



Review

Modulation of dendritic cell and T cell cross-talk during aging: The potential role of checkpoint inhibitory molecules

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ABSTRACT

Dendritic cells (DCs) undergo continuous changes throughout life, and there is evidence that elderly DCs have a reduced capacity to stimulate T cells, which may contribute to impaired anti-tumour immune responses in elderly people with cancer. Changes in checkpoint inhibitory molecules/pathways during aging may be one mechanism that impairs the ability of elderly DCs to activate T cells. However, little is currently known regarding the combined effects of aging and cancer on DC and T cell inhibitory molecules/pathways. In this review, we discuss our current understanding of the influence of aging and cancer on key DC and T cell inhibitory molecules/pathways, the potential underlying cellular and molecular mechanisms contributing to their modulation, and the possibility of therapeutically targeting inhibitory molecules in elderly cancer patients.

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Abbreviations: APC, antigen-presenting cell; cDC, conventional dendritic cell; CTLA-4, cytotoxic T lymphocyte antigen-4; DC, dendritic cell; DUSP, dual-specific phosphatase; GAL-9, galectin-9; ICOS, inducible T cell co-stimulator; ICOSL, inducible T cell co-stimulator ligand; IFN- γ , interferon- γ ; IL, interleukin; LAG-3, lymphocyte activation gene-3; LPS, lipopolysaccharide; mDC, myeloid dendritic cell; MDSC, myeloid-derived suppressor cell; mTOR, mammalian target of rapamycin; NF κ B, nuclear factor- κ B; PD-1, programmed cell death protein-1; pDC, plasmacytoid dendritic cell; PD-L1, programmed cell death ligand-1; PD-L2, programmed cell death ligand-2; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TCR, T cell receptor; TGF- β , transforming growth factor- β ; Th1, T helper-1; TIM-3, T cell immunoglobulin and mucin-domain containing-3; TLR, toll-like receptor; TNF- α , tumour necrosis factor- α ; Treg, regulatory T cell.

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1. Introduction: dendritic cells and aging

Most of our current understanding of dendritic cells (DCs), which are specialised antigen-presenting cells (APCs), comes from studies using young adult mice and humans. However, similar to other immune cells, DCs reflect the age of their host. In the very young (fetuses/neonates), DCs have not fully developed all their functions (Willems et al., 2009), whilst at the other end of the age spectrum, there is evidence that the ability of elderly DCs to induce T cell responses is compromised (Agrawal and Gupta, 2011; Gupta, 2014). The latter may contribute to impaired anti-tumour T cell responses thereby enabling cancer progression, and affecting responses to anti-cancer therapies involving the immune system in elderly people with cancer. Age-related changes in inhibitory/checkpoint molecules/pathways may be one mechanism that impairs the ability of elderly DCs to activate T cells. This is an important consideration in elderly hosts with cancer, as studies in young hosts show that tumours exploit inhibitory pathways to thwart anti-tumour immunity (Mittal et al., 2014; Pardoll, 2012; Topalian et al., 2015; Zarour, 2016). Moreover, checkpoint blockade is proving to be a promising strategy for restoring anti-tumour immune responses (Pardoll, 2012; Topalian et al., 2015). This review will discuss our current understanding of the effects of aging on DC and T cell inhibitory/checkpoint pathways, as well as the potential contribution of age-associated cellular and molecular changes. We consider how changes in DC and T cell inhibitory molecules/pathways might impact anti-tumour immunity in the elderly, and the potential for using checkpoint blockade strategies in elderly cancer patients.

Studies first performed in young adult mice, and later confirmed in humans, showed that DCs are a heterogeneous population comprised of several subsets, which can be broadly divided into conventional/myeloid DCs (cDCs/mDCs) and plasmacytoid DCs (pDCs; Merad et al., 2013). DCs play a key role in the generation of antigen-specific T cell immune responses against pathogens and tumours, due to their ability to prime antigen-specific effector T cells (Steinman and Banchereau, 2007). Once an immune response has occurred and the initiating agent eliminated, immune attenuation must occur to prevent damage to host tissues (Hubo et al., 2013; Murakami and Riella, 2014). To facilitate this, DCs and T cells up-regulate inhibitory/checkpoint molecules and anti-inflammatory cytokines, resulting in a greater summation of negative signals during DC/T cell interactions, leading to T cell suppression (Chen and Flies, 2013; Hubo et al., 2013; Murakami and Riella, 2014). Expression of inhibitory molecules and negative DC/T cell cross-talk also allows DCs to maintain immune tolerance under healthy steady state conditions (Hawiger et al., 2001; Hubo et al., 2013).

The effects of aging on DCs are currently not well-defined, and a consensus is yet to be reached, as the limited studies to-date report conflicting results (reviewed by Agrawal et al., 2007b; Agrawal et al., 2008; Agrawal and Gupta, 2011; Gupta, 2014; Shaw et al., 2013; Wong and Goldstein, 2013). Furthermore, those studies focused mainly on the impact of aging on aspects of the DC lifecycle such as antigen uptake and presentation, migration, responses to maturation stimuli, and expression of antigen-presenting and co-stimulatory molecules and pro-inflammatory cytokines involved in priming/activation of effector T cells, which has been summarised in several excellent reviews (Agrawal et al., 2007b, 2008; Agrawal and Gupta, 2011; Agrawal et al., 2012; Gupta, 2014; Gupta and Agrawal, 2013; Shurin et al., 2007; Wong and Goldstein, 2013). However, very little is known regarding the influence of aging on expression of inhibitory molecules by DCs and negative DC/T cell interactions leading to attenuation of immune responses. Recently, immune inhibitory pathways have been gaining increasing attention, particularly in the field of cancer immunotherapy, where they

represent therapeutic targets (Pardoll, 2012; Topalian et al., 2015). Given that cancer is a disease that predominantly affects elderly populations (Derhovanessian et al., 2008; Fulop et al., 2010), it is important to understand if molecules involved in inhibitory DC/T cell cross-talk are affected by aging, as this may impact on anti-cancer immune responses in elderly patients.

2. Inhibitory molecules in DC/T cell cross-talk and aging

Currently, a major focus in the field of cancer immunology/immunotherapy is immune inhibitory pathways, how they are hijacked by tumours to evade immune destruction, and how they can be therapeutically targeted using checkpoint blockade to improve anti-tumour immunity (Pardoll, 2012; Topalian et al., 2015). Many of these inhibitory pathways involve negative DC/T cell cross-talk, although the influence of aging is poorly characterised. This section provides an overview of our current understanding of the effects of aging on several key inhibitory molecules/pathways involved in DC/T cell cross-talk, and discusses conflicting results in the literature, as well as our viewpoint on which studies are most informative for immunity and aging research in the context of checkpoint molecules.

2.1. Programmed cell death ligand-1 (PD-L1), PD-L2 and programmed cell death protein-1 (PD-1)

PD-1 (expressed on T cells) binds its ligands, PD-L1 and PD-L2 (expressed on DCs and other APCs), leading to inhibition of T cell proliferation and activation, T cell anergy, and promotion of regulatory T cell (Treg) development. PD-L1-PD-1 signalling has reciprocal negative effects on DCs by inhibiting DC activation, and promoting immunosuppressive IL-10-producing DCs (Keir et al., 2007).

Surface expression of PD-1 has been shown to increase with aging on murine CD8⁺ and CD4⁺ T cells in spleens, lymph nodes and/or blood (Channappanavar et al., 2009; Decman et al., 2012; Inoue et al., 2014; Lages et al., 2010; Lee et al., 2016; McClanahan et al., 2015; Norrie et al., 2014; Shimada et al., 2009; Shimatani et al., 2009), as well as on human blood CD8⁺ T cells (Dolfi et al., 2013), and human CD4⁺ T cells in skin (Vukmanovic-Stojic et al., 2015). Increased PD-L1 expression has also been reported on elderly murine splenic CD8⁺ T cells (Mirza et al., 2010). However, two conflicting studies showed no age-related changes in PD-1 expression on human blood (Canaday et al., 2013) and murine splenic CD8⁺ and CD4⁺ T cells (Mirza et al., 2010).

The effect of aging on PD-L1/PD-L2 expression on DCs is even less clear, with one study reporting increased expression of PD-L1 and PD-L2 on elderly murine splenic and lung DCs (Lages et al., 2010), and two contrasting studies showing no age-related differences in PD-L1 and PD-L2 in splenic, lymph node and lung DCs (Mirza et al., 2010; Tan et al., 2012). Another study demonstrated that splenic pDCs in middle-aged (12 months old) mice infected with *E. cuniculi* have increased PD-L1 expression, and these pDCs suppressed the capacity of cDCs to induce effector CD25⁺IFN-γ⁺CD8⁺ T cells (Gigley and Khan, 2011). Despite the contradictory reports, collectively, these studies suggest that suppressive DC/T cell interactions via the PD-L1/PD-L2-PD-1 pathway are likely to be increased in the elderly (Table 1).

2.2. CD80, CD86 and cytotoxic t lymphocyte antigen-4 (CTLA-4)

CD80 and CD86 expressed on DCs engage two ligands on T cells: the positive co-stimulatory molecule CD28, and the negative regulatory molecule CTLA-4. CD28-CD80/CD86 interaction leads to enhanced T cell activation, proliferation and cytokine production,

Table 1

Changes in DC and T cell inhibitory pathways during healthy aging.

Inhibitory pathway	Changes on DCs with aging	Changes on T cells with aging	Potential effects on elderly DC/T cell inhibitory cross-talk
PD-L1/PD-L2–PD-1	<u>PD-L1/PD-L2:</u> <i>Increased</i> (Gigley and Khan, 2011; Lages et al., 2010) <i>Maintained</i> (Mirza et al., 2010; Tan et al., 2012)	<u>PD-1:</u> <i>Increased</i> (Channappanavar et al., 2009; Decman et al., 2012; Dolfi et al., 2013; Inoue et al., 2014; Lages et al., 2010; Lee et al., 2016; McClanahan et al., 2015; Norrie et al., 2014; Shimada et al., 2009; Shimatani et al., 2009; Vukmanovic-Stejic et al., 2015) <i>Maintained</i> (Canaday et al., 2013; Mirza et al., 2010) <u>CTLA-4 (negative ligand):</u> <i>Increased</i> (Canaday et al., 2013; Channappanavar et al., 2009; Inoue et al., 2014; Leng et al., 2002) <i>Maintained</i> (Channappanavar et al., 2009; Gregg et al., 2005; Hwang et al., 2009; Lages et al., 2008) <u>CD28 (positive ligand):</u> <i>Decreased</i> (Boucher et al., 1998; Effros et al., 1994; Fagnoni et al., 1996; Leng et al., 2002; Vallejo et al., 1998; Warrington et al., 2003; Weng et al., 2009; Weyand et al., 1998)	Likely increased negative PD-L1/PD-L2–PD-1 interactions
CD80/CD86 – CTLA-4	<u>CD80/CD86:</u> <i>Increased</i> (Della Bella et al., 2007; van Dommelen et al., 2010) <i>Maintained</i> (Agrawal et al., 2007a; Ciaramella et al., 2011; Grolleau-Julius et al., 2008, 2006; Jiang et al., 2009; Li et al., 2012a; Lung et al., 2000; Ordemann et al., 2002; Pashenkov et al., 2000; Pereira et al., 2011; Pietschmann et al., 2000; Prakash et al., 2013; Qian et al., 2011; Shen et al., 2009; Tan et al., 2012; van Dommelen et al., 2010; Wong et al., 2010; You et al., 2014, 2013) <u>GAL-9:</u> Not yet reported	<u>CTLA-4 (negative ligand):</u> <i>Increased</i> (Canaday et al., 2013; Channappanavar et al., 2009; Inoue et al., 2014; Leng et al., 2002) <i>Maintained</i> (Channappanavar et al., 2009; Gregg et al., 2005; Hwang et al., 2009; Lages et al., 2008) <u>CD28 (positive ligand):</u> <i>Decreased</i> (Boucher et al., 1998; Effros et al., 1994; Fagnoni et al., 1996; Leng et al., 2002; Vallejo et al., 1998; Warrington et al., 2003; Weng et al., 2009; Weyand et al., 1998)	Decreased positive CD28–CD80/CD86 interactions and increased negative CD80/CD86–CTLA-4 interactions
TIM-3 – GAL-9	<u>GAL-9:</u> Not yet reported	<u>TIM-3:</u> <i>Increased</i> (Channappanavar et al., 2009; Lee et al., 2016) <i>Maintained</i> (Canaday et al., 2013; Channappanavar et al., 2009) <i>Decreased</i> (Canaday et al., 2013)	Retained or increased capacity of T cells to respond to negative signals via TIM-3, depending on conditions
LAG-3 – MHC-II	<u>MHC-II:</u> <i>Maintained</i> (Agrawal et al., 2007a; Ciaramella et al., 2011; Gardner et al., 2014a,b; Grolleau-Julius et al., 2008, 2006; Lung et al., 2000; Pashenkov et al., 2000; Ping et al., 2003; Prakash et al., 2013; Qian et al., 2011; Shen et al., 2009; Steger et al., 1996; Tan et al., 2012; van Dommelen et al., 2010; Wong et al., 2010) Other LAG-3 ligands, e.g. LSECtin: Not yet reported	<u>LAG-3:</u> <i>Increased</i> (Channappanavar et al., 2009; Decman et al., 2012; Dolfi et al., 2013)	Likely increased negative LAG-3–MHC-II interactions
ICOS – ICOSL	<u>ICOSL:</u> Not yet reported	<u>ICOS:</u> <i>Increased</i> (Channappanavar et al., 2009) <i>Maintained</i> (Canaday et al., 2013; Mirza et al., 2010) <i>Decreased</i> (Canaday et al., 2013)	Together with reduced positive CD28–CD80/CD86 interactions, likely increased negative ICOS–ICOSL signalling
Adenosine (CD39, CD73, adenosine receptors)	<u>CD39, CD73, adenosine receptors:</u> Not yet reported	<u>CD39:</u> <i>Increased</i> (Fang et al., 2016) <u>CD73:</u> <i>Maintained</i> (Hesdorffer et al., 2012) <i>Decreased</i> (Boss et al., 1980) A2A receptor: <i>Maintained</i> (Hesdorffer et al., 2012) Other adenosine receptors: Not yet reported	Likely increased immunosuppression via CD39 for T cells Possible maintenance of T cell suppressive CD73 activity and ability to respond to adenosine via A2A receptor
Anti-inflammatory cytokines: IL-10 and TGF-β	<u>IL-10:</u> <i>Increased</i> (Grolleau-Julius et al., 2006; Zanca et al., 2015) <i>Maintained</i> (Agrawal et al., 2007a; Della Bella et al., 2007; Jing et al., 2009; Pashenkov et al., 2000; Pereira et al., 2011; Prakash et al., 2013; You et al., 2014, 2013) <i>Decreased</i> (Ciaramella et al., 2011; Myer et al., 2010; Wong et al., 2010) <u>TGF-β:</u> <i>Increased</i> (Myer et al., 2010) <i>Maintained</i> (You et al., 2014, 2013)	<u>IL-10:</u> <i>Increased</i> (Garg et al., 2014; Hobbs et al., 1994; Lee et al., 2016; Tatari-Calderone et al., 2012) <i>Maintained</i> (Lages et al., 2008) <u>TGF-β latency-associated peptide:</u> <i>Increased</i> (Santiago et al., 2011)	Likely increased DC and T cell regulatory activity and suppressive cross-talk

whilst ligation of CTLA-4 to CD80/CD86 attenuates effector T cells and induces Treg development (Gardner et al., 2014a).

Most studies to-date have reported that CD80 and CD86 expression is retained or increased on elderly human blood DCs (Della Bella et al., 2007; Pashenkov et al., 2000; Pietschmann et al., 2000), human monocyte-derived DCs (Agrawal et al., 2007a; Ciaramella et al., 2011; Lung et al., 2000; Prakash et al., 2013; Qian et al., 2011), human DCs from a low-density PBMC fraction (You et al., 2013, 2014), as well as elderly murine splenic DCs (Jiang et al., 2009; Li et al., 2012a; Shen et al., 2009; Tan et al., 2012; van Dommelen et al., 2010; Wong et al., 2010), lymph node DCs (Pereira et al., 2011; Tan et al., 2012), thymic DCs (van Dommelen et al., 2010) and in vitro bone marrow-derived DCs (Grolleau-Julius et al., 2006, 2008; Ordemann et al., 2002); Table 1. In contrast, CD28 expression on human CD8⁺ and CD4⁺ T cells declines during aging (Boucher et al., 1998; Effros et al., 1994; Fagnoni et al., 1996; Leng et al., 2002; Vallejo et al., 1998; Warrington et al., 2003; Weng et al., 2009; Weyand et al., 1998), and this is paralleled by increased CTLA-4 expression on elderly human blood CD8⁺ and CD4⁺ T cells (Canaday et al., 2013; Leng et al., 2002), as well as on murine splenic, lymph node and blood CD4⁺ T cells (Channappanavar et al., 2009; Inoue et al., 2014); Table 1. Elderly Tregs from human peripheral blood (Gregg et al., 2005; Hwang et al., 2009; Lages et al., 2008) and murine spleens and lymph nodes (Channappanavar et al., 2009; Lages et al., 2008) also maintain or increase their CTLA-4 levels. Taken together, the reduction/absence of CD28 and concomitant increase in CTLA-4 means that there will be no other possibility but for CD80/CD86 to bind CTLA-4, leading to increased negative DC/T cell cross-talk via this pathway in the elderly.

2.3. T cell immunoglobulin and mucin-domain containing-3 (TIM-3) and galectin-9 (GAL-9)

A model for the role of TIM-3-GAL-9 interactions in attenuation of T cell responses has been proposed in which activated, terminally differentiated CD4⁺ T helper (Th)-1 and CD8⁺ T cells secrete IFN- γ as part of their effector function, and up-regulate TIM-3 expression. IFN- γ promotes GAL-9 expression on DCs/APCs, which interact with TIM-3-expressing effector T cells, triggering T cell apoptosis, inducing tolerance and promoting Treg expansion (Kuchroo et al., 2008).

The three studies examining age-related changes in TIM-3 on T cells report conflicting results, with TIM-3 increasing on elderly murine splenic CD8⁺ T cells (Lee et al., 2016) and conventional CD4⁺ T cells (Channappanavar et al., 2009), remaining unaltered on elderly murine Tregs (Channappanavar et al., 2009) and non-activated elderly human CD8⁺ and CD4⁺ T cells (Canaday et al., 2013), and decreasing on elderly human CD8⁺ T cells following mitogen stimulation (Canaday et al., 2013); Table 1. This suggests that elderly steady state/non-activated T cells may have increased capacity to respond to negative signals via the TIM-3 pathway. The effects of aging on GAL-9 expression on DCs have not yet been reported.

2.4. Lymphocyte activation gene-3 (LAG-3) and MHC-II

Ligation of LAG-3 on (i) CD4⁺ T cells to MHC-II on DCs, and (ii) CD8⁺ T cells to LSECTin on DCs negatively regulates T cell activation by inhibiting T cell receptor (TCR) signalling, and preventing T cell proliferation, IL-2 production and Th1 polarisation (Anderson et al., 2016).

Most studies observed that MHC-II expression is maintained on elderly human blood DCs (Pashenkov et al., 2000), and monocyte-derived DCs (Agrawal et al., 2007a; Ciaramella et al., 2011; Lung et al., 2000; Prakash et al., 2013; Qian et al., 2011; Steger et al., 1996), as well as on murine splenic and lymph node DCs (Gardner

et al., 2014b; Ping et al., 2003; Shen et al., 2009; Tan et al., 2012; van Dommelen et al., 2010; Wong et al., 2010), thymic DCs (van Dommelen et al., 2010), and in vitro bone marrow-derived DCs (Grolleau-Julius et al., 2006; Grolleau-Julius et al., 2008); Table 1. No studies have yet examined age-related changes in other LAG-3 binding partners, such as LSECTin, on DCs. Three studies described an age-related increase in LAG-3 on elderly CD8⁺ T cells in mouse blood and spleen (Decman et al., 2012), elderly murine splenic conventional CD4⁺ T cells and Tregs (Channappanavar et al., 2009) and elderly human blood CD8⁺ T cells (Dolfi et al., 2013), suggesting increased potential for T cell inhibition (Table 1).

2.5. Inducible T cell co-stimulator (ICOS) and ICOS ligand (ICOSL)

If ICOS on activated T cells binds ICOSL on DCs at the same time as CD80/CD86-CD28 interactions, the two pathways co-operate to promote differentiation, proliferation and cytokine production by effector T cells. In the absence of CD28 stimulation, ICOS-ICOSL signals are tolerogenic and enhance the ability of T cells to respond to IL-10 leading to T cell anergy and Treg development (Hubo et al., 2013). As described in section 2.2 and Table 1, CD28 stimulation may be diminished in the elderly, which may skew DC/T cell cross-talk via ICOS-ICOSL towards a tolerogenic outcome.

There are conflicting reports regarding the effects of aging on ICOS expression (Table 1). Two studies have shown that ICOS expression is retained on elderly murine splenic CD8⁺ and CD4⁺ T cells (Mirza et al., 2010), and human blood CD8⁺ T cells (Canaday et al., 2013). An age-related decline in ICOS expression has been reported for human blood CD4⁺ T cells (Canaday et al., 2013), and murine splenic DCs (Mirza et al., 2010). In contrast, one study observed increased numbers of ICOS⁺ conventional CD4⁺ T cells and ICOS⁺ Tregs in elderly murine spleens (Channappanavar et al., 2009). ICOS⁺ Tregs have potent suppressive activity (Herman et al., 2004; Vocanson et al., 2010), suggesting that Treg-mediated suppression may increase with age. No studies have yet examined the effects of aging on ICOSL expression on DCs.

2.6. CD39, CD73, adenosine and adenosine receptors

Regulation of immune cell function by the adenosine pathway is gaining interest. DCs and T cells express the adenosine-producing enzymes CD39 (ecto-nucleoside triphosphate diphospho-hydrolase-1) and CD73 (ecto-5'-nucleotidase), and can thus generate immunosuppressive adenosine. DCs and T cells can respond to adenosine through four adenosine-binding receptors: A1, A2A, A2B and A3, leading to inhibition of effector T cell differentiation, proliferation and cytotoxic activity, Treg expansion, and skewing DCs towards regulatory function (Antonioli et al., 2013).

Only three studies have examined the adenosine pathway and elderly T cells (Boss et al., 1980; Fang et al., 2016; Hesdorffer et al., 2012); Table 1. One showed an age-related decline in CD73 activity in human lymphocytes (Boss et al., 1980). In contrast, another demonstrated that elderly human T cells activated in vitro via TCR stimulation produced higher quantities of adenosine compared to young T cells, although expression of CD73 and the A2A receptor were comparable between the two age groups (Hesdorffer et al., 2012). Recently, Fang et al. (2016) showed that CD39 was up-regulated to a greater extent on memory CD4⁺ T cells from elderly humans following antigenic stimulation by DCs, relative to their young counterparts. Moreover, elderly CD39⁺ CD4⁺ T cells were more susceptible to apoptosis (Fang et al., 2016). Whilst no studies have reported age-related changes in molecules associated with the adenosine pathway on DCs, adenosine-mediated immunosuppres-

sion appears to represent a potent immunosuppressive mechanism in the elderly.

2.7. Anti-inflammatory cytokines: IL-10 and transforming growth factor (TGF)- β

DCs and T cells can secrete a range of anti-inflammatory and immunosuppressive cytokines/mediators; two key mediators are IL-10 and TGF- β . IL-10 and TGF- β inhibit activation and function of effector T cells, induce T cell anergy, suppress DC maturation, and drive differentiation of Tregs and regulatory DCs (Couper et al., 2008; Li et al., 2006).

Several studies have shown that there are no age-related changes in IL-10 production by healthy steady state human blood DCs (Della Bella et al., 2007; Pashenkov et al., 2000), human monocyte-derived DCs (Prakash et al., 2013), and murine lymph node DCs (Pereira et al., 2011). However, following stimulation, IL-10 secretion has been reported to be maintained at levels equivalent to their younger counterparts in elderly human blood DCs (Della Bella et al., 2007; Jing et al., 2009), monocyte-derived DCs (Agrawal et al., 2007a), and DCs from a low-density PBMC fraction (You et al., 2013, 2014). In contrast, others have shown that elderly murine splenic DCs (Zacca et al., 2015) and in vitro bone marrow-derived DCs (Grolleau-Julius et al., 2006) produce higher levels of IL-10 than their younger counterparts following stimulation with Toll-like receptor (TLR) agonists. Yet, others have shown that elderly TLR-activated human monocyte-derived DCs (Ciaramella et al., 2011), murine splenic DCs (Wong et al., 2010) and murine bone marrow-derived DCs (Myer et al., 2010) secrete less IL-10 than their equivalent young DC counterparts. Only three studies have examined DC TGF- β production following stimulation (Myer et al., 2010; You et al., 2013, 2014), and reported conflicting results. Myer et al. (2010) reported an increase in TGF- β mRNA in elderly murine bone marrow-derived DCs stimulated with lipopolysaccharide (LPS) and a TLR-7 agonist, whilst You et al. (2014, 2013) reported that LPS-stimulated elderly human DCs produced similar levels of TGF- β to their younger counterparts.

Elderly T cells may also have increased immunosuppressive capacity, as IL-10 production is increased in elderly murine Tregs (Garg et al., 2014), as well as elderly murine splenic CD8 $^{+}$ and CD4 $^{+}$ T cells stimulated with anti-CD3 alone (Hobbs et al., 1994), PMA/ionomycin alone (Lee et al., 2016), and the combination of anti-CD3 and PMA/ionomycin (Tatari-Calderone et al., 2012). In contrast, one study has shown that young and elderly effector T cells isolated from lymph nodes and co-cultured in vitro with bone marrow-derived DCs infected with the parasite *L. major* produced similar quantities of IL-10 (Lages et al., 2008). Percentages of CD4 $^{+}$ T cells expressing TGF- β latency-associated peptide are also increased in elderly murine mesenteric lymph nodes, Peyer's patches and intestinal lamina propria (Santiago et al., 2011). Collectively, a consensus is yet to be reached regarding the effect of aging on elderly DC and T cell IL-10 and TGF- β production.

2.8. Understanding the current state of the art regarding aging and DC and t cell inhibitory molecules

As described in Sections 2.1–2.7, and summarised in Table 1, there are several discrepancies in the literature regarding age-related changes in DC and T cell expression of inhibitory molecules. It is important to understand reasons for these discrepancies and which studies are most relevant and informative for understanding human immune system aging and its role in age-related diseases. It is now understood that the human and mouse immune systems age differently (Nikolicich-Žugich, 2014; Shaw et al., 2013). Other confounding factors include differences in: (i) age ranges of human volunteers or mice; (ii) criteria used to select human volunteers

(such as nutritional status, medications and socioeconomic status); (iii) mouse strains; and (iv) examination of immune cells isolated ex vivo versus those generated or stimulated in vitro. In this section, we discuss our viewpoint on the relative importance of human and murine studies examining age-related changes in inhibitory molecules.

With regards to literature on DCs and aging, a main reason for conflicting reports is use of different DC subsets. We propose that ex vivo studies of freshly isolated DC subsets from human tissues, that have had minimal in vitro manipulation, are likely to best reflect age-related changes to checkpoint molecules that would be most relevant for understanding how these age-related changes in DCs contribute to diseases, such as cancer; these studies have been highlighted in bold in Table 1. However, one issue is the difficulty associated with obtaining DCs from human tissues. Most studies examining human ex vivo DCs use peripheral blood DCs, as they are easier to obtain. Very few studies have examined human DCs from other tissues, such as natural and ultraviolet-exposed skin (Bhushan et al., 2004; Ghersetich and Lotti, 1994; Grewe, 2001; Kurban and Bhawan, 1990; Thiers et al., 1984), thymus (Nakahama et al., 1990; Varas et al., 2003) and tonsils (Indrasingh et al., 1999), and these studies did not examine age-related changes in inhibitory molecules. Nonetheless, age-related checkpoint molecular changes in human blood DCs could represent changes occurring in tissue DCs, as recent studies have shown that human blood DC subsets have equivalent tissue-resident counterparts, and accurately reflect tissue-resident DC subsets (Haniffa et al., 2012; Mittag et al., 2011; Segura et al., 2013; Segura et al., 2012). Due to the difficulties in isolating human DCs, many studies use DCs differentiated from monocytes in vitro, although they may not reflect age-related changes occurring in vivo. An in vivo counterpart of monocyte-derived DCs has yet to be identified (Harman et al., 2013), and recent transcriptional studies have shown that monocyte-derived DCs share more similarities with monocytes than in vivo DC subsets (Harman et al., 2013; Lundberg et al., 2013). Furthermore, the in vitro generation of DCs may not accurately reflect the complexity of the aged microenvironment in vivo. Thus, it is not yet clear whether studies using in vitro monocyte-derived DCs provide useful age-related information regarding checkpoint molecules.

Similar to DCs, studies using T cells freshly isolated from human tissues and analysed directly with minimal in vitro manipulation could be most representative of age-related changes occurring in vivo when examining suppressive/checkpoint molecules; these studies have also been highlighted in bold in Table 1. An important issue to consider with aging T cell studies is the current debate as to whether T cell immunosenescence is a result of intrinsic age-related changes, or life-long exposure to antigens, resulting in depletion of the naïve T cell pool, and a concomitant increase in antigen-experienced effector/memory T cells (Pawelec, 2012). Early studies often did not consider the latter, and their observations may be indicative of the prevalence of antigen-experienced T cells in elderly individuals. Recent studies have compared specific T cell populations (for example, naïve T cells, or memory T cells) and have found that there is a specific influence of aging on T cell functional status in the elderly (Goronzy et al., 2015; Moskowitz et al., 2017; Pawelec, 2012). Age-related cellular/molecular changes may contribute to age-related differences in inhibitory molecules/pathways (Goronzy et al., 2015; Pawelec, 2014); this is discussed further in section 3. We propose that studies examining: (i) specific T cell sub-populations; and (ii) age-related changes in inhibitory molecules/pathways in conjunction with associated cellular/molecular changes, will be the most informative.

Despite differences in aging, mice remain an important widely used biological model. In particular, murine cancer models are extensively used to study the complexity of the tumour microenvironment, and for pre-clinical assessment of anti-cancer therapies.

Thus, it is necessary to understand the effects of aging and cancer on murine immune cells, and their translational potential. Studies examining freshly isolated ex vivo murine DC and T cell subsets are likely to be more informative for checkpoint profile studies, compared to DCs and T cells generated/stimulated *in vitro*, as the latter are not subject to the complex influence of the *in vivo* healthy aging environment and aging tumour microenvironments. Recent identification of human and murine *in vivo* DC subset equivalents (Crozat et al., 2010; Merad et al., 2013; Robbins et al., 2008) suggests that aging murine DC studies will provide insight into the human situation.

In summary, there is evidence suggesting that inhibitory DC/T cell interactions may be increased with aging. Studies examining ex vivo human DC and T cell subsets will be the most relevant and informative. The studies highlighted in bold in Table 1 show that the PD-L1-PD-1, CD80/CD86-CTLA-4 and LAG-3 pathways on DCs and T cells, and the adenosine pathway on T cells (Fig. 1), are key inhibitory pathways that increase with age. This implies that the outcome of DC/T cell cross-talk is likely to be suppression of effector T cells, as well as induction of Tregs and DCs with regulatory function, preventing the generation of effective anti-tumour T cell responses in the elderly (Fig. 1).

3. Potential mechanisms contributing to changes in DC and t cell inhibitory pathways during aging

There are several factors that could modulate DC and T cell inhibitory molecules/pathways during aging, including changes in the aging microenvironment, and changes at the cellular and molecular levels, with the latter representing an area of increasing interest and importance in the field of immune aging research. Alterations in the aging microenvironment which could affect elderly DC/T cell inhibitory cross-talk include: (i) increases in circulating pro- and anti-inflammatory cytokines (Franceschi and Campisi, 2014), hormones (Chahal and Drake, 2007) and lipids (Lawton et al., 2008); (ii) changes in the microbiome (Magrone and Jirillo, 2013); and (iii) the influence of previous infections and chronic antigenic stimulation as a result of latent infections, particularly cytomegalovirus (Tu and Rao, 2016). In elderly hosts with cancer, secretion of immunosuppressive factors by tumours, such as adenosine, indoleamine 2,3-dioxygenase, IL-10 and TGF- β may further modulate inhibitory molecules/pathways on elderly DCs and T cells, as these tumour-derived factors are known to induce DCs and T cells with immune inhibitory functions in young hosts (Nishikawa and Sakaguchi, 2010; Tran Janco et al., 2015).

The response of elderly DCs and T cells to changes in the aging milieu will ultimately occur at the cellular and molecular levels. Therefore, it is important to understand age-related changes in genetic and epigenetic modifications, and alterations in signalling and metabolic pathways as they will not only influence expression of inhibitory molecules, but also impact downstream events following engagement of DC/T cell inhibitory pathways. Furthermore, the processes of cellular signalling, metabolism and gene expression are intertwined, which means that age-related changes in one of these compartments will have reciprocal effects on the others. This section will relate what we currently know about changes at the cellular/molecular levels in DCs and T cells during aging to the potential impact this could have on inhibitory molecules/pathways in elderly DCs and T cells.

3.1. The potential influence of age-related cellular/molecular changes on inhibitory molecules in elderly DCs

The few studies performed to-date examining cellular/molecular changes to DCs during aging have shown that

these changes impact upon DC functions involved in T cell priming/activation. Age-related changes in DC inhibitory molecules (Table 1) may be attributed to changes in gene expression. One study has shown that young and elderly human monocyte-derived DCs have different gene expression profiles, although inhibitory molecules were not examined (Cao et al., 2014). Alterations in histone methylation patterns have been observed in elderly DCs, and shown to affect transcription factor binding and expression of IFN- α and λ (Prakash et al., 2013) and IL-23 (El Mezayen et al., 2009). Thus, age-related alterations in histone methylation and chromatin configuration may affect expression of DC inhibitory molecules.

Age-related alterations in several signalling pathways, and their regulators and associated transcription factors have been reported in elderly DCs, specifically: (i) reduced phosphorylation of AKT leading to decreased induction of the phosphatidylinositol 3-kinase (PI3 K) pathway (Agrawal et al., 2007a); (ii) alterations in nuclear factor- κ B (NF κ B) pathway activity due to increased activation of the transcription factor interferon regulatory factor (IRF)-3 (Agrawal et al., 2009) and altered phosphorylation kinetics of I κ B kinase (Zacca et al., 2015); (iii) impaired induction and/or phosphorylation of IRF-1 and 7 (Qian et al., 2011; Sridharan et al., 2011; Stout-Delgado et al., 2008); and (iv) reduced phosphorylation and expression of signal transducer and activator of transcription (STAT)-1 and STAT-3 (Qian et al., 2011; Wong et al., 2010). Defects in these pathways have been linked to reduced DC functions involved in T cell activation/priming, such as antigen phagocytosis (Agrawal et al., 2007a) and migration (Agrawal et al., 2007a), as well as impaired production of the pro-inflammatory anti-viral cytokines IFN- α and IFN- λ (Qian et al., 2011; Sridharan et al., 2011; Stout-Delgado et al., 2008). Several of these pathways and their associated factors (IRF-1, AKT, PI3 K, STAT-3 and/or NF κ B) are involved in mediating signalling through negative regulatory molecules, including PD-L1 (Keir et al., 2007), the A2A receptor (Bono et al., 2015), and CD39 (Antonioli et al., 2013). Thus, it is possible that age-related changes in DC signalling pathways influence negative signalling and inhibitory function in elderly DCs.

Metabolic changes in elderly DCs may affect inhibitory signalling pathways. Increased levels of reactive oxygen species (ROS), leading to accumulation of oxidatively modified proteins and lipids in elderly DCs (Cannizzo et al., 2012) may affect signalling through inhibitory pathways, as metabolically modified (acetylated, methylated and glycosylated) proteins and lipids are important mediators of intracellular signalling events in DCs (Pearce and Everts, 2015). Furthermore, DC activation involves a complex series of metabolic changes which are linked to simultaneous changes occurring in signalling pathways, such as NF κ B, PI3K and AKT (O'Neill and Pearce, 2016; Pearce and Everts, 2015). These signalling pathways are altered in elderly DCs; thus, age-related changes in DC signalling and metabolism may have reciprocal effects, leading to altered inhibitory function. Additionally, changes in DC energy profile and mitochondrial function with aging may affect the development of an immune-activating versus regulatory/tolerogenic status in elderly DCs. Increased glycolysis and mitochondrial activity have been linked to DC activation, whilst autophagy and fatty acid oxidation are associated with tolerogenic DC function (O'Neill and Pearce, 2016). With aging, DCs demonstrate mitochondrial dysfunction, specifically, reduced ATP synthesis and mitochondrial membrane potential (Chouquet et al., 2015), and increased accumulation of autophagosomes (Cannizzo et al., 2012); these changes suggest a metabolic profile less conducive to activation, and more conducive to regulatory function. Taken together, it can be speculated that age-related changes in DC gene expression and epigenetic regulators, signalling pathways and metabolism are likely to influence DC inhibitory function.

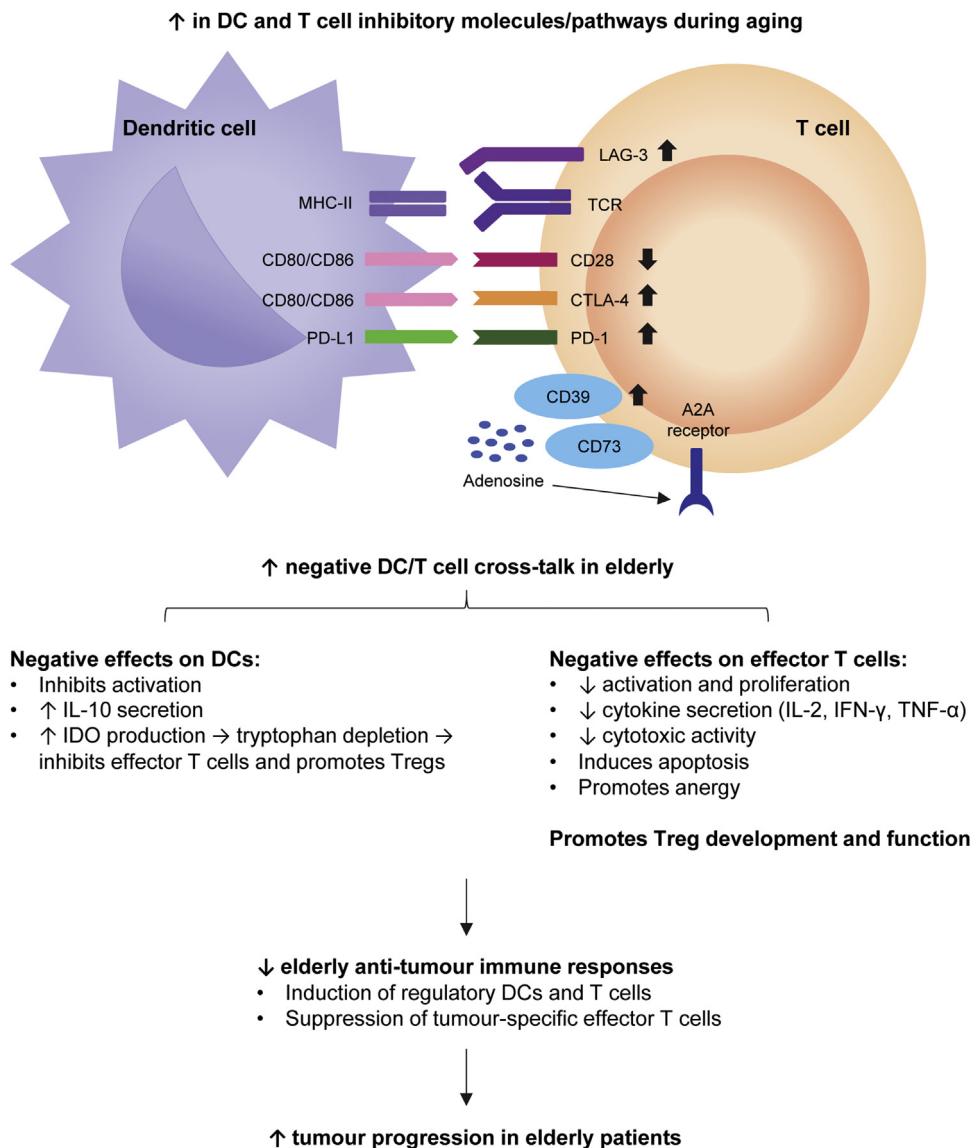


Fig. 1. Inhibitory DC/T cell interactions may be increased during aging, which may impair anti-tumour immunity in the elderly. This figure summarises results from recent studies showing increased expression of inhibitory molecules on ex vivo human dendritic cells (DCs) and/or T cells during aging (shown in bold in Table 1). This may result in a greater summation of negative signals during elderly DC/T cell cross-talk, leading to negative effects on DCs and effector T cells, and promotion of regulatory T cells (Tregs). As a consequence, the generation of effective anti-tumour immune responses may be compromised in the elderly. CTLA-4: cytotoxic T lymphocyte antigen-4, IDO: indoleamine 2,3-dioxygenase, IFN- γ : interferon- γ , IL: interleukin, LAG-3: lymphocyte activation gene-3, MHC-II: major histocompatibility complex II, PD-1: programmed cell death protein-1, PD-L1: programmed cell death ligand-1, TCR: T cell receptor, TNF- α : tumour necrosis factor- α .

3.2. The potential influence of age-related cellular/molecular changes on inhibitory molecules in elderly T cells

As discussed in Section 2.8, increased expression of inhibitory markers on elderly T cells may reflect accumulation of antigen-experienced T cells, however, recent studies examining cellular and molecular changes in elderly T cells provide support for a specific influence of aging. A few studies have shown that elderly human (Bektaş et al., 2013; Cao et al., 2010; Moskowitz et al., 2017; Tserel et al., 2015) and murine (Decman et al., 2012; Mirza et al., 2011) T cells have different gene expression profiles to young T cells, including increased expression of genes for the inhibitory molecules CTLA-4 (Bektaş et al., 2013), IL-10 (Decman et al., 2012; Mirza et al., 2011), LAG-3 (Decman et al., 2012) and PD-1 (Decman et al., 2012). Decman et al. (2012) and Mirza et al. (2011) showed increased expression of inhibitory marker genes in naïve T cells from elderly relative to young non-antigen-exposed mice, sug-

gesting a specific effect of aging, and that increased inhibitory marker expression is not exclusively due to expansion of antigen-experienced T cells. Epigenetic modifications in aged T cells may contribute to their increased expression of inhibitory molecules. Two recent studies have shown that elderly human CD8⁺ T cells have different methylation patterns (Tserel et al., 2015) and patterns of chromatin openness (Moskowitz et al., 2017), compared to their younger counterparts. Tserel et al. (2015) observed DNA hypermethylation of genes involved in T cell differentiation in elderly CD8⁺ T cells, and hypothesised that this may direct the gene expression profile of elderly T cells towards a terminally differentiated state, a hallmark being increased expression of inhibitory molecules (Tu and Rao, 2016). Moskowitz et al. (2017) compared elderly human naïve and central memory T cell populations to their younger counterparts, thereby removing the confounding influence of age-associated oligoclonal T cell expansions. This study observed that aged naïve T cells had an overall loss in chromatin

accessibility at gene promoters, leading to altered transcription factor binding and expression of respiratory chain genes (Moskowitz et al., 2017); alterations in chromatin accessibility could affect gene expression of inhibitory molecules.

It is well-established that T cell signalling is altered during aging, due to changes in: (i) expression and/or activity of intracellular signalling molecules and their regulators; (ii) membrane lipid composition; and (iii) cytoskeletal organisation (reviewed by Garcia and Miller, 2011; Larbi et al., 2011). These changes lead to reduced signalling through the TCR, CD28 and IL-2 receptor pathways, which may contribute to a concomitant increase in signalling through inhibitory pathways (reviewed by Larbi et al., 2011). A possible mechanism may be via age-associated alterations in phosphatases, as elderly human CD4⁺ T cells have increased expression of dual-specific phosphatase (DUSP)-4 (Yu et al., 2012) and DUSP-6 (Li et al., 2012b), the latter due to a decline in levels of the regulator microRNA-181a (Li et al., 2012b). The inhibitory mechanisms of PD-1 and CTLA-4 on T cells are mediated by recruitment of phosphatases to the immunological synapse, leading to de-phosphorylation of signalling molecules involved in T cell activation (Chen and Flies, 2013). Therefore, increases in T cell phosphatases with aging may lead to increased inhibitory activity through the PD-1 and CTLA-4 pathways. In addition, changes in the membrane lipid composition and cytoskeleton of elderly T cells may lead to increased recruitment of inhibitory molecules to the immunological synapse; as demonstrated for the inhibitory molecule CD43 (leukosialin; Garcia and Miller, 2011). This may also apply to the inhibitory molecules discussed in this review. An increase in inhibitory molecules at the immunological synapse may skew the balance away from positive and towards negative signalling, leading to inhibition of elderly T cells (Garcia and Miller, 2011).

Age-related changes in T cell metabolism may also affect inhibitory pathways, although there is a scarcity of information on the impact of metabolic changes on T cell function during aging (reviewed by Goronzy et al., 2015). Mitochondrial dysfunction that occurs with aging may contribute to altered elderly T cell function (Ron-Harel et al., 2015), as effector T cell activation requires intact mitochondrial function (Sena et al., 2013). There is also emerging evidence for a role of mitochondrial metabolism in T cell inhibitory pathways, as a recent study in young mice has shown that PD-1-mediated T cell apoptosis is dependent on mitochondrial fatty acid oxidation and ROS generation (Tkachev et al., 2015). However, the influence of age-related mitochondrial changes on specific T cell inhibitory pathways is yet to be determined. There may be a link between the mammalian target of rapamycin (mTOR) pathway and T cell inhibitory status with aging. Pharmacologic inhibition of the mTOR pathway has been shown to reduce the frequency of PD-1⁺ T cells and improve vaccination responses in elderly humans (Mannick et al., 2014), and reduce numbers of PD-1⁺ and LAG-3⁺ T cells in elderly mice (Hurez et al., 2015), although the exact mechanisms, and whether this is due to removal of antigen-experienced cells, require further investigation.

In summary, age-related genetic and epigenetic changes, as well as alterations in signalling and metabolic pathways are complex and interconnected, and are likely to influence DC and T cell inhibitory molecules/pathways. These changes may not only alter expression of inhibitory molecules by DCs and T cells, but also alter downstream activity through inhibitory pathways, increasing the likelihood of negative DC/T cell interactions, leading to DC and T cell suppression in the elderly. However, the links between age-related cellular/molecular changes and their potential influence on DC and T cell inhibitory molecules/pathways are largely speculative at this stage, and this represents an important area for future research.

4. Aging, cancer and DC/T cell inhibitory molecules

Age-associated changes in DCs and T cells that lead to increased inhibitory DC/T cell cross-talk are likely to affect anti-tumour immune responses in the elderly (Fig. 1). From studies in young hosts with cancer, it is known that tumours and their secreted factors modulate immune cells, with a major effect being skewing of immune cells, including DCs and T cells, towards immunosuppressive function (Motz and Coukos, 2013; Zong et al., 2016). It is possible that aging exerts additive effects leading to exacerbation of immune suppression/dysfunction in elderly hosts with cancer. However, few studies have examined the combined effects of aging and cancer on DC and T cell function, and of those that have, the majority focused on DC and T cell functions involved in the activation/effector phase of anti-tumour immunity (Flood et al., 1998; Flood et al., 1981; Gravkamp et al., 2009; Grizzle et al., 2007; Grolleau-Julius et al., 2006, 2008; Li et al., 2002; Norian and Allen, 2004; Sharma et al., 2006; Shi et al., 2005; Urban and Schreiber, 1984; Win et al., 2002; Young et al., 2001). This means that the combined influence of aging and cancer on DC and T cell inhibitory molecules remains a largely unexplored area, with only two studies to-date examining T cells (McClanahan et al., 2015; Mirza et al., 2010). McClanahan et al. (2015) identified that aging has a confounding effect and contributes to the tumour-induced increase in PD-1 expression on elderly splenic CD8⁺ T cells in a murine chronic lymphocytic leukaemia model. Mirza et al. (2010) reported higher PD-L1 expression on elderly proliferating and resting splenic CD8⁺ T cells relative to their younger counterparts, in healthy and tumour (leukaemia)-bearing mice; in this case the presence of a tumour did not exacerbate age-related differences in PD-L1 expression.

Taken together, the limited evidence available suggests that age-related increases in T cell PD-1 are exacerbated in elderly tumour-bearing hosts. This means that elderly T cells are likely to be hyper-responsive to inhibitory PD-1-PD-L1 signals, thereby impairing elderly anti-tumour immunity. However, the PD-1-PD-L1 pathway represents one mechanism by which tumour-specific T cells can be suppressed. It is important to consider a more complete picture which takes into account the combined influence of aging and cancer on other inhibitory mechanisms that can suppress tumour-specific T cells, including: (i) the inhibitory molecules and cytokines discussed in this review, as well as other tumour-derived inhibitory cytokines/factors, such as IL-6, arginase and inducible nitric oxide synthase, and (ii) other immune cell subsets known to exert immunosuppressive function, such as tumour-associated macrophages and myeloid-derived suppressor cells (MDSCs). The additional influence of cancer on the age-associated reduction in T cell co-stimulatory molecules (such as CD28) should be considered, as this represents another mechanism by which the generation of tumour-specific effector T cells may be thwarted in the elderly. This will allow greater understanding as to how immune-inhibitory pathways/mechanisms might be therapeutically targeted in elderly cancer patients.

5. Targeting inhibitory molecules/pathways in aging and cancer

Targeting inhibitory/checkpoint pathways may be a promising strategy for restoring immune function in elderly people with cancer. This is supported by studies showing that checkpoint blockade can be effective in restoring certain aspects of elderly murine T cell function in vitro (Lages et al., 2010; Mirza et al., 2010). PD-1 blockade has been shown to improve IFN- γ secretion by aged murine splenic CD8⁺ and CD4⁺ T cells stimulated with anti-CD3 antibody (Lages et al., 2010), and PD-L1 blockade restored the ability of elderly murine CD8⁺ T cells to proliferate in response to anti-CD3

and anti-CD28 stimulation (Mirza et al., 2010). Additionally, targeting age-related changes at the cellular/molecular level may help to reduce the inhibitory status of elderly immune cells, as one study has shown that in elderly mice treated with rapamycin, an inhibitor of the mTOR pathway, the frequency of splenic CD8⁺ and CD4⁺ T cells positive for PD-1 and LAG-3 is reduced, and this is associated with partial restoration of elderly PD-1⁺ T cell proliferative capacity (Hurez et al., 2015). Pharmacologic inhibition of mTOR in elderly humans has also been associated with reduced percentages of circulating PD-1⁺ CD8⁺ and CD4⁺ T cells, and improved responses to influenza vaccination (Mannick et al., 2014).

There is currently little information regarding the use of immune checkpoint blockade in elderly tumour-bearing hosts. One study evaluated anti-CTLA-4 and anti-PD-L1 in an elderly murine melanoma model and showed that treatment with anti-PD-L1 monotherapy was less effective in elderly compared to young mice, however combining both agents partially restored treatment efficacy in elderly mice (Hurez et al., 2017). Another study demonstrated that blockade of PD-L1 in elderly leukaemia-bearing mice improved elderly CD8⁺ T cell cytotoxic activity and survival to similar levels as young mice (Mirza et al., 2010). Despite varied results from the limited number of murine studies, a few recent studies evaluating CTLA-4 and PD-1 blockade in patients with melanoma, non-small cell lung cancer or renal cancer included elderly patients (aged 65 years and above). These studies reported that elderly patients showed similar survival benefits and ability to tolerate treatments, compared to younger patients (Chiarion Sileni et al., 2014; Elias et al., 2016; Freeman and Weber, 2015; Helissey et al., 2016; Nishijima et al., 2016; Sgambato et al., 2017). Although these studies did not perform in-depth analysis of immune cells in elderly patients treated with checkpoint blockade, the promising results provide support for use of checkpoint blockade strategies in elderly patients. Antibodies to block other inhibitory molecules (including several discussed in this review, such as CD39, CD73, A2A and A2B receptors, LAG-3 and TIM-3) are under development and pre-clinical and clinical testing for several types of cancer (Anderson, 2014; Antonioli et al., 2016; Bastid et al., 2013; Bonnefoy et al., 2015; Goldberg and Drake, 2011; Leone et al., 2015; Pardoll, 2012; Sepúlveda et al., 2016). However, the specific effects of aging have not yet been considered. Additionally, simultaneous blockade of multiple inhibitory molecules (e.g. checkpoint blockade), combined with other strategies to alleviate immunosuppression should be considered, as blocking a single inhibitory pathway may not show an effect, as it may be overwhelmed by other immune-suppressing pathways.

Several inhibitory molecules and cytokines/factors discussed in this review are expressed by other suppressive immune cells, such as M2 macrophages and MDSCs (Lu et al., 2016; Ugel et al., 2015); in particular MDSCs strongly express PD-L1 (Lu et al., 2016). The frequency of lymphoid M2 macrophages (Jackaman et al., 2014; Jackaman et al., 2013) and MDSCs in tumours increases with age (Chandra et al., 2013; Grizzle et al., 2007). Therefore, eliminating M2 macrophages and/or MDSCs may restore anti-tumour T cell immunity; the latter has been demonstrated by two studies in elderly tumour-bearing mice (Grizzle et al., 2007; Hurez et al., 2012). Thus, eliminating multiple immune cell types which have high expression of inhibitory molecules may alleviate immunosuppression in elderly cancer patients.

6. Conclusions

In summary, there is a small amount of evidence suggesting that elderly DCs and T cells have increased expression of inhibitory molecules, which may lead to increased inhibitory DC/T cell cross-talk and T cell suppression in the elderly, likely impairing the

generation of effective anti-tumour immune responses (Fig. 1). However, this area requires further investigation, as there are conflicting reports in the literature. We propose that examination of ex vivo human DC and T cell subsets is likely to be most informative for human immune aging research on suppressive/checkpoint molecules. Comparisons of specific naïve and memory T cell subpopulations will be necessary to identify the influence of aging, and uncouple changes resulting from the accumulation of antigen-experienced T cells. Another important consideration is identifying how age-associated genetic/epigenetic, signalling and metabolic changes contribute to expression of inhibitory molecules/pathways in elderly DCs and T cells, as this is largely unexplored to date. Studying age-related changes in DC and T cell inhibitory molecules/pathways in combination with the potential underlying mechanisms allows for a more complete understanding of alterations in elderly DC/T cell cross-talk, and how this may impact immune responses in age-related diseases, such as cancer. Additionally, the role of age-related immune modulation in cancer immunotherapy is largely ignored. Since most cancer patients are elderly, and the importance of cancer immunotherapy is increasing, studying immune responses to cancer, including DC/T cell interactions, in the older age groups will be crucial to improve the success of cancer immunotherapy. Nonetheless, the literature suggests targeting inhibitory molecules/pathways represents a promising strategy to complement other approaches for restoring DC and T cell function in elderly cancer patients.

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