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Age-related modifications of macrophages influenced by "inflamm-ageing" in graft *vs.* host disease

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Abstract: Most studies focus on the adaptive immune cells in the GVHD pathogenesis, while little is known about innate immune cells in GVHD occurrence and development, especially macrophages. Meanwhile, a higher incidence of graft versus host disease (GVHD) is also found in the elderly patients. Though advances have been made in the modification of macrophages influenced by the inflamm-ageing, there is still no review on the role of macrophages in GVHD and the association between GVHD and the altered macrophages by inflamm-ageing. In this review, we focus on the potential age-related modifications of macrophage in GVHD, which contributes to the change of morbidity and mortality of GVHD. Via literature review, we found that the infiltration of macrophages is associated with GVHD and macrophages are modified in inflamm-ageing state, including the proliferation, migration, phagocytosis, antigen presentation, interaction with other immune cells, and pro-fibrosis. We suppose that altered macrophage functions in inflamm-ageing state contribute to GVHD in elderly patients.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has become an advantageous therapeutic choice for many hematological diseases (Gratwohl *et al.*, 2010, 2015). Graft versus host disease (GVHD), one of the major complications of HSCT, contributes to non-relapse mortality (Arai *et al.*, 2015; Pasquini *et al.*, 2010). GVHD occurs when host tissues in immunocompromised allogeneic recipients are recognized and attacked by the immunocompetent cells of the donor. GVHD can be classified as either acute or chronic GVHD. Acute GVHD usually occurs within 100 days of stem cell transplantation and the three main targeted organs are skin, gastrointestinal tract and liver with disease severity ranging from degree I to degree IV. The severity of chronic GVHD is also evaluated via examination of the mouth, eyes, lungs, genital tract, fasciae, and joints and can be classified as mild, moderate, or severe based on NIH consensus criteria (Jagasia *et al.*, 2015).

Pathogenesis of GVHD has not been fully characterized but some potential theories have been developed. Acute GVHD is possibly initiated by inflammation caused by transplantation conditioning regimens or other diseases, such as infection or tissue damage. These mechanisms consequently promote the release of damage-associated molecular pattern (DAMP) molecules and pathogen-associated molecular pattern (PAMP) molecules, following the initiation of innate and adaptive immune responses. Three steps are conceptualized, including activation of the APCs; activation, proliferation, differentiation and migration of donor T cells; and the destruction of target tissue (Ferrara, 2000; Zeiser and Blazar, 2017).

Patients with a prior episode of acute GVHD are higher susceptible to chronic GVHD development (Afram *et al.*, 2018; Chen *et al.*, 2017; Eisner and August, 1995). The mechanism of chronic GVHD is characterized by fibrosis with little inflammation (Blazar *et al.*, 2012; Zeiser and Blazar, 2017), and is different from acute GVHD (Flowers *et al.*, 2011). Cooke *et al.* (2017) has proposed a three-phase model for the initiation and development of chronic GVHD. In phase 1, early inflammation and tissue injury prepares for the migration of donor immune cells into secondary lymphoid organs. In phase 2, chronic inflammation, thymic injury, and dysregulated immunity increase the immune response and lower the immune tolerance. In phase 3, aberrant tissue repair with fibrosis is evident. Many immune components are involved in this complicated process, including T cells, B cells, NK cells, dendritic cells (DC),

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macrophages and so on. Among them, macrophage infiltration contributes to the occurrence and development of GVHD.

Ageing and GVHD

Among the risk factors of GVHD, recipient age is one of the most important for disease development. elderly recipients have higher chances of developing GVHD than younger patients (Afram *et al.*, 2018; Carlens *et al.*, 1998; Eisner and August, 1995; Ferrara, 2000; Flowers *et al.*, 2011; Hahn *et al.*, 2008; Stewart *et al.*, 2004; Storb *et al.*, 1983). Kasamon *et al.* (2015) studied 271 patients aged 50–75 years who received a nonmyeloablative regimen, a treatment related to human leukocyte antigen -haploidentical blood or marrow transplantation (haplo-BMT) (Kasamon *et al.*, 2015). Their comparison of patients aged 50–59 years with those aged 60–75 years showed that patient age is remarkably associated with an increased risk of grade 2 to 4 acute GVHD Macrophages infiltration plays an important role in GVHD. Exploring the role of age-related macrophage is helpful to improve of HSCT in older patients. Cellular senescence is chromatin-associated and may permanently stop the cell cycle via mainly senescence-associated secretory phenotype Cellular senescence accompanied by changes from immunosenescence will result in inflamm-ageing, which in turn may contribute to the function alteration in the elderly (Fulop *et al.*, 2018; Vicente *et al.*, 2016). Inflamm-ageing is a chronic low-grade inflammatory state associated with advanced age (Franceschi *et al.*, 2000; Xia *et al.*, 2016). Studies have found a relationship between inflamm-ageing and innate immune cells, such as neutrophils, NK and NKT cells, macrophages and DC (Boe *et al.*, 2017; Shaw *et al.*, 2013). Macrophages contribute to inflamm-ageing, which in turn alters macrophages. When these two reciprocal mechanisms are combined, macrophages in an inflamm-ageing state manifest changed functions, including phagocytosis, antigen presentation, interaction with other immune cells, and pro-fibrosis, which is achieved with the help of cytokines release (Fei *et al.*, 2016; Minhas *et al.*, 2019).

Development and Migration of Macrophages Influenced by Inflamm-Ageing in GVHD

Macrophage infiltration is increased in inflamm-ageing and is an important feature in GVHD pathogenesis. It has been believed that macrophage populations are mainly replenished by monocytes from the bone marrow (BM), but a partial tissue-resident macrophage which stem cells from embryonic hematopoiesis is able to self-maintain independently of BM (Ginhoux and Guilliams, 2016; Hume *et al.*, 2019). Of interest, factors related to ageing, such as sirtuin-1 (Sirt-1), which is essential in organismal longevity, stem cells function and self-renewal by regulating acetylation/deacetylation of transcription factors and co-regulators, were also confirmed to affect macrophage self-renewal ability (Imperatore *et al.*, 2017). Notably, the regulator can be blocked by inhibitors such as ex-527 and thus attenuate acute GVHD with a reduction in T-cell pathogenicity, and alleviate ongoing chronic GVHD by diminishing follicular T-helper generation, B-cell activation and plasma cell differentiation in murine models (Daenthanasanmak *et al.*, 2019). Factors related to ageing, such as Sirt-1, may also influence the proliferation and aging of macrophages in GVHD pathogenesis. However, contrary to most studies, Hashimoto and Merad (2011) indicated that the administration of CSF-1 before transplantation that ameliorates the GVHD morbidity and mortality via expending the host macrophage pool and subsequently reducing donor T cells expansion. It may be explained that the final effect of reducing donor T cells expansion plays an dominant role in ameliorating GVHD. Notably, increased infiltration of macrophages in inflamm-ageing state regulate many disease development (Maeso-Díaz *et al.*, 2019; Noble *et al.*, 2019; Xu *et al.*, 2015). Given the above information, altered macrophages infiltration in inflamm-ageing may contribute to GVHD.

Age-related macrophage polarization in GVHD

Macrophages can polarize to different subgroups when stimulated by different microenvironment. Under the stimulation of lipopolysaccharide, interferon (IFN), tumor necrosis factor (TNF) , M1 macrophages are induced to produce pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-12, and IL-23, as well as nitric oxide (NO) and reactive oxygen species (ROS), While stimulated by cytokines, such as IL-4, IL-10, and IL-13, glucocorticoids, and immune complexes, M2 macrophages polarization plays an important role in anti-inflammatory action by secreting anti-inflammatory cytokines, such as IL-10 and TGF-β, and promoting the differentiation into M2a, M2b, M2c, M2d (Becker *et al.*, 2018; Li *et al.*, 2018; Mohammadi *et al.*, 2019). Macrophages are usually polarized to M2a that expresses mannose receptors/CD206, IL-R, and CCL17; produces profibrotic factors; and participates in wound healing when induced by IL-4 and IL-13. Macrophages are polarized either to M2b, which regulates immune response and inflammation when stimulated by LPS, IL-1β, and immune complex or to M2c, which secretes pro-fibrotic factors and expresses Mer receptor tyrosine kinase, which is important for phagocytosis under IL-10 stimulation. Stimulation with IL-6, Toll-like receptor (TLR) ligands, and A2 adenosine receptor agonists polarizes macrophages to M2d, which prompts adenosines to engage in angiogenesis and cancer metastasis (Ferrante and Leibovich, 2012; Wang *et al.*, 2018). Most studies support that the pro-inflammatory M1 macrophage contributes to acute GVHD, meanwhile, it is M2 macrophage primarily works in chronic GVHD. However, results about M1 macrophage /M2 macrophage ratio influenced by inflamm-ageing were not uniform in different studies.

Cytokines secreted by macrophages and receptors play important roles in GVHD. However, cytokines are pleiotropic and complicated, and cytokine network works through an antagonistic and synergistic effect. Many animal studies focused on the inhibition of IL-6. Though IL-6 can be secreted by M1 macrophage, IL-6 can promote transformation of M1 macrophages to M2 macrophages subgroup, which plays an important role in chronic GVHD.

Murine sclerodermatous graft-versus-host disease (Scl GVHD), characterized by skin thickening and lung fibrosis with infiltration of macrophage and TGF-β, is a mature model (Yang *et al.*, 2017). Using a Scl GVHD model, Beyer *et al.* (2015) showed that the activation of LXRs, are able to inhibit fibroblast activation and collagen release via interfering with the release of interleukin-6 from macrophages (Beyer *et al.*, 2015). Maier *et al.* (2017) suggested that the inhibition of phosphodiesterase 4 (PDE4), may block inflammation-driven fibrosis by reducing inflammatory cell activity and profibrotic cytokines released from M2 macrophages, such as IL-6, thereby reducing fibroblast activation and collagen release in murine Scl GVHD. A study by Shao *et al.* (2015) reported that Stat1 can regulate lupus-like chronic GVHD severity through interactions with Stat3, as evidenced by the increase in IL-6 and IFN-γ secretion and macrophage infiltration in the Stat1- knockout mice. Advances have been achieved in clinical drug administration to ameliorate chronic GVHD. Du *et al.* (2017) found that pirfenidone is able to ameliorate murine chronic GVHD by inhibiting the infiltration of macrophages and the production of TGF-β, another important cytokine secreted by macrophages in chronic GVHD. Findings on the changes in cytokines secreted are inconsistent. Macrophage-derived cytokines are elevated in the ageing state, but detailed changes are complicated because the elevation of cytokines varies in different diseases and conditions (Xu *et al.*, 2015). In addition, increased oxidizing compounds are detected in elder patients (Suchy *et al.*, 2014). Hence, the increased levels of pro-inflammatory cytokines secreted by age-related macrophages and the increased levels of oxidizing compounds aggravate acute GVHD. Meanwhile, TGF-β and IL-6 may tend to exaggerate chronic GVHD.

On the other hand, cytokine alteration in inflamm-ageing should be considered in regulating macrophage activation. Generally, the factors contributing to activation of macrophage include not only the pattern recognition receptor (PRR) signals but also the growth and survival factors to increase macrophages (Du *et al.*, 2017). Colony stimulating factor (CSF) is necessary for the proliferation and polarization of macrophage and an anti-CSF-1R monoclonal antibody is effective in reducing chronic GVHD by depleting macrophages (Wen *et al.*, 2019). Also, IL-21 and IL-17A generated by T cells drive monocyte-macrophage differentiation. Cutaneous macrophages infiltrate in acute GVHD (Terakura *et al.*, 2015). Alexander *et al.* (2014) also confirmed that macrophages derived from donor bone marrow (F4/80+CSF-1R+CD206+iNOS-) infiltrate in cutaneous chronic GVHD and subsequently revealed that it is CSF-1/CSF-1R-dependent in the IL-17-dependent chronic GVHD model (Alexander *et al.*, 2014).

Recognition, Phagocytosis and Clearance of Macrophages in Regulating GVHD

Tissue damage and infection can release PAMPs and DAMPs, which interact with PRPs and promote APCs activation, which is the first step to initiate acute GVHD (Akira *et al.*, 2006; Shin and Harris, 2011). A reduced response to PRR agonists and cytokines dysregulation was observed in the peripheral blood mononuclear cells between old individuals and adults (Metcalf *et al.*, 2015). Notably, Nod-like receptor 3 (NLRP3) inflammasome, a class of PRPs, can mediate IL-1β production and subsequently GVHD, furthermore, the early blockade of IL-1β could reduce GVHD severity (Jankovic *et al.*, 2013; Yao *et al.*, 2017). In an inflamm-aging state, an increased ROS release was observed, which can activate NLRP3. This means that macrophages in inflamm-ageing may show an upregulated expression in NLRP3, which promotes CTL-induced IL-1β secretion and contributes to GVHD in elder patients. Another important receptor participating in macrophage to recognize PAMPs and DAMPs is Toll-like receptor (TLR) (Shin and Harris, 2011). TLR expression is decreased in ageing (Renshaw *et al.*, 2002). TLR-4 gene mutations was reported to be an risk for severe acute GVHD (Elmaagacli *et al.*, 2006) Also, study investigating the role of TLR-4 in triggering acute GVHD by Imado *et al.* (2010). Imado *et al.* (2010) showed increased levels of serum lipopolysaccharide, increased infiltration of donor cell and CD68+ cell, increased expression of TNF-α mRNA, increased apoptotic cells, and TLR-4 expression in TLR-4-mutant recipients who developed significantly more severe GVHD. Thus, it is possible that the expression of recognition receptors in aged macrophages regulate GVHD.

Reduced phagocytosis and clearance ability in inflamm-ageing are supposed to aggravate GVHD. Phagocytosis and clearance are basic functions that allow macrophages to eliminate harmful materials and dead cells to maintain a balanced microenvironment. Macrophages can also digest antigens into peptides and present them to adaptive immune cells. The main classes of phagocytic receptors include Fc receptors, complement receptors, integrins, C-type lectins and Scavenger receptors (Linehan and Fitzgerald, 2015). The macrophages of old individuals have a decreased capacity for phagocytosis and clearance (Aprahamian *et al.*, 2008; Kim *et al.*, 2017). A diminished ability to phagocytose apoptotic bodies was showed in ageing macrophages *in vivo* (Aprahamian *et al.*, 2008). Increased macrophages phagocytosis and clearance is related to attenuate GVHD because macrophages can engulf apoptotic T cells, release TGF-β, and promote the expansion of regulatory T cells (Bonnefoy *et al.*, 2018; D'Aveni *et al.*, 2015). Bonnefoy *et al.* (2018) reported that SuperMApo, the pro-resolving factors within the supernatant of macrophages, are able to enhance the phagocytic capacity of macrophages to eliminate apoptotic cells or bacteria and modify APC homeostasis by accelerating expression loss of co-stimulatory and MHC-II molecule. SuperMApo treatment strongly limited GVHD clinical scores and improved survival in xenogeneic GVHD model. The TGF-β within SuperMApo is critical to enhance the phagocytic capacity of macrophages. Interestingly, increased macrophage and TGF-β contribute to chronic GVHD. The opposite response of TGF-β between acute and chronic GVHD may owing to aforementioned differences in GVHD pathogenesis. Despite enhancing the phagocytic capacity of macrophages, TGF-β produced early after HSCT is able to attenuate acute GVHD by regulating the proliferation, expansion, and differentiation, especially inducing Treg

cells, while the production of TGF-β which contributes to tissue fibrosis late after HSCT mediates chronic GVHD (Banovic *et al.*, 2005; McCormick *et al.*, 1999; Yang *et al.*, 2017; Zhang *et al.*, 2018; Zhang *et al.*, 2003). Therefore, it is possible that macrophages play an important role in controlling acute GVHD and limiting GVHD clinical score by eliminating apoptotic cells, releasing TGF-β and inducing regulatory T cell.

Macrophages Promote Activation, Proliferation, Differentiation and Migration of Donor T Cells

Antigen presentation ability was decreased in aged macrophages with reduced autophagy; decreased surface antigen expression; decreased expression of TLR, major histocompatibility complex (MHC) II, and coreceptor CD86 in macrophages; and high ROS level (Stranks *et al.*, 2015). Antigen presentation plays an important role in connecting the innate and adaptive immune responses. Macrophages, as one of antigen presentation cells, participate in the initiation of GVHD after antigen exposure (van Balen *et al.*, 2018). As we mentioned before, tissue damage promotes the release of alarmins, including DAMPs, MAMPs, and inflammatory cytokines. Alarmins can be engulfed directly or recognized via interacting with PRRs through APC. Antigens digested into peptides and assembled with MHC molecules to form the peptide-MHC complex are presented on the membrane of macrophages to provide a signal for recognition of T cells. Stimulated by TCR-peptide-MHC complex, and some adhesion molecules with co-stimulatory signals, activated T cells proliferate and polarize. Activated macrophages secrete cytokines and promote activation, proliferation, differentiation, and migration of donor T cells.

There are some therapies based on this process. Saric *et al.* (2016) reported that mechanistic target of rapamycin (mTOR) controls lysosome tabulation and antigen presentation in macrophages and dendritic cells. Interestingly, studies showed that efficacy of mTOR inhibitors, such as sirolimus and everolimus, correlates with prophylaxis and therapy of GVHD (Lutz and Mielke, 2016). The potential mechanism is that mTOR is activated through signaling pathways, such as the P3K–Akt–mTOR pathway, and is then engaged to regulate cell metabolism, proliferation, and survival. While mTOR inhibitors can target and block mTOR, it is possible that mTOR inhibitors function to inhibit the antigen presentation of macrophages and prevent GVHD in a certain degree, but direct evidence is still lacking. Generally, though mTOR signaling increases with age in mice (Baar *et al.*, 2016), it is possible that mTOR complex in an inflamm-ageing state aggravates GVHD.

Notably, some molecules related to predicting GVHD are also expressed in macrophages. Recently, PD-1 and CTLA-4 have become popular targets in immunotherapy. Tim-3, expressed on IFN-γ-producing T cells, FoxP3+ Treg cells, macrophages, and dendritic cells, are also co-inhibitory receptors in regulating immune responses (Lee *et al.*, 2016). Surprisingly, studies found that Tim-3 was predictive of grade 3-4 GVHD. Other informative plasma biomarkers, including IL6, sTNFR1, and ST2, can predict GVHD (McDonald *et al.*, 2015). A study by Blazar *et al.* (2016) demonstrated that IL-33–mediated ST2 signaling can activate p38 MAPK to expand Treg cells, IL-33–expanded Treg cells then control macrophage activation and reduce M1 macrophage generation, thus preventing the accumulation of effector T cells in GVHD-target tissue and protects recipients from GVHD. Besides, as a member of the tumor necrosis factor receptor (TNFR) family, glucocorticoid-induced tumor necrosis factor receptor family-related gene (*GITR*)s was found to regulate alloreactive responses during GVHD (Muriglan *et al.*, 2004). A study suggested that GITR functions as a costimulatory signal for the proliferation and polarization of the antigen-driven proliferation of T cells. What is more, mouse GITR ligand (GITRL) was expressed in dendritic cells, macrophages, and B cells constitutively. Macrophages are also able to increase the production of pro-inflammatory factors, such as NO, MMP-9, and COX-2 under the stimulation of soluble GITR via the Rel–NFκB pathway. CD30 is another TNFR family member and regulates CD4+T Cell-mediated GVHD (Blazar *et al.*, 2004). CD30L (CD153) is its ligand and expressed by activated T cells, B cells, and macrophages. Ferritin is a macrophage activation-linked acute-phase protein as well as an iron storage marker, but it fails to be a candidate biomarker for acute or chronic GVHD in pediatric HSCT (Großekatthöfer *et al.*, 2013). Also, IL-10 was shown to suppress antigen presentation (Mittal and Roche, 2015), but in a mice model of GVHD, IL-10 inhibited macrophage function and inflammatory cytokines production without significant improvement in morbidity and mortality (Emmanouilides *et al.*, 1996). Thus, more studies are needed to determine candidate markers, and further reduce GVHD with the help of macrophage-targeted therapies.

Macrophages Influenced by Inflamm-Ageing in Chronic GVHD

Macrophages participate in aberrant tissue repair with fibrosis. A study on oral chronic GVHD manifesting basal cell squamatization, lichenoid inflammation, sclerosis, apoptosis, and lymphocytic exocytosis also showed a higher expression of CD3, CD4, CD8, CD103, CD163, and FoxP3and demonstrated that oral chronic GVHD is largely T-cell-driven with macrophage participation (Motta *et al.*, 2018). Some factors expressed on macrophages were indicated to be a potential target in fibrosis. Allograft inflammatory factor-1, a protein expressed by macrophages, is essential in the mechanism of lung fibrosis by stimulating macrophages to secrete TGF-β and promoting the migration and proliferation of lung fibroblasts (Nagahara *et al.*, 2018). A study by Yamakawa *et al.* (2018) described a cascade of fibrosis in chronic GVHD that TGF-β produced by macrophages that mediates fibroblast differentiation to HSP47+ myofibroblasts and results in the production of collagen.

With a Scl-GvHD model manifesting skin thickening, increased collagen synthesis and tissues fibrosis, including skin, kidney, and lung, the infiltration of F4/80+ macrophage as well as increased CD4+ T cells were detected (Yang *et al.*, 2017). The Scl-GvHD model is a represented chronic GVHD model. Chronic GVHD is associated with elevated M2 macrophage markers and endoplasmic reticulum stress which contributes to chronic inflammation

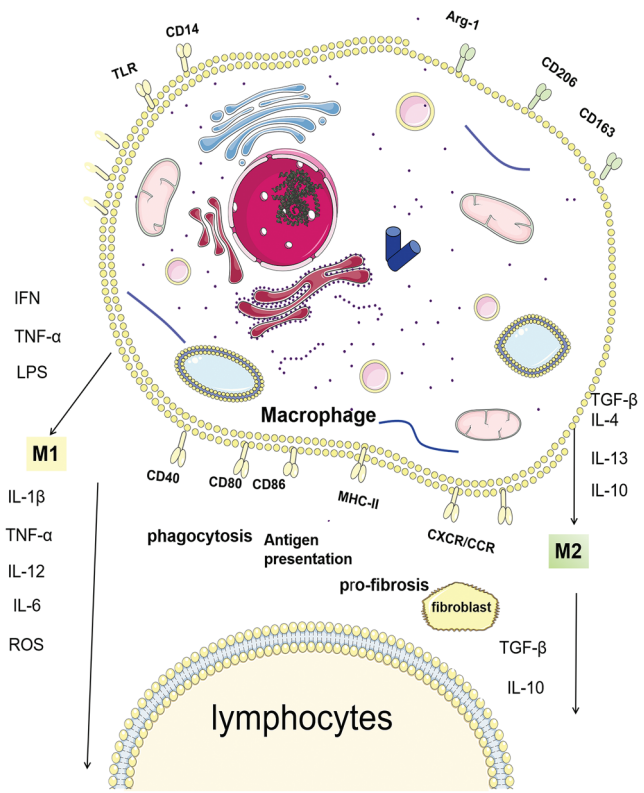

FIGURE 1. Macrophage in GVHD. Macrophages can be stimulated and polarized into M1 or M2 macrophages and thus produce different cytokines.
Both M1 and M2 macrophages play an important role in GVHD. Macrophages generally interact with lymphocytes and regulate GVHD. Functions of macrophage in GVHD can be modified, including polarization, phagocytosis, antigen presentation and pro-fibrosis. M1: M1 macrophage; M2: M2 macrophage.

and age-related diseases (Brown and Naidoo, 2012; Garg *et al.*, 2012; Mukai *et al.*, 2017). As a scavenger receptor, CD163 is mostly expressed on M2 macrophages (Law *et al.*, 1993). A retrospective study by Nishiwaki *et al.* (2009) counted the total number of CD8+ T cells, CD163+ macrophages, and CD1a+ dendritic cells in 104 biopsy specimens of previously untreated skin acute GVHD and found that the infiltration of CD163+ macrophages was the only significant predictor for refractory GVHD. Overall survival was significantly lower in patients with many CD163+ macrophages. Additionally, a recent study by Inamoto *et al.* (2017). also showed that the cumulative incidence of de novo-onset chronic GVHD is higher in patients with higher plasma soluble CD163 concentrations at day 80 than those with lower concentrations. Nishiwaki *et al.* (2009) also indicated that overall survival of patients with infiltration of more CD163+ macrophages is significantly lower than that of those who are infiltrated with less CD163+ macrophages in acute refractory GVHD. Therefore, CD163+ macrophages may be a new target for treating GVHD. With regard to the association of CD163 and GVHD, anti-inflammatory cytokines are able to increase the expression of CD163 in macrophages that the expression of CD163 is positively related to anti-inflammatory macrophages which contributes to chronic GVHD. Interestingly, Nishiwaki *et al.* (2009) also detected the elevated expression of CD163 in oxidative conditions, and suspected that the activation of monocyte or macrophage or increased oxidative stress plays an important role in the pathogenesis of chronic GVHD. Surprisingly, it was confirmed that inflamm-aging is closely associated with oxidative stress. Vida *et al.* (2017) studied the role of macrophages in "oxi-inflamm-aging", and found an increased xanthine oxidase activity and expression, higher levels of ROS, and an increase in GSSG, which represents an intracellular oxidized compound, lower catalase activity and a higher GSSG/GSH ratio as well as a higher lipofuscin accumulation in total peritoneal leukocytes and isolated macrophages of older patients. These markers are all indicators of oxidative stress and damage. Consequently, phagocytic capacity and digestive capacity were also assessed and shown to be decreased. Thus, the increased expression of CD163 macrophages caused by oxidative stress may explain the difference in chronic GVHD between elderly and young patients in chronic GVHD. However, it is infiltrated macrophages with a pro-inflammatory phenotype, not M2

TABLE 1

Age-related modification of macrophage

Function	Ageing	References
Phagocytosis	↓	(Antonini *et al.*, 2001; Aprahamian *et al.*, 2008; Kim *et al.*, 2017; Mancuso *et al.*, 2001; Minhas *et al.*, 2019; Wong *et al.*, 2017)
Polarization	M1/M2↑	(Becker *et al.*, 2018; Gonzalez *et al.*, 2015; Ma *et al.*, 2015; Minhas *et al.*, 2019; Pajarinen *et al.*, 2019; Ray, 2017)
	M1/M2↓	(Cui *et al.*, 2019; Strohacker *et al.*, 2012)
Antigen presentation	MHC class II ↓	(Herrero *et al.*, 2001; Strohacker *et al.*, 2012)
	TLR ↓	(Renshaw *et al.*, 2002; Strohacker *et al.*, 2012; van Duin *et al.*, 2007)
Cytokines secretion	Pro-inflammatory (IL-6, TNF-α, etc.) ↑	(Bruunsgaard and Pedersen, 2003; Franceschi *et al.*, 2007)
	Pro-inflammatory (IL-6, etc.) ↓	(Higashimoto *et al.*, 1993; Renshaw *et al.*, 2002)
	TGF-β ↑	(Carrieri *et al.*, 2004; Forsey *et al.*, 2003)
Metabolism production	NO ↓	(Antonini *et al.*, 2001; Koike *et al.*, 1999)

Arrows (↑ and ↓) indicate increased or decreased levels in the elderly compared with young; M1/M2: the ratio of M1 macrophage and M2 macrophage; MHC class II: major histocompatibility complex class II molecule; TLR: Toll-like receptor; IL-6: interleukin 6; TGF-β: transforming growth factor-β; NO: nitric oxide.

macrophage subset that is related to a more severe fibrosis of old mice in a liver fibrosis model (Delire *et al.*, 2016). Several disagreements still exist, Therefore, further studies with GVHD models are needed to understand alteration of ageing macrophages in GVHD.

Conclusion

This review addresses the issue by discussing the following: role of macrophages in GVHD, modifications of macrophages influenced by "inflamm-ageing", and whether the modified macrophages aggravate or attenuate GVHD. Infiltration of macrophages plays an important role in GVHD pathogenesis mainly through cytokines secreted by macrophages and molecules expression on macrophages. The increased migration and infiltration of macrophages in inflamm-ageing state and elevated oxidation products may aggravate GVHD. In acute GVHD, pro-inflammatory macrophage filtrations show an advantage as a potential therapeutic target, while in chronic GVHD, anti-inflammatory macrophages prominently contribute to fibrosis. Macrophage in GVHD is presented in Fig. 1 and age-related alteration of macrophage is showed in Tab. 1.

Reviews in macrophages in GVHD is lacking, though advances have been achieved in inflamm-ageing, age-related modifications of macrophages influenced by inflamm-ageing in GVHD are rarely studied, and no direct related work have been reported. This is an interesting area requiring further study. Aiming to figure out the age-related modifications of macrophages influenced by "inflamm-ageing" in GVHD, we connect modifications of macrophages in inflamm-ageing with macrophages in GVHD. An expansion of research in this field, as well as more direct evidences and further studies about the age-related modifications of macrophages influenced by "inflamm-ageing" in GVHD are needed.

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References

Afram G, Simón JAP, Remberger M, Caballero-Velázquez T, Martino R, Piñana JL, Ringden O, Esquirol A, Lopez-Corral L, Garcia I, López-Godino O, Sierra J, Caballero D, Ljungman P, Vazquez L, Hägglund H (2018). Reduced intensity conditioning increases risk of severe cGVHD: identification of risk factors for cGVHD in a multicenter setting. *Medical Oncology* **35**: 79. DOI 10.1007/s12032-018-1127-2.

Akira S, Uematsu S, Takeuchi O (2006). Pathogen recognition and innate immunity. *Cell* **124**: 783–801. DOI 10.1016/j.cell.2006.02.015.

Alexander KA, Flynn R, Lineburg KE, Kuns RD, Teal BE, Olver SD, Lor M, Raffelt NC, Koyama M, Leveque L, Le Texier L, Melino M, Markey KA, Varelias A, Engwerda C, Serody JS, Janela B, Ginhoux F, Clouston AD, Blazar BR, Hill GR, MacDonald KP (2014). CSF-1-dependant donor-derived macrophages mediate chronic graft-versushost disease. *The Journal of clinical investigation* **124**: 4266–4280. DOI 10.1172/JCI75935.

Antonini JM, Roberts JR, Clarke RW, Yang HM, Barger MW, Ma JY, Weissman DN (2001). Effect of age on respiratory defense mechanisms: pulmonary bacterial clearance in Fischer 344 rats after intratracheal instillation of Listeria monocytogenes. *Chest* **120**: 240–249. DOI 10.1378/chest.120.1.240.

Aprahamian T, Takemura Y, Goukassian D, Walsh K (2008). Ageing is associated with diminished apoptotic cell clearance *in vivo*. *Clinical & Experimental Immunology* **152**: 448–455. DOI 10.1111/j.1365-2249.2008.03658.x.

Arai S, Arora M, Wang T, Spellman SR, He W, Couriel DR, Urbano-Ispizua A, Cutler CS, Bacigalupo AA, Battiwalla M, Flowers ME, Juckett MB, Lee SJ, Loren AW, Klumpp TR, Prockup SE, Ringdén OTH, Savani BN, Socié G, Schultz KR, Spitzer T, Teshima T, Bredeson CN, Jacobsohn DA, Hayashi RJ, Drobyski WR, Frangoul HA, Akpek G, Ho VT, Lewis VA, Gale RP, Koreth J, Chao NJ, Aljurf MD, Cooper BW, Laughlin MJ, Hsu JW, Hematti P, Verdonck LF, Solh MM, Norkin M, Reddy V, Martino R, Gadalla S, Goldberg JD, McCarthy PL, Pérez-Simón JA, Khera N, Lewis ID, Atsuta Y, Olsson RF, Saber W, Waller EK, Blaise D, Pidala JA, Martin PJ, Satwani P, Bornhäuser M, Inamoto Y, Weisdorf DJ, Horowitz MM, Pavletic SZ (2015). Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the center for international blood and marrow transplant research. *Biology of Blood and Marrow Transplantation* **21**: 266–274. DOI 10.1016/j.bbmt.2014.10.021.

Baar EL, Carbajal KA, Ong IM, Lamming DW (2016). Sex- and tissue-specific changes in mTOR signaling with age in C57BL/6J mice. *Aging Cell* **15**: 155–166. DOI 10.1111/acel.12425.

Banovic T, MacDonald KPA, Morris ES, Rowe V, Kuns R, Don A, Kelly J, Ledbetter S, Clouston AD, Hill GR (2005). TGF-β in allogeneic stem cell transplantation: friend or foe? *Blood* **106**: 2206–2214. DOI 10.1182/blood-2005-01-0062.

Becker L, Nguyen L, Gill J, Kulkarni S, Pasricha PJ, Habtezion A (2018). Age-dependent shift in macrophage polarisation causes inflammation-mediated degeneration of enteric nervous system. *Gut* **67**: 827–836. DOI 10.1136/gutjnl-2016-312940.

Beyer C, Huang J, Beer J, Zhang Y, Palumbo-Zerr K, Zerr P, Distler A, Dees C, Maier C, Munoz L, Krönke G, Uderhardt S, Distler O, Jones S, Rose-John S, Oravecz T, Schett G, Distler JH (2015). Activation of liver X receptors inhibits experimental fibrosis by interfering with interleukin-6 release from macrophages. *Annals of the Rheumatic Diseases* **74**: 1317–1324. DOI 10.1136/annrheumdis-2013-204401.

Blazar BR, Levy RB, Mak TW, Panoskaltsis-Mortari A, Muta H, Jones M, Roskos M, Serody JS, Yagita H, Podack ER, Taylor PA (2004). CD30/CD30 ligand (CD153) interaction regulates CD4+ T cell-mediated graft-versus-host disease. *Journal of Immunology* **173**: 2933–2941. DOI 10.4049/jimmunol.173.5.2933.

Blazar BR, Matta BM, Reichenbach DK (2016). Peri-alloHCT IL-33 administration expands recipient T-regulatory cells that protect mice against acute GVHD. *Blood* **128**: 427–439.

Blazar BR, Murphy WJ, Abedi M (2012). Advances in graft-versus-host disease biology and therapy. *Nature Reviews: Immunology* **12**: 443–458. DOI 10.1038/nri3212.

Boe DM, Boule LA, Kovacs EJ (2017). Innate immune responses in the ageing lung. *Clinical and Experimental Immunology* **187**: 16–25. DOI 10.1111/cei.12881.

Bonnefoy F, Gauthier T, Vallion R, Martin-Rodriguez O, Missey A, Daoui A, Valmary-Degano S, Saas P, Couturier M, Perruche S (2018). Factors produced by macrophages eliminating apoptotic cells demonstrate pro-resolutive properties and terminate ongoing inflammation. *Frontiers in Immunology* **9**: 2586. DOI 10.3389/fimmu.2018.02586.

Brown MK, Naidoo N (2012). The endoplasmic reticulum stress response in aging and age-related diseases. *Frontiers in Physiology* **3**: 263.

Bruunsgaard H, Pedersen BK (2003). Age-related inflammatory cytokines and disease. *Immunology and Allergy Clinics of North America* **23**: 15–39. DOI 10.1016/S0889-8561(02)00056-5.

Carlens S, Ringden O, Remberger M, Lonnqvist B, Hagglund H, Klaesson S, Mattsson J, Svahn BM, Winiarski J, Ljungman P, Aschan J (1998). Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. *Bone Marrow Transplantation* **22**: 755–761. DOI 10.1038/sj.bmt.1701423.

Carrieri G, Marzi E, Olivieri F, Marchegiani F, Cavallone L, Cardelli M, Giovagnetti S, Stecconi R, Molendini C, Trapassi C, De Benedictis G, Kletsas D, Franceschi C (2004). The G/C915 polymorphism of transforming growth factor beta1 is associated with human longevity: a study in Italian centenarians. *Aging Cell* **3**: 443–448. DOI 10.1111/j.1474-9728.2004.00129.x.

Chen YB, Wang T, Hemmer MT, Brady C, Couriel DRal, Alousi A, Pidala J, Urbano-Ispizua A, Choi SW, Nishihori T, Teshima T, Inamoto Y, Wirk B, Marks DI, Abdel-Azim H, Lehmann L, Yu L, Bitan M, Cairo MS, Qayed M, Salit R, Gale RP, Martino R, Jaglowski S, Bajel A, Savani B, Frangoul H, Lewis ID, Storek J, Askar M, Kharfan-Dabaja MA, Aljurf M, Ringden O, Reshef R, Olsson RF, Hashmi S, Seo S, Spitzer TR, MacMillan ML, Lazaryan A, Spellman SR, Arora M, Cutler CS (2017). GvHD after umbilical cord blood transplantation for acute leukemia: an analysis of risk factors and effect on outcomes. *Bone Marrow Transplantion* **52**: 400–408. DOI 10.1038/bmt.2016.265.

Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH, van den Brink MRM, Hansen JA, Parkman R, Miklos DB, Martin PJ, Paczesny S, Vogelsang G, Pavletic S, Ritz J, Schultz KR, Blazar BR (2017). The biology of chronic graft-versus-host disease: a task force report from the national institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. *Biology of Blood and Marrow Transplantation* **23**: 211–234. DOI 10.1016/j.bbmt.2016.09.023.

Cui CY, Driscoll RK, Piao Y, Chia CW, Gorospe M, Ferrucci L (2019). Skewed macrophage polarization in aging skeletal muscle. *Aging Cell* **18**: e13032. DOI 10.1111/acel.13032.

Daenthanasanmak A, Iamsawat S, Chakraborty P, Nguyen HD, Bastian D, Liu C, Mehrotra S, Yu XZ (2019). Targeting Sirt-1 controls GVHD by inhibiting T-cell allo-response and promoting Treg stability in mice. *Blood* **133**: 266–279. DOI 10.1182/blood-2018-07-863233.

D'Aveni M, Rossignol J, Coman T, Sivakumaran S, Henderson S, Manzo T, Santos e Sousa P, Bruneau J, Fouquet G, Zavala F, Alegria-Prevot O, Garfa-Traore M, Suarez F, Trebeden-Negre H, Mohty M, Bennett CL, Chakraverty R, Hermine O, Rubio MT (2015). G-CSF mobilizes CD34+ regulatory monocytes that inhibit graft-versus-host disease. *Science Translational Medicine* **7**: 281ra242.

Delire B, Lebrun V, Selvais C, Henriet P, Bertrand A, Horsmans Y (2016). Aging enhances liver fibrotic response in mice through hampering extracellular matrix remodeling. *Sedentary Life and Nutrition* **9**: 98–113.

Du J, Paz K, Flynn R, Vulic A, Robinson TM, Lineburg KE, Alexander KA, Meng J, Roy S, Panoskaltsis-Mortari A, Loschi M, Hill GR, Serody JS, Maillard I, Miklos D, Koreth J, Cutler CS, Antin JH, Ritz J, MacDonald KP, Schacker TW, Luznik L, Blazar BR (2017). Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-beta production. *Blood* **129**: 2570–2580. DOI 10.1182/blood-2017-01-758854.

Eisner MD, August CS (1995). Impact of donor and recipient characteristics on the development of acute and chronic graft-versus-host disease following pediatric bone marrow transplantation. *Bone Marrow Transplantation* **15**: 663–668.

Elmaagacli AH, Koldehoff M, Hindahl H, Steckel NK, Trenschel R, Peceny R, Ottinger H, Rath PM, Ross RS, Roggendorf M, Grosse-Wilde H, Beelen DW (2006). Mutations in innate immune system NOD2/CARD 15 and TLR-4 (Thr399Ile) genes influence the risk for severe acute graft-versus-host disease in patients who underwent an allogeneic transplantation. *Transplantation* **81**: 247–254. DOI 10.1097/01.tp.0000188671.94646.16.

Emmanouilides CE, Luo J, Baldwin G, Buckley D, Lau P, Lopez E, Tabibzadeh S, Yu J, Wolin M, Rigor R, Territo M, Black AC (1996). Murine IL-10 fails to reduce GVHD despite inhibition of alloreactivity *in vitro*. *Bone Marrow Transplantation* **18**: 369–375.

Fei F, Lee KM, McCarry BE, Bowdish DME (2016). Age-associated metabolic dysregulation in bone marrow-derived macrophages stimulated with lipopolysaccharide. *Scientific Reports* **6**: 22637. DOI 10.1038/srep22637.

Ferrante CJ, Leibovich SJ (2012). Regulation of macrophage polarization and wound healing. *Advances in Wound Care* **1**: 10–16. DOI 10.1089/wound.2011.0307.

Ferrara JLM (2000). Pathogenesis of acute graft-versus-host disease: cytokines and cellular effectors. *Journal of Hematotherapy & Stem Cell Research* **9**: 299–306. DOI 10.1089/15258160050079407.

Flowers MED, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, Pereira SE, Nash RA, Mielcarek M, Fero ML, Warren EH, Sanders JE, Storb RF, Appelbaum FR, Storer BE, Martin PJ (2011). Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to national institutes of health consensus criteria. *Blood* **117**: 3214–3219. DOI 10.1182/blood-2010-08-302109.

Forsey RJ, Thompson JM, Ernerudh J, Hurst TL, Strindhall J, Johansson B, Nilsson BO, Wikby A (2003). Plasma cytokine profiles in elderly humans. *Mechanisms of Ageing and Development* **124**: 487–493. DOI 10.1016/S0047-6374(03)00025-3.

Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000). Inflamm-aging: an evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* **908**: 244–254. DOI 10.1111/j.1749-6632.2000.tb06651.x.

Franceschi C, Capri M, Monti D, Giunta S, Olivieri Fal, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E,

Castellani GC, Salvioli S. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of Ageing and Development* **128**: 92–105. DOI 10.1016/j.mad.2006.11.016.

Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, Witkowski JM, Franceschi C (2018). Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? *Frontiers in Immunology* **8**: 1960. DOI 10.3389/fimmu.2017.01960.

Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P (2012). ER stress-induced inflammation: does it aid or impede disease progression? *Trends in Molecular Medicine* **18**: 589–598. DOI 10.1016/j.molmed.2012.06.010.

Ginhoux F, Guilliams M (2016). Tissue-resident macrophage ontogeny and homeostasis. *Immunity* **44**: 439–449. DOI 10.1016/j.immuni.2016.02.024.

Gonzalez OA, Novak MJ, Kirakodu S, Stromberg A, Nagarajan R, Huang CB, Chen KC, Orraca L, Martinez-Gonzalez J, Ebersole JL (2015). Differential gene expression profiles reflecting macrophage polarization in aging and periodontitis gingival tissues. *Immunological Investigations* **44**: 643–664. DOI 10.3109/08820139.2015.1070269.

Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, Szer J, Lipton J, Schwendener A, Gratwohl M, Frauendorfer K, Niederwieser D, Horowitz M, Kodera Y (2010). Hematopoietic stem cell transplantation: a global perspective. *JAMA* **303**: 1617–1624. DOI 10.1001/jama.2010.491.

Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, Gratwohl M, Bouzas LF, Confer D, Frauendorfer K, Gluckman E, Greinix H, Horowitz M, Iida M, Lipton J, Madrigal A, Mohty M, Noel L, Novitzky N, Nunez J, Oudshoorn M, Passweg J, van Rood J, Szer J, Blume K, Appelbaum FR, Kodera Y, Niederwieser D (2015). One million haemopoietic stem-cell transplants: a retrospective observational study. *Lancet Haematology* **2**: e91–100. DOI 10.1016/S2352-3026(15)00028-9.

Großekatthöfer M, Güclü ED, Lawitschka A, Matthes-Martin S, Mann G, Minkov M, Peters C, Seidel MG (2013). Ferritin concentrations correlate to outcome of hematopoietic stem cell transplantation but do not serve as biomarker of graft-versus-host disease. *Annals of Hematology* **92**: 1121–1128. DOI 10.1007/s00277-013-1737-x.

Hahn T, McCarthy PL Jr., Zhang MJ, Wang D, Arora M, Frangoul H, Gale RP, Hale GA, Horan J, Isola L, Maziarz RT, van Rood JJ, Gupta V, Halter J, Reddy V, Tiberghien P, Litzow M, Anasetti C, Pavletic S, Ringden O (2008). Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. *Journal of Clinical Oncology* **26**: 5728–5734. DOI 10.1200/JCO.2008.17.6545.

Hashimoto D, Merad M (2011). Harnessing dendritic cells to improve allogeneic hematopoietic cell transplantation outcome. *Seminars in Immunology* **23**: 50–57. DOI 10.1016/j.smim.2011.01.005.

Herrero C, Marques L, Lloberas J, Celada A (2001). IFN-gamma-dependent transcription of MHC class II IA is impaired in macrophages from aged mice. *Journal of Clinical Investigation* **107**: 485–493. DOI 10.1172/JCI11696.

Higashimoto Y, Fukuchi Y, Shimada Y, Ishida K, Ohata M, Furuse T, Shu C, Teramoto S, Matsuse T, Sudo E (1993). The effects of aging on the function of alveolar macrophages in mice. *Mechanisms of Ageing and Development* **69**: 207–217. DOI 10.1016/0047-6374(93)90024-L.

Hume DA, Irvine KM, Pridans C (2019). The mononuclear phagocyte system: the relationship between monocytes and macrophages. *Trends in Immunology* **40**: 98–112. DOI 10.1016/j.it.2018.11.007.

Imado T, Iwasaki T, Kitano S, Satake A, Kuroiwa T, Tsunemi S, Sano H (2010). The protective role of host Toll-like receptor-4 in acute graft-versus-host disease. *Transplantation* **90**: 1063–1070. DOI 10.1097/TP.0b013e3181f86947.

Imperatore F, Maurizio J, Vargas Aguilar S, Busch CJ, Favret J, Kowenz-Leutz E, Cathou W, Gentek R, Perrin P, Leutz A, Berruyer C, Sieweke MH (2017). SIRT1 regulates macrophage self-renewal. *EMBO Journal* **36**: 2353–2372. DOI 10.15252/embj.201695737.

Inamoto Y, Martin PJ, Paczesny S, Tabellini L, Momin AA, Mumaw CL, Flowers MED, Lee SJ, Carpenter PA, Storer BE, Hanash S, Hansen JA (2017). Association of plasma CD163 concentration with de novo-onset chronic graft-versus-host disease. *Journal of the American Society for Blood and Marrow Transplantation* **23**: 1250–1256. DOI 10.1016/j.bbmt.2017.04.019.

Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, Palmer J, Weisdorf D, Treister NS, Cheng GS, Kerr H, Stratton P, Duarte RF, McDonald GB, Inamoto Y, Vigorito A, Arai S, Datiles MB, Jacobsohn D, Heller T, Kitko CL, Mitchell SA, Martin PJ, Shulman H, Wu RS, Cutler CS, Vogelsang GB, Lee SJ, Pavletic SZ, Flowers ME (2015). National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. *Biology of Blood and Marrow Transplantation* **21**: 389–401.e381. DOI 10.1016/j.bbmt.2014.12.001.

Jankovic D, Ganesan J, Bscheider M, Stickel N, Weber FC, Guarda G, Follo M, Pfeifer D, Tardivel A, Ludigs K, Bouazzaoui A, Kerl K, Fischer JC, Haas T, Schmitt- Gräff A, Manoharan A, Müller L, Finke J, Martin SF, Gorka O, Peschel C, Ruland J, Idzko M, Duyster J, Holler E, French LE, Poeck H, Contassot E, Zeiser R (2013). The Nlrp3 inflammasome regulates acute graft-versus-host disease. *Journal of Experimental Medicine* **210**: 1899–1910. DOI 10.1084/jem.20130084.

Kasamon YL, Bolanos-Meade J, Prince GT, Tsai HL, McCurdy SR, Kanakry JA, Rosner GL, Brodsky RA, Perica K, Smith BD, Gladstone DE, Swinnen LJ, Showel MM, Matsui WH, Huff CA, Borrello I, Pratz KW, McDevitt MA, Gojo I, Dezern AE, Shanbhag S, Levis MJ, Luznik L, Ambinder RF, Fuchs EJ, Jones RJ (2015). Outcomes of nonmyeloablative HLA-haploidentical blood or marrow transplantation with high-dose post-transplantation cyclophosphamide in older adults. *Journal of Clinical Oncology* **33**: 3152–3161. DOI 10.1200/JCO.2014.60.4777.

Kim OH, Kim H, Kang J, Yang D, Kang YH, Lee DH, Cheon GJ, Park SC, Oh BC (2017). Impaired phagocytosis of apoptotic cells causes accumulation of bone marrow-derived macrophages in aged mice. *BMB Reports* **50**: 43–48. DOI 10.5483/BMBRep.2017.50.1.167.

Koike E, Kobayashi T, Mochitate K, Murakami M (1999). Effect of aging on nitric oxide production by rat alveolar macrophages. *Experimental Gerontology* **34**: 889–894. DOI 10.1016/S0531-5565(99)00061-3.

Law SK, Micklem KJ, Shaw JM, Zhang XP, Dong Y, Willis AC, Mason DY (1993). A new macrophage differentiation antigen which is a member of the scavenger receptor

superfamily. *European Journal of Immunology* **23**: 2320–2325. DOI 10.1002/eji.1830230940.

Lee KA, Shin KS, Kim GY, Song YC, Bae EA, Kim IK, Koh CH, Kang CY (2016). Characterization of age-associated exhausted CD8+ T cells defined by increased expression of Tim-3 and PD-1. *Aging Cell* **15**: 291–300. DOI 10.1111/acel.12435.

Li C, Xu MM, Wang K, Adler AJ, Vella AT, Zhou B (2018). Macrophage polarization and meta-inflammation. *Translational Research: Journal of Laboratory and Clinical Medicine* **191**: 29–44. DOI 10.1016/j.trsl.2017.10.004.

Linehan E, Fitzgerald DC (2015). Ageing and the immune system: focus on macrophages. *European Journal of Microbiology and Immunology* **5**: 14–24. DOI 10.1556/EuJMI-D-14-00035.

Lutz M, Mielke S (2016). New perspectives on the use of mTOR inhibitors in allogeneic haematopoietic stem cell transplantation and graft-versus-host disease. *British Journal of Clinical Pharmacology* **82**: 1171–1179. DOI 10.1111/bcp.13022.

Ma Y, Chiao YA, Clark R, Flynn ER, Yabluchanskiy A, Ghasemi O, Zouein F, Lindsey ML, Jin YF (2015). Deriving a cardiac ageing signature to reveal MMP-9-dependent inflammatory signalling in senescence. *Cardiovascular Research* **106**: 421–431. DOI 10.1093/cvr/cvv128.

Maeso-Díaz R, Ortega-Ribera M, Lafoz E, Lozano JJ, Baiges A, Francés R, Albillos A, Peralta C, García-Pagán JC, Bosch J, Cogger VC, Gracia-Sancho J (2019). Aging influences hepatic microvascular biology and liver fibrosis in advanced chronic liver disease. *Aging and Disease* **10**: 684–698. DOI 10.14336/AD.2019.0127.

Maier C, Ramming A, Bergmann C, Weinkam R, Kittan N, Schett G, Distler JHW, Beyer C (2017). Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of interleukin-6 from M2 macrophages. *Annals of the Rheumatic Diseases* **76**: 1133–1141. DOI 10.1136/annrheumdis-2016-210189.

Mancuso P, McNish RW, Peters-Golden M, Brock TG (2001). Evaluation of phagocytosis and arachidonate metabolism by alveolar macrophages and recruited neutrophils from F344xBN rats of different ages. *Mechanisms of Ageing and Development* **122**: 1899–1913. DOI 10.1016/S0047-6374(01)00322-0.

McCormick LL, Zhang Y, Tootell E, Gilliam AC (1999). Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderma. *Journal of Immunology* **163**: 5693–5699.

McDonald GB, Tabellini L, Storer BE, Lawler RL, Martin PJ, Hansen JA (2015). Plasma biomarkers of acute GVHD and nonrelapse mortality: predictive value of measurements before GVHD onset and treatment. *Blood* **126**: 113–120. DOI 10.1182/blood-2015-03-636753.

Metcalf TU, Cubas RA, Ghneim K, Cartwright MJ, Grevenynghe JV, Richner JM, Olagnier DP, Wilkinson PA, Cameron MJ, Park BS, Hiscott JB, Diamond MS, Wertheimer AM, Nikolich-Zugich J, Haddad EK (2015). Global analyses revealed age-related alterations in innate immune responses after stimulation of pathogen recognition receptors. *Aging Cell* **14**: 421–432. DOI 10.1111/acel.12320.

Minhas PS, Liu L, Moon PK, Joshi AU, Dove C, Mhatre S, Contrepois K, Wang Q, Lee BA, Coronado M, Bernstein D, Snyder MP, Migaud M, Majeti R, Mochly-Rosen D, Rabinowitz JD, Andreasson KI (2019). Macrophage de novo NAD(+) synthesis specifies immune function in aging and inflammation. *Nature Immunology* **20**: 50–63. DOI 10.1038/s41590-018-0255-3.

Mittal SK, Roche PA (2015). Suppression of antigen presentation by IL-10. *Current Opinion in Immunology* **34**: 22–27. DOI 10.1016/j.coi.2014.12.009.

Mohammadi A, Blesso CN, Barreto GE, Banach M, Majeed M, Sahebkar A (2019). Macrophage plasticity, polarization and function in response to curcumin, a diet-derived polyphenol, as an immunomodulatory agent. *Journal of Nutritional Biochemistry* **66**: 1–16. DOI 10.1016/j.jnutbio.2018.12.005.

Motta A, Zhan Q, Larson A, Lerman M, Woo SB, Soiffer R, Murphy G, Treister N (2018). Immunohistopathological characterization and the impact of topical immunomodulatory therapy in oral chronic graft-versus-host disease: a pilot study. *Oral Diseases* **24**: 580–590. DOI 10.1111/odi.12813.

Mukai S, Ogawa Y, Urano F, Kudo-Saito C, Kawakami Y, Tsubota K (2017). Novel treatment of chronic graft-versus-host disease in mice using the ER stress reducer 4-phenylbutyric acid. *Scientific Reports* **7**: e64724. DOI 10.1038/srep41939.

Muriglan SJ, Ramirez-Montagut T, Alpdogan O, Van Huystee TW, Eng JM, Hubbard VM, Kochman AA, Tjoe KH, Riccardi C, Pandolfi PP, Sakaguchi S, Houghton AN, Van Den Brink MRM (2004). GITR activation induces an opposite effect on alloreactive CD4(+) and CD8(+) T cells in graft-versus-host disease. *Journal of Experimental Medicine* **200**: 149–157. DOI 10.1084/jem.20040116.

Nagahara H, Seno T, Yamamoto A, Obayashi H, Inoue T, Kida T, Nakabayashi A, Kukida Y, Fujioka K, Fujii W, Murakami K, Kohno M, Kawahito Y (2018). Role of allograft inflammatory factor-1 in bleomycin-induced lung fibrosis. *Biochemical and Biophysical Research Communications* **495**: 1901–1907. DOI 10.1016/j.bbrc.2017.12.035.

Nishiwaki S, Terakura S, Ito M, Goto T, Seto A, Watanabe K, Yanagisawa M, Imahashi N, Tsukamoto S, Shimba M, Ozawa Y, Miyamura K (2009). Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease. *Blood* **114**: 3113–3116. DOI 10.1182/blood-2009-03-209635.

Noble KV, Liu T, Matthews LJ, Schulte BA, Lang H (2019). Age-related changes in immune cells of the human cochlea. *Frontiers in Neurology* **10**: 1851. DOI 10.3389/fneur.2019.00895.

Pajarinen J, Lin T, Gibon E, Kohno Y, Maruyama M, Nathan K, Lu L, Yao Z, Goodman SB (2019). Mesenchymal stem cell-macrophage crosstalk and bone healing. *Biomaterials* **196**: 80–89. DOI 10.1016/j.biomaterials.2017.12.025.

Pasquini MC, Wang Z, Horowitz MM, Gale RP (2010). Report from the center for international blood and marrow transplant research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. *Clinical Transplants* **2010**: 87–105.

Ray K (2017). A shift in macrophage phenotype underlies age-related ENS degeneration. *Nature Reviews Gastroenterology & Hepatology* **14**: 196.

Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S (2002). Cutting edge: impaired toll-like receptor expression and function in aging. *Journal of Immunology* **169**: 4697–4701. DOI 10.4049/jimmunol.169.9.4697.

Saric A, Hipolito VEB, Kay JG, Canton J, Antonescu CN, Botelho RJ (2016). mTOR controls lysosome tubulation and antigen presentation in macrophages and dendritic cells. *Molecular Biology of the Cell* **27**: 321–333. DOI 10.1091/mbc.e15-05-0272.

Shao WH, Gamero AM, Zhen Y, Lobue MJ, Priest SO, Albandar HJ, Cohen PL (2015). Stat1 regulates lupus-like chronic graft-versus-host disease severity via interactions with stat3. *Journal of Immunology* **195**: 4136–4143. DOI 10.4049/jimmunol.1501353.

Shaw AC, Goldstein DR, Montgomery RR (2013). Age-dependent dysregulation of innate immunity. *Nature Reviews: Immunology* **13**: 875–887. DOI 10.1038/nri3547.

Shin OS, Harris JB (2011). Innate immunity and transplantation tolerance: the potential role of TLRs/NLRs in GVHD. *Korean Journal of Hematology* **46**: 69–79. DOI 10.5045/kjh.2011.46.2.69.

Stewart BL, Storer B, Storek J, Deeg HJ, Storb R, Hansen JA, Appelbaum FR, Carpenter PA, Sanders JE, Kiem HP, Nash RA, Petersdorf EW, Moravec C, Morton AJ, Anasetti C, Flowers MED, Martin PJ (2004). Duration of immunosuppressive treatment for chronic graft-versus-host disease. *Blood* **104**: 3501–3506. DOI 10.1182/blood-2004-01-0200.

Storb R, Prentice RL, Sullivan KM, Shulman HM, Deeg HJ, Doney KC, Buckner CD, Clift RA, Witherspoon RP, Appelbaum FA, Sanders JE, Stewart PS, Thomas ED (1983). Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. *Annals of Internal Medicine* **98**: 461–466. DOI 10.7326/0003-4819-98-4-461.

Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJP, Puleston DJ, Shenderov K, Watson AS, Veldhoen M, Phadwal K, Cerundolo V, Simon AK (2015). Autophagy controls acquisition of aging features in macrophages. *Journal of Innate Immunity* **7**: 375–391. DOI 10.1159/000370112.

Strohacker K, Breslin WL, Carpenter KC, McFarlin BK (2012). Aged mice have increased inflammatory monocyte concentration and altered expression of cell-surface functional receptors. *Journal of Biosciences* **37**: 55–62. DOI 10.1007/s12038-011-9169-z.

Suchy D, Łabuzek K, Bułdak Ł., Szkudłapski D, Okopień B (2014). Comparison of chosen activation markers of human monocytes/macrophages isolated from the peripheral blood of young and elderly volunteers. *Pharmacological Reports* **66**: 759–765. DOI 10.1016/j.pharep.2014.04.008.

Terakura S, Martin PJ, Shulman HM, Storer BE (2015). Cutaneous macrophage infiltration in acute GvHD. *Bone Marrow Transplantation* **50**: 1135–1137. DOI 10.1038/bmt.2015.114.

van Balen P, van der Zouwen B, Kruisselbrink AB, Eefting M, Szuhai K, Jordanova ES, Falkenburg JHF, Jedema I (2018). Tissue damage caused by myeloablative, but not non-myeloablative, conditioning before allogeneic stem cell transplantation results in dermal macrophage recruitment without active t-cell interaction. *Frontiers in Immunology* **9**: 331. DOI 10.3389/fimmu.2018.00331.

van Duin D, Mohanty S, Thomas V, Ginter S, Montgomery RR, Fikrig E, Allore HG, Medzhitov R, Shaw AC (2007). Age-associated defect in human TLR-1/2 function. *Journal of Immunology* **178**: 970–975. DOI 10.4049/jimmunol.178.2.970.

Vicente R, Mausset-Bonnefont AL, Jorgensen C, Louis-Plence P, Brondello JM (2016). Cellular senescence impact on immune cell fate and function. *Aging Cell* **15**: 400–406. DOI 10.1111/acel.12455.

Vida C, de Toda IM, Cruces J, Garrido A, Gonzalez-Sanchez M, De la Fuente M (2017). Role of macrophages in age-related oxidative stress and lipofuscin accumulation in mice. *Redox Biology* **12**: 423–437. DOI 10.1016/j.redox.2017.03.005.

Wang LX, Zhang SX, Wu HJ, Rong Xl, Guo J (2018). M2b macrophage polarization and its roles in diseases. *Journal of Leukocyte Biology* **106**: 345–358. DOI 10.1002/JLB.3RU1018-378RR.

Wen Q, Kong Y, Zhao HY, Zhang YY, Han TT, Wang Y, Xu LP, Zhang XH, Huang XJ (2019). G-CSF-induced macrophage polarization and mobilization may prevent acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplantation* **54**: 1419–1433.

Wong CK, Smith CA, Sakamoto K, Kaminski N, Koff JL, Goldstein DR (2017). Aging impairs alveolar macrophage phagocytosis and increases influenza-induced mortality in mice. *Journal of Immunology* **199**: 1060–1068. DOI 10.4049/jimmunol.1700397.

Xia S, Zhang X, Zheng S, Khanabdali R, Kalionis B, Wu J, Wan W, Tai X (2016). An update on inflamm-aging: mechanisms, prevention, and treatment. *Journal of Immunology Research* **2016**: 1–12. DOI 10.1155/2016/8426874 2016.

Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, White TA, Johnson KO, Stout MB, Mezera V, Giorgadze N, Jensen MD, LeBrasseur NK, Kirkland JL (2015). JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proceedings of the National Academy of Sciences of the United States of America* **112**: E6301–E6310. DOI 10.1073/pnas.1515386112.

Yamakawa T, Ohigashi H, Hashimoto D (2018). Vitamin a-coupled liposomes containing siRNA against HSP47 ameliorate skin fibrosis in chronic graft-versus-host disease. *Blood* **131**: 1476–1485.

Yang X, Liu C, Fujino M, Yang J, Li XK, Zou H (2017). A modified graft-versus-host-induced model for systemic sclerosis, with pulmonary fibrosis in Rag2-deficient mice. *FEBS Open Bio* **7**: 1316–1327. DOI 10.1002/2211-5463.12268.

Yao Y, Chen S, Cao M, Fan X, Yang T, Huang Y, Song X, Li Y, Ye L, Shen N, Shi Y, Li X, Wang F, Qian Y (2017). Antigen-specific CD8(+) T cell feedback activates NLRP3 inflammasome in antigen-presenting cells through perforin. *Nature Communications* **8**: 15402. DOI 10.1038/ncomms15402.

Zeiser R, Blazar BR (2017). Pathophysiology of chronic graft-versus-host disease and therapeutic targets. *New England Journal of Medicine* **377**: 2565–2579. DOI 10.1056/NEJMra1703472.

Zhang Q, Fu L, Liang Y, Guo Z, Wang L, Ma C, Wang H (2018). Exosomes originating from MSCs stimulated with TGF-β and IFN-γ promote Treg differentiation. *Journal of Cellular Physiology* **233**: 6832–6840. DOI 10.1002/jcp.26436.

Zhang Y, McCormick LL, Gilliam AC (2003). Latency-associated peptide prevents skin fibrosis in murine sclerodermatous graft-versus-host disease, a model for human scleroderma. *Journal of Investigative Dermatology* **121**: 713–719. DOI 10.1046/j.1523-1747.2003.12517.x.