INTERNATIONAL TRENDS IN IMMUNITY VOL.1 NO.4 OCTOBER 2013
ISSN 2326-3121 (Print) **ISSN** 2326-313X (Online) http://www.researchpub.org/journal/iti/iti.html

An Overview of T Cell Subsets and Their Potential Use as Markers of Immunological Ageing

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***Abstract* – Until recently, T cells were divided into two main categories, the helpers, expressing the CD4, and the cytotoxic, expressing the CD8 molecule. Their origin and differentiation have been well documented, leading to numerous discoveries and new therapies. But with time, immunologists identified T cell complexity. Step by step, scientists have identified more than ten different T cell subsets with their own lineage, role and specificity. For instance, the helpers T cells can now be divided at least into six subpopulations based on their general function. Additionally, each subset is further discriminated based on surface/intracellular markers. In addition of the classical αβ T cells, γδ T cells are specialized cells recognizing mainly phospho-antigens. All T cell differentiate after antigen recognition into different subsets of memory cells and ultimately may become senescent. In the present review we summarize the latest information about T cell development and differentiation as well as the particularities of each subset and discuss how this evolves over age.**

Index terms – ageing, development, differentiation, T cells, Thymus, Senescence

I. INTRODUCTION

THE adaptive immunity, one of the most advanced defense mechanism known, is also probably the most complex system in biology. The ability to memorize the type of antigen encountered and its specificity for virtually any foreign organism are unique characteristics of adaptive immunity. The system relies on lymphocytes that act as a cornerstone that defined the type, the intensity and the duration of the immune response.

The lymphocyte pool comprises T cells expressing CD3 (helper, cytotoxic, γδ, Natural Killer T cells, invariant Natural Killer T cells, Mucosa-Associated Invariant T cells) and B cells expressing CD19.

Submitted 13 September 2013
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The B cells are mainly known for being the cells responsible for the production and secretion of antibodies. On the other hand, the T cells are considered as the soldiers of immunity as they can support and lead the immune response. Here, we will describe the fate of T cells from release of precursors from the bone marrow to their ultimate state of differentiation, senescence.

In the bone marrow, some hematopoietic stem cells can initiate the acquisition of a common lymphoid progenitor (CLP) phenotype that will start dividing and differentiating. These still very progenitor-like cells will migrate to the thymus following a gradient of chemo-attractant released to sustain lymphopoiesis. There, they undergo very complex selection processes that will eliminate more than 90% immature T cells. This demonstrates that thymic maturation is very efficient in eliminating unwanted T cells but also its metabolic cost. After selection, mature naïve T cells will enter the blood circulation and reach the secondary lymphoid organs (SLO) where antigen presentation allows differentiation into a selection of memory subsets and clonal T cell proliferation (Figure 1).

The differentiation is dependent on the stimulation provided by the antigen-presenting cell (APC). The helper T (T_H) cells population is more heterogeneous than the cytotoxic T (T_C) cells. The diversity of the T_H population (beyond the T_H1, 2 and T regulatory (T_{Reg}) phenotypes) is a recent discovery that dramatically increases the complexity of our understanding of the T_H response. Until recently, the T_H response was divided into the T_H1, in response to viral infection (the cellular immunity) and the T_H2 more prominent to antibody secretion (the humoral immunity). Later, regulatory T cells and their ability to suppress the immune response were discovered. This tripartite concerto (T_H, T_C and T_{Reg}) lasts until the discovery and understanding of the function and role of the T_H3, T_H17, T_H9, T_{FH} (for follicular helpers), NKT and γδ subsets.

In this review we will cover the steps leading to generation of the various T cell populations, including their differentiation. How adaptive immunity and especially T cells may predict the aging of the immune system will be discussed.

II. T CELL DEVELOPMENT IN HUMANS

Like any type of blood cell, lymphocytes originate from pluripotent hematopoietic stem cells (HSC) located in the

ISSN 2326-3121 (Print) **ISSN** 2326-313X (Online) http://www.researchpub.org/journal/iti/iti.html

bone marrow, especially in the pelvis and iliac crest. These cells are able to divide asymmetrically: the daughter cell is the replication of the parent cell, while the parent cell keeps the capacity to generate more daughter cells without differentiation, the daughter cell will differentiate into the desired cell type. This property allows the bone marrow to keep a constant pool of self-renewing stem cells. The HSC, originating from the aorta-gonad-mesonephros in the embryo, expand in the fetal liver and then colonize the bone marrow [1].

depending on their lymphoid/myeloid ratio ($^{L}/_{M}$), the balanced HSC ($3< {}^{L}/_{M} <10$), the lymphoid-biased HSC ($^{L}/_{M} >10$) and the myeloid-biased HSC ($0< {}^{L}/_{M} <3$) [3]. Under the influence of some cytokines and growth factors like SCF, Interleukin-3 (IL3) and GM-CSF, the HSC will differentiate into a common myeloid progenitor (CMP). This CMP will then differentiate into either a megakaryocyte and erythroid progenitor (MEP) or a granulocyte and macrophage progenitor (GMP). After a succession of division/differentiation steps, MEP will

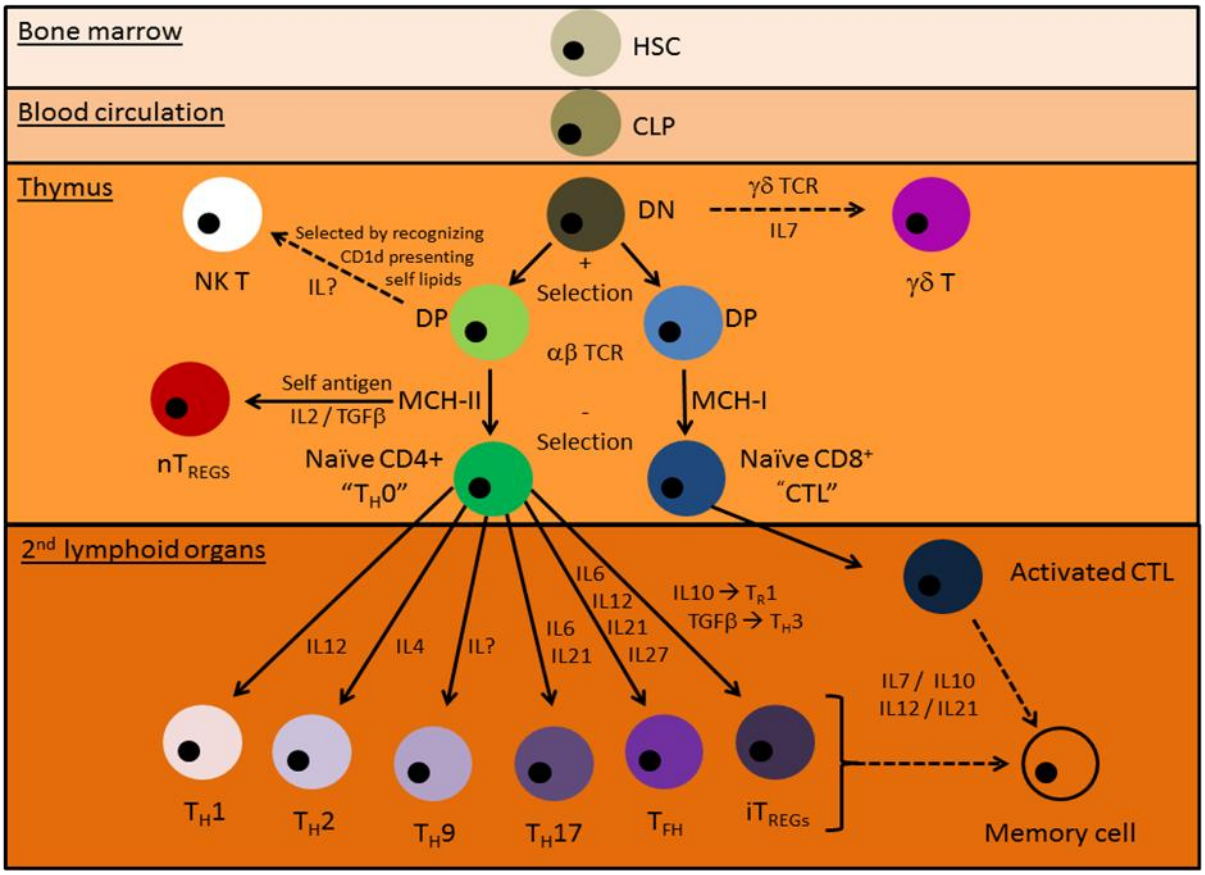

Figure 1. General and non-exhaustive overview of T cell development and classification. HSC: hematopoietic stem cells; CLP: common lymphoid progenitor; CTL: cytotoxic T lymphocyte; TCR: T cell receptor; DN: double negative; T_H: T helper; T_{FH}: T folicular helper; MHC: major histocompatibility complex; IL: interleukin

Pluripotent HSC, identified as $CD34^+$ $CD59^+$ $Thy1/CD90^+$ $CD38^{low/-}$ $C\text{-}kit/CD117^+lin^-$, can lead to the generation of the lymphoid and the myeloid lineage [2]. It was accepted for long that HSC were homogeneous and follow their path in a stochastic manner. As for other immune-related phenomenon, stochasticity has it limits. In fact, the HSC population can be divided into 3 subsets

generate platelets and erythrocytes whereas GMP will generate monocytes (which may become macrophages or dendritic cells), neutrophils, basophils and eosinophils [4].

Concerning the lymphoid lineage, the main mechanism is very similar [5]. Under IL3 stimulation, the pluripotent HSC will start to asymmetrically differentiate into long term-HSC then short-term HSC to reach a multipotent

progenitor state (MPP, that may lead to either a CMP or a CLP). When this progenitor is stimulated by IL7, it will shift toward a lymphoid lineage and become a CLP that will lose its myeloid potential. When the CLP is stimulated by SCF and IL2, it will follow the NK lineage, if stimulated by IL7, it will follow either the T or the B differentiation. If the "pro-B or pro-T" CLP expresses regulators such as Notch-1 and GATA-3, it will shift to T lineage and if it expresses EBF, E2A and Pax-5 it will follow the B lineage. The future B cells reside in the bone marrow until they express IgM, and then join the secondary lymphoid organs to finish their differentiation in mature naïve B cells. The T cells development will take place nearly exclusively in the thymus [6]. At an undetermined stage, a very early T cell precursor leaves the bone marrow, enters the blood circulation, reaches the thymus and will be called thymocytes. Thymic T cell differentiation is driven by thymic stromal cells and the factors they are secreting (cytokines and growth factors). It is an incredibly complex fine-tuned and well-regulated phenomenon composed by a succession of selection steps that will end for 97% of developing T cells to apoptosis. To face the number of antigens an organism can encounter during its lifespan, the chosen strategy is to generate the highest variety of TCR specificities without compromising the quality of antigen recognition and avoiding anti-self responses.

When a CLP enters the T lineage pathway, it will migrate from the bone marrow to the thymus. Once arrived in the thymic cortical area, under the influence of the stroma, it will become an early thymocyte progenitor (ETP) but still able to differentiate into myeloid cells [7]. This stage is very transient, as the ETP will quickly differentiate into the T lineage. The early T cells are $CD44^+$ and negative for the common T lineage markers ($CD3^-CD4^-CD8^-CD25^-TCR^-$) and are called double negative 1 (DN1). As the differentiation progresses, the DN1 cells will start to express the adhesion molecule CD44 and CD25 (the α chain of the IL2 receptor) [8]. At this stage the cells will initiate T cell receptor (TCR) rearrangement. The conventional T cells will rearrange a αβ T cell receptor while the non-conventional T cells will rearrange a γδ TCR. The VDJ recombination is the mechanism that allows the generation of a huge diversity of T cell specificities. During the DN2 stage T cells lose CD117 expression and are fully committed T cells [9]. At the DN3 stage ($CD4^-CD8^-CD24^+CD25^+CD44^{low}CD117^{low}$), T cells are located in the subcapsular zone, are definitely engaged in αβ or γδ fate, while still rearranging combinations for the β, γ and δ chains [10]. Between the DN3a ($CD27^{low}$) and DN3b phases ($CD27^{high}$), a very important checkpoint occurs, the β selection. The correct rearrangement of the β chain is verified thanks to an invariant α chain and the pre-TCR signaling [11]. All cells that fail the β selection will enter apoptosis. Then, cells maturate to the DN4 stage ($CD4^-CD8^-CD24^+CD25^-CD44^-CD117^-$), during which T cells move to the medulla. During their migration, DN4 cells will upregulate both CD4 and CD8 to become double positive (DP) ($CD4^+CD8^+CD24^+CD25^-CD44^-CD117^-$) and will rearrange their TCR α chain [8]. Here, cells will be positively selected depending on their TCR avidity for MHC molecules. Once the TCR in finalized, depending on which MHC molecule it recognizes, the DP cell will become single positive (SP) $CD4^+$, if the TCR binds a MHC-II molecule, or $CD8^+$, if the TCR binds a MHC-I molecule. Once in the medulla, the SP cells will undergo a negative selection step where all of the self-reactive T cells will enter apoptosis [12]. The surviving selected cells are now mature naïve T cells (T_H0) and are ready to leave the thymus.

Homing is a very important part of T cell development: from bone marrow to thymus, through the different thymic areas, to the secondary lymphoid organs (SLO) and later, to the site on infection. Mice studies helped to dissect the succession of factors that drive cells through their path. It is not clear which factors attract CLP to thymus through the cortico-medullary blood vessels but CCR9 (chemokine receptor 9)-ligand seems to be involved [13]. Inside the thymus, ETP migrate to the subcortical zone via the CCR7 [14]. It is highly expressed in DN1/2 population. CCR7 is also required for DP cells migration in the medulla [15]. The mature naïve T cells emigration from thymus to the SLO requires, in newborn mice, CCL19-CCR7 interactions [16]. Another receptor is engaged in SLO homing, the sphingosine-1-phosphate receptor 1 ($S1P_1$) [17]. It is expressed on both type of SP and naïve cells and is also involved in emigration from SLO to lymphatic vessels.

III. GENERATION OF MEMORY T CELLS

Memory T cells are antigen-experienced, long-lived T cells that are different from naïve cells in numbers and functions due to their previously encounter with antigens following infection or vaccination. They can mediate protection by mounting a faster and stronger immune response to subsequent encounters with the invader. More than 90% of responding cells die after infection while for the 10% surviving, IL7 [18], IL12 [19] and an IL21/IL10/STAT3 pathway [20] seem to play a decisive role in their differentiation/maturation/maintenance, at least in the $CD8^+$ memory T cells.

Memory T cells may be divided into three subpopulations based on their homing capacity, namely central memory cells (T_{CM}), effector memory (cells T_{EM}), and tissue-resident memory cells (T_{RM}) [21]. While the majority of memory cells are left behind following the massive apoptosis of effector T cells, a significant

proportion (easily detectable in blood) remains after an immune response. T_{CM} express CCR7 and CD62L (L-selectin) as well as secrete IL2 however lack the capacity to produce interferon-gamma (IFNγ) and IL4. Because T_{CM} display higher self-renewal capacity, they are associated with a memory stem cells capacity -that still need a consensus, and regarded as superior to T_{EM}. On the other hand, T_{EM} do not express CCR7 or CD62L, are less proliferative, but produce higher levels of IFNγ and IL4. The $CD4^+$ and $CD8^+$ T_{CM} mainly reside in secondary lymphoid organs, while T_{EM} can be found in peripheral compartment [22]. After infection, populations of memory T cells can also reside in peripheral tissues, and recently designated as tissue-resident memory T cells (T_{RM}) expressing CD103 and CD69 molecules [23]. T_{RM} are present in various tissues, such as brain, lung, vagina, gut, and skin. In mice it has been shown that skin infection generates T_{RM} that provide skin-specific immunity against further infection. Likewise, a similar population of T cells was found in human skin [24]. The generation of memory T cells can be fated at different stages of T cell life, and is influenced by complex variables such as antigen, costimulatory molecules, cytokines, chemokines, metabolism, and transcription factors. For example, chronic infection whereby antigen and inflammation are present at high and constant levels, generation of effector cells is favored against memory cells [25]. On the impact of the soluble factors influencing T cell differentiation it was shown that STAT3- SOCS and IL10 signaling favors memory T cell; while IL12 and also IL2 may exert negative regulation of memory T cell formation [26, 27]. Furthermore, transcription factors such as T-bet and Blimp1 favor effector T cells fate [28, 29]; while transcription regulators Bcl6, Id2 and Id3 as well as Wnt-β-catenin signaling pathway influence memory T cell generation in positive manner [30-32]. This suggests a complex mechanism, including signaling crosstalks and negative feedback loops, has been developed to fine-tune the generation of memory cells.

IV. THE DIVERSITY IN HELPER T CELLS DIFFERENTIATION

Following recognition of foreign antigens, the antigen-presenting cell (APC) is activated and will migrate to the SLO (Figure 1). When naïve T cell are activated by APC, they acquire effector functions while differentiating into T_H1, T_H2, T_H3, T_H9, T_H17, T_{FH}, $T_{Reg,}$ or cytotoxic T cells [33]. $CD4^+$ T cell fate will be highly influenced by the cocktail of cytokines present in the milieu during antigen presentation. After activation, cytotoxic T lymphocytes (CTL) become fully functional (cytotoxic and then memory) and dispose of their entire arsenal (granzyme, Fas, perforin) to eliminate infected cells.

Activated CD4+ T cells 'help' to modulate the function of B cells and cytotoxic T cells via cytokine secretion and cell-cell contact. In addition, T_H cells also participate in the regulation, enhancement, and recruitment of innate cells such as macrophages, neutrophils, mast cells, and monocytes [34]. Activation of naïve CD4 T cells by APC involves binding of TCR with MHC-II as well as binding of B7 co-stimulatory molecule to T cells' CD28 receptor [35]. In addition, signals provided by distinct cytokines will program naïve CD4 T cells into different T_H subsets. A defined T_H subset should have a signature cytokine profile and distinct transcription factor(s) that regulates its development into terminal differentiation.

The first two subsets of T_H cells discovered, T_H1 and T_H2, were categorized based on cytokine secretion [36]. T_H1 cells produce IFNγ and are associated in cell-mediated immune responses against intracellular pathogens; while T_H2 cells produce IL4, IL5, IL13, and IL10 (Figure 1) and are thought to drive humoral immune responses against parasites [34, 37, 38]. To induce T_H1 cell differentiation, IL12 secretion from DC has been identified as the key cytokines required to upregulate T-bet as master regulator [39, 40]. Meanwhile, IL4 drives T_H2 subset via GATA-3 transcription factor induction, which leads to IL4, IL5, and IL13 secretion [34, 37, 38]. The main effector cell for T_H1 immunity comprise of macrophages, CTL, IgG B cell, and IFNγ producing CD4 T cell; while the main effector cells for T_H2 immunity are eosinophils, basophils, mast cells, IgE B cells, and IL4/IL5 producing CD4 T cells.

The restricted T_H1/T_H2 hypothesis has been re-evaluated because of the identification of another T_H subset, the T_H17 cells. This third T_H subset was discovered through autoimmune disorder studies and has been shown to develop independently from T_H1 and T_H2 lineages [41, 42]. Transcription factors such as T-bet and gata-3 that are important for T_H1 / T_H2 differentiation are negative regulators of T_H17 differentiation [43, 44]. T_H17 cells secreted IL17, IL1, TNFα, IL21 and IL22 to mediate protection against extracellular bacteria and fungal infection instead of secreting T_H1/T_H2 cytokines IFNγ or IL4 [34, 45]. Additionally, T_H17 cells also mediate B cell responses by inducing proliferation and isotype switching [46]; as well as drive the differentiation of plasma cells via IL21 secretion [47]. T_{Regs} and T_H17 cells balance is tightly regulated especially in the mucosa as both require TGFβ for their development [48]. However the involvement of IL6 and IL21 upregulates RORγt, the typical T_H17 transcription factor, and drive the cells towards T_H17 differentiation [49, 50]. Factors such as vitamin A, retinoic acid, and IL6 were shown to promote T_{Regs} differentiation, while IL6 inhibition promotes T_H17 formation [51].

Another subset, the T_H9 cells has been identified after a population of CD4 T cells was reported to secrete substantial amounts of IL9 while failing to secrete T_H2 cytokines upon TGFβ and IL4 stimulation [52]. For T_H9 cells development, the PU.1 and IRF4 transcription factors are required [53, 54]. Functionally, T_H9 cells are thought to play significant role in extracellular parasite infection and allergy disorders [55]. However, T_H17 and T_{Regs} are also reported to secrete IL9 [56], and hence future studies are needed to further elucidate the function and characteristic of T_H9 cells (Figure 1).

Recently, a distinct subset of human skin homing memory T cells was shown to produce IL22, IL26, and IL13, while failed to secrete IL17 and IFNγ [57], and was coined as the T_H22 subset. These cells are thought to have an important role in skin immunity and in a variety of autoimmune diseases [58]. Distinctively, T_H22 express CCR6, CCR4, CCR10, and characteristic transcription factor aryl hydrocarbon receptor (Ahr) [57]. However, T_H17, T_H1, NK, and NK T cells are also known to secrete IL22 cytokine.

Additionally, the T follicular helper (T_{FH}) cells are a T_H subset that is essential in assisting B cells to maintain a long-lived antibody response in the germinal centers of secondary lymphoid organs. They interact with matured B cells that differentiate into high affinity plasmocytes or memory B cells that produce long-lasting antibodies [59]. T_{FH} are distinct from other T_H subset by the signature expression of BCL-6 and CXCR5 [60]. T_{FH} cells also produce high levels of IL21 that serves as germinal center B cell survival and differentiation factor, and low levels of IL4, IFNγ, and IL17 [61]. Their development, far from being fully understood, seems very dependent of STAT3 and of IL21-inducing cytokines such as IL6 [62], IL12 [63], IL21 [64] and IL27 [65]. This enlightens even more the role of the cytokines present in the microenvironment of developing T_H.

V. GENERATION OF OTHER T CELL POPULATIONS

A) REGULATORY T CELLS

Regulatory T cells (T_{Regs}) were discovered by Sakaguchi et al. in 1995 [66]. They participate in the immune response by suppressing immunity to contain its duration and its intensity that prevents septic shocks. They are $CD4^+$ T cells characterized by a constitutive expression of CD25 (IL2Rα) and a specific transcription factor, FoxP3 [67]. T_{Regs} population was thought to be homogeneous but it appears that at least 2 subpopulations coexist: the natural T_{Regs} (nT_{Regs}) and the induced T_{Regs} (iT_{Regs}) [68]. While nT_{Regs} originate from thymic maturation the iT_{Regs} undergo a post-thymic maturation (Figure 1).

The nT_{Regs} develop in the thymus from autoreactive T cells with a TCR having a medium to high affinity for self-antigens [69]. The selection process seems to begin when the TCR avidity for self-antigens is comprised between the ones that influence positive and negative selection steps [70]. Moreover, the repertoire of classical T_H cells is different from that of nT_{Reg} cells with only a little overlap and the latter is much more autoreactive [71]. To underline the importance of nT_{Regs}, it has been shown that their TCR-dependent selection seems to be quite permissive as it is possible that a part of the autoreactive T cell has its regulatory doppelganger [69]. Downstream of the TCR signaling, Akt (Protein kinase B)-mammalian Targent of Rapamycin and NF-κB pathways (particularly the transcription factor cREL) are deeply implicated in the nT_{Regs} differentiation with the latter supposed to be necessary and sufficient for regulatory fate [72]. After the TCR-dependent step, a TCR-independent step occurs where IL2 and IL15 [73] definitely drive the cell toward the nT_{Reg} state.

The iT_{Regs} develop in the thymus as naïve conventional T_H cells until they meet their antigen in the periphery. They can be divided in 2 subsets: the T_r1 cells, producing IL10 but not expressing FoxP3 [74], and T_H3, producing TGFβ and expressing FoxP3 [75]. It seems that a weak TCR avidity for antigen is a determining feature for differentiation into a regulatory state [76] and CD28 co-stimulation is not required [77]. Recently, it has been shown that beads coated with PD-L1 on their membrane were able to induce iT_{Regs} [78]. iT_{Regs} play their regulatory role by secreting the cytokine that induce their differentiation, i.e. Tr1 mainly secrete IL10 and T_H3 mainly secrete TGFβ (although each of them can secrete both molecules).

Despite the fact regulatory T cells are mainly known as $FoxP3^+$ cells and that FoxP3 mutations lead to the IPEX syndrome (Immunodysregulation Polyendocrinopathy and Enteropathy X-linked syndrome) [79], the fact that Tr1 cells are $FoxP3^-$ widens the spectrum of regulatory activities by T cells.

B) γδ T CELLS

Unlike the majority of T lymphocytes, the γδ T cells bear a non-conventional TCR made up of one γ and one δ chain that recognizes a restricted antigen diversity [80]. They represent 5-10% of the T cells but are more abundant in the gut mucosa within the intraepithelial lymphocytes [81]. Due to their locations (tongue, lung, guts, or skin) they act as a first line of defense and are a bridge between innate and adaptive immunity. Their TCR repertoire is much more limited than the conventional T cells' and is variable

depending on their localization. This may be an adaptation to the pathogens they meet in the environment they reside in [82]. As they rearrange and display a TCR, they are part of the adaptive immune system; although the TCR acts more like a Toll-Like Receptor recognizing pathogen-associated molecules, as it is limited in the repertoire. Finally, the γδ T cells were shown to be capable of phagocytosis [83].

In the thymus the cells that are successful in rearranging a γδ TCR in a very early state will become γδ T cells and will not rearrange a αβ TCR. Those who failed will begin rearrangement of their β chain and become αβ T cells, if successful. The mechanism(s) influencing the choice of a γδ or αβ fate are still unknown but it seems that the TCR itself and the way it is stimulated plays a very important role [84]. For γδ T cells, the intensity of the TCR avidity will favor the differentiation toward γδ if the interaction is strong or αβ if the interaction is weak

Moreover, several cytokines are able to modulate the γδ lineage development. Knock-out mice for IL7 are not able to generate γδ T cells [85]. It has also been shown that IL4 was able to promote both growth and differentiation of thymocytes toward αβ and γδ T cells with a preference for the latter [86]. IL15 is also very important for γδ T cells as it acts as a growth factor and is essential for survival of the γδ dermal subset [87]. It has been demonstrated that IL2Rβ was crucial for the γδ T cell development, as it is part of the IL15R. IL10 may also play a role during the fetal development of γδ T cells [88]. The addition of very low concentrations of IL10 in a fetal thymic organ structure was shown to increase the generation of γδ T cells.

The biology of γδ T cells are not yet fully understood but their role in immunity, quick responsiveness and their link between innate and adaptive immunity make them a very interesting target for immunotherapies in numerous infections, cancers and autoimmune diseases.

C) NK T CELLS

In the early 1990s, several groups discovered subsets of α β -DN and $CD4^+$ T cells that had intermediate TCR level and were potent cytokines producers while expressing NK-cells marker NK1.1[89]. Their development do not require MHC class II expression, but dependent on the non-polymorphic MHC class-I molecule CD1d, a non-classical antigen-presenting molecule that binds to glycolipids and associates with β_2-microglobulin (β_2m) [90]. These cells also express higher frequency of TCR V β 11 in human than conventional T cells [91] and skewed to usage of invariant TCR α chain V α 14-J α 281 (V α 14-J α 18) in mice and V α 24-J α Q (V α 24-J α 18) in humans [92]. Hence, Natural killer T (NK T) cells first emerged as a term to describe a subset of T cells that express NK1.1 marker in the mouse (CD161 in human) [93]. However, this initial definition is rather simplistic as a broader NK T-cell family consisting of different types of T cells were discovered, some of which do not express NK1.1 [94]. Therefore, it is now more appropriate to designate NK T cells as CD1d-dependent NK-like T cells.

NK T cells differ from conventional T cells that interact with MHC class I and II peptide complex and are distinguished from NK cells by the expression of TCR α / β with restricted repertoire. Upon activation, NK T cells exert innate-like rapid response to self and foreign glycolipid antigens and produce Th1 and Th2 cytokines such as IFN ɣ , IL4, and GM-CSF to bridge adaptive immunity [95]. NK T cells have been found to be essential in infections, tumor immunity, allergy and autoimmune diseases such as asthma, diabetes and atherosclerosis [96].

In general, NK T cells are categorized into type I and type II NK T cells [94]. Type I NK T cells are the ones expressing the invariant V α 24-J α 18 in humans and are well known as invariant NK T cells (iNK T cells). The transcription factor promyelocytic leukemia zinc finger (PLZF) was found to direct the development of iNK T cells [97]. Recently, Olszak et al. demonstrated that the absence of microbial exposure in neonatal mouse led to pathological accumulation of mucosal iNK T cells and immune morbidity [98]. These cells are CD1d-dependent and vary in CD4 and CD8 expression. Two subpopulations are defined within the type I NK T cells: $CD4^+$ and DN population. Type II NK T cells (non-invariant) consist of all other $NK1.1^-$ cells that are CD1d-dependent. These cells mostly express CD4 but are not reactive to α -GalCer-although they are also restricted by CD1d [96]. This subset was found in the thymus of both human and mice and was shown to produce higher IL4 and lower IFN ɣ level in comparison to type I NK T cells [99]. Additionally, CD1d-independent NK T-like cells have been identified [100], which most likely are T cells that express $NK1.1^+$ but otherwise are not related to NK T cells.

NK T cells develop in the thymus from the same precursor than conventional T cells [101] but follow a different path. They are selected by interacting with their TCR with DP thymocytes expressing endogenous glycolipids presented by CD1d molecules [102]. Many pathways are involved in NK T cells differentiation like Src kinase Fyn [103] signaling mediated through $Slamf^l$ and Slamf6 receptors [104]. NK T cells were shown to receive a stronger TCR signal during their development [105], proving that they were positively selected by self-lipid. It is still unknown if they undergo a negative selection step or if they are more resilient to the apoptosis this step would induce. During the maturation, the expression of cytokines

such as IL4, IFNγ, IL2 and IL15R β-chain varies until cells divide and begin to express NK markers such as NK1.1, Ly49s and CD94 [106]. During this expansion, T-bet [107], GATA3 [108] and IL15 signaling [109] are required.

Contrary to conventional T cells, NK T cells become immediately functionally active after the positive selection by expressing PLZF [97] and respond to TCR stimulation by expressing high levels of IL4 and low levels of IFNγ. Mature NK T cells express CD44, a marker of antigen experience, and CD69, an early activation marker, showing that they are not naïve when they leave the thymus. Altogether, these findings demonstrate that NK T cells possess a unique way to differentiate sharing both naïve and effector cells capacities.

VI. T CELLS AS MARKERS OF IMMUNOLOGICAL AGEING AND LONGEVITY?

All the cells described previously will, as any other cell of the body, age. Cellular turnover can be very intense in some tissues such as in the digestive tract or very slow to inexistent such as in the brain and the heart. In the case of immune cells, cellular turnover varies from cell type to cell type. With the ageing of the cell some dysfunction may occur and this phenomenon is called immunosenescence [110]. Innate cells such as neutrophils have a very short live (approximately 24h) that can be expanded following response to antigens (via GMSCF). While antibody-producing B cells (plasma cells) may operate for several weeks before decreasing their activity and numbers of the antigen-specific memory T cells may survive for years and decades. This provides an essential protection against invaders during lifespan.

However, older individuals display a higher susceptibility to different infections, often lose immunity against latent infection and are more susceptible to Influenza and its adverse effects. One of the key features of an aged immune system is the decline of naïve T cell frequency as well as the deterioration in functions of T cell subsets. Diminished capacities of the hematopoietic stem cells to generate lymphoid progenitors as well as the involution of thymus are accounted for the observed decrease in T cell frequencies [111]. In addition, aged T cells displayed a more advanced memory differentiated phenotype.

Apart from the classical T_H/T_C classification, memory cells are also distinguished by their surface markers expression; namely the $CCR7^+CD45RA^-CD45RO^+CD28^+CD27^+$ T_{CM}, $CCR7^-CD45RA^-CD45RO^+CD28^{+/-}CD27^{+/-}$ T_{EM}, and the $CCR7^-CD45RA^+CD45RO^{low}CD28^-CD27^-$ late differentiated cells. Memory T cells display reduced telomere length and upregulate senescence markers such as CD57, KLRG-1, CTLA-4, and PD-1 [112]. The proportion of $CD28^-$ T cells which comprise both the effector memory and T_{EM} re-expressing CD45RA (TEMRA) cells are significantly increased in the elderly in comparison to younger subjects [113]. In particular, the cytomegalovirus (CMV)-specific memory $CD8^+$ T cells tend to expand more than those specific of Influenza or HIV, with majority expressing the senescence markers CD57 and/or KLRG-1 (Figure 2) [114]. As the consequence, the naïve T cell pool is reduced in elderly but this is strongly initiated and driven by CMV that may accelerate immunosenescence [115]. Conflicting reports are published regarding whether CMV infection, additionally to affecting the T cell phenotypes, could also affect responses to other viruses in the elderly; hence, further work is required [116]. This impact of persistent/latent infections is relevant in the elderly, as the number of late-differentiated T cells and level of expression of senescence markers may be proportionate to the number of antigenic challenge it encounters through an individual's life (Figure 2). Hence subsequently, the ageing of the immune system is not only determined by age but by the immunological history. A hierarchy in the pathogens that may drive the ageing of the immune system exists. However, much work is needed to classify the most common pathogens and understand how poly-infection is dealt with

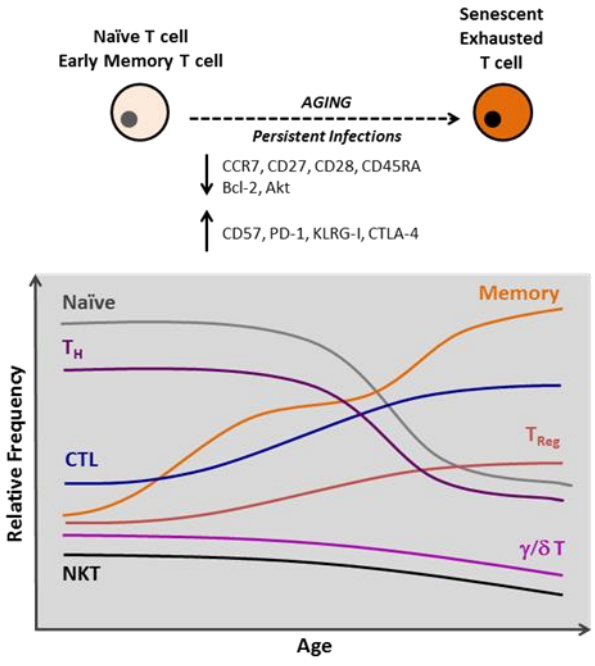

Figure 2. T cell subset changes during ageing. An increased frequency of senescent T cells is observed during ageing. The expression of CD57, Program Death-1 (PD-1), Killer Lectin Receptor G-1 (KLRG-1] are hallmarks of changes in T cell functionality.

in this regard.

Senescence [110, 117] and exhaustion [25] can be seen as the T cells ultimate state of development and differentiation. Senescent and exhausted T cells will lose expression of activatory molecules such as CD27 and CD28 and upregulate inhibitory molecules such as CD57, KLRG-1 and PD-1 (Figure 2). Their resistance to apoptosis is decreased by the decline of Bcl-2 and Akt expression. Their proliferation capacity [118], as well as their capacity to secrete IL2 [119] is inhibited whereas their inflammatory/cytotoxic potentials are increased (upregulation of IFNγ, TNFα, granzyme and perforin) [120]. A consensus for alteration describing immunosenescence exists and includes a deregulation of intracellular signal transduction [121], a shrinkage of the TCR repertoire [122], a decrease of the cytotoxic activity of the some subsets [123], an accumulation and a clonal expansion of memory and effector T-cells [119] and a decreased immunity against viral pathogens, especially by cytotoxic $CD8^+$ T cells [124]. STC and ETC appear during ageing and chronic diseases like AIDS, hepatitis C, CMV and Epstein-Barr Virus infection. As senescence can be interpreted as an anti-oncogenic process by turning off the proliferation of aged cells, exhaustion could be interpreted as a way to shorten long immune response to avoid collateral damages.

Furthermore, several changes were reported in other subsets of T cells such as the NKT cells and the γδ T cells in the elderly. Ageing has been shown to affect the frequency of iNKT cells, alter their subset distribution, proliferation capacity, as well as cytokines response in the human peripheral blood [125]. The reduction of iNKT cell number is suggested as the result of thymic involution in ageing [101], or redistribution of these cells in different tissues. Increase of $CD4^+$ iNKT cells were observed in line with decrease of $CD4^-CD8^-$ DN subset; and cytokine profile of iNKT cells was shifted from Th1 to Th2 in the elderly [125]. Similarly, impairment in cytotoxicity and IFN ɣ production was reported in old mice and human; although very old mice and human centenarians do not suffer this defect and have satisfactory number of NKT cells [126, 127]. Likewise, majority of the reports also demonstrated reduced frequency of γδ T cells in the elderly [128]. The reduction of $V\delta2^+$ subset mainly accounts for the decline in number while the $V\delta1^+$ population remains stable [128]. The reduction of naïve and central memory subsets ($CCR7^+CD27^+$) and a shift into more differentiated phenotypes ($CCR7^{+/-}CD27^{+/-}$) were accounted for this observation [128]. Recently, CMV has also been associated with age-related alterations in the γδ T cells [129]. Consequently, defects in these T cell subsets upon ageing could dampen efficient tumor immunosurveillance, contribute to the deregulation of the cytokine network, and translate to age-related disease.

The common point between every subset of T cells affected by senescence is that their ability to divide will be inhibited and they will turn into a proinflammatory state. This could be the result of a continuous exposure to external agents [130]. With the thymic output running low [131, 132] and the accumulation of memory T cells resulting from the past infections [133], the possibility to generate new cells to fight newly met pathogens is reduced dramatically. It is one of the reasons behind yearly "epidemic of deaths" due to new strains of influenza. Moreover, as the tissue/organs of the body ages quite homogeneously, the dampening of the immune response can be seen as an adaptation to an old body composition and function as it is possible that a "young" immune response may not be adapted to an "old" environment. But, as life expectancy has been increased in most countries this provides statistically higher chances to meet new infectious agents. Ageing can be seen as a consumption of reserves due to these various encounters and responses. In a more realistic model, each infection contracted throughout life consumes a certain amount of reserve, depending on the weight of the disease. This can lead to a quicker depletion of the reserve and induces a higher exhaustion prior to clinical outcomes such as death. Although quite simplistic, as other factors could induce a decrease of the reserve (stress, depression, lifestyle, genetics, etc.), this model could explain why some elderly respond and survive to influenza infection and others not. This raises a very interesting possibility: using the T cells pool as a reserve gauge. By analyzing the phenotype of T cell subsets of a given elderly individual, it is possible to draw a portrait of his immunological history (at least quantitatively). The gap that needs to be filled with evidence is how to translate this into reserves that may not only predict the robustness of his immune system (be it adaptive) but also identify individuals at risk. A classification of immunological challenges and diseases based on their impact of the reserves is needed. While acute infections such as Influenza and benign surgery may minorily affect the reserves, diseases such as AIDS, hepatitis C, CMV infection or cancer would substantially reduce them. Recently, a moderate abdominal fat tissue was found to provide a survival advantage [134] for elderly, which corroborates the theory of reserves. Fat is a source for energy and hormones secretion that may be of critical help during infections and their adverse events. This theory is also plausible when considering the impact of stress on lifespan [135], inflammation [136] and the immune system in general [137]. A comprehensive implementation of the reserve may prove difficult at the clinical level and also for researchers. Indeed, fat, muscle, the antioxidative potential, the metabolism rate or even the thymus involution could be components of a reserve index.

Currently, the frequency of $CD28^-$ and $CD27^-$ T cells correlate with ageing as well as seropositivity to persistent infections such as CMV. The frequency of CD8+CD28- T cells was associated to an Immune Risk Profile (IRP) that predicted survival over a 2, 4, 6 and 8-years period in the elderly. Because of the feasibility and non-invasiness of assessing T cell phenotypes and functions in humans, further identifying biomarkers of immunological ageing in T cells will provide an advantage. A better stratification of the elderly individuals will also allow to better correlate immunological ageing, health, reserves and longevity.

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