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Author: Jon Hazeldine Janet M. Lord Peter Hampson

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Immunesenescence and inflammaging: a contributory factor in the poor outcome of the geriatric trauma patient

Jon Hazeldine[1], Janet M. Lord[1], Peter Hampson[1,2*] P.Hampson@bham.ac.uk
[1]NIHR Surgical Reconstruction and Microbiology Research Centre, School of Immunity and Infection, Birmingham University Medical School, Birmingham, B15 2TT.
[2]Healing Foundation Centre for Burns Research, Queen Elizabeth Hospital, Birmingham, B15 2WB, UK.
[*]Correspondence author at: School of Immunity and Infection, Birmingham University Medical School, Birmingham, B15 2TT, UK, Tel.: +44 121 371 3264, Fax: +44 121 371 3203.

Highlights

- Aging is associated with a dysregulated immune and inflammatory response.
- Older adults have worse outcome post-trauma, including increased infection rates.
- Impaired immunity and delayed resolution of inflammation may underlie poor outcome.
- A detailed understanding of the mechanism(s) responsible has yet to be determined.

Abstract

Compared to younger patients, traumatic injury in older patients is associated with increased mortality and a range of adverse outcomes such as higher rates of infectious episodes, longer length of hospital stay and poor functional outcome at follow up. Data emerging from human and murine-based studies suggest age-related changes in immune function, collectively termed immunesenescence, and the chronic sub-clinical systemic inflammatory state of older adults, termed inflammaging, may contribute to these poor outcomes. Here, we review the findings of these studies, whose results demonstrate that the geriatric trauma patient elicits an immune response to injury that is distinct to that of younger adults, being characterised by reduced immune cell activation, impaired function and abnormal haematopoiesis, defects that are accompanied by an altered inflammatory response that fails to return to a homeostatic baseline in the days following injury. Although considerable evidence is accumulating that demonstrates clear and significant age-related differences in the immune and inflammatory response to traumatic injury, our current understanding of the mechanism(s) that underlie these changes is limited. Future studies that provide a mechanistic explanation for the unique immune and inflammatory response of older adults to traumatic injury are therefore essential if we are to determine whether manipulation of the immune system has potential as a future therapeutic strategy by which to improve the outcome of the geriatric trauma patient.

Keywords: Ageing; innate immunity; immunesenescence; infection; trauma.

1.0 Introduction

Recent advancements in medical care and public health policies, as well as improvements in socio-economic status mean we are now living longer than ever before. By 2050, it is estimated that the proportion of older adults (aged 60 years and over), who accounted for 9.2 and 11.7 percent of the global population in 1990 and 2013 respectively, will constitute 21.1 percent of the total world's population by 2050 (*United Nations World Population Healthy Ageing Report 2013*). In this section of society, traumatic injury is a major cause of morbidity and mortality. Compared to younger adults, geriatric trauma patients exhibit increased mortality rates *(Nacionales et al. 2015;Newell et al. 2009;Taylor et al. 2002;Vanzant et al. 2015*), a longer length of hospital stay *(McKevitt et al. 2003;Taylor et al. 2002;Vanzant et al. 2015)*, higher rates of infectious complications *(Butcher et al. 2003;Vanzant et al. 2015)*, a greater need for rehabilitation *(McKevitt et al. 2003;Nacionales et al. 2015;Shinoda-Tagawa and Clark 2003;Vanzant et al. 2015)* and worse functional outcomes (*Mosenthal et al. 2002*) following mild and severe traumatic injury, resulting in greater resource usage and management costs *(MacKenzie et al. 1990;Newell et al. 2009;Ross et al. 1989)*. With the shift that is anticipated to occur in global population demographics in the coming years (*United Nations World Population Healthy Ageing Report 2013*) and the fact that older adults represent an increasing proportion of trauma centre admissions *(Clark and Chu 2002;Hannan et al. 2004;Shinoda-Tagawa and Clark 2003)*, geriatric trauma is expected to become a major future public healthcare issue.

Recently, in an effort to improve clinical decision making and management of the geriatric trauma patient, prospective and retrospective studies have been conducted with the aim of developing robust scoring systems capable of predicting patient outcome *(Brooks et al. 2014;Joseph et al. 2014a;Joseph et al. 2014b)*. From these studies, a measure of frailty, defined by a series of variables that exhibit strong association for unfavourable discharge

such as existing co-morbidities, daily activity patterns and nutrition *(Joesph et al. 2014a)* has emerged as a strong independent predictor of both mortality and long-term hospitalisation following traumatic injury, proving to be superior to both age and injury severity as a predictor of patient outcome (*Joseph et al. 2014b*). However, despite these studies providing novel predictive models that have the potential to influence patient care, a greater understanding of the mechanism(s) that underlie the poor outcomes of the geriatric trauma patient is needed. Reduced physiological reserve and pre-existing co-morbidities are often cited as being primarily responsible. However, independent of injury severity and after adjustment for co-morbidities, older adults still exhibit significantly worse prognosis post trauma when compared to their younger counterparts *(Bruijns et al. 2013;Jones et al. 2014)*.

Physiological ageing is associated with remodelling of the immune system, a phenomenon termed immunesenescence. Evident in both the innate and adaptive arms of the immune system (Table 1), immunesenescence encompasses the age-related changes that occur in the composition, phenotype and function of the peripheral immune cell pool as well as the chronic sub-clinical systemic inflammatory state of older adults, termed inflammaging, which is characterised by elevated levels of serum pro-inflammatory cytokines and reduced levels of anti-inflammatory cytokines *(Bartlett et al. 2012;Stowe et al. 2010;Wei et al. 1992)*. Following both mild and severe traumatic injury, older adults suffer from an increased incidence and severity of nosocomial infection *(Blot et al. 2014;Bochicchio et al. 2001;Butcher et al. 2003;Vanzant et al. 2015)*, which has adverse effects on outcome in respect of increased mortality rates and longer length of hospital stay *(Brusselaers et al. 2010; Colohan2010;Richards et al. 2013)*. These observations coupled with the future changes that are expected in population demographics (*United Nations World Population Healthy Ageing Report 2013*) has led a number of groups to investigate the impact of age on the immune and inflammatory response to traumatic injury. Here, we review the findings of

these studies, which taken together, suggest that by exacerbating the changes in immune function that occur as a direct consequence of traumatic injury, immunesenescence contributes to the poor outcome of the geriatric trauma patient by increasing their susceptibility to infection and subsequent mortality.

2.0 The impact of immunesenescence and inflammaging on the immune and inflammatory response to traumatic injury in the geriatric trauma patient

2.1 Immunesenescence

Through two observational longitudinal studies in the setting of mild traumatic injury, defined as an injury severity score (ISS) <15, we were the first group to comprehensively investigate whether immunesenescence could influence patient susceptibility to infection post trauma *(Butcher et al. 2003;Butcher et al. 2005)*. Focussing exclusively upon the microbicidal function of neutrophils, which serve as the first line of defence against pathogenic infection, we found reactive oxygen species (ROS) production by neutrophils isolated from older adults within 24 hours of sustaining a fractured neck of femur as well as five weeks post injury was significantly reduced when compared to ROS generation by neutrophils obtained from healthy older adults *(Butcher, et al. 2003;Butcher et al. 2005)*. Importantly, when the cohort was split into those elderly hip fracture (HF) patients who went on to develop infection and those who remained infection free during follow-up, ROS production was significantly lower in the former group *(Butcher et al. 2003)*. In contrast, in a cohort of young trauma patients that had sustained a single limb fracture, none of whom reported post traumatic infection, neutrophil ROS generation was comparable to that of young healthy controls *(Butcher et al. 2003)*. Thus, these two studies were the first to suggest that age and not the traumatic injury per se was responsible for driving the aberrant neutrophil function observed in elderly HF patients and the subsequent development of post traumatic complications. Following on from this work, Baehl et al *(Baehl et al. 2015)* have

recently shown neutrophil ROS generation remains suppressed for up to six months post HF in older adults, a defect they attributed to reduced activation of $p47^{phox}$, a critical subunit of the ROS generating enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase *(Baehl et al. 2015)*. Accompanying this reduction in ROS generation was a short-term impairment in the ability of neutrophils to phagocytose immunoglobulin (Ig)-opsonised Escherichia coli (*E.coli*) *(Baehl et al. 2015)*. The group also observed during this time-period a significant reduction in the surface density of CD16, an Fcγ receptor that facilitates neutrophil recognition of Ig-coated pathogens and whose expression we have shown is reduced during "healthy" ageing *(Butcher et al. 2001)*. It may be therefore, that akin to our findings with ROS generation *(Butcher et al. 2003;Butcher et al. 2005)*, immunesenescence exacerbates the effect of mild trauma on the phagocytic activity of neutrophils via a reduction in CD16 expression.

Alongside the more renowned defensive mechanisms of ROS production and phagocytosis, activated neutrophils release into the extracellular environment their nuclear DNA decorated with a range of granule-derived proteases and peptides *(Brinkmann et al. 2004)*. Termed neutrophil extracellular traps (NETs), these structures are considered the "last resort" in neutrophil anti-microbial defence and play a key role in preventing the dissemination of invading pathogens (Meng et al. 2012; Yipp et al. 2012). Two studies have recently demonstrated a significant impairment in NET generation during "healthy ageing". Using murine neutrophils, Tseng et al found *in vitro* NET formation by neutrophils from young mice was approximately 2.5-fold higher than that of neutrophils from aged mice following treatment with either the diacylglycerol mimetic phorbol 12-myristate 13-acetate (PMA) or methicillin-resistant *Staphylococcus aureus (S.aureus) (Tseng et al. 2012)*. Furthermore, in an *in vivo* model of MRSA infection, whilst observing extracellular DNA networks that contained entrapped bacteria in tissue extracted from young mice, the group found no

evidence of any NET-derived extracellular DNA in areas of infected skin tissue in aged mice *(Tseng et al. 2012)*. In line with these observations, we recently demonstrated that human ageing is also accompanied by an impairment in NET production, with tumour-necrosis factor alpha-primed neutrophils from older adults (≥65 years) generating significantly fewer NETs upon IL-8 and LPS stimulation when compared to those from younger adults (≤35 years) *(Hazeldine et al. 2014)*. To date, only one study has examined NET production in the context of geriatric trauma. Comparing PMA-induced NET generation by neutrophils isolated from severely-injured young (≤65 years) and old (≥65 years) trauma patients with a group of age-matched healthy controls, Itagaki et al observed similar NET production by neutrophils isolated from young trauma patients and their controls, suggesting no detrimental effect of trauma on this neutrophil function *(Itagaki et al. 2015)*. In contrast, PMA-induced NET release by neutrophils from traumatically-injured older adults was negligible, mirroring the response of neutrophils isolated from healthy older adults *(Itagaki et al. 2015)*. Thus, as outlined above for ROS production *(Butcher et al 2003)*, it appears that age and not trauma per se is responsible for the aberration in NET formation in the geriatric trauma patient.

Severe blunt traumatic injury (ISS > 15) in older adults has recently been shown to elicit a unique genomic response in peripheral leukocytes. As part of a prospective observational trial, Vanzant and colleagues *(Vanzant et al. 2015)* characterised and compared the transcriptomic responses of circulating neutrophils isolated from severely injured young (<55 years) and older (>55 years) adults with evidence of haemorrhagic shock and observed significant age-related alterations in gene expression *(Vanzant et al. 2015)*. Restricting their analysis to those patients with complicated outcome (defined as a stay in intensive care for longer than 14 days with evidence of ongoing organ dysfunction or death) the group reported that in the acute phase post injury (12 hours and 1 day), gene expression patterns were significantly less perturbed in neutrophils from older donors when compared to their younger

counterparts *(Vanzant et al. 2015)*. However, at day 4 post injury, a time-point at which gene-fold changes in neutrophils from younger patients had begun to return to baseline levels, neutrophils from older adults still demonstrated significant gene alterations when compared to healthy controls. Detailed analysis revealed these alterations were in genes involved in decreased chemotaxis of neutrophils (e.g. interleukin-8), up-regulation of myeloid derived suppressor cells (e.g. arginase 1), increased inflammation (e.g. matrix metalloprotease 8 and 9) and decreased innate and adaptive immunity *(Vanzant et al. 2015)*. Interestingly, these alterations in gene expression mirror functional and compositional observations that have been reported to occur during "healthy" ageing, with recent immune gerontological studies reporting ageing to be associated with decreased neutrophil chemotaxis *(Sapey et al. 2014)* and an increased frequency of circulating myeloid derived suppressor cells (*Verschoor et al. 2013)*, which occur alongside the chronic sub-clinical systemic inflammatory state of older adults *(Stowe et al. 2010;Wei et al. 1992)*.Thus, the prolonged neutrophil transcriptome response observed by Vanzant et al (*Vanzant et al. 2015*) in injured older adults may be a consequence of trauma-induced changes superimposed on the background of age-related immunesenescence.

Following thermal injury, mortality rates are greater in aged mice when compared to young mice and are accompanied by a greater degree of immune suppression *(Kovacs et al. 2004a;Kovacs et al. 2002;Kovacs et al. 2004b)*. Following a 15% total body surface area (TBSA) burn, aged mice exhibit impaired delayed type hypersensitivity (DTH) responses, significantly reduced immune cell proliferation and a greater shift in the T helper (T_H)-1-T_H-2 cytokine profile when compared to the response of young mice subjected to injuries of comparable size and depth *(Kovacs et al. 2004a;Kovacs et al. 2004b)*. Interestingly, estrogen supplementation in aged mice prior to injury partially restored both the thermal-induced defective DTH response and aberrant T_H1-T_H2 cytokine profile, which was accompanied by a

28% increase in survival rates *(Kovacs et al. 2004a;Kovacs et al. 2004b)*. Despite the success of these studies, no study to our knowledge has examined whether estrogen supplementation post burn can elicit the same beneficial effects upon immune function and survival rates.

In murine models of polytrauma and subsequent Pseudomonas pneumonia infection, Nacionales et al *(Nacionales et al. 2015)* have shown aged mice exhibit increased mortality rates when compared to their younger counterparts. Interestingly, no evidence of any age-related differences in mortality rates were found following polytrauma alone, demonstrating that infection is driving the higher rates of mortality amongst aged mice in this combined model of trauma and secondary infection *(Nacionales et al. 2015)*. Proposing a role for immunesenescence in this poor outcome, the group studied the microbicidal activity of lung-resident neutrophils and reported a significant age-related impairment in phagocytosis, a defect they traced back to the level of the transcriptome, where during the acute phase of trauma it was found that aged mice failed to up-regulate genes involved in phagocytosis as efficiently as young mice *(Nacionales et al. 2015)*. Additional observations made in this study were that there were significantly fewer short-term hematopoietic stem cells (HSCs) in the bone marrow (BM) of aged mice post trauma and that lineage$^-$ sca-1$^+$ c-kit$^+$ cells (LSKs) isolated from aged BM exhibited impaired proliferation *(Nacionales et al. 2015)*. Again, as with lung-resident leukocytes, age-related differences were observed at the level of the HSC transcriptome, with those obtained from elderly mice post trauma failing to upregulate expression of innate immunity genes related to chemotaxis and toll-like receptors as efficiently as HSCs from their younger injured counterparts *(Nacionales et al. 2015)*. Based on these latter findings, it has been proposed that following severe trauma, failure of aged HSCs to undergo adequate rapid myelopoiesis culminates in the release into the circulation of granulocytes with suboptimal function, thereby leaving aged mice susceptible to secondary infections and ultimately death *(Nacionales et al. 2015)*.

A common complication in hospitalised older adults is the development of sepsis, with nearly 60% of sepsis cases occurring in patients aged 65 years and over, which in the setting of critical care is associated with worse clinical outcome *(Mann et al. 2012;Martin et al. 2006)*. The results of recent human (*Inoue et al. 2014;Inoue et al. 2013)* and murine *(Gentile et al. 2014;Nacionales et al. 2014)* based studies suggest this susceptibility is attributable in part to age-related defects in innate (*Gentile et al. 2014;Nacionales et al. 2014)* and adaptive *(Inoue et al. 2014;Inoue et al. 2013)* immunity. As early as day one post septic insult, both monocytes and neutrophils isolated from aged mice exhibit impaired ROS generation and phagocytosis when compared to the response of cells from young septic mice *(Nacionales et al. 2014)*. Analysis of blood leukocyte gene expression has revealed an attenuated early genomic response in aged mice with the up-regulation of genes relating to neutrophil-mediated immunity, chemotaxis and chemokine binding significantly lower in aged septic mice, suggesting that the early aberration observed in immune function can be explained at the level of the leukocyte transcriptome *(Nacionales et al. 2014)*. Interestingly, and in line with the abovementioned human study in severely injured patients with haemorrhagic shock *(Vanzant et al. 2015)*, gene expression in septic aged mice fails to return to homeostasis at day 3 post septic insult, which is in contrast to young septic mice, whose leukocyte gene profiles are near comparable to those of healthy young controls *(Nacionales et al. 2014)*. This sustained response was suggested to reflect the inability of the aged immune system to efficiently clear the microbial infection, culminating in an ongoing infectious insult that elicited continual immune cell stimulation and gene upregulation *(Nacionales et al. 2014)*.

If immunesenescence exacerbates trauma-induced changes in immune function and thereby contributes to the poor outcome of the geriatric trauma patient by increasing their susceptibility to infection, is it amenable to therapeutic targeting for patient benefit? To address this question, we must first understand the mechanism(s) that underlie the more

severe immunoparesis in the older trauma patient. It is well established that ageing is associated with a reduction in the circulating levels of DHEA (*Orentreich et al. 1992)*, an immune enhancing steroid hormone that counteracts the potent immune suppressive properties of corticosterone, whose production is maintained with age (*Hazeldine et al. 2010)*. Thus, healthy older adults possess a raised circulating cortisol:DHEAS ratio *(Orentreich et al. 1992)*. Following traumatic injury, corticosterone levels rise, whilst DHEA levels are unaltered *(Wade et al. 1988)*. Thus, given the increased cortisol:DHEAS ratio that occurs naturally with age, one would expect trauma in older adults would result in a more severe corticosterone excess than would be observed in the younger trauma patient, resulting in a state of peripheral immune suppression. In line with this hypothesis, we found the cortisol:DHEAS ratio in elderly HF patients was significantly higher than that measured in healthy elderly subjects and a cohort of young orthopaedic trauma patients *(Butcher et al. 2005)*. Moreover, we noted that the ratio was significantly higher in those individuals who subsequently developed an infection in the 6 weeks following injury, suggesting a raised cortisol:DHEAS ratio had increased patient susceptibility to infection, presumably via reducing the microbicidal activity of circulating neutrophils *(Butcher et al. 2005)*. As *in vitro* studies have shown DHEAS can prevent cortisol-mediated inhibition of formyl-methionine-leucine-phenylalanine (fMLP)-induced ROS production by primed neutrophils *(Butcher et al. 2005)*, DHEA replacement could potentially reduce infection risk in the elderly trauma patient by counteracting trauma-induced glucocorticoid excess, thereby creating an environment that favours immune enhancement over immune suppression *(Butcher et al. 2003;Butcher et al. 2005;Butcher and Lord 2004)*.

2.2 Inflammaging

Numerous studies in healthy subjects have demonstrated that advanced age is associated with a hyper-inflammatory state. Termed “inflammaging”, this state is characterised by an age-

related chronic low grade increase in basal circulating levels of a number of pro-inflammatory cytokines, most notably interleukin (IL)-6 *(Bartlett et al. 2012;Stowe et al. 2010;Wei et al. 1992)* and tumor necrosis factor-alpha (TNF-α) *(Bartlett et al. 2012;Paolisso et al. 1998;Stowe et al. 2010)*. Importantly, "inflammaging" is recognised as an important pathogenic factor in the development of age-related pathologies including type-2 diabetes *(Paolisso et al. 1998)* and cardiovascular disease *(Bruunsgaard et al. 2000;Jenny et al. 2002;Libby et al. 2010)*, and has also been shown to be a strong predictor of all-cause mortality risk in the elderly in a number of longitudinal cohort studies *(Bruunsgaard et al. 2003;Harris et al. 1999;Reuben et al. 2002)*.

Traumatic injury leads to a systemic inflammatory response syndrome (SIRS), characterised by an acute increase in serum levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α *(Bone et al. 1992)*. Importantly, circulating levels of pro-inflammatory cytokines following traumatic injury are associated with mortality. For example, in a cohort of 230 patients who had sustained a thermal injury, Jeschke and colleagues *(Jeschke et al. 2014)* showed that individuals who did not survive their injury had significantly higher levels of IL-6, IL-8 and granulocyte colony stimulating factor (G-CSF) compared to survivors, and similar findings have been made in the case of TNF-α *(Marano et al. 1990;Zhang et al. 1998)*. In addition, in a cohort of 29 intensive care unit patients with severe sepsis or septic shock, IL-6, IL-8 and MCP-1 levels were significantly higher in those patients with a fatal outcome *(Andaluz-Ojeda et al. 2012)*. SIRS is accompanied by a compensatory anti-inflammatory response syndrome (CARS) *(Xiao et al. 2011)* designed to counteract the ongoing inflammation. However, CARS can lead to a state of immune suppression, including a reduction in neutrophil function *(Parment et al. 2007)* predisposing the patient to infections and sepsis. Indeed, IL-10 levels have been shown to be significantly higher in those patients with poor outcome *(Andaluz-Ojeda et al. 2012;Jeschke et al. 2014)*.

Given that advanced age is accompanied by elevated circulating basal levels of pro-inflammatory cytokines, and pro-inflammatory cytokine levels are associated with complicated outcome post-trauma, it is reasonable to suggest that the SIRS response may be exaggerated in older patients and that this may, at last in part, underlie the poor prognosis of the elderly trauma patient. In support of this idea, work from the Kovacs laboratory has shown that similar to elderly humans, aged mice have increased mortality in response to burn injury *(Kovacs et al. 2002)*. In a subsequent study, where mice were subjected to a 15% TBSA burn, the group found that aged mice had double the serum levels of IL-6 when compared to young mice *(Kovacs et al. 2004b*). Furthermore, when old mice were given estrogen replacement therapy, which began 7 days prior to burn injury, diminished IL-6 release was observed post-burn, which was accompanied by improved survival rates *(Kovacs et al. 2004b)*. Similar observations of elevated cytokine levels in the aged trauma patient have been made following the relatively mild trauma of HF. In a recent study, Vester et al *(Vester et al. 2014)* demonstrated that old (>70 years) HF patients had elevated serum levels of IL-6 on admission compared to old healthy controls, which increased further following surgery. In contrast, IL-6 levels were not elevated above the levels seen in healthy controls for young (<50 years) patients with long bone fractures, nor were they elevated following surgery *(Vester et al. 2014)*. Baëlh and colleagues also documented increased levels of pro-inflammatory cytokines in old patients following HF, including IL-6 and TNF-α *(Baehl et al. 2015)*. Interestingly, the group found that the levels of TNF-α were still significantly elevated 6 months post-fracture, suggesting an inability of old hip fracture patients to resolve the inflammation *(Baehl et al. 2015)*.

In contrast to the aforementioned studies, murine models of polytrauma, in which mice are subjected to haemorrhagic trauma and long bone fracture, have yielded conflicting results. Nacionales et al (Nacionales et al. 2015) found no difference in serum levels of IL-6 or TNF-

α between old and young mice 1 day after polytrauma. In fact, the data showed a trend towards reduced levels in the old mice although this was not statistically significant (Nacionales et al. 2015). Published by the same group, data from the Inflammation and the Host Response to Injury Collaborative Program, has demonstrated a similar pattern in human severe trauma. In a cohort of 244 patients who had suffered blunt trauma and were in haemorrhagic shock, the authors found that old (≥55 years) patients with complicated outcomes (ICU hospitalization for longer than 14 days with evidence of ongoing organ dysfunction, or death) had decreased plasma cytokine and chemokine (IL-6, IL-8, IL-10, TNF-α and monocyte-chemoattractant protein-1) levels when compared to young (<55 years) patients with complicated outcomes (Vanzant et al. 2015). Taken together, the results of these two studies suggest that "inflammaging" does not lead to an exaggerated systemic inflammatory response to injury in the elderly. These studies did suggest however, that the inflammatory response in the old takes longer to resolve to baseline levels when compared to the young. Interestingly, a study of 50 critically ill blunt trauma patients found significantly lower plasma IL-6 and IL-10 levels in geriatric patients compared to young patients, and the development of a secondary infection was associated with lower presenting IL-6 and IL-10 levels in the geriatric group *(Ottinger et al. 2014).*

Inter study differences in the severity and mechanisms of traumatic injury, as well as the different age cut-offs used to define a geriatric trauma patient are likely to have contributed to the conflicting observations described above. Directly comparing data obtained from murine and human models of trauma may also be a source of variation given the difficulty that exists in replicating the type, severity and clinical course of human injury in mice as well as the species differences in circulating leukocyte populations. Indeed, at the genomic level it has been suggested that murine models of burn and trauma poorly mimic the human condition (Gentile et al. 2014b; Seok et al. 2013). However, this has recently been challenged by Takao

and colleagues who reported strong similarities in the genomic response of mice and humans following both thermal and traumatic injury *(Takao et al . 2015)*. Thus the debate continues as to how suitable murine models are at recapitulating in its entirety the human response to traumatic injury.

Despite the reported differences in respect of the inflammatory response elicited by young and old trauma patients, the general consensus is that older adults elicit a sustained inflammatory response that takes longer to resolve to baseline levels, leading to prolonged immune suppression and increased susceptibility to infection. Further studies are required in order to better understand the age-related changes in the inflammatory response to injury, and the mechanism(s) underlying its persistence, as this may provide therapeutic targets which would allow a stratified treatment strategy in the geriatric trauma patient.

3.0 Impaired resolution of inflammatory responses and accumulation of damage associated molecular patterns (DAMPs): an underlying cause of immunoparesis in the geriatric trauma patient?

3.1 The effect of DAMPs on innate immune cells

The systemic inflammatory response syndrome that occurs following traumatic injury is thought to be initiated in part by damage associated molecular patterns (DAMPs). Actively secreted by immune cells and released from damaged and necrotic tissue *(Gardella et al. 2002;Kaczmarek et al. 2013),* nuclear and mitochondrial DAMPs have been shown *in vitro* and *in vivo* to trigger a range of immune responses, which include: (i) neutrophil ROS production, degranulation, chemokine secretion and extracellular trap generation *(Hazeldine et al. 2015;Itagaki et al. 2015),* (ii) pro-inflammatory cytokine production *(Collins et al. 2004)* and (iii) monocyte activation *(Crouser et al. 2009).* This immune cell activation is driven through pattern recognition receptors (PPR) such as TLR-9 in the case of

mitochondrial DNA and formyl peptide receptors (FPR) in the case of mitochondrial-derived formyl peptides (Zhang, 2010). The fact that these DAMPs bind to the same PRRs as pathogen-associated molecular patterns (PAMPs) is attributable to structural similarities. For example, the CpG repeats that define mitochondrial DNA are also present in bacterial DNA and bind to TLR-9.

3.2 DAMP induced immune tolerance

Human ageing has recently been reported to be associated with marked elevations in circulating DAMPs, with plasma levels of the mitochondrial DAMP, mtDNA, found to be significantly higher in healthy older adults *(Itagaki et al. 2015;Pinti et al. 2014)*. In the context of physiological ageing, an age-related accumulation of DAMPs has been proposed as a factor driving the chronic low-grade inflammatory state of healthy older adults *(Kapetanovic et al. 2015)*. Interestingly, as with healthy ageing, plasma levels of mtDNA have been found to be significantly higher in the geriatric trauma patient when compared to the levels measured in younger trauma subjects matched for injury severity *(Itagaki et al. 2015)*. Whether simply an additive effect of trauma and age-induced DAMP release or the result of other mechanisms such as reduced clearance rates, *could an increased amount of circulating DAMPs contribute to the more severe immunoparesis experienced by the geriatric trauma patient?* Results of a handful of recent studies suggest this could be the case.

Due to structural similarities, N-formyl peptides released from ruptured mitochondria post trauma activate the same intracellular signalling cascades as the bacterial peptide fMLP. Prior exposure to fMLP has been shown to markedly suppress the chemotactic response of neutrophils upon secondary stimulation *(Li et al. 2015)*. Based on this result and *ex vivo* data showing neutrophil chemotaxis is significantly reduced post trauma *(Li et al. 2015)*, the phrase “DAMP-induced tolerance” has been coined, which proposes that circulating

mitochondrial-derived DAMPs released following traumatic injury may tolerise neutrophils to subsequent pathogenic danger signals *(Li et al. 2015).* Thus, in the context of the geriatric trauma patient, it is conceivable that not only would the levels of circulating DAMPs be higher in the immediate aftermath of trauma *(Itagaki et al. 2015)* but such levels would be retained for a longer period of time given the apparent age-related impairment in DAMP clearance *(Itagaki et al. 2015;Pinti et al. 2014).* Consequently, the tolerising effects of DAMPs could be one factor underlying both the short *(Butcher et al. 2003;Butcher et al. 2005) and long (Baehl et al. 2015)* term suppression of neutrophil microbicidal function that has been reported in the aged trauma patient, leaving them at risk of subsequent infectious episodes. In addition to influencing the functional activity of neutrophils, DAMPs have been found to: (i) induce endotoxin tolerance in human monocytes *(Fernandez-Ruiz et al. 2014),* (ii) drive the functional exhaustion of murine monocytes *(Liesz et al. 2015)* and (iii) induce a state of systemic lymphopenia *in vivo* (*Liesz et al. 2015*). Thus, a higher abundance of circulating DAMPs could be one mechanism that contributes to the significantly impaired innate *(Baehl et al. 2015;Butcher et al. 2003;Butcher et al. 2005)* and adaptive *(Kovacs et al. 2004a;Kovacs et al. 2004b)* immune responses of the geriatric trauma patient. Interestingly, a recent study by Yao et al found that estrogen treatment 15 minutes following a 40% TBSA burn injury, was able to protect rats from trauma-induced cardiac damage, and that this effect was the result of decreased mtDNA release *(Yao et al. 2014).* This data may also explain the mechanism underlying the protective effects (reduced IL-6 release and mortality) of estrogen treatment in murine models of burn injury, as published by the Kovacs laboratory *(Kovacs et al. 2004b).* If it is the case that DAMPs are at least in part responsible for the reduction in neutrophil function seen post injury, then could this provide a potential therapeutic avenue to help reduce infections post-trauma? Whilst this is possible in theory at least, a better understanding of kinetics of DAMP release post-trauma is required to ensure that preventing

the DAMP induced tolerance of immune cells can be achieved, without unnecessarily supressing the immune response that may be required to deal with the immediate aftermath of the injury itself.

3.3 Do elevated circulating DAMP levels drive sustained inflammation and immunoparesis in old trauma patients?

Transcriptomic analysis of neutrophils isolated from severely injured patients with haemorrhagic shock has revealed advanced age is associated with a unique genomic response *(Vanzant et al. 2015)*. Of interest, it was found that at day 4 post injury, a time-point where gene-fold changes in neutrophils from younger patients had begun to return to baseline levels, neutrophils from older adults still demonstrated significant alterations in genes related to decreased chemotaxis, increased inflammation and decreased innate immunity when compared to healthy controls *(Vanzant et al. 2015)*. Interestingly, evidence from a murine model of burn injury suggests that following a traumatic insult, resolution of inflammation is attenuated with age. Following a 15% TBSA, full thickness burn, Nomellini et al *(Nomellini et al. 2008)* measured inflammatory cell accumulation in the lungs at 24 hours post injury and found that whilst the number of neutrophils remained significantly elevated in aged, burn-injured mice when compared with sham controls, neutrophil numbers were comparable in the lungs of young burn-injured and non-injured mice *(Nomellini et al. 2008)*, suggesting an age-related impairment in neutrophil clearance. If replicated in humans, one can envisage how failure to clear apoptotic neutrophils would result in cellular necrosis and the release of DAMPs into the extracellular environment. This would result in sustained stimulation of neighbouring cells, which could explain both the transcriptome data that has revealed gene expression in aged leukocytes fails to return to baseline as quickly as leukocytes from younger subjects *(Vanzant et al. 2015)*, as well as the *in vitro* and *in vivo* immune tolerance that has been observed in aged mice and humans post injury *(Baehl et al. 2015;Butcher et al.*

2003;Butcher et al. 2005;Kovacs et al. 2004a;Kovacs et al. 2004b). Thus, aberrant resolution of inflammation in the geriatric trauma patient could initiate a vicious cycle of DAMP release, immune cell stimulation and eventual exhaustion, culminating in more severe immunoparesis and an increased susceptibility to infection.

4.0 Concluding remarks

Understanding the impact of age on the immune and inflammatory response to traumatic injury is an emerging area of research in the field of immune gerontology. Studies have shown the geriatric trauma patient elicits an immune response that is distinct to that of the younger trauma patient, with evidence of reduced immune cell activation, impaired function and abnormal haematopoiesis, which together leave the elderly patient at risk of subsequent infection and mortality (Figure 1). Moreover, genomic analysis has revealed age-related differences in both the magnitude and duration of the inflammatory response post trauma, with the elderly patient initiating an attenuated transcriptomic response in the acute phase, which fails to return to a homeostatic baseline in the days following injury, suggesting aberrant resolution that would culminate in long-term inflammation and immune suppression.

Currently, very little is known in respect of the mechanism(s) that underlie the dysregulated immune and inflammatory response to trauma in older adults. Our own work suggests that age-associated changes in the endocrine system exacerbate trauma-induced changes in the circulating levels of immune suppressing and enhancing hormones, culminating in a systemic state of immune suppression that is more profound than that witnessed in younger patients with similar injuries (Butcher et al. 2005;Butcher and Lord 2004). To our knowledge, this observation coupled with the results of a handful of murine-based studies *(Kovacs et al. 2004a;Kovacs et al. 2004b;Nomellini et al. 2008)* are the only data that exists that provides a potential mechanistic explanation for the altered immune response that accompanies

traumatic injury in older adults. Results of future studies that address this issue will provide much needed information in respect to determining whether targeting certain aspects of the immunesenescent profile could serve as a potential therapeutic strategy by which to reduce the risk of infection and subsequent mortality in the geriatric trauma patient.

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Figure Captions

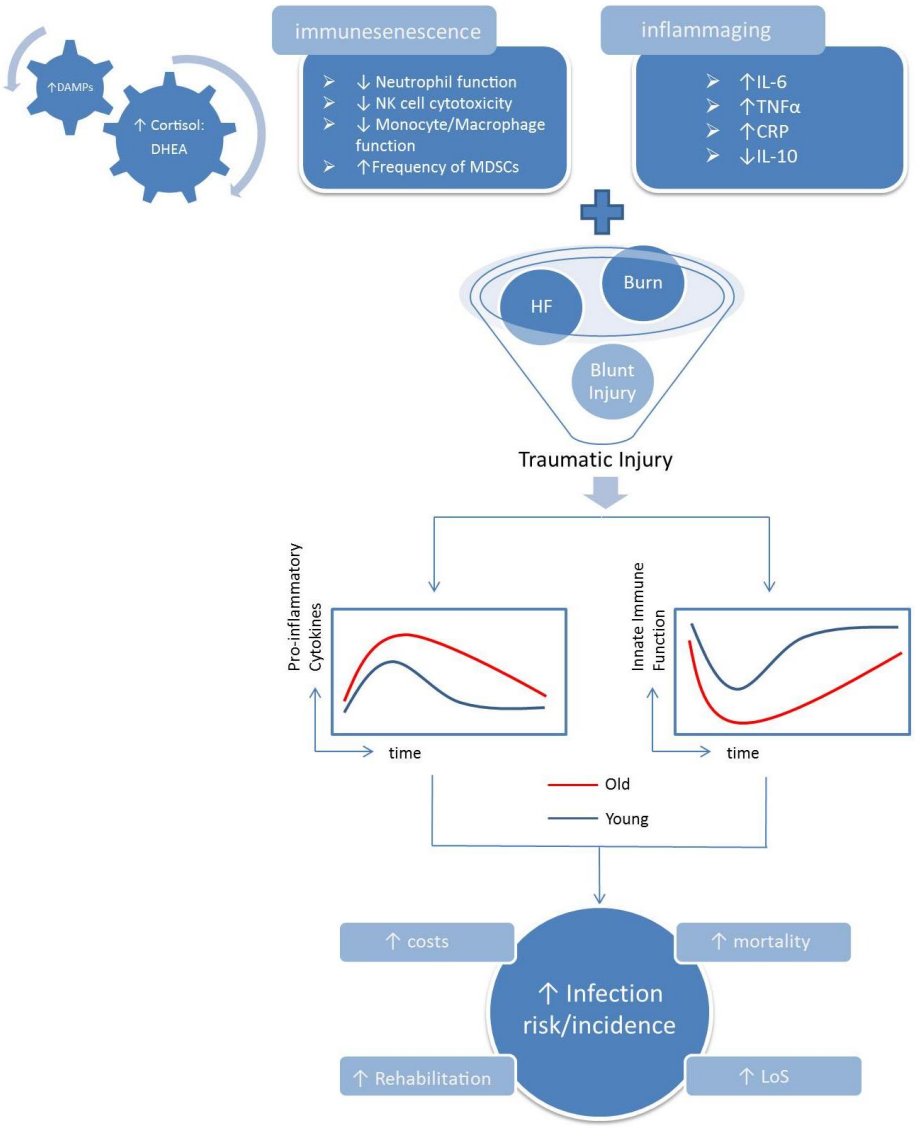

Figure 1. Age-associated differences in the innate immune and inflammatory response to trauma. Relative to young individuals, older adults elicit a unique immune and inflammatory response to traumatic injury. Age-associated immunesenescence and inflammaging, driven by elevated levels of circulating DAMPs and a raised cortisol:DHEA ratio, are proposed to underlie these changes, which manifest as an exaggerated inflammatory response to injury and a greater and more sustained innate immunoparesis. These factors are proposed to underlie poor-outcome in the geriatric trauma patient which includes increased infection incidence. Abbreviations: CRP, C-reactive Protein; DAMPs, Damage Associated Molecular Patterns; DHEA, Dehydroepiandrosterone; HF, Hip Fracture; HSC, Haematopoietic Stem Cell; IL, Interleukin; LoS, Length of Stay; PMN, Polymorphonuclear leukocytes; ROS, Reactive Oxygen Species; TNF-α, Tumour Necrosis Factor-Alpha.

Tables

Table 1. Features of innate and adaptive immunesenescence

	Composition	Phenotype	Function	References
Neutrophils	• No change in circulating numbers.	• No change in CD11a, CD11b expression	• Reduced chemotaxis *in vitro.* • Reduced phagocytosis. • Impaired NET formation. • Increased/decreased ROS formation. • Impaired receptor recruitment into lipid rafts.	*Hazeldine et al. 2014;* *Sapey et al. 2014;* *Tseng et al. 2012;* *Fulop et al.2004;* *Wenisch et al. 2000;* *Born et al 1995.*
Monocytes/ Macrophages	• No change in circulating % and/or absolute number. • Increased % of $CD14^{+}$ 16^{++} non-classical monocytes. • Reduced % of $CD14^{+}$ 16^{-} classical monocytes.	• Reduction in CD62L, TLR1/4 expression. • No change in TLR2 expression. • Increase in CD11b, TLR5 expression.	• Decreased monocyte phagocytosis. • Decreased LPS-induced ROS production. • Increased TLR4-driven TNF-α production. • Decreased TLR1/2-induced production of IL-6 and TNF-α. • Increased TLR5-induced IL-8 production.	*Hearps et al.2012;* *Qian et al. 2012;* *Nyugen et al.2010;* *Seidler et al.2010;* *Van Duin et al.2007;* *McLachlan et al.1995*
Natural killer (NK) cells	• Increase in $CD56^{DIM}$ % and/or number. • Reduction in $CD56^{BRIGHT}$ % and/or number. • Increased $CD56^{DIM}$:$CD56^{BRIGHT}$	• Comparable NKG2D, CD2, CD16 expression. • Decreased CD94, KLRG1, NKp46 expression.	• Decreased NKCC. • Impaired perforin release. • Reduced cytokine/chemokine secretion. • Impaired NK cell maturation. • Impaired proliferation *in vivo.*	*Shehata et al. 2015;* *Hazeldine et al 2012;* *Lutz et al 2011;* *Almedia-Oliveira et al.2011;* *Hayhoe et al.2010;* *Lutz et al.2005;* *Mariani et al. 2002;* *Mariani et al.2001*
MDSCs	• Increased frequency of circulating MDSCs in older adults and frail elderly.	• Not known	• Not known	*Verschoor et al. 2013*

Abbreviations: IL, Interleukin; KLRG1, Killer cell lectin-like receptor subfamily G member 1; LPS, lipopolysaccharide; MDSCs, Myeloid Derived Suppressor Cells; NETs, Neutrophil extracellular traps; NKp46, Natural killer cell p46-related protein; ROS, Reactive oxygen species; TLR, Toll-like receptor, TNF-α, Tumour-necrosis factor alpha.