

The innate immune response is responsible for fast, efficient and non-specific clearance of infection and cancers. The clearance function is largely attributed to the activity of phagocytic cells that express pattern recognition receptors (PRR). These PRR recognise generic molecular patterns present within or on the surface of microbes, some of which are common with oxidised lipids (12). On recognising a broad class of pathogens, phagocytes rapidly migrate to the site of infection, ingest and kill pathogens within a phagolysosome (Figure 1). NADPH oxidase (NOX) activity is critical for microbial killing by phagocytic cells by generating reactive oxygen (ROS) that may also react with nitric oxide to produce cytotoxic reactive nitrogen (RNS) species within the phagolysosome (66) (Figure 2). The production of ROS in response to stress are variably reported to be increased in macrophages and dendritic cells (from mitochondria) but decreased in neutrophils with age (from NADPH oxidase). However, the net effect for immune defence is a decline in the ability to effectively remove pathogen (14,121). This could be interpreted to mean that ROS have a minimal role to play in pathogen removal. Alternatively, these observations may indicate that an overall decrease in immune function with age may relate to the dysregulation of ROS production (wrong time and/or in the wrong place), for example, increased mitochondrial ROS production has been linked to immunosenescence (76) or aberrant regulation of the hydrogen donating antioxidant peptides and enzymes that results in lower reducing capacity with age. Table 1 summarises current knowledge of changes in phagocyte ROS production with age in the respiratory burst and from mitochondria.

Opposing the oxidising side of the redox equation is the capacity to generate reducing equivalents, in part through glycolysis but also via activation of antioxidant gene expression. Nrf2 is a transcription factor that acts as the master regulator of antioxidant and phase II protective genes. It is responsible for the upregulation of the biosynthetic genes required for synthesis of the major cellular antioxidant glutathione, including the rate limiting γ-glutamylcysteine ligase (γ-GCL), It has been proposed that ageing leads to decreased Nrf2 activity, but that it remains sensitive to activation by the electrophilic activator sulphoraphane (59), indicating a potential target for restoring age-related immune decline.

This conundrum of variable ROS response and ineffective immunity during ageing is the focus of the present review as we explore the relationship between NADPH oxidase activity, ROS production, thiol donors and phagocyte function. We highlight how this knowledge may be used to improve the specific, adaptive immune response featuring B and T cells (required for memory e.g. of vaccination) during ageing.

Author	Cell type	Differences in ROS production with age	Species	Reference

Alvarez	neutrophil	decreased respiratory burst to fMLP	rat	(4)
Alvarez	monocyte	decreased in response to PMA	human	(5)
Baehl	neutrophil	decreased NADPH oxidase-dependent ROS after hip fracture	Human	(6)
Braga	neutrophil	decreased ROS after soluble but not particulate stimuli	Human	(13)
	monocyte	decreased response to PMA	Human	(14)
Bruce	neutrophil	increased in response to PMA	Human	
Chougnet	DCs	increase in mitochondrial ROS	Mouse	(21)
Hazeldine	neutrophil	decreased NADPH oxidase-dependent ROS in response to IL-8 and LPS	Human	(49)
Kayaalti		increased with CC genotype in p22phox C242T SNP	Human	(56)
Kim	BMSC	increased activity that correlated with increased bone erosion	Mouse	(61)
Mege	phagocyte	decreased in response to particulate stimuli	Human	(71)
Pararasa	monocytes	increase in mitochondrial ROS	Human	(89)
Qin	microglia	increased NOX-dependent ROS	Mouse	(95)
Scott	neutrophil	increased in response to PMA	Human	(22)
Tomilov	macrophage	increased NADPH oxidase-dependent ROS in short-lived animals	Mouse	(112)
Vitlic	neutrophil	decreased NADPH oxidase-dependent ROS after bereavement	Human	(121)
Weinisch	neutrophil	decrease in ROS in response to S. aureus uptake	Human	(123)
Wiley	PBMC	decreased mitochondrial ROS with lower survival	human	(126)
Zhang	macrophages	increase in response to injury	Mouse	(140)

Table 1: Change in ROS production by phagocytes with age

Cells of the innate immune system and the effects of ageing

A range of immune cells play a non-specific role in engulfing pathogenic or damaged materials in a process known as phagocytosis. There are three major families of professional phagocytes: monocytes/macrophages, granulocytes and dendritic cells (DCs). Neutrophils are the first

granulocyte responder cells that arrive at sites of infection within minutes with the goal of killing pathogens through a combination of ROS-mediated oxidative damage and proteolytic degradation (47,127). They are followed within hours by monocytes that are induced to differentiate into macrophages e.g. by the presence of bacterial fragments such as the peptide formyl-methionine-leucine-phenylanane (fMLP) and lipopolysaccharide (LPS). However, in the chronic inflammatory disease periodontitis, which increases in frequency with age, neutrophil hyperactivity was observed with low expression of antioxidant genes suggesting a deficiency in an Nrf2-dependent pathway (25).

Each family of phagocytes has some tissue specific members (some common examples are shown in Table 2). The exception to this is for the bone marrow-derived cells that are found both within the blood and in tissue. For each of these cell types, emigration from blood into tissue is an important part of their surveillance role to seek out foreign material and tumours.

During ageing and in the absence of disease there is no evidence for change in numbers of macrophages or granulocytes although functional deficits in directional migration to infection are observed (105,123)and these are explored later in sections 5 and 7. There are also several subtypes of the phagocytic cells and the classical definitions e.g. of M1 and M2 macrophages describe M1 as proinflammatory and M2 as playing a role in tissue repair and tumorigenesis. Detailed discussion of these cells is beyond the scope of this review although it is worth noting that polarisation between subtypes is likely to be a continuous process and may, at least indirectly, be ROS-dependent (46).

Studies DC frequency number with age are conflicting. Some authors have reported that age is associated with an increase in frequency and maturity of myeloid DC (mDC, recognise a range of pathogens) (24) whereas others have reported no difference in mDC numbers with age (2). The numbers of plasmacytoid DC (pDC) that mediate antiviral responses have been suggested by some authors to be unaffected by age (24). However, others showed an age-associated decrease in pDCs (103). A similar effect is reported for DCs in the spleens of aged mice which showed reduced frequency of pDC (128). pDCs play an important role in neutralising virally infected cells by secreting interferons, increasing antigen presenting capacity and activating a specific T cell subset, NK cells, to kill virally infected cells. Overall, a consistent picture is emerging of a functional decline and decrease in frequency of pDC, but not mDC, in healthy elderly subjects. However, declining health in the elderly associates with loss of mDC although whether this is cause or consequence has not been explored (54).

Decreases in human Langerhan cell densities have been described in the epidermis of the ageing skin (9) again with polarisation towards a more mature phenotype (137). Others have shown that follicular dendritic cells (FDC) in old mice were smaller with lower chemokine CXCL13 expression in

response to challenge (117). The FDCs and homing DCs mediate T cell activation and initiate the first steps of the specific and adaptive immune response. They typically present antigens from invading pathogens to T cells in order to stimulate the adaptive immune response. To achieve an antigen specific response, T cells differentiate into effector Th1 cells, Th2 cells and cytotoxic T lymphocytes (CTL) after contact with antigen presenting DCs. Effector T cells are important mediators of vaccination responses. Several studies have examined T cell deficits during ageing, however, it is possible that defects in DC function with age may underpin the poor vaccination responses typically seen in older adults (116). While increased mitochondrial ROS production during ageing may contribution to DC senescence has not been explored to date, it has been shown that mitochondrial overexpression of catalase inhibited TLR7 mediated activation and cross-presentation e.g. of viral proteins by pDCs to $CD8^+$ T cells without affecting other immune functions (81).

Table 2: Major types and residency of specific phagocytes

Tissue	Macrophages	Granulocytes	Dendritic cells
Lung	Alveolar macrophages (lung)	Pulmonary resident neutrophils	plasmacytoid dendritic cells (p)DCs and conventional (c)DC
Blood	Monocytes	Basophils, neutrophils, eosinophils	Myeloid (m)DCs, plasmacytoid dendritic cells (p)DCs
Liver	Kupffer cells	Eosinophils (blood and tissue)	pDCs
Brain	Microglia		
Bone marrow	Osteoclasts	Neutrophils	Myeloid (m)DCs, plasmacytoid dendritic cells (p)DCs
Lymphoid tissue			Follicular dendritic cells
Skin	Langerhans cells		Epidermal langerhans cells and cDCs

To develop a specific immune response, molecules from foreign pathogens must be presented to the immune system. Macrophages and DCs can serve as antigen presenting cells (APCs). They phagocytose the foreign material, process and transport antigenic molecules e.g. derived from ingested pathogens to the lymphoid tissue. They act as a bridge between innate and adaptive immune systems by presenting antigens to naïve B and T cells in lymph nodes in association with co-stimulatory molecules such as MHC. T and B cell activation mediate the later adaptive immune response and retain memory of specific antigens from each pathogen that is encountered. Age-associated changes in uptake, processing, cytokine, chemokine and interferon production, as well as lower expression of co-stimulatory molecules such as CD86 could contribute to the blunted immune responses and therefore impaired memory B- and T-cell to vaccines and infection (10,24,72).

Pathogen recognition by the innate immune system - the effects of ageing

The innate immune response is activated when pathogens and pathogen-associated molecular patterns (PAMPs) are recognized by PRR-bearing cells. They include those with C-type lectin receptor- (CLR), scavenger receptor- (SR), and Toll-like receptors (TLRs) that are expressed by cells of the innate immune system and on epithelial cells (Table 3). SRs responsible for bacterial clearance also bind oxidised LDL e.g. CD36, MARCO and SR1. Due to the similarities between oxidatively damaged host derived lipids, lipoproteins and the structural motifs of microbial pathogens there is often overlap between PAMP and damage associated molecular pattern (DAMP) recognition (73). In the same way that PAMPs on bacteria are recognised and engulfed, oxidized low-density lipoprotein is also taken up by CD36 and triggers the NLRP3 inflammasome to activate caspase-1, pro-IL-1β/pro-IL-18 in macrophages (102). Readers are referred to recent review that explores redox regulation of the NLRP3 inflammasome (1).

Oxidised lipids frequently induce an inflammatory response through TLR4 (12,73,74). This is at least in part through the binding of oxidised cholesterol to MD-2 an LPS-binding receptor that interacts with TLR4, causing it to dimerize and recruit necessary adaptor proteins (20,90). This interaction has been shown to induce TLR4 dependent IL-6 and IL-4 production by macrophages (68,73) and is likely to be a bystander effect of an essential pathway that is normally used for pathogen removal. In contrast, the oxidised phospholipid oxPAPC inhibits the ability of innate immune cells to phagocytose and clear bacteria, increasing disease severity (70).

TLRs are found both as cell surface receptors and as intracellular endosomal membrane receptors e,g, TLR7. Pathogen recognition by surface receptors triggers dimerization and signals are transduced in the intracellular environment to increase expression of chemokines such as CCL2 and CCL7, thereby facilitating chemotaxis of incoming immune cells to inflammatory sites and phagocytosis.

Table 3. TLR family distribution between phagocytic cells and key ligands that act as microbial pathogen signals – effect of age

PAMPs	PRR	Cells	Expression with↑age	Reference
Bacterial triacyl lipopeptides e.g. Pam3CSK4	TLR1/TR2 heterodimer	Neutrophils, monocytes, dendritic cells,	↓ ↔	(87,93,119)

		Basophils, eosinophils		
Mycoplasma diacyl lipopeptides; gram positive bacterial lipoteichoic acid	TLR2/TLR6	Neutrophils, monocytes, dendritic cells, Basophils, eosinophils		
Fungal zymosan; fungal β-glycan; protozoal GPI-mucin; viral envelope glycoproteins; Neisseria porins; mycobacterial lipoarabinomannan; Candida phospholipomannan;	TLR2	Dendritic cells	↔	(27)
Viral dsRNA	Endosomal TLR3	Dendritic cells Monocytes	 ↔	(78,87) (72)
Gram negative bacterial LPS; viral envelope glycoproteins; protozoal glycoinsoitolphospholipids; fungal mannan; HSP70; oxidised lipids; oxysterols	TLR4 (plus CD14 and MD-2)	mDendritic cells Monocytes Neutrophils Macrophages eosinophils	↔ ↔ ↔	(27) (27) (49)
Bacterial flagellin	TLR5	Monocytes Epithelial cells	↑	(87,94)
Viral ssRNA	Endosomal TLR7/8	Monocytes pDC, eosinophils	↔ ↓	(87) (27) (54)
CpG DNA from bacteria, protozoa and viruses	Endosomal TLR9	pDC, eosinophils, basophils epithelial cells	↓	(54)

We have previously reviewed the effect of ageing on TLRs in macrophages and did not find any substantive evidence for a change in receptor density with age (30). We proposed that downstream signalling from the macrophage TLRs may be diminished with ageing in support of the evidence for age-associated increases in infection (30). Following receptor dimerisation after binding to pathogen, intracellular signalling proceeds via Toll/IL-1-like homology receptor (TIR) domain, TIRAP, TRIF, MyD88 and TRAM. Again, evidence is lacking for any age-specific differences in expression of these adaptor molecules during ageing (30).

Increased ROS production is widely described during ageing and this associates with an increase in macromolecular oxidation. The lipid oxidation product 4-hydroxynonenal forms adducts with TLR4 and the intracellular MD2 domain cysteine residues so preventing the essential dimerization for TLR4 receptor activation. This would be expected in turn to reduce or delay the effector functions of macrophages and impair host defence to infectious agents.

Evidence for the importance of TLR7 cysteine residues for pathogen removal and the negative effect of NOX2-dependent hydrogen peroxide production (presumably following the dismutation of superoxide anion) in macrophages has been reported by To et al (2017). Endosomal hydrogen peroxide generation was shown to suppress antiviral and humoral signalling networks via modification of Cys98 on TLR7 (111) which inhibits viral products e.g. RNA from binding to TLR7. The authors highlighted an important paradox that emerges from these findings – endosomal ROS are essential for killing but appear to promote viral pathogenicity. Why does such a system exist? The authors suggest that oxidative modification to TLR7 may be necessary to prevent autoimmunity to self-RNA.

Most innate immune cells are short-lived, however, engagement of PAMPs with TLR1 in neutrophils prevents apoptosis. In the elderly, reduced expression of TLR1 has been described, implicating a defect in apoptotic regulation with age (93). Cells continue towards apoptosis in the presence of TLR1 agonists but were defective in MAPK signalling and chemokine expression. This was attributed to an impairment in glucose uptake and cellular bioenergetics. These findings suggest that neutrophils in the elderly will be less able to kill bacteria based on their shorter lifespan alone, even if all other functions are intact (13).

Production of reactive oxygen and nitrogen species (RONS) by NADPH oxidases, nitric oxide synthase (NOS) and myeloperoxidase (MPO) is central to microbial killing. It is now recognised that PRR activation contributes to ROS production.

Pattern rich receptors and NADPH oxidase activation

An adhesion family of G-protein coupled PRRs has been implicated in pathogen uptake and results in the activation of NADPH oxidases by macrophages through Rac1/2 and p47 phosphorylation (11). In monocytes, protein kinase c theta has been identified as the downstream signalling kinase that activated NADPH oxidase and phagocytosis of zymosan (fungal) particles after binding to the CLR, dectin 1 (32). Following TLR activation, the downstream signalling molecule TRAF6 is pivotal. TRAF6 lies upstream of MAPK and NFkB activation pathways, both of which are ROS-dependent, implicating TRAF6 indirectly in ROS generation. NFkB activation is also required for expression of inducible NOS expression in phagocytes. A seminal paper by West et al in 2011 illustrated how TLR signalling via TRAF6 augments ROS by directing mitochondria to phagosomes (124). TRAF6 first translocates to mitochondria, binds to ECSIT (evolutionarily conserved signalling intermediate in Toll pathways), is ubiquitinated and enhances mitochondrial ROS generation at the phagosome in an Mst1 and MSt2 kinase specific manner (42,124). These studies highlight the complexity and potential redundancy of multiple receptors, pathways and activating signals in different cell types to support effective pathogen removal through ROS production by NOX2 and mitochondria.

RONS production in the ageing innate immune system

The NADPH oxidase, NOX2, was discovered by Babior in neutrophils in 1979 (39). Since the initial discovery of the multiple component enzyme, NOX2, six further NOX isoforms (NOX1, 3, 4, 5 and DUOX1 and 2) have been described. The organisation and activation of the NOX enzyme family has been extensively reviewed and they share similarities in structure and capacity to produce ROS (8). The expression of different NOX isoforms is cell- and tissue-specific. The first ROS to be identified was the superoxide anion radical which can spontaneously dismutate into hydrogen peroxide under physiological conditions. Hydrogen peroxide potentially regulates the target molecules through reversible or irreversible oxidation of redox-sensitive cysteine residues (97) (Figure 3). On the other hand, other NOX family members such as NOX4 and DUOX produce hydrogen peroxide directly.

NOX2 was described as essential for the killing of bacterial pathogens by professional defenders in the innate immune system, particularly in neutrophils and macrophages (96). Of the NOX isoforms, NOX2 remains the most significant in terms of quantity and duration of ROS production (84). Despite this, NOX2 has a relatively low Km for oxygen in its resting state. The phosphorylation of different serine residues in p47 by protein kinase c alpha, beta II, delta, and zeta in neutrophils promotes migration to gp91 phox in the membrane, then assembly of the complex (37). Production of the primary radical species from NOX2, superoxide anion radical, is dependent on a supply of NADPH and oxygen (Figure 1). Superoxide anion radicals are released to the extracellular face of the membrane where they may dismutate to hydrogen peroxide and diffuse across lipid bilayers to

mediate intracellular signalling and activate MAPK (129). Conversely, after uptake of pathogen and membrane internalisation, superoxide anion radicals are released into the phagosome.

The importance of NOX2 in preventing bacterial infections is highlighted in the disease chronic granulomatous disease (CGD), where patients exhibit persistent bacterial infections (O'Neill, 2016). Several mutations have been described in different proteins within the NOX2 complex that give rise to CGD, but ultimately, they all share the same deficit – a failure to produce ROS and to develop an effective phagocytic response.

Bacterial killing would normally take place inside the phagosome. Subsequent fusion with granules rich in enzymes facilitates killing (47). MPO is released from the azurophilic granules into phagosomes where it catalyses the production of hypohalous acid from hydrogen peroxide and halide ions. Associated with the generation of ROS is an immediate rise followed by a sustained rise of pH in the phagosome which has been suggested to be an essential part of the killing process (80,101). An ineffective NOX2 complex, as seen in CGD patients, leads to accumulation of bacteria and the formation of granulomas within tissue.

The literature concerning innate immunity and ROS production by innate immune cells during ageing is conflicting as summarised in Table 1 (5,6,9,14,24,121). This may relate to study design. There is an inherent difficulty in studying ageing *in vivo* due to the length of time for an ageing phenotype to be evident. Most human studies take a cross-sectional snapshot of different ageing populations do not follow the same individual over time. Instead, studies frequently compare groups of older adults with younger adults with the assumption that older people would ne physiologically worse off. However, humans that live to older age when surrounded by pathogens may be considered the survivors of biological ageing and this is likely to be a consequence of their genes and environmental mix. Older survivors may express a unique survivor phenotype and this may not be typical of changes seen during ageing in the majority of the population. Animal model studies of ageing enable longitudinal studies to be performed and at the very least, use animals of the same genotype within the same environment. However, despite numerous studies designed to explore NOX2 knockout on the ageing process, some describing benefit and others showing increased risk, only two have looked at effects on the immune system. One showed that joint inflammatory and immune responses were enhanced with age in the absence of NOX2, supporting a crucial role for NOX2-dependent ROS generation in the modulation of Th17/Treg cell development during ageing (65). In contrast, another study showed the ablation of NADPH oxidase promoted neutrophil recruitment in the lung but reduced inflammation (139). A third study showed that NOX2-dependent ROS activation was higher

in older animals and was enhanced by mitochondrial ROS, suggesting intracellular cross-talk via ROS (63). However, functional outcomes were not reported.

Mitochondrial and NOX-derived ROS in the phagosome during ageing

Our understanding of how TLR signalling to ROS production is affected during ageing is limited. One study has shown that TRAF6 is less ubiquitinated in lung macrophages from older mice which are less able to activate NFkB and phagocytose stimuli (50). This might also be expected to reduce endosomal ROS released because the efficiency of mitochondrial trafficking to the phagosome is impaired, however, mitochondria themselves release more ROS in monocytes with ageing (88). The inter-play between mitochondrial ROS and NOX activation in phagocytes could contribute to increases in phagosomal ROS during ageing (63) which may impede antigen processing. Taken together, these studies suggest a complex interplay between TLR activation, ROS production and signalling that may be cell and receptor specific (57).

ROS and infection in older adults

In human cross-sectional studies it has been shown that with age, immune defence is compromised. In older adults, there have been conflicting reports about the extent to which impaired ROS production might contribute to an increase in infection rate and failure to kill pathogens effectively. In the neutrophil, few studies have reported an increase in ROS production (14) (86) (100) (15), with no change in MPO products (14) whereas the majority have reported a decrease in ROS production in response to some (e.g. fMLP) but not to other (e.g. zymosan) ligands (13) with ageing (see Table 1). In a rat model, a similar deficit in neutrophil respiratory burst in response to the bacterial peptide fMLP was reported. This was attributed to an increase in membrane fluidity due to declining cholesterol/phospholipid ratio and increasing PUFA, although it is difficult to appreciate why this might affect the fMLP activation response exclusively (4). In older adults, infections frequently develop during hospitalisation e.g. after a fall. Baehl et al explored whether the stress associated with hip fracture had any influence on neutrophil function. They showed the respiratory burst was impaired shortly after the event and that this effect was sustained despite restoration of phagocytic ability (6).

In monocyte/macrophages, a mixed picture has emerged of a decrease in stimulus-induced ROS production by monocyte NADPH oxidase (5,14) with age although low level activation may persist with others describing an increase in ROS from mitochondria and from NADPH oxidase (89,138).

Circulating monocytes are versatile precursors with the ability to differentiate into a variety of macrophages (43). Different macrophage subsets develop in response to the local cytokine milieu

and play a role in the regulation of innate immune responses to acute and chronic inflammatory stimuli. The M1 macrophage is associated with chronic inflammation whereas an M2 phenotype promotes healing, tissue remodelling but also cancer. Mitochondrial derived ROS have been shown as early differentiation signalling to yield M2 tissue associated macrophages, and it has been proposed that inhibition of mitochondrial ROS , which is elevated during ageing, may be a useful cancer target. In summary, dysregulation of macrophage differentiation may lead to defects in wound healing, development of autoimmunity and cancer (35), all common traits in older adults.

Redox regulation of phagocyte function

Innate immune cells have the capacity to sense danger and migrate in a directional manner to pathogens. This is achieved through tethering of leukocytes to the vascular wall local to the inflammatory site. This process is enhanced by circulating oxidised LDL (74). Oxidised phospholipids can modulate vascular endothelial cell adhesion molecule expression whereas 7-ketocholesterol increases leukocyte MAPK activation and expression of adhesion molecule ligands (45,110). Following adhesion to the endothelium, leukocytes undergo diapedesis into tissue. The first step towards limiting infection or removing damaged tissue is phagocytosis of foreign material. To achieve this, neutrophils exhibit active actin dynamics involving local polymerisation and de-polymerisation, by a ROS-dependent mechanism and oxidation of thiol moieties (99). The homeostatic control of thiol redox state is supported by transient mixed disulphide formation between cytoskeletal thiols and glutathione (GSH). To restore thiols and function to actin, de-glutathionylation is catalysed by the enzyme glutaredoxin (Grx, Figure 3). Grx is a small thiol transferase that removes GSH adducts from proteins (protein -S-SG) using either a monothiol or dithiol mechanism and participates in redox signalling.

$$\text{Grx-SH} + \text{protein-S-SG} \rightarrow \text{Grx-S-SG} + \text{protein-SH}$$

Using Grx1-deficient mice, which do not remove GSH from mixed disulphides, it has been shown that NOX2-dependent ROS generation negatively regulates actin polymerisation in neutrophils through irreversible glutathionylation (99). Similarly, Grx1-negative neutrophils showed impaired actin polymerisation and chemotaxis to sites of inflammation. In addition, adhesion, phagocytosis and bactericidal activity were all inhibited when Grx1 was absent. In another example of RONS-dependent changes in actin polymerisation, Garcia Ortiz et al (41) have studied the immune synapse, which is a localised clustering of receptors in lipid rafts within different types of immune cells that are required to communicate for an effective immune response. After receptor crosslinking between

immune cells, the recruitment of a serine/threonine-specific protein kinase C theta is necessary for the formation of signalling complexes and downstream cytokine transcription. It has been elegantly demonstrated that nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) controls the clustering of protein kinase C-theta in the immune synapse. The molecular mechanism that underpinned the effect of NO was due to S-nitrosylation of beta-actin on Cys374 which prevented effective polymerisation and translocation of protein kinase C (PKC)-theta to the immune synapse. Actin binding to profilin-1 was impaired and PKC theta activation, necessary for NOX activation, was diminished. Collectively, these examples illustrate how a reducing environment may favour phagocytosis and immune activation.

GSH is a cysteine-containing tripeptide that is common to all mammalian cells. Combined with NADH and NADPH, it forms the major source of cellular reducing potential. Under oxidising conditions, it is rapidly oxidised to form glutathione disulphide (GSSG) or to form mixed disulphides with redox sensitive thiols in cellular proteins. Through a sequence of reactions with other reducing enzymes/proteins, it is largely responsible for repairing reversibly oxidised thiols (see Figure 3). These are largely under the regulatory control of redox-sensitive transcription factors such as Nrf2. Normally, Nrf2 has a short half-life because it is maintained in a complex with KEAP1 and Cullin 3 which ubiquitinates Nrf2 and transports it to the proteasome for degradation. Under oxidative stress, KEAP1 becomes oxidised, dissociates and Nrf2 accumulates, migrating to the nucleus to promote expression of antioxidant proteins. As intracellular reducing potential declines in phagocytic cells(122), Nrf2 is activated and more reducing proteins related to the GSH cycle are synthesised, however, this process is impaired during ageing (31). GSH and GSH-related enzyme activities decrease with age (19). Glutathione reductase (GSR) expression is regulated by Nrf2 and catalyses the reduction of glutathione disulphide back to glutathione. Interestingly, it has been shown that GSR-null mice exhibit elevated sensitivity to *Staphylococcus aureus*. Consistent with the findings in Grx1 deficient mice, neutrophil phagocytosis and bacterial killing was impaired in GSR-null mice (130).

In human studies of opsonized bacteria including *Staphylococcus aureus*, yeast and zymosan uptake, a significant reduction in phagocytic ability was observed in neutrophils from older adults (33,71,123). One study has shown that phagocytosis of Candida is unaffected by ageing (27). None of the studies in humans have explored whether s-nitrosylation or glutathionylation of actin or other cytoskeletal regulatory proteins in neutrophils may contribute to impaired phagocytosis.

Once taken up into a phagosome, microbes are killed by ROS which serve an additional role in processing of antigenic peptides for surface display by antigen presenting cells (3,62). Killing is

achieved by fusion of the phagosome with lysosomes that release enzymes such as MPO, lysozyme and elastase in concert with ROS generated by NOX activity (47,75). In addition to producing cytotoxic ROS, the acidification of the vacuole to around pH4 is bacteriostatic and may provide an optimal pH for lysosomal cysteine protease activity required for processing of antigens and development of the adaptive immune response (23,34).

A more recently described phenomenon which operates as the neutrophil dies is the release of neutrophil extracellular traps (NETs). NETs comprise of nuclear material and granule enzymes that entrap bacteria and promote the clearance of microorganisms (104). It has been shown that hypochlorous acid is required and mediates the formation of NETs in humans (85). It is therefore not unexpected that in older adult neutrophils, which may produce less ROS, LPS- and interleukin-8-induced NET formation exhibited a significant age-related decline (49). Furthermore, GSR-deficient neutrophils display a marked impairment in NET formation. The latter studies suggest that GSR-mediated redox regulation is required for bacterial clearance via NETs (130). The induction of genes that are responsible for glutathione homeostasis such as GSR, Grx1 and gamma-glutamyl cysteinyl synthase e.g. by Nrf2 activators may provide an important strategy for restoration of neutrophil mediated killing efficacy during ageing (58,104).

NOX is required for macrophage polarisation

ROS generation by NOX2 has been implicated in the polarization to M2 macrophages, but not M1 macrophages in the mouse (129,138). As discussed earlier, the activation of MAPK enzymes is important in immune cell activation and they are involved in macrophage polarisation. These include the kinases JNK and ERK, which are redox sensitive. Macrophage differentiation in NOX1/2 knockout animals was impaired with an inability to polarise towards an M2 macrophage phenotype (129). This was associated with a delay in wound healing and the inhibition of tumour growth and metastasis. In summary, these authors suggested that NOX1/2 KO enhances tumour growth. In contrast, a study of p47phox-/- mice showed an increase in production of M2 macrophages that was associated with decreased ROS production (131).

To gain a better physiological understanding of the importance of NOX-dependent ROS signalling for ageing and longevity, knockout models of the adapter protein p66Shc have been developed. P66Shc is an adapter protein that links activation of the growth factor receptor and the downstream MAPK cascade and can be directly activated by H_2O_2. The $p66Shc^{-/-}$ mouse is unresponsive to growth factor activation and is long-lived. It is characterised by a decrease in macrophage ROS production and is associated with a reduction in inflammation and increased longevity (112). Lower NOX activity in macrophages was suggested to contribute to increased longevity. Further work will be necessary to

unravel why different knockdowns that inhibit NOX2 can on the on hand promote a reduction in inflammation and longevity but on the other hand, impair M2 polarisation.

Table 4 summarises our present understanding of the cellular localisation of different NOX isoforms within cells that are responsible for immunity. No systematic study has been undertaken to explore whether expression of NOX and DUOX changes in immune cells during ageing , however, studies in endothelial cells suggest that NOX4 is upregulated with age and my contribute to paracrine oxidative stress (67,120).

Table 4. NOX localisation in cells involved in immune defence – the effect of ageing

NOX	Immune cell types	References
NOX1	Astrocytes, microglia, macrophages, epithelial cells	(18,52,79,129)
NOX2	Macrophages, granulocytes, DCs	(62,80,106)
NOX3	ND	
NOX4	Macrophages?	(52)
NOX5	Spleen, thymus, lymph nodes, monocytes/macrophages	(7,8,69)
DUOX1	Mucosal cells	(48)
DUOX2	Mucosal cells	(48)

Dendritic cells – at the interface between the innate and adaptive immune system

Dendritic cells (DCs) are one of a few professional APCs. In common with other phagocytes, they recognise PAMPs and DAMPs from exogenous pathogens and tumour cells in the periphery and phagocytose them or take them into the cytoplasm by pinocytosis. DC activation by TLR4 ligands induces their homing to lymph nodes where they engage with naïve T cells and promote adaptive immunity (60). Antigen presentation in the lymph nodes is critically controlled to ensure that self-antigens are tolerated and foreign antigens are targeted.

DCs in ageing

Three key signals are necessary to induce a T cell response and these are provided by DCs. These are antigenic peptide-loaded onto one of three major histocompatability complexes (MHC I-III), proinflammatory cytokine secretion and expression of the costimulatory surface antigens, CD80 and CD86. To produce these signals, DCs must first phagocytose and process the antigen (Figure 2).

DCs from elderly individuals have a significantly reduced capacity to phagocytose pathogens, an impaired chemotactic response to SDF1, increased proinflammatory cytokine secretion after TLR engagement (2), impaired IL-12 and IFN-α production by mDCs and pDCs respectively (24,103). TLR-mediated expression of CD80 and CD86 is critical for vaccine immunity. Older adult TLR-induced CD80 expression in monocytes is lower than in young adults and is associated with an impaired response to vaccination (118). These studies highlight the need to better understand the biology of DC maturation to support strategies that can improve immune response.

After pathogen uptake into a phagolysosome, proteins are degraded by cathepsins, lysozyme and elastase and ROS, then the digested peptides may become ligands for MHC II receptors within the phagosome membrane. This type of proteolysis creates peptides of 14 to 20 amino acids in length that are alternatively loaded into MHC II complexes (3) Peptide-loaded MHC-II molecules are delivered to the cell surface either directly or via an intermediate tubular MHC-II storage compartment where they will ultimately activate the classical CD4+ T cell response.

MHC I molecules are usually loaded with peptides derived from cytosolic proteolysis of intracellular antigens for activation of effector CD8+ cytotoxic T lymphocytes to promote the killing of virally infected cells. Foreign antigens within the cytoplasm are degraded by the immunoproteasome into peptides of between 8 and 10 amino acids. These are then loaded into MHC I complexes within the ER and presented on the cell surface. However, phagosomes within a subclass of DCs are specialized for cross-presentation, an unusual process by which phagolysosomal protein antigens provide ligands for MHC-I for activation of effector CD8+ cytotoxic T lymphocytes. In older adults, cross-presentation is defective (21,134). However, in vitro scavenging of ROS partially restores cross-presentation by aged DCs suggesting that excess phagolysosomal ROS may prevent cross-presentation. In support of an important role for reducing activity in the endosomal lumen, gamma-interferon-inducible lysosomal thiol reductase is a thioredoxin-related oxidoreductase found in DCs. It reduces disulphide bonds, promotes unfolding and assists in MHC class II-restricted antigen processing, e.g. through exposing cryptic antigens that are normally present in disulphide crosslinked proteins and also in MHC class I-restricted cross-presentation (125).

Redox regulation of antigen presentation by DCs

The development of a CD8 cytotoxic T cell response through cross presentation of cytoplasmic peptides is important for tumour immunity and for overcoming viral evasion tactics. In contrast to the inhibitory effects on cross-presentation ascribed to ROS in ageing DCs, NOX2 has been proposed to promote cross-presentation that are essential for defence against viral infection. An elegant study of dengue virus infected DCs using genome-wide transcription analysis showed three early and discrete responses to infection, STAT1 signalling, NFkB activation and an Nrf2 dependent oxidative stress response that arose following an increase in NOX2-dependent ROS production. ROS were essential both for inducing apoptosis in infected cells and also for activation of bystander cells (83). ROS production by DUOX has also been identified as host-protective in mucosal cells against influenza infection, by inhibiting the spliceosome required for RNA processing (107).

In DCs, NOX2 activation changes the endosomal lumen pH to inhibit lysosomal proteases and prevents excessive antigen degradation (62). ROS-mediated oxidation of redox sensitive thiols on cathepsins will also impede endosomal peptide processing (3), promote endosomal membrane lipid peroxidation and release of antigen into the cytosol for proteasomal degradation (26). Similarly, Kagatani has described how DC maturation is enhanced by sensitisers that oxidise cell surface thiols but inhibited by the reducing agent, N-acetyl cysteine (55). DC maturation under oxidising conditions was associated with an increase in p38 MAPK signalling.

In contrast, others have shown using $p47^{phox}$ and catalytic subunit NOX2-deficient DC that ROS are required to regulate DC IL-12p70 expression and inhibit inflammation, reducing CD4+Th1 activation. Similarly, GSH depletion in APCs was correlated with impaired antigen processing and reduced secretion of Th1 cytokines, while an increase in intracellular GSH content increased the expression of inflammatory IL-12, and increased differentiation of naive CD4+ T cells to Th1 cells. Moreover, GSH inhibited the replication of viruses and bacteria (38). $O_2^{\bullet -}$ produced within phagolysosomes rapidly dismutates to H_2O_2 and regulates PI3K and p38-MAPK signalling pathways, so reducing IL-12p70 expression (53). Zanoni *et al* have demonstrated that oxidised 1-palmitoyl-2-arachidonyl-sn- glycero-3-phosphorylcholine (oxPAPC) and LPS bind to separate domains of caspase 11 in DCs which leads pro-IL-1β cleavage yielding IL-1β and other synergistic inflammasome-mediated events. LPS alone is able dimerize TLR-4 and activate pyroptosis, however, the addition of oxPAPC increases DC viability and is thought to prime the inflammasome in such a way as to hyperactivate DCs and increase T cell activation through IL-1β. It is noteworthy to mention that oxPAPC failed stimulate macrophages, this could in part be explained by DC existing in more highly primed state than macrophages (136).

Antigen processing is therefore affected by the extent and locality of ROS production or oxidised phospholipid. There is a suggestion that ROS production in DCs may be important at least for cross-

presentation in health but there is ongoing debate about the importance of ROS in these processes (106). In a recent study using gp91$^{-/-}$ mice and the cytochrome inhibitor apocynin, NOX2-dependent ROS formation was not required for maturation of bone marrow derived dendritic cells or for T cell activation Similarly, functional analysis of the role of NOX2 in human mDCs showed that NOX2 dependent superoxide anion production did not play a role in DC differentiation, maturation, cytokine production or induction of T cell proliferation, but was essential for intracellular bacterial killing (44) (106).

Some of these seemingly contradictory studies may be due to a focus on ROS derived from NOX2 only. As described earlier, mitochondrial recruitment and enhancement of ROS production within the endosomal compartment is proving to be of great significance to macrophage function and polarisation. As mitochondria leak increasing amounts of ROS with age but without the corresponding pH regulatory potential of NOX2, excessive antigen processing may occur in the endosome, so minimising cross-presentation by DCs in older adults. The other key component to redox state is the extent of reduced cofactors such as NADPH and thiol protective glutathione present in cell compartments. These are dependent both on metabolism and on Nrf2 dependent gene expression.

In an ageing animal model with reduced contact hypersensitivity, Kim et al showed that poor immune response was likely to be due to poor presentation by DCs through decreased Nrf2-mdeiated antioxidant enzyme expression and GSH synthesis. While transfer of old DCs to young mice impaired the immune response, both the presence of N- acetyl cysteine or sulforaphane to induce GSH synthesis restored the in vivo challenge response (59), highlighting the potential to boost Nrf2 during ageing to enhance immune response.

T cells in ageing

The interface between the innate and adaptive immune responses is at the immune synapse which is formed between T cells and antigen presenting cells, such as DCs (see graphical abstract). An impaired synapse in the elderly could explain increased risk for autoimmunity, infection and poor vaccination response. Several studies have shown that the ability of macrophages and DCs to activate T cells from older adults and to stimulate T cell proliferation is reduced with ageing whether the DCs were from young or older adults suggesting an inherent T cell deficit (72,133). An effective and sustained T cell response requires a reducing environment (38,113).

T cell recognition of presented antigen

Once naive T cells recognise foreign antigenic peptides within an MHC complex on the surface of APCs, they mature into effector T cells. One of the major subsets activated by MHC I carrying peptides is the CD8+ T cell. Activated CD8+ T cells do not prevent infection but target infected cells to reduce, control and clear intracellular pathogens, either directly by killing infected cells through release of perforin, or indirectly by killing infected cells through antimicrobial cytokine release. Similarly, CD4+ Th1 cells do not prevent but help to reduce, control and clear extra- and intracellular pathogens by producing IFN-γ, TNF-α/-β, IL-2 and IL-3 and supporting the activation and differentiation of antibody producing B cells, CD8+T cells and macrophages. CD4+ Th2 cells produce IL-4, IL-5, IL-13, IL-6 and IL-10 and support B cell activation and differentiation. Once activated, T cells no longer home to the lymph node but instead they circulate to the area of infection where they will be triggered by recognition of antigen to release the cytolytic molecule perforin or effector cytokines depending on the T cell type. The decision to proliferate and differentiate into Th1 or Th2 effector T cells is dependent on energy supply and the cytokines released by DCs. IL-12 and IL-27 for example are Th1 polarizing signals whereas the chemokine CCL2 is a Th2 signal, and both yield an antibody response. Lipid metabolism plays a key role in the switch to proinflammatory and autoreactive T cells, in manner that is intrinsically linked to redox state (16) and ageing (113). Knockout of thioredoxin inhibitory protein (TxNIP), i.e. the reducing protein thioredoxin (linked to GSH, see Figure 3) was active, resulted in inhibition of lipid metabolism (82). In addition, fatty acid metabolism at the mitochondrion which is a net consumer of reducing potential would be expected to yield increased mitochondrial ROS production, particularly with age. Modulating lipid metabolism, which is intrinsically linked to redox state, is an intense area of investigation for repurposing drugs to modulate aberrant immune responses (51).

Redox regulation of T cell proliferation in response to antigen

The ability of T cells to generate specific response to antigen is dependent on the oxidation state of cell surface and cytoplasmic protein-thiols (91). Intracellular thiols are maintained in their reduced state by a network of redox regulating peptides, proteins and enzymes such as glutathione, thioredoxin (Trx) and thioredoxin reductase (Figure 3). Yodoi's group were the first to clone cloned human Trx as adult T cell leukemia derived factor produced by HTLV-I transformed cells and then to demonstrate that overexpression of Trx results in resistance to oxidative stress and a possible extension of life span (77,109). Later studies have shown that overall lifespan is not affected by overexpression of Trx1, but that the early part lifespan is improved, particularly in males (92). Further studies that focussed on calorie restriction, known to slow ageing in animal models, confirmed that the decline in Trx and GSH observed with age are mitigated by calorie restriction, indicating that age-

dependent redox change can be mitigated by calorie restriction (19). We have previously shown that healthy older adults have reduced lymphocyte surface expression of Trx1 and lower circulating plasma Trx1 concentrations. This associated with reduced proliferation and IL-2 production. Correspondingly, it would be anticipated that more T cell surface cysteines would be oxidised (17). This has been investigated in a systems biology approach to examine the immunopeptidome. It revealed an over-representation of cysteine-containing peptides as T cell epitopes. They comprise between 5–10% of the immunopeptidome from a study of 70,000 peptides. Many of the cysteine residues in peptides were oxidised and expressed as *S*-glutathionylated peptides. This was more prevalent for presentation to virus-specific T cells and may represent a mechanism for evading the virus-specific T cell response (115). Similarly, it might be predicted that increased peptide glutathionylation would occur due to an increasingly oxidative environment in ageing DCs and that this might also reduce T cell recognition and response to pathogen. Indeed, persistent pathogens like cytomegalovirus that are common in older adults may also evade the effector pathway through peptide glutathionylation. An increase in intracellular DC GSH content stimulated IL-12 and/or IL-27, which in turn induced differentiation of naive CD4+ T cells to Th1 cells (38).

Together these lines of evidence support the importance of using agents that modulate intracellular redox state in the innate immune dendritic cell to improve antigen specific immunity at the immune synapse. An additional benefit of increased Nrf2 activation for APCs at inflammatory sites, is the ability to resist oxidative damage and cell death (122). The timing and extent of reducing versus oxidising environment are thus predicted to exert a strong influence on and the effectiveness of adaptive immunity.

Therapeutic targets for redox modulation in vaccination

If dysregulation of redox state plays a role in an impaired immune response during ageing, it is anticipated that restoration of the reducing and oxidising potential at specific sites and times may improve innate immune defence (Figure 4). Cells of the innate immune system derive from bone marrow where increased ROS production is attributed to resident stromal cells in the ageing niche (108). In bone marrow derived stem cells (BMSC), increased expression of NADPH oxidase during ageing is a major source of ROS and associates with senescence. Apocynin, a free radical scavenger and NOX inhibitor, restored mitogenic potential in BMSCs and in vivo (108). Dysregulation of endothelial redox state may also influence BMSCs. This raises the possibility that cells of the immune system are a bystander for ageing effects of ROS generated by vascular cells. However, it is important to remember that ROS are inherently unstable with a half-life of nanoseconds, with limited capacity to diffuse beyond their site of production. Nevertheless, peroxynitrite and hypochlorous acid produced by endothelial cells may diffuse within blood vessels and potentially

may oxidise migrating immune cells. Distant effects of senescent endothelial cells on BMSCs have also been reported, reducing both their stemness and proliferative capacity. Ageing endothelial cells express elevated NOX4, produce more ROS, show enhanced activation of NFkB and secrete proinflammatory cytokines resembling a senescence associated secretory phenotype (SASP) (28,29). Co-culture of senescent human umbilical vein endothelial cells enforced proinflammatory cytokine secretion by bone marrow -MSCs and increased expression miR-126a-3p, that targets the stemness gene, SOX2 (64). The antioxidant TEMPOL has been used effectively to maintain endothelial function in ageing mice by reducing NOX4 expression and decreasing ROS production, however, whether TEMPOL's protective effects either directly or indirectly via endothelial cells can preserve BM MSC function during ageing is unknown (36). Similarly, activation of endothelial SIRT1 in ageing mice by SRT1720 was also effective in reducing inflammation, although the mechanism was independent of NOX4 expression but related to increased antioxidant enzyme expression and enhancing cyclooxygenase 2 signalling (40). Targeting the ROS production by NOX4 in the endothelium during ageing as a systemic driver of paracrine ageing e.g. of vascular or immune cells is an underexplored area that merits further study (67).

After exiting bone marrow and entering the circulation, phagocytes recognise and take up pathogens via PRR. This leads to activation of NADPH oxidase-dependent intracellular signalling and inflammatory cytokine production (124). There is evidence for molecular mimicry between DAMPs and PAMPs such that endogenously produced oxidised lipids are also potent PRR activators (74). Stimulation of macrophages by oxidised cholesteryl esters has been shown to cause lipid build-up through macropinocytosis and the release inflammatory cytokines (20). This is at least in part through the binding of oxidised cholesteryl to MD-2 an LPS-binding receptor that interacts with TLR-4 causing it to dimerize and recruit necessary adaptor proteins (20,90), then to increase IL-6 and IL-4 production by macrophages (68,73). As the purpose of vaccination is to increase the production of cytokines from phagocytes, one potential redox target that could be manipulated to enhance vaccination responses is to present any desired antigen within an oxidised lipid formulation (135). Liposomes synthesised using oxidised lipids could be explored in vaccine adjuvant design, particularly for the development of Th2-biased vaccines for production of neutralising antibodies.

Another opportunity for redox manipulation in vaccine design is to tackle the role of NOX2 activity in antigen presentation. NOX2 generates superoxide anion radicals in the endosomal lumen of DCs that dismutate to hydrogen peroxide and inhibit proteolytic activity (98), so limiting excessive degradation of peptides. Moreover, in ageing increased mitochondrial superoxide production (21) may impair antigen presenting function due to disulphide crosslink formation (114). In this case, the thiol donor N-acetyl cysteine is protective (21). After activation and homing to lymph nodes for

encounter with naïve lymphocytes, increased GSH also positively regulates the later adaptive phase of immune function through increasing IL-12 and IL-27 production, thus promoting a Th1 mediated immune response (38) and viral protection.

Looking Forward

The importance of ROS in adaptive immunity is well documented, however, the redox modulatory potential of adjuvants merits of further exploration. From a vaccinology standpoint, oxidised lipids are appealing new areas of adjuvant development and discovery. The oxidation state of the delivery system of vaccine antigens such as liposomes and virosomes could yield more potent adjuvants able to elicit a greater immune response particularly in older adults.

Using adjuvants, timely manipulation of the redox state with reducing agents, enzymes such as Trx1 (132) or Nrf2 activators may guide the immune response through to T cell interactions with APCs. In combination with other vaccine adjuvant strategies to further extend antigen release times, improve antigen uptake or prolong depot effects, the addition of redox modulators could offer a new redox-tailored approach to adjuvants.

Abbreviations

APC	antigen presenting cell
BMSC	bone marrow derived stem cell
cDC	conventional dendritic cell
CGD	chronic granulomatous disease
CLR	C-type lectin receptor
CTL	cytotoxic T lymphocyte
DAMP	damage associated molecular pattern
DC	dendritic cell
eNOS	endothelial nitric oxide synthase
ERK	extracellular receptor kinase
FDC	follicular dendritic cell
fMLP	formyl methionine leucine phenylalanine
γGCL	γ-glutamyl cysteinyl ligase
GRX	glutaredoxin
GSH	glutathione
GSR	glutathione reductase
GSSG	glutathione di-sulphide
IFNγ	interferon gamma
IL	interleukin
JNK	jun kinase
LPS	lipopoylsaccharide
M1	m1 macrophage
M2	m2 macrophage
MAPK	mitogen activated protein kinase
mDC	dendritic cell
MHC	major histocompatibility complex
MPO	myeloperoxidase
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NETs	neutrophil extracellular traps
NFkB	nuclear factor kappa B
NLR	NOD like receptor
NO	nitric oxide
NOS	nitric oxide synthase

NOX	NADPH oxidase
oxPAPC	oxidised 1-palmitoyl-2-arachidonyl-sn- glycero-3-phosphorylcholine
PAMPs	pathogen associated molecular patterns
pDC	plasmacytoid dendritic cell
PKC	protein kinase C
PRR	pattern recognition receptor
RNS	reactive nitrogen species
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
SASP	senescence associated secretory phenotype
SR	scavenger receptor
Th	T helper
TLR	toll like receptor
TNFα	tumour necrosis factor alpha
Trx	thioredoxin
TXNip	thioredoxin inhibitory protein

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No competing financial interests

Legends

Figure 1: NOX2 (gp91phox) construction from subunits.

A(1) Intracellular vesicles contain NOX2 and p22phox when in a resting state. These proteins co-stabilise one another and are found in close proximity. (2) When a neutrophil becomes activated Rac trades GDP for GTP triggering its activation.

B(3) Cytosolic p47phox is phosphorylated changing its conformation and allowing association with p22phox. This enables the assembly of the full NOX2 complex in the intracellular vesicle. (4) This vesicle then makes its way to the plasma or the phagosomal membrane where NOX2 transports electrons from cytoplasmic NADPH across the membrane to oxygen creating the superoxide anion radical $O_2^{-\cdot}$

Figure 2: Phagosomal degradation of bacteria

(1) Bacteria presenting fMet-Leu-Phe peptides activate Rac2 and are engulfed through phagocytosis into the neutrophil. **(2)** Primary and secondary granules fuse with the phagosome bringing p22 and gp91 phox into the phagosomal membrane. **(3)** Rac2, p22 and gp91 phox are used in the assembly of NADPH oxidase. This generates $O_2^{\cdot-}$ within the phagosome. **(4)** Ion influx due to acidification discharges granule proteases from the granule matrix.

Figure 3: The critical role of H_2O_2 in modulating redox state.

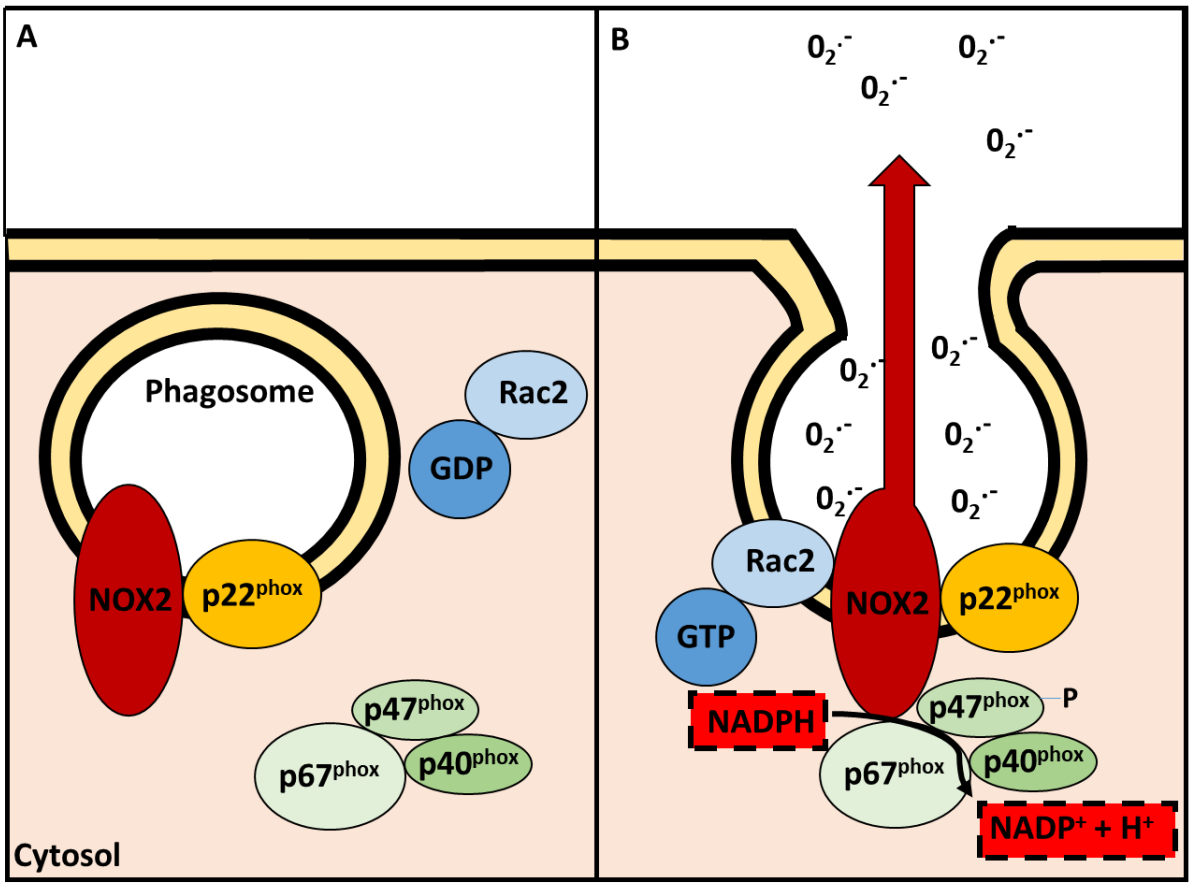
Reducing agents are shown in blue, bold and oxidising in red, italics.

Figure 4: Altered redox state during ageing affects the immune response

Developing stem cells in the bone marrow are exposed to higher NOX2 and mitochondrial ROS that reduce their stemness and increases proinflammatory cytokine secretion. Stemness was protected and inflammatory cytokine production was decreased by apocynin, which inhibits iron-sulphur cluster enzymes such as found in NADPH oxidase.

In the circulation, antigen presenting cells and neutrophils circulate and are exposed to increased concentration of oxidants produced by senescent endothelial cells as they emigrate into tissue. An impaired directional response to chemotactic stimuli has been described in neutrophils from older adults, which subsequently show an impaired ability to produce NETS and to kill pathogens. Specific ROS-producing chemical entities localised to neutrophils may improve bacterial killing.

A persistent deficit in Nrf2 activation has been reported in ageing dendritic cells that impairs their ability to regulate antigen processing and present to Th1 cells. The Nrf2 activator sulforphane and N-acetyl cysteine have both been shown to improve antigen presenting capacity of DCs from ageing donors. Finally, the T cell proliferative response depends on reduced T cell proteins involved in the immune synapse and signalling. Activators of Nrf2 may improve T cell help for B cell antibody production within the adaptive arm of the immune response.


A
B
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Rac2
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NOX2
p22phox
p47phox
p67phox
p40phox
Cytosol
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Rac2
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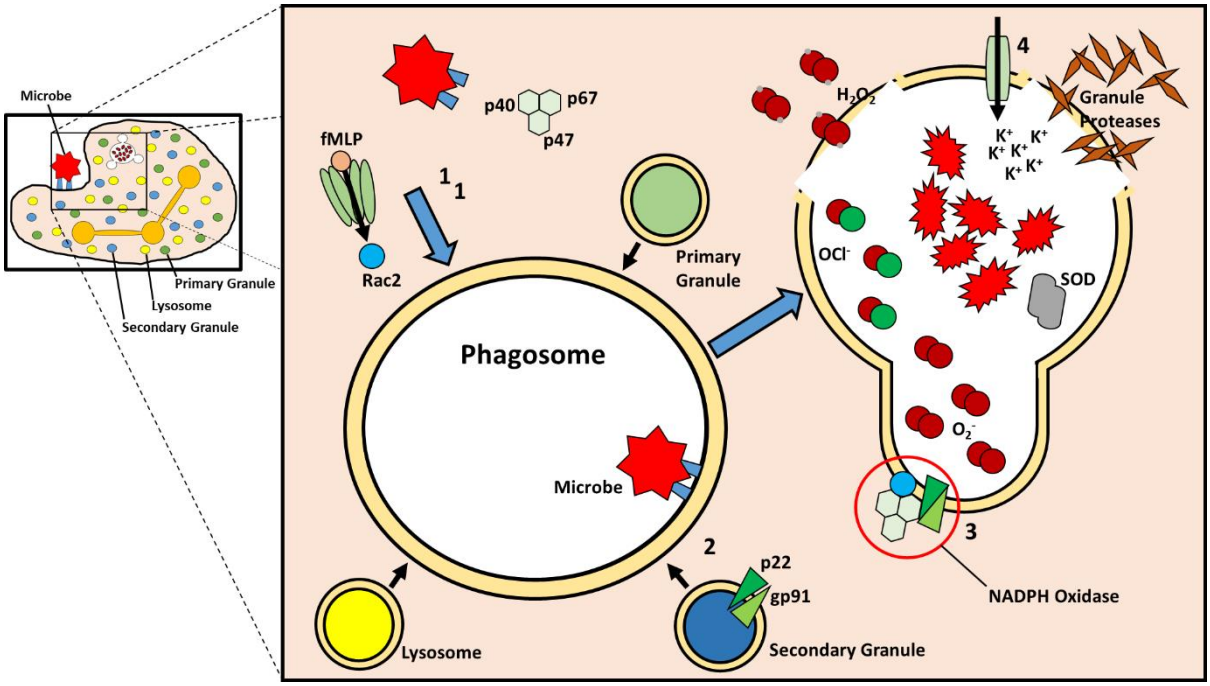
$NADP^+ + H^+$

Figure 1


Microbe
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1
Primary Granule
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p22
gp91
Lysosome
Secondary Granule
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H_2O_2
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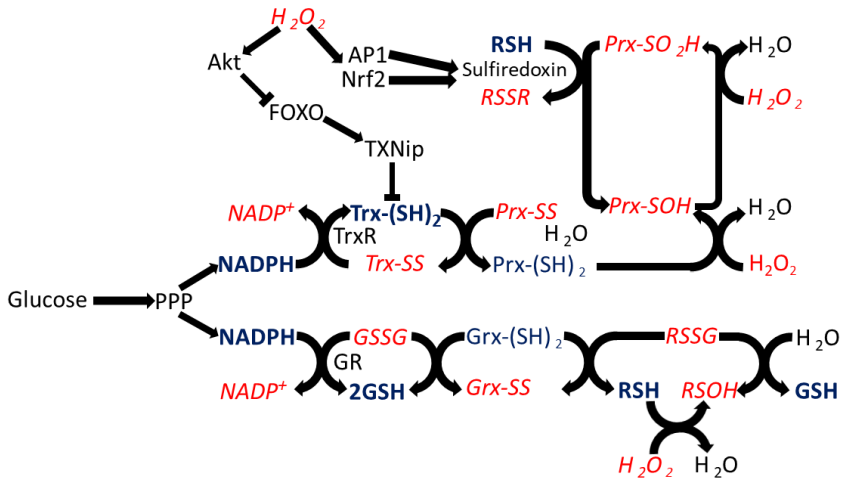
OCl^-
SOD
O_2^-
3
NADPH Oxidase

Figure 2



GSH	glutathione
GSSG	glutathione disulphide
GR	glu-tathione reductase
Grx	glutaredoxin
Prx	peroxiredoxin
PrxSOH	peroxiredoxinsulphenate
PrxSOOH	peroxiredoxin sulphinate
PrxSS	oxidised peroxiredoxin
RSH	protein
RSSG	glutathionylated protein
RSOH	protein sulphenate
RSSR	protein disulphide
trx	thioredoxin
TrxR	thioredoxin reductase

Figure 3

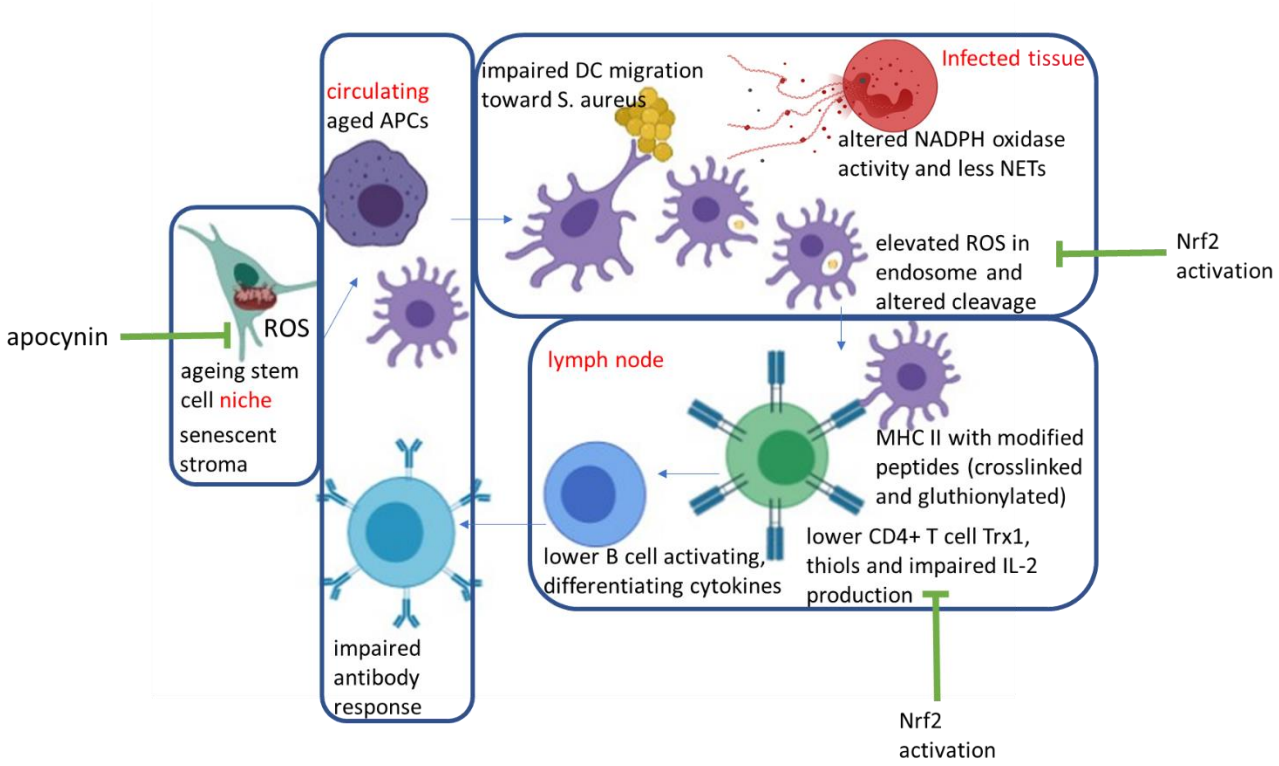

circulating
aged APCs
impaired DC migration
toward S. aureus
Infected tissue
altered NADPH oxidase
activity and less NETs
elevated ROS in
endosome and
altered cleavage
Nrf2
activation
apocynin
ROS
ageing stem
cell niche
senescent
stroma
lymph node
MHC II with modified
peptides (crosslinked
and gluthionylated)
lower B cell activating,
differentiating cytokines
lower CD4+ T cell Trx1,
thiols and impaired IL-2
production
impaired
antibody
response
Nrf2
activation

Figure 4