Thymus involution sets the clock of the aging T-cell landscape: Implications for declined immunity and tissue repair

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\textbf{Abstract}

Aging is generally characterized as a gradual increase in tissue damage, which is associated with senescence and chronic systemic inflammation and is evident in a variety of age-related diseases. The extent to which such tissue damage is a result of a gradual decline in immune regulation, which consequently compromises the capacity of the body to repair damages, has not been fully explored. Whereas CD4 T lymphocytes play a critical role in the orchestration of immunity, thymus involution initiates gradual changes in the CD4 T-cell landscape, which may significantly compromise tissue repair. In this review, we describe the lifespan accumulation of specific dysregulated CD4 T-cell subsets and their coevolution with systemic inflammation in the process of declined immunity and tissue repair capacity with age. Then, we discuss the process of thymus involution—which appears to be most pronounced around puberty—as a possible driver of the aging T-cell landscape. Finally, we identify individualized T cell-based early diagnostic biomarkers and therapeutic strategies for age-related diseases.

1. Introduction: chronic inflammation and declined repair with aging

Aging can be viewed as a gradual increase in cell and tissue damage, which accompanies a decline in repair processes. In other words, challenges—such as nutritional or physical—can cause either no or substantial tissue damage, based on the capacity of the body to repair itself (Lopez-Otin et al., 2013; Mahmoudi et al., 2019) (Fig. 1). As any cell or tissue damage elicits an immune response, the repair process is, to a great extent, orchestrated by the molecular and cellular components of the damaged tissue and the tissue-specific inflammatory process (Eming et al., 2014, 2017), both of which are substantially altered with aging (Franceschi et al., 2018b; Larbi et al., 2008; Lopez-Otin et al., 2013). For example, compared to young animals, an acute skin injury in old animals heals poorly due to a persistent inflammatory milieu and intrinsic age-dependent changes in various immune cell subsets (Keyes et al., 2016; Mahmoudi et al., 2019). Similarly, it was shown that inflammatory mediators contribute to reduced regenerative capacity and barrier dysfunction of the intestinal tract with aging (Jasper, 2020). The escalating chronic inflammatory state in aging also appears to directly affect tissue stemness (Jasper, 2020; Wells and Watt, 2018), a phenomenon that was evident in various tissues—including bone (Josephson et al., 2011; Liu et al., 2011), intestinal tract (Jasper, 2020), muscle, liver, and skin (Jurk et al., 2014) and that was shown not only to compromise tissue repair, but also to facilitate the aging process (Jurk et al., 2014).

Cellular senescence is an additional robust process that is associated with chronic inflammation and with reduced tissue stemness and repair. It is characterized by irreversible division arrest, apoptosis resistance, metabolic alterations (Franceschi et al., 2018b; Jeon et al., 2017), and increased synthesis of pro-inflammatory mediators—referred to as a senescence-associated secretory phenotype (SASP). Cellular senescence is enhanced in various tissues with aging (Rodier et al., 2009; Tchkonia et al., 2013) and further sustains systemic inflammation by producing pro-inflammatory cytokines, such as IL-1\(\beta\), IFN-\(\iota\), and IL-6 (De Cecco et al., 2019; Gorgoulis et al., 2019). Notably, reduced clearance of dysfunctional cells, as part of immunosurveillance (Burton and Stolzing, 2018; Kale et al., 2020), was recently shown to accelerate senescent cell accumulation along with aging-related tissue dysfunction and reduced life expectancy (Ovadya et al., 2018). Senescent cells, not only inhibit their clearance by immune cells similar to some tumors, but their accumulation was also shown to correlate with dysfunctional CD4 T cells in the skin of elderly individuals (Waaijer et al., 2019).

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https://doi.org/10.1016/j.arr.2020.101231
Received 22 July 2020; Received in revised form 15 November 2020; Accepted 20 November 2020
Available online 25 November 2020

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Taken together, a dysregulated immune system is thus likely to compromise repair processes and, consequently, to facilitate low-grade chronic inflammation. This process supports a vicious cycle of chronic inflammation—called ‘inflammaging’ (Franceschi et al., 2000)—which further alters leukocyte properties, cell senescence, and tissue stemness (Desdin-Mico et al., 2020). At a certain point in this process, tissue dysfunction leads to clinical manifestations in the form of various age-related chronic diseases (Franceschi and Campisi, 2014; Franceschi et al., 2018b; Furman et al., 2019) and affects longevity (De Martinis et al., 2005; Desdin-Mico et al., 2020; Larbi et al., 2008). Key questions regarding the age-dependent dysregulation of the immune system include (1) what triggers the initiation of this process and when does it occur? (2) Can it predict the risk for age-related diseases and treatment efficacy? And (3) Does interfering with the process of immune dysregulation offer novel therapeutic approaches for age-related diseases?

The model shown in Fig. 1 suggests that the decline in thymic naïve T-cell output with age and the accumulation of dysfunctional T cells directly compromise the inflammatory responses that are required for tissue repair. Since thymus involution reaches a significant functional outcome only at puberty, the impact of this process may not be substantially evident at the reproduction phase of life; rather, it may manifest only when changes in the T-cell landscape become more robust and co-emerge with chronic inflammation and initial signs of tissue erosion (Coder et al., 2015; Desdin-Mico et al., 2020). As we and others have shown, significant changes in CD4 T-cell subsets gradually increase with aging (Elyahu et al., 2019; Moro-Garcia et al., 2013; Thome et al., 2014). Notably, whereas this aging T-cell landscape represents the circulating T-cell repertoire, it may also affect the maintenance, turnover, and function of the tissue-resident memory T-cell (TmRM) population, cell subsets that may play a key role in tissue inflammation and repair (Gray et al., 2018). The proportion of TmRM in various tissues was shown to increase with age (Farber et al., 2014; Szabo et al., 2019a; Szabo et al., 2019b; Thome and Farber, 2015), albeit the mechanisms by which they contribute to aging-associated pathologies are only starting to emerge (Moreau et al., 2017). Whereas a similar pattern of changes in the T-cell landscape appears to be part of the aging process, its evolution with age is highly variable among individuals (Elyahu et al., 2019; Nussey et al., 2012; Thome et al., 2014). Thus, clinical manifestations of aging may largely depend on the extent to which aging-related T-cell subsets develop, accumulate, and function, generating a whole new ecosystem that, from an evolutionary perspective, does not favor repair. The interaction between this new immune ecosystem and genetic and/or environmental factors may thus dictate the risk for age-related diseases and longevity. As evident in various age-related diseases, a higher incidence of cancer in older individuals is attributed, at least in part, to impaired immunosurveillance, dysregulated leukocytes, and enhanced chronic inflammation (Fane and Weeraratna, 2020; Manser and Uhrberg, 2016; Seluanov et al., 2018; Wang and DuBois, 2015), and it was demonstrated in various types of tumors, both in human and in mice (Golomb et al., 2015; Loaiza and Demaria, 2016; Rielland et al., 2014; Wang and DuBois, 2015; Zinger et al., 2017). Notably, the low-grade systemic inflammation evident in aging represents primarily innate immunity (e.g., tissue senescent cells and activated macrophages), which, at least in part, is manifested along with the accumulation of damage load and leads to the development of age-related diseases and mortality. Damage load can be reduced by intervention (e.g., through nutrition (Davinelli et al., 2019; de Cabo and Mattson, 2019; Franceschi et al., 2018c; Mattison et al., 2012; Mercken et al., 2013) and physical activity (Calvani et al., 2017; Conte et al., 2020; Duggal et al., 2019; Graziosi et al., 2017; graph, top right) and/or by genetic factors that better maintain repair with age (graph, bottom right). Both the reduction of damage load and the genetic factors impact life expectancy.
dysregulated T cells (Hearps et al., 2012; Oishi and Manabe, 2016). For example, microglia—the macrophages of the brain—exhibit a reduced phagocytic capacity and enhanced production of innate cytokines, which overall augment amyloid pathology in a mouse model of Alzheimer disease (AD) that lacks a CD4 T-cell compartment (Mittal et al., 2019). The following sections detail changes in CD4 T cells that occur with aging and may underlie chronic inflammation and declined immunity. We then discuss thymus involution as a key process that generates a shift in the T-cell landscape with aging, and we describe therapeutic implications aiming to re-equip the repair capacity of the immune system.

2. The gradual accumulation of dysregulated CD4 T-cell subsets during aging

T cells generate adaptive immunity via their ability to recognize a vast array of antigens and to undergo a tightly controlled activation process, which changes their phenotype from naive to effector or memory cells (Zhu et al., 2010). These capabilities rely, on the one hand, on a highly diverse T-cell receptor (TCR) repertoire that allows the specific recognition of peptide-loaded MHC class I (MHC-I) or class II (MHC-II) in antigen presenting cells (APCs). On the other hand, these capabilities rely on a set of signals (e.g., cytokine and chemokine receptors, pathogen and/or damage-associated molecular patterns, hormonal receptors, adhesion molecules, and costimulatory molecules) that direct their differentiation to various functionalities, such as killing, inflammatory, or regulatory (Zhu et al., 2010). Integrated with the innate arm of the immune system, T cells provide protection against pathogens and orchestrate tissue maintenance and repair, such as wound-healing processes and clearance of cancer and senescent cells in a regulated fashion, which prevents destructive autoimmunity and inflammation (Kang et al., 2011; Pellicoro et al., 2014; Prata et al., 2018). With aging—that is accompanied by thymic involution, intrinsic senescence pathways, and a gradually developing systemic inflammation—these key immune orchestrators change their phenotypes and functions, thereby facilitating vulnerability to age-related diseases.

2.1. From naive to dysregulated memory T cells: a gateway to immunosenescence

In young rodents and humans, naïve T cells leave the thymus and circulate in blood, lymph, and secondary lymph nodes to ultimately provide long-lasting protection from pathogens and tissue damage. The number of naïve T cells progressively declines with age (Elyahu et al., 2019; Thome et al., 2014), mainly due to the process of thymus involution (Goronzy and Weyand, 2017; Lynch et al., 2009) and, to a lesser extent, the destruction of lymph node (LN) architecture, which delays the maturation and proliferation of naïve cells (Becklund et al., 2016; Thompson et al., 2019). Yet, a subset of naïve cells is maintained with aging mainly due to the extra-thymic proliferation of T-cell clones—a phenomenon that is markedly more evident in humans than in rodents (Goronzy and Weyand, 2017) and relies more on remnants of thymic function (Goronzy and Weyand, 2017; Hogan et al., 2015). The remaining naïve T cells exhibit a diverse TCR repertoire, which is sufficient to recognize newly encountered antigens as well as to elicit a proper immune response (Goronzy and Weyand, 2017; Qi et al., 2014). In parallel to the reduction in the number of naïve T cells, memory cells gradually occupy the T-cell compartment (Elyahu et al., 2019; Kumar et al., 2018; Saule et al., 2006; Thome et al., 2014), a shift that is generally evident as a reduced expression of naïve cell markers, such as CD62L (in mice), CCR7 (in mice and humans) and CD45RA (in humans), and an increased expression of memory cell markers, such as CD44 (in mice), CD45RO (in humans), and integrin beta1 (in mice) (Elyahu et al., 2019; Nikolich-Zugich, 2014; Thome et al., 2014). These memory cells gain intrinsic defects, along with a loss of quiescence state, which may contribute to the process of declined immunity and chronic inflammation (Geltink et al., 2018; Goronzy and Weyand, 2019; Muller et al., 2019). Among the key defects described in memory T cells are (1) an increased expression of co-inhibitory receptors (e.g., PD1, LAG3,) (Elyahu et al., 2019; Larbi and Fulop, 2014; Shimatani et al., 2009), (2) a decreased expression of co-stimulatory molecules, such as CD28 (Esensten et al., 2016; Weng et al., 2009), and (3) a disruption in the proper activation process of the cells. These changes converge to disruptions in key signaling pathways (Larbi et al., 2011) reflected by reduced TCR sensitivity, reduced IL-2 production (Larbi et al., 2011) and proliferation capacity (Li et al., 2012), and a dysregulated production of pro-inflammatory cytokines (Akbar et al., 2016; Harpaz et al., 2017). Although especially evident in T cell subsets with memory phenotypes (Haynes et al., 2003; Nikolich-Zugich, 2018), similar changes in functional properties were also reported in naïve cells from aged mice (Clise-Dwyer et al., 2007), presumably due to chronic systemic inflammation, as detailed in the following sections.

The dysregulated properties of CD4 T cells that accumulate with age accompany changes in metabolic pathways, which may cause and/or facilitate the intrinsic defects of the T cells. A recent study by Desdin-Mico et al. reported that a defect in mitochondrial activity, confined to the CD4 T-cell compartment, caused systemic inflammation, senescent cell accumulation, and multiple features of early-onset age-related morbidities (Desdin-Mico et al., 2020). In addition, CD4 T cells from old mice exhibited dysregulated mitochondrial function, which was associated with a T helper 17 (Th17) effector function (e.g., IL-17, IL-6) (Bharath et al., 2020). Intriguingly, by improving mitochondrial functionality, the T cells presented a more balanced cytokine profile (Bharath et al., 2020). Impairments in the mitochondrial respiration process were recently evident also in CD4 T cells from elderly individuals (Bekta et al., 2019), highlighting the connection between proper mitochondrial function in T cells and healthy aging. As compared with memory CD4 T cells of young individuals, the activation of memory CD4 T cells of aged individuals showed a higher mitochondrial activity and increased production of ATP and reactive oxygen species (ROS), which has led to a higher state of activation of the cells (Geltink et al., 2018; Yanes et al., 2019). Other metabolic pathways were also shown to be involved; Laana et al. showed that the activation of mitogen-activated protein kinases (MAPKs) may provide a link between metabolic dysfunction and the dysregulated properties of CD4+CD27-CD28- T cells (Laana et al., 2017)—a well-defined exhausted subset that accumulates with aging (Laana et al., 2014; Moro-Garcia et al., 2013; Weng et al., 2009). The study by Laana et al. further revealed that ablatting the sestrin family of proteins, which supports and regulates MAPK activation, restored antigen-specific proliferation and cytokine production in vitro (Laana et al., 2017). Other studies have shown that increased CD39 expression by CD4 T cells in elderly individuals promotes the activation of the metabolic master regulator AMP-activated protein kinase (AMPK), which, in turn, causes an aberrant effector function of the cells, characterized by increased expression of pro-inflammatory cytokines and predisposition to apoptosis (Fang et al., 2016). The changes in the metabolic properties of CD4 T cells is heterogeneous among and within defined CD4 T-cell subsets, but is generally more substantial in memory than in naïve subsets. A recent study by Kared et al. suggested that alteration in the Wnt signaling pathway underlines the heterogeneous and inefficient response to stimuli of human CD4 T memory stem cells (TSRM) (Kared et al., 2020), a self-renewal subset that supports long immunological memory (Gattinoni et al., 2011). Together, since leukocytes sharply respond to triggers in a process that is tightly regulated and has high energetic demands (Chang and Pearce, 2016; Mills et al., 2017; Wang et al., 2019), age-related metabolic changes may play a key role in the dysregulated properties of the cells. Such metabolic defects in CD4 T cells—which are the orchestrators of immunity—may cause a substantial damage to immunity and repair. In a broader view, these alterations in the CD4 T-cell compartment are part of the global deterioration of the immune system with aging, referred to as immunosenescence (Fulop et al., 2017; Goronzy and Weyand, 2013; Pawelec,
2.2. Inflammaging contributes to changes in the CD4 T-cell landscape

Molecular changes in immunological niches, caused primarily by evolving systemic inflammation, may also constitute a factor that shapes CD4 T-cell functionality in aging individuals. Compelling evidence indicates that increased levels of circulating inflammatory mediators may change the phenotypic properties of T cells to favor effecter memory differentiation, exhaustion, and senescence (Pulop et al., 2016; Goronzy and Weyand, 2019; Schluns and LeFrancois, 2003). Notably, these effects are not necessarily dependent on TCR stimulation and can be mediated by cytokine signaling (Goldrath et al., 2000; Polonsky et al., 2018; Sprent and Surh, 2011). For example, Cope et al. demonstrated that chronic exposure to TNF compromises T-cell responses in vitro (in mice) and in vivo (cope et al., 1997). In addition, Bryl et al. showed that a chronic exposure of blood-derived CD4 T cells from elderly individuals to TNF, as part of low-grade systemic inflammation, decreases the levels of CD28 on T cells and contributes to the development of CD27–CD28− T cells that exhibit exhausted properties (Bryl et al., 2001). These findings are further supported by a more recent study, which showed in vitro that TNF induces the expression of PD1—one of the key exhaustion molecules—in blood-derived human CD4 T cells (lim et al., 2016). Notably, by activating key signaling pathways (such as the JAK-STAT system), chronic systemic inflammation may alter the activation threshold and/or the effector functions of CD4 T cells; thereby, such inflammation contributes to exhausted and dysregulated phenotypes, which, in turn, promote cancer, autoimmunity, and other age-related diseases (Furman et al., 2017; Rao et al., 2017; Shirakawa et al., 2016; Tsukamoto et al., 2018). Chronic inflammation may also accelerate thymus involution and impair the hematopoietic stem cell (HSC) niche (He et al., 2020), thereby causing a more abundant loss of circulating naïve T cells. This process, in parallel, can nurture the accumulation of dysfunctional T cells (e.g., cells exhibiting increased levels of co-inhibitory molecules, reduced co-stimulatory molecules, reduced proliferation, reduced TCR sensitivity, and metabolic dysfunction) resulting from clonal expansion, as in the case of chronic infection with cytomegalovirus (CMV) (Karrer et al., 2003; Koch et al., 2007; Sylwester et al., 2005; Wertheimer et al., 2014), Epstein-Barr virus (EBV) (Koch et al., 2007; Tanaka and Sakuguchi, 2017) and other latent viruses (Araujo Carvalho et al., 2018) which affect both CD4 and CD8 cells (Wetlen et al., 2018; Wertheimer et al., 2014).

Taken together, available data indicate that low-grade chronic systemic inflammation, which accompanies and/or is caused by processes such as tissue senescence and altered metabolism, may act as an additional component that contributes to the dysfunctional properties of age-related T-cell subsets. Considering the very low turnover of naïve T cells in aging (as discussed in Section 3, below), such dysfunctional properties may not only facilitate the pro-inflammatory environment, but they may also disrupt immunity and repair processes and give rise to a variety of age-associated pathologies in a personalized manner.

2.3. Increased heterogeneity of CD4 T cells with aging

The aging process is markedly different across individuals (Chaleckis et al., 2016; Lopez-Otin et al., 2013; Mahmoudi et al., 2019) and could be represented as an individualized and continuum trajectory (Francesch et al., 2018a). Among the various factors that can impact the aging process, the change of the immune ecosystem with age appears to impact the individual’s aging trajectory. A recent development in sequencing technologies enables researchers nowadays to characterize the dynamic cellular changes that the immune system undergoes with aging in unprecedented resolution. Work done by Alper et al. described dynamic changes—within and across individuals—in immune cell subsets with age (Alper et al., 2019). Using gene expression profiling and high-throughput cellular phenotyping, the authors analyzed over 100 healthy individuals across nine years and demonstrated that certain lymphocyte subsets (e.g., CD8+CD28− T cells and CD4+CD28− T cells) correlate with age and may at least partially explain differences in aging trajectories. By combining the transcriptomic and proteomic data, the authors extrapolated the biological age of individuals that could effectively predict the potential risk of all-cause mortality in the elderly (Alper et al., 2019). Other studies demonstrated increased variation of chromatin modifications in the immune cells (e.g., memory T cells and NK cells) of elderly individuals (Cheun et al., 2018). At the functional level, Martinez et al. showed, at a single-cell resolution, an elevated cell-to-cell transcriptional variability in the activation program of CD4 T cells, which affects the functional synchronization between T cells (Martin et al., 2017) and may compromise the immune fitness required to fight aggressive pathogens, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Mehta et al., 2020; Qin et al., 2020). These phenomena, namely, the increased variation in chromatin modifications and the functional variability within and between cell subsets, may explain, at least partially, an inter-individual heterogeneity in the overall process of declined immunity and repair with aging.

To better understand the individualization of immune-aging, extensive efforts have been made to explore, in high resolution, the dynamics of age-related changes in immune cell phenotypes. A recent study by our group examined the changes in CD4 T-cell phenotypes with aging. We showed that CD4 T-cell subsets gradually rearrange with age into four main subsets: two subsets of effector memory T cells, either with or without an exhausted phenotype; a subset of CD4 T cells with cytotoxic traits, which produce highly inflammatory and cytolytic cytokines; and regulatory T cells (Tregs) with activated phenotypes, which exhibit a higher suppressive capacity of effector T cells in vitro (Elyahu et al., 2019). Although high variation in subset frequency was observed between individual old mice, the subsets, together, occupied around 30% of the CD4 T-cell population. Of note, the aging-related T-cell subsets that we identified were previously described in mice and humans under conditions involving chronic inflammatory states, such as cancer, autoimmune disorders, and chronic viral infections (Matto et al., 2017; Patil et al., 2018; Zheng et al., 2017). Particularly relevant is the marked increase in the frequency of cytotoxic CD4 T cells, which was recently evident in supercentenarians and suggested that this subset plays a beneficial role in cancer and chronic inflammation (Hashimoto et al., 2019). These changes in the CD4 T-cell landscape (Fig. 2) are variable among individuals and likely to impact immune fitness; yet, the differentiation cues of the various subsets, their accumulation over a life span, and their contribution to age-related pathologies have yet to be explored.

3. Thymus involution sets the clock of the aging T-cell landscape

3.1. Thymus development, structure, and function

The thymus, which exists in nearly all vertebrates, is a primary lymphoid organ essential for the maturation of bone marrow (BM)-
derived T lymphoid precursors. The thymus co-evolved with the emergence of T lymphocytes, and its anatomical structure and function are preserved across vertebrate species (Ge and Zhao, 2013). Perhaps one of the most primitive forms of the thymus can be found in jawless fish, such as lampreys (Bajoghli et al., 2011; Ge and Zhao, 2013). In mammals, the thymus consists of two main anatomical sites, the cortex and the medulla, where T lymphocytes undergo positive, negative, and agonist selections (Kadouri et al., 2020; Petrie, 2002; Takahama, 2006). T-cell progenitors initially undergo somatic recombination in DNA segments encoding the variable domain [which consists of Variable, Diversity, and Joining (VDJ) sequences] of the TCR, which gives rise to a wide variety of clones ($>10^7$), each expressing a single combination of recombined $\alpha/\beta$ or $\gamma/\delta$ TCRs (Nikolich-Zugich et al., 2004). The positive selection of MHCI-restricted CD3+$\alpha/\beta$+CD8+ and MHCII-restricted CD3+TCR$\alpha/\beta$+CD4+ cells is mediated by cortical epithelial cells (Stritesky et al., 2012)—a quality control process that subjects the majority (>90%) of entering precursor cells to apoptosis, referred to as “death by neglect” (Palmer, 2003). Consequently, negative and agonist selections occur in the medulla and are mediated by medullary epithelial cells and BM-derived myeloid cells (Starr et al., 2003). Antigen presentation in this compartment leads to either deletion of high-affinity single-positive (SP) CD4 or CD8 T cells, or to an agonist selection of Tregs (Jordan et al., 2001; Takaba and Takayanagi, 2017). Ultimately, the maturation and selection processes of T cells in the thymus generate a pool of circulating naïve SP CD4 and CD8 T cells. Such a pool allows the generation of immunity, on the one hand, and the ability to maintain tolerance to self-antigens, on the other hand. A proper thymic output may thus be considered a crucial first stage that generates a highly diverse T-cell repertoire, which is required to maintain plasticity, protection, and repair, while minimizing the risk of pathogenic autoimmunity. Since CD4 T cells evolved to play a key role in orchestrating these functions, they may be considered keystone in the ecosystem of properly regulated immunity (Zhan et al., 2004; Zhu et al., 2010).

### 3.2. Thymus involution

Regardless of the seemingly crucial role of the thymus in preserving homeostasis, its involution in humans and other mammals begins in

![Fig. 2. T-cell heterogeneity develops with aging.](image_url)

The CD4 T-cell compartment undergoes remodeling with age. Whereas the CD4 T-cell compartment is dominated by naïve cells in young rodents and humans, new CD4 T-cell phenotypes emerge and accumulate with age, increasing the variability of CD4 T cells within and between individuals (lower box). Intrinsic changes that occur in CD4 T cells with aging feed forward the differentiation of dysregulated CD4 T cells (upper right box). The major CD4 T-cell phenotypes that accumulate with aging, and their main molecular markers, are shown in the lower box. The resulting CD4 T-cell heterogeneity between individuals is a key feature that may reflect and shape the individual’s aging trajectory.
childhood and peaks around puberty, resulting in an almost completely non-functional organ in aging (Nasi et al., 2006). Thymus involution gradually reduces the output of naïve T cells with age (Palmer, 2013), a process that is manifested in a decrease in tissue cellularity—primarily in the cortical and medullary areas of the thymus—along with enlarged perivascular areas and accumulating adipocytes (Rezzani et al., 2014). Dynamic changes in the thymic T lymphocytes include a reduced number of undifferentiated T cells and an increase in terminally differentiated cells (e.g., T cells with memory phenotypes) (Park et al., 2020). A hint to the effect of thymus involution can be found in the context of early life (before puberty) thymectomy (Deya-Martinez et al., 2020). In young humans, thymectomy results in T-cell changes that are similar to those related to immunosenescence (Deya-Martinez et al., 2020). Specifically, it was shown that the number of both CD4 and CD8 naïve T cells decreases while the number of memory and exhausted phenotypes increases (Elder et al., 2015; Gudmundsdottir et al., 2016). Moreover, Tregs expanded by peripheral proliferation and gained an effector memory phenotype following a young-age thymectomy (Schadenberg et al., 2014). Thymectomized human individuals were also more prone to infections, cancer, autoimmune diseases, and premature immune aging (Elder et al., 2015; Gudmundsdottir et al., 2016; Sauce and Appay, 2011). Notably, however, not all thymectomized individuals exhibit immune defects such as those observed in DiGeorge syndrome, where T-cell maturation and selection in the thymus is often severely impaired in utero (Kuo et al., 2018; Sauce and Appay, 2011). In fact, a small residual normally functioning thymic tissue appears to be sufficient to minimize immune complications following early-life thymectomy, at least in the first 2–3 decades of life (Deya-Martinez et al., 2020); thereafter, at least certain thymectomized patients exhibit an altered T-cell compartment reminiscent of that seen in elderly individuals (Sauce et al., 2009). Similarly, thymectomized mice presented various immune alterations (Miller, 1965), including defects in naïve T cells (Tsukamoto et al., 2009) and Tregs (Vianna et al., 2016). Taken together, these studies may imply a causal link between thymus involution and accumulating defects in the immune system with aging, which prone to age-related diseases. Future research with thymectomized mice or similar models, in combination with advanced research tools, may help to gain additional insights into the impact of thymus involution on T-cell alteration, chronic inflammation, and age-related diseases.

3.3. Factors that induce thymus involution

The thymic output is coordinated by both the stromal (i.e., cortex and medulla) and lymphoid compartments. Mechanistically, several factors were found to regulate these compartments and impact the involution process. For example, early studies have shown that the sex hormones testosterone and estrogen facilitate the involution process mainly by affecting the thymus epithelial cells (TECs), and to a lesser extent, via direct signaling in thymocytes (Dudakov et al., 2009; Olsen and Kovacs, 1996; Simpson, 1974). Castrating rodents before puberty or reducing the levels of sex hormones (e.g., by using Lupron, which desensitizes the luteinizing hormone-releasing hormone (LH-RH) receptors) can attenuate or markedly recover the involution process in aging mice (Chaudhry et al., 2016; Dudakov et al., 2012; Rossi et al., 2007). Inflammation and stress, which activate the hypothalamus-pituitary-adrenal (HPA) axis and induce the production of cortisol, may also play a role in thymus functionality due to the abundant expression of the glucocorticoid receptor (GR) on thymocytes (Purton et al., 2006; Taves et al., 2019). For example, acute Trypanosoma infection in mice was shown to induce a marked thymus atrophy, which was partially reversed following the blockade of the GRs (Roggero et al., 2006). Whether chronic stress and inflammation in humans facilitate thymus involution or regulate its functionality so that it is more prone to premature lymphocyte aging (Bauer, 2005) is yet to be investigated. Recently, additional molecular pathways, which play a role in maintaining the thymus function, have been explored, including, but not limited to, the keratinocyte growth factor (KGF), the pro-longevity factor fibroblast growth factor (FGF) 21, and growth hormone (GH) (Ashwell et al., 2006; Boehm and Swann, 2013; Chaudhry et al., 2016; Dorshkind and Horsemann, 2000; Lynch et al., 2009; Montecino-Rodriguez et al., 2013; Rossi et al., 2007; Velardi et al., 2015), the transcription factor Forkhead box (FOX)N1 (Bredenkamp et al., 2014), and the cytokines IL-7 (Tan et al., 2001) and IL-22 (Lynch et al., 2009). FGF21 and its receptor were shown to be expressed in thymic stromal cells and to play a key role in maintaining the levels of thymic progenitors, cortical epithelial cells, and circulating naïve T cells in aging (Erickson et al., 2002; Youm et al., 2016). The FOXN1 transcription factor is expressed in TECs and regulates their development and function (e.g., antigen presentation and T-cells selection) (Bredenkamp et al., 2014; Gordon et al., 2001; Zuklys et al., 2016). Its downregulation with aging accelerates thymic atrophy (Chen et al., 2009; Reis et al., 2015; Rode et al., 2015), a process that, at least in part, is regulated by the bone morphogenetic protein 4 (BMP4) expressed by thymic endothelial cells (Lepletier et al., 2019; Wortheimer et al., 2018). Reduced levels of IL-22 with age was implicated in dysfunctional TECs and reduced thymopoiesis (Boehm and Swann, 2013; Chaudhry et al., 2016). The IL-7 cytokine, produced mainly by TECs, is also reduced with age and is involved in the migration, maturation, and maintenance of lymphocytes within the thymus (Lynch et al., 2009; Rodrigues et al., 2018; Tan et al., 2001). Such discoveries of the molecular pathways involved in maintaining thymus functionality may allow a partial recovery of the naïve T-cell pool in aging and, therefore, of the aging-related T-cell landscape.

3.4. Why does the thymus involute?

Strikingly, a dynamic similar to that of thymus involution occurs across vertebrate species, suggesting a strong evolutionary pressure (Boehm, 2011; Shanley et al., 2009; Torroba and Zapata, 2003). While the reason behind this evolutionary pressure is yet unknown, there are several assumptions: (1) having a sufficiently large repertoire of specialized memory T cells, and maintaining these cells over the production of new naïve ones, may increase immune fitness to previously encountered antigens; (2) thymus involution, which inhibits lymphoid progenitor cell migration from the BM, may decrease the possibility of blood cancers; and (3) shifting energy expenditure from the growing phase, which involves the learning of both the intrinsic and the extrinsic environments (e.g., self-antigens, pathogens, allergens), to the reproduction phase of life. In that sense, thymus involution is, in fact, part of a life-span program that shifts from a postnatal development and learning phase to the reproductive phase; while the former requires learning and plasticity, the latter has to do with more focused actions (hunting, reproduction, etc.). As such, the brain and the immune system can be regarded as two compartments that continuously process inputs to outputs, and thus, overall, reduced plasticity in both systems may allow the reduction of ‘noise’ and the more effective performance of established memory-based effector functions. Intriguingly, post-pubertal changes also occur in the brain, in the form of reduced dentate gyrus neurogenesis and increased myelination (Bercury and Macklin, 2015; Takei, 2019).

4. Therapeutic implications

The overall process that leads to immune failure in old age is apparently the sum of the aforementioned parallel processes, namely, thymus involution, intrinsic T-cell defects, chronic inflammation, and cell senescence. Targeting these components may shed light on the impact of each process and its therapeutic potential for the restoration of immunity and its repair in elderly.

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4.1. Thymus rejuvenation

Although the ability to recover the aged thymus and reconstitute peripheral T cells is impressive and has been demonstrated in both rodents and humans (Chaudhry et al., 2016; Legrand et al., 2007), the possibility that such an approach, as a sole therapy, can be effective in the rehabilitation of the immune system is still under investigation. For example, the overexpression of FOXN1 in the thymus of old mice re-generated thymic function and attenuated memory CD4 T cells accumulation in the spleen (Zook et al., 2011). As detailed in Section 3.3, the recovery of stromal cell proliferation and function in the aged thymus, such as via FGF21 signaling, appears to be a promising approach that alters the molecular microenvironment required to support T-cell progenitor homing and maturation (Youm et al., 2016). Conversely, a recent study by Thompson et al. demonstrated that, despite the effective capacity of KGF to recover thymic structure and function in old mice, the number of T cells in peripheral blood does not increase and the survival under the West-Nile challenge is not improved (Thompson et al., 2019). The authors demonstrated that the new thymic immigrants generated in old mice do not improve immunity to the West-Nile virus, presumably due to LN fibrosis and loss of function (Thompson et al., 2019). Therefore, thymus rejuvenation, by itself, may not suffice to affect immunity to pathogens in aging, as it is only part of the overall immunosenescence process; for example, the reduction in hematopoietic stem cells (HSCs), their reduced potency, and their myeloid differentiation preference are likely not only to skew the immune system to a more myeloid-dominant one, but also to cause a significant decline in the number of lymphoid progenitors that reside in the thymus (de Haan and Lazare, 2018; Den- kinger et al., 2015; Montecino-Rodríguez et al., 2013). However, since thymus involution begins early in life—much earlier than the age-related changes in HSCs and senescent lymphocytes are evident—it is yet to be determined whether thymus rejuvenation performed at earlier time points during aging, or in combination with other therapies, are more effective, at least when considering chronic inflammation-related illnesses (Campisi et al., 2019; Furman et al., 2019; Palmer et al., 2018). For example, combining cancer immunotherapy, such as PD1 blockade, with thymus rejuvenating factors may alter the tumor microenvironment (Zakrzewski et al., 2006; Zhang et al., 2018) toward a more effective infiltration and activation of CTLs (Johansson-Pericval et al., 2018).

4.2. Modulating dysregulated T cells

The identification and tracking of the aging immune milieu could serve as reproductive grounds for the development of biomarkers and personalized treatments for age-related diseases. The high-resolution analysis that we recently published (Elyahu et al., 2019) revealed CD4 T-cell subsets (Fig. 2) that, at least partially, feed the processes of chronic inflammation and declined immunity with aging. Notably, whereas the cellular reorganization that we observed occurred in all the mice that we analyzed, there was a substantial degree of variation in the frequency and accumulation dynamics of each subset between individual mice. As all these mice shared an identical genetic background, such significant variation may reflect a robust impact of environmental factors and suggests that therapeutic targeting of the various age-related CD4 subsets needs to be evaluated in a disease- and individual-specific manner.

4.2.1. Exhausted and regulatory CD4 T cells

Both Tregs and exhausted T cells in the tumor microenvironment have been directly implicated as cells that can attenuate tumor immunity (Guo et al., 2018; Tanaka and Sakaguchi, 2017; Zheng et al., 2017), and targeting these subsets was found to be highly efficacious in cancer immunotherapy (e.g., PD1 and CLTA-4 blockade) (Topalian et al., 2020; Wei et al., 2017). A compelling key question is whether targeting these subsets can also be considered for the treatment of other age-related diseases. As described below, seminal studies show promising results in animal models, but they still need to be examined in humans. For example, although peripheral infection and chronic inflammation have been implicated in brain inflammation and cognitive decline in people with AD and in animal models of the disease (Cunningham, 2013; Heneka et al., 2015; Perry and Holmes, 2014), Treg depletion or the blockade of the checkpoint ligand PD1 have recently been found to facilitate the migration of leukocytes into the brain, which was accompanied by reduced pathology and cognitive deficits (Baruch et al., 2016). Another example is the potential therapeutic effect on heart diseases and other conditions that involve the accumulation of atherosclerotic plaques (e.g., ischemic stroke) (Saigusa et al., 2020); a recent study by Fernandez et al. used scRNA-seq to examine the leukocyte populations within atherosclerotic plaques of symptomatic patients and demonstrated, as in tumors, high levels of exhausted cells (Fernandez et al., 2019). Other studies have shown that CD134+- (TNFRSF4) T cells and other T cells with exhausted characteristics accumulate in athero-sclerotic plaques (Dumitriu et al., 2012; Qiu et al., 2015), and that blocking CD134 on these cell subsets reduced their inflammatory and cytotoxic potential after activation in vitro and may, overall, contribute to plaque stabilization (Dumitriu et al., 2012). Although this strategy has not been sufficiently tested in animal models (Saigusa et al., 2020), such intervention may improve acute coronary syndrome (ACS) and patient survival. Targeting exhausted cells may prove beneficial in increasing the potency of the immune system against pathogens and in improving the efficacy of vaccines in the elderly population (Goronzy and Weyand, 2013); such approaches, however, appear to enhance pathogenic autoimmunity and/or inflammation (Boutros et al., 2016; Nishimura et al., 1999) and should be further investigated to improve efficacy—especially considering the dysregulated properties of the immune system in the elderly population.

4.2.2. Memory and cytotoxic CD4 T cells

The increase in the frequency of CD4 T cells with memory characteristics with aging opens additional directions for intervention, although cell subset complexity and their heterogeneity in aging individuals makes it a challenging approach. Nevertheless, targeting defined subsets of memory cells based on their antigen specificity or on defined phenotypic properties (e.g., cytokine profile, exhaustion state) could prove beneficial. The cytotoxic CD4 T-cell subset (CD4 CTLs), which exhibits memory traits, appears as a unique lineage that accumulates in aging and apparently occupies up to 40 % of the CD4 population, both in humans (Hashimoto et al., 2019) and in mice (Elyahu et al., 2019). The CD4 CTLs appear to be MHCII-restricted T cells that express the entire cytotoxic molecular machinery of CTLs (Fig. 3). Specifically, it has been reported that CD4 CTLs produce the cytokines IFNγ, Granzyme B, and perforin, which are regulated mainly via the Eomesoderin (EOMES), T-box21 (Tbet), and Runt-related transcription factor 3 (RUNX3) transcription factors (Elyahu et al., 2019; Hashimoto et al., 2019). These cells were previously observed and therapeutically utilized in mouse models of colitis (Mucida et al., 2013), multiple types of cancer (Guo et al., 2018; Oh et al., 2020; Slezinska et al., 2020; Zheng et al., 2017), and chronic viral infection (Crawford et al., 2014; Juno et al., 2017; Ma et al., 2015; Patil et al., 2018); whereas they mostly accumulate in the course of chronic inflammation, the differentiation cues of these cells may vary between tissues (e.g., gut versus CNS) (Matoth et al., 2016; Mucida et al., 2013) and inflammatory contexts (e.g., inflamed versus sterile inflammation, or aging) (Giam et al., 2016; Weiskopf et al., 2015). Our studies in old mice further demonstrated that CD4 CTLs are not only functionally active, but that their frequency positively correlates with circulating inflammatory cytokines (Elyahu et al., 2019). Finally, a study by Raveney et al. has shown that CD4+ Eomes + CTLs differentiate in the CNS during the late phase of experimental autoimmune encephalomyelitis (EAE)—with a key role played by infiltrating prolactin-expressing myeloid and B cells—and promote neuroinflammation (Raveney et al., 2015). This and other
studies from this group suggest that CD4 CTLs accumulate in the periphery and in the CSF of people with multiple sclerosis in the secondary progressive phase of the disease, which is characterized by a more pronounced neurodegenerative process (Raveney et al., 2015; Zhang et al., 2019a). Clearly, additional studies are required to unveil the antigen specificities, differentiation, and clonal expansion, of CD4 CTLs in aging and disease. At the functional level, their accumulation may represent a risk profile in the form of shift from the inverted CD4:CD8 ratio, which has been described as an immune risk profile (e.g., immune failure, chronic inflammation, and declined tissue repair (Strindhall et al., 2007)), towards an increased CD4/CD8 ratio observed at late stages of aging primarily after 85 years of age (Adriaensen et al., 2015). Alternatively, the accumulation of CD4 CTLs may represent a stage in late aging with a robust clonal expansion of CD4 memory cells directly implicated in longevity as adjusted and effective anti-viral and anti-tumor cells (Hashimoto et al., 2019). Exploring these aspects will likely open new immune-based therapeutic avenues.

Taken together, when considered in each individual—based on the assessment of the overall balance between age-related CD4 T-cell subsets, their change over time, and their co-evolution with chronic inflammation and disease—the detailed molecular patterns of each of the subsets, and of the environmental cues that induce their differentiation, may offer a more precise and therapeutically efficacious targeting of cell dysfunction.

4.3. Fighting inflammaging and reducing senescent cell load

Reducing the inflammatory load in elderly individuals has long been studied as a general strategy to treat age-related diseases (Chambers and Akbar, 2020; Ferrucci and Fabbri, 2018). In AD, for example, anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), were shown to decrease plaque burden in animal models (Daniels et al., 2016) but were unsuccessful in clinical trials (Group et al., 2008). In mouse model of chronic inflammation (nfb1/−/−), treatments with NSAIDs (COX-2 inhibitors) resulted in reduced cell senescence, telomere dysfunction, and increased regenerative potential (Jurk et al., 2014). Another approach for reducing inflammation is through the inhibition of mTOR activity by compounds such as rapamycin, sirolimus, and metformin. These drugs were shown to improve immunity (Mannick et al., 2014), reduce senescence and SASP (Herranz et al., 2015; Laberge et al., 2015), and, possibly, induce changes in CD4 T-cell differentiation to favor immune regulation (Chi, 2012; Zeng et al., 2013). Whereas accumulating evidence suggests that rapamycin or its analogs are beneficial for a variety of age-related diseases, such as cancer and AD, adverse effects were also reported (Aliper et al., 2017) and, therefore, prescribing them as a prophylactic treatment requires careful, personalized dosing (Blagosklonny, 2019). Reducing inflammation by targeting specific pro-inflammatory mediators (e.g., IL-1β, TNF, or IL-6) has also been tested. For example, TNF is a potent pro-inflammatory cytokine whose levels in the plasma of elderly individuals increases as part of inflammaging (Gerli et al., 2000). Targeting this cytokine or its receptors in aging showed relative improvement in AD pathology (Steeland et al., 2018; Tobinick and Gross, 2008) and reduced both cardiac events (Shaaban and Al-Mutairi, 2018) and the known elevation in rheumatoid arthritis pathology in the elderly (Rechman et al., 2020). Interestingly, TNF-α was recently found in mice as a key cytokine that causes HSC aging (He et al., 2020) and mediates premature aging in mice with a CD4 T-cell metabolic defect
(Desd-Micò et al., 2020). Thus, TNF-α affects CD4 T cells not only directly but also indirectly, acting in concert to facilitate the aging process. Although such therapies have shown relatively positive effects in diverse age-related diseases, more evidence for their therapeutic potential is needed, especially with regard to their effect on the repair process in aging. Likewise, these approaches did not consider the environmental context in aging, such as continued immune dysregulation and increased senescent cells. The growing evidence that senescent cells are an elemental part of damage accumulation in aging and in the pathogenesis of aging and age-related diseases has advanced the notion that the clearance of these cells can be beneficial to reduce inflammation and accelerate repair (Childs et al., 2015; He and Sharpless, 2017; Ogrodnik et al., 2019). Recent studies have shown that the elimination of senescent cells in mouse models of AD markedly mitigated the hallmark pathology of the disease, including the accumulation of misfolded proteins, neuroinflammation, and cognitive decline (Bussian et al., 2018; Zhang et al., 2019c). Such senolytic interventions have also shown promising results in atherosclerotic diseases, renal function, and frailty in aging (Baar et al., 2017; Roos et al., 2016; Xu et al., 2018). Moreover, a recent report showed that the clearance of senescent cells by a senolytic treatment restored stem cell function in the BM of old mice (Chang et al., 2016), and other studies found that such treatments can be beneficial for the restoration of T-cell function in mouse models of increased senescence load (irradiated mice) (Palacio et al., 2019). Together, these studies provide preliminary evidence for new ways to restore immunity with aging. Of note, the ability to differentiate between senescent and non-senescent cells is still not sufficient for the precise elimination of these cells (Gorgoulis et al., 2019); progress in this avenue will accelerate the development of improved senolytic treatment. Moreover, since chronic diseases are assumed to propagate even 1–2 decades before the appearance of clinical symptoms, an early intervention in the low-grade inflammation and senescence processes appears to be therapeutically promising (Baker et al., 2016; Karin et al., 2019). As the clearance of senescent cells requires a properly functional immune system, it could be interesting to examine the combined effect of thymus rejuvenation and senolytic treatments on age-related diseases.

5. Conclusions

Since the beginning of mankind, life expectancy has increased from about 40 years to 80 years, on average. Assuming that thymic involution is advantageous for reproduction, the consequences it has on aging were presumably unpredictable from evolutionary perspectives. The recent discoveries in the field of T-cell aging may offer early diagnosis of immunological trajectories, which are prone to enhanced aging and age-related disease susceptibility. Such biomarkers for early diagnosis are promising avenues to treat individuals much earlier than clinical manifestation. Moreover, since chronic diseases are assumed to propagate even 1–2 decades before the appearance of clinical symptoms, an early intervention in the low-grade inflammation and senescence processes appears to be therapeutically promising (Baker et al., 2016; Karin et al., 2019). As the clearance of senescent cells requires a properly functional immune system, it could be interesting to examine the combined effect of thymus rejuvenation and senolytic treatments on age-related diseases.
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