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Review

CD4+/CD8+ double-positive T cells: more than just a developmental stage?

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ABSTRACT

CD4+/CD8+ DP thymocytes are a well-described T cell developmental stage within the thymus. However, once differentiated, the CD4+ lineage or the CD8+ lineage is generally considered to be fixed. Nevertheless, mature CD4+/CD8+ DP T cells have been described in the blood and peripheral lymphoid tissues of numerous species, as well as in numerous disease settings, including cancer. The expression of CD4 and CD8 is regulated by a very strict transcriptional program involving the transcription factors Runx3 and ThPOK. Initially thought to be mutually exclusive within CD4+ and CD8+ T cells, CD4+/CD8+ T cell populations, outside of the thymus, have recently been described to express concurrently ThPOK and Runx3. Considerable heterogeneity exists within the CD4+/CD8+ DP T cell pool, and the function of CD4+/CD8+ T cell populations remains controversial, with conflicting reports describing cytotoxic or suppressive roles for these cells. In this review, we describe how transcriptional regulation, lineage of origin, heterogeneity of CD4 and CD8 expression, age, species, and specific disease settings influence the functionality of this rarely studied T cell population. *J. Leukoc. Biol.* 97: 31–38; 2015.

Introduction

T cells are key components of the adaptive immune system. Mature T cells are generally considered to express either the CD4 or CD8 coreceptor, in addition to their TCR, and consequently, the T cell pool is commonly divided into two subsets, based on expression of either CD4 or CD8. The CD4 molecule, a member of the IgR family, is encoded by a single gene and expressed on the surface as a transmembrane monomer [1, 2]. CD4 interacts with the β2-domain of MHC class II molecules and has also been shown to act as an important chemotactic receptor for IL-16 [3]. In contrast to the CD4 monomer, the CD8 coreceptor exists as a CD8$\alpha\alpha$ homodimer or a CD8$\alpha\beta$ heterodimer; yet, in both cases, the Ig domains of the CD8 molecule bind MHC class I [4]. No substantial difference in MHC class I affinity binding has been observed between murine CD8$\alpha\alpha$ and CD8$\alpha\beta$ molecules [5], despite that the sequences for the CD8 homo- and heterodimer are shown to be <20% identical [4]. The intracellular regions of CD4 and CD8 interact with the tyrosine kinase, *lck* [6]. By inducing intracellular signaling through *lck*, the CD8 coreceptor is an important factor affecting TCR-mediated T cell activation and modulation of immune responses, both in terms of immunosuppression and cytotoxicity [7, 8].

TRANSCRIPTIONAL REGULATION OF CD4 AND CD8 EXPRESSION

During development, T cell progenitors migrate from the bone marrow into the bloodstream and subsequently enter the thymus. As they migrate to the subcapsular zone, they expand and pass through the DN stages of differentiation and begin to express TCR. As they pass back toward the medulla, they begin to express CD4 and CD8 [9]. At this stage, DP thymocytes have a limited lifespan of 3–4 days [10]. Appropriate TCR signaling is crucial for survival of the DP cells during this period and ultimately determines whether developing T cells are positively or negatively selected [11]. At the time of negative selection in the corticomedullary junction, thymocytes differentiate to $CD8^+$ or $CD4^+$ SP T cells after passing through a $CD4^+CD8^{lo}$ stage [12]. It is generally believed that coexpression of CD4 and CD8 is possible only during T cell development, and once past the negative selection checkpoint, T cells are committed to the CD4 or the CD8 lineage [13].

The tight regulation of CD4 and CD8 expression involves several transcription factors, particularly ThPOK and Runx3 (**Fig. 1**). ThPOK negatively regulates expression of *Runx3* and CD8 lineage genes, including CD8 and the cytotoxic effector genes perforin and granzyme B [14], and hence, directs commitment of MHC class II-reactive thymocytes toward the $CD4^+$ lineage [15]. ThPOK-mediated suppression of CD8α

Abbreviations: DN = double-negative, DP = double-positive, FasL = Fas ligand, GVHD = graft-versus-host-disease, LCMV = lymphocytic choriomeningitis virus, MAZR = Myc-associated zinc finger protein-related factor, NKT cell = NK T cell, Runx3 = runt-related transcription factor 3, SP = single-positive, ThPOK = Th inducing POZ-Kruppel factor

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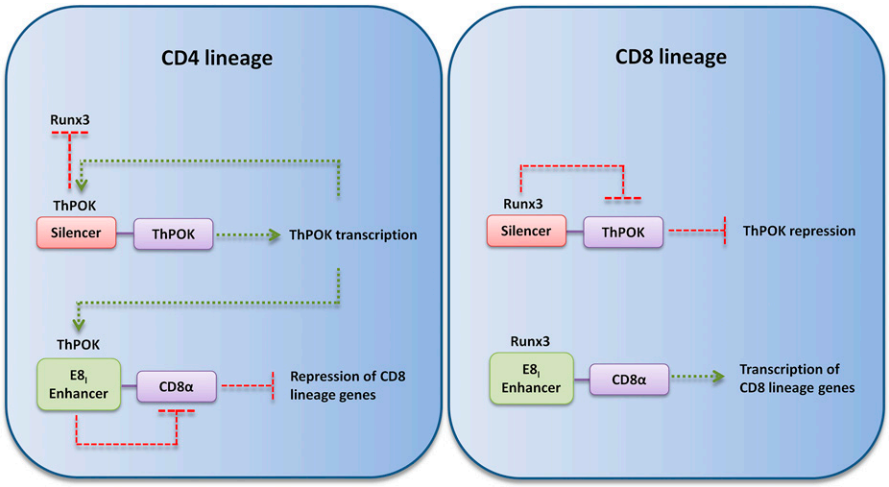

Figure 1. ThPOK and RunX3 tightly regulate CD4 and CD8 expression. The two transcription factors, ThPOK and Runx3, are of high importance during transition of DP thymocytes to mature SP T cells. (Left) ThPOK binds to its own silencer element located upstream, hence preventing Runx3-mediated suppression, resulting in a positive-feedback loop with continuous expression of *ThPOK*. ThPOK binds to the $E8_I$ enhancer element of the *Cd8*α gene locus and represses transcription of CD8 and CD8 lineage genes, resulting in a $CD4^+$ T cell fate. (Right) Alternatively, Runx3 binds to the *ThPOK* silencer element, hence preventing expression of *ThPOK*. Runx3 is then free to bind to the $E8_I$ enhancer element of the *Cd8*α gene locus and induces the transcription of CD8 lineage genes, resulting in a $CD8^+$ T cell fate.

expression appears to be a result of direct suppression of the $E8_I$ enhancer element located in the *Cd8a* gene locus [16]. The *ThPOK* locus, however, contains a silencing element to which Runx3 can bind, consequently allowing Runx3 to counter-inhibit the expression of *ThPOK* [12, 17]. In MHC class II-restricted thymocytes, however, ThPOK itself is capable of binding to its own silencer sequence, hence preventing Runx3-mediated suppression and resulting in the continuous expression of *ThPOK* [16].

ThPOK is crucial in determining the lineage commitment of CD4/CD8 DP thymocytes; in the absence of ThPOK, MHC class II-restricted thymocytes in ThPOK-deficient mice are redirected to the CD8 lineage [15], whereas constitutive expression of *ThPOK* redirects commitment of MHC class I-restricted thymocytes into the $CD4^+$ lineage [17]. Conversely, Runx3 is of crucial importance for full commitment to the $CD8^+$ lineage, as MHC class I-restricted thymocytes are redirected into the $CD4^+$ lineage in the absence of the *Runx3* gene [17].

Interestingly, thymocytes in ThPOK and Runx3 double-deficient mice can generate $CD4^+$ T cells, even in the absence of ThPOK, suggesting an additional, potential upstream mechanism for directing thymocytes into the CD4 lineage. Other transcription factors likely play a role in CD4 versus CD8 commitment, including the zinc-finger protein MAZR. MHC class I-restricted thymocytes are partially redirected to a CD4 fate following loss of MAZR, and ThPOK expression is not inhibited in $MAZR^{-/-}$ mice [18]. This redirection was nicely shown by crossing $MAZR^{-/-}$ mice with OT-I transgenic mice, resulting in $CD4^+$ T cells expressing the OT-I TCR-α chain specific for MHC class I [18]. The same study reported that MAZR plays a dual role in the decision between CD4 and CD8 commitment, providing genetic evidence that MAZR directly regulates *ThPOK* gene expression by binding the upstream silencer, while at the same time, confirming MAZR to be a negative regulator of the *Cd8* gene locus during transition from DN to DP stages. Thus, Runx3 and ThPOK are essential molecules involved in the decision between $CD4^+$ or $CD8^+$ lineage commitment of thymocytes, and MAZR is one of potentially several important upstream regulators of these two transcription factors [18].

The generation of $CD4^+$ and $CD8^+$ T cells from a common CD4/CD8 DP precursor pool and the subsequent migration of SP $CD4^+$ or $CD8^+$ T cells to peripheral tissues following negative selection have, for a long time, been largely the sole models used to explain the development of the $CD4^+$ and $CD8^+$ T cell lineages. Runx3 and ThPOK expression is believed to be mutually exclusive outside of the thymus, and consequently, coexpression of the CD4 and CD8 molecule in mature peripheral T cells would therefore not be expected [19]. As shown in **Table 1**, however, mature CD4/CD8 DP T cells in the periphery have been described in multiple disease settings in humans.

ORIGIN OF CD4/CD8 DP T CELLS IN THE PERIPHERY

Mature T cells, expressing CD4 and CD8 coreceptors, have been observed in the periphery, hence, bringing into question the mutually exclusive expression of ThPOK and Runx3. Interestingly, when $CD4/CD8\alpha^+$ DP T cells are generated in vitro by coculturing mature $CD4^+$ T cells with TGF-β, IL-7, and IFN-γ, Runx3 expression is increased, along with ThPOK being reduced [38], thus providing support for the idea that ThPOK and Runx3 expression may, in fact, be more plastic outside of the thymus in mature T cells than thought previously. In vivo, it was reported recently that mature $CD4^+$ intraepithelial lymphocytes in the intestine concurrently express ThPOK and Runx3 [39]. Mature $CD4^+$ T cells, isolated from the spleens of *Runx3*-yellow fluorescent protein and *Thpok*-GFP reporter mice and transferred into $Rag1^{-/-}$ immune-deficient mice, up-regulated Runx3 and *CD8αα* expression and subsequently, down-regulated ThPOK expression after migrating to the intestinal environment [39].

With the use of ThPOK-GFP reporter mice to examine expression, Mucida et al. [16] also showed that the fate of mature $CD4^+$ T cells is not necessarily fixed and that re-expression of *CD8α* occurs following down-regulation of ThPOK. For example, in the small intestine of ThPOK-GFP mice, $CD8^+$ T cells were found to be GFP^-, as expected; however, a prominent proportion of the $CD4^+$ T cells was also GFP^-, hence, indicating a lack of

TABLE 1. CD4/CD8 DP T cells have been described in multiple human disease settings

Disease condition	Isolation site	References
Infectious disease		
HIV	Blood	[20, 21]
Chronic hepatitis B	Blood, liver	[22]
Chronic hepatitis C	Blood, liver	[22]
CMV infection	Blood	[23, 24]
Lepromatous leprosy	Blood	[25]
Chagas disease	Blood	[26, 27]
Cancer		
Cutaneous T cell lymphoma	Cutaneous tumor	[28]
Melanoma	Tumors, lymph nodes	[29]
Nodular lymphocyte predominant Hodgkin lymphoma	Lymph nodes	[30]
Colorectal cancer	Colonic mucosa	[31]
Human T cell leukemia virus type 1-associated leukemia	Blood	[32]
Breast cancer	Tumors, pleural effusions	[33]
Autoimmune disease		
Atopic dermatitis (eczema)	Skin, blood	[34]
Rheumatoid arthritis	Blood, synovium	[35]
Inflammatory bowel disease	Blood, intestinal mucosa	[36]
Cutaneous systemic sclerosis	Skin	[1]
Other		
GVHD	Skin	[37]

CD4/CD8 DP T cells have been described in various diseases, including infectious disease, cancer, autoimmune disease, and GVHD.

ThPOK expression. This lack of GFP expression in $CD4^+$ T cells correlated with re-expression of the CD8 coreceptor, thus changing the phenotype of these cells to CD4/CD8 DP. Therefore, the authors support the hypothesis that CD4/CD8 DP T cells can originate from MHC class II-restricted $CD4^+$ T cells. However, in contrast to the previous study, which suggests that re-expression of CD8α is a result of Runx3 up-regulation and subsequent ThPOK down-regulation [39], Mucida et al. [16] suggest that the re-expression of the CD8 coreceptor is a result of the loss of ThPOK expression, with such down-regulation mediated by reactivation of the silencer upstream of the ThPOK gene locus. Taken together, these studies agree in suggesting that mature CD4/CD8 DP T cells in the intestine are derived from the $CD4^+$ lineage; however, it is important to keep in mind that both of these studies focused on the intestinal environment and that the origin of CD4/CD8 DP T cells may be influenced by tissue location. For example, whereas adoptive transfer of $CD4^+$ T cells into SCID mice results in CD4/CD8 DP T cells forming in the small intestine, no CD4/CD8 DP T cells are observed in the mesenteric lymph nodes [40].

Whereas several studies support mature $CD4^+$ T cells as the source of CD4/CD8 DP T cells [1, 41–44], evidence for CD8 as the lineage of origin for CD4/CD8 DP T cells also exists. For example, expression of CD4 on $CD8^+$ T cells occurs following in vitro activation of human PBMCs [45], subsequently rendering them susceptible to HIV infection [46, 47]. Expression of CD4 on $CD8^+$ T cells is thought to be associated mainly with activation of naive rather than memory $CD8^+$ T cells and may affect the distribution of $CD8^+$ T cells by acting as a chemotactic receptor for IL-16 [3]. Surface ligation of CD4 on $CD8^+$ T cells has also been linked to increased expression of IFN-γ and FasL, as well as to increased antiviral effector function [48, 49].

Whereas many of the pathways involved have yet to be fully elucidated, evidence suggests that the emergence of CD4/CD8 DP T cells can be attributed to transcriptional reprogramming of mature, SP T cells, following egress from the thymus [16]. Importantly, unlike CD4/CD8 DP thymocytes in the thymus, which display a limited lifespan of 3–4 days [10], peripheral CD4/CD8 DP T cells can express memory markers [50] and retain CD4/CD8 DP coexpression in culture for >1 year [28]. Given the evidence, neither $CD4^+$ T cells nor $CD8^+$ T cells can be excluded as a potential source of peripheral CD4/CD8 DP T cells. A high degree of heterogeneity exists within the CD4/CD8 DP T cell population, as described in more detail below, and it may be that different origins of CD4/CD8 DP T cell populations potentially explain some of the observed diversity in coreceptor expression levels and the presence or absence of CD8$\alpha\alpha$ homodimers versus -$\alpha\beta$ heterodimers.

CD4/CD8 DP T CELLS ARE PRESENT IN THE PERIPHERAL TISSUES OF A VARIETY OF SPECIES

Peripheral CD4/CD8 DP T cells were first described in humans by Blue et al. [41]; however, since then, extrathymic CD4/CD8 DP T cells have been reported in a variety of other species, including mice, rats, chickens, monkeys, as well as swine (**Table 2**). Across species, CD4/CD8 DP T cells do not appear to

have highly restricted tissue localization; rather, they appear to be present at multiple sites. The abundance of CD4/CD8 DP T cells also varies substantially between species; whereas rare in humans and mice compared with swine, chickens, and monkeys [62], the high percentage of CD4/CD8 DP T cells (~60%) in swine underlies their recognition as a major lymphocyte population in these animals. CD4/CD8 DP T cell numbers also vary depending on tissue distribution and other factors, such as health status and age; CD4/CD8 DP T cell numbers increase with age in humans [63] and in cynomolgus monkeys [64], and their increase has been shown to coincide with thymic involution [64]. Conversely, however, CD4/CD8 DP T cell numbers appear to decrease with age in rats [19, 55].

HETEROGENEITY OF CD8 HOMODIMER/HETERODIMER AND CD3 EXPRESSION ON CD4/CD8 DP T CELLS

Considerable differences in the expression level of the CD4, as well as the CD8, coreceptor, as defined by use of fluorescently conjugated antibodies, have been reported within CD4/CD8 DP T cell populations [50, 65, 66]. Differences in expression levels of CD4 and CD8 might reflect differences in the lineage of origin, and at least one study correlates low expression of CD4 in vitro to a $CD8^+$ T cell origin [67]. The components of the CD8 dimer expressed (i.e., $\alpha\alpha$ or $\alpha\beta$ chains) have been linked to the level of surface expression of CD8 on CD4/CD8 DP T cells. Tonutti et al. [65] divided human peripheral blood CD4/CD8 DP T cells into two groups, based on CD8 coreceptor expression. The $\alpha\alpha$ homodimer appeared to be linked to $CD8^{dim}$ cells, whereas the $\alpha\beta$ heterodimer was linked to the $CD8^{bright}$ phenotype [68]. A similar correlation has been described in rhesus macaques [42]. However, differences in lineage of origin, tissue location, function, and disease association, as well as species, may all influence the form of CD8 expressed. For example, in mice, rats, and chickens, CD4/CD8 DP T cells derived from the CD4 lineage have been reported to express the CD8$\alpha\alpha$ homodimer exclusively [38, 44, 52, 69]. In humans, within the CD4/CD8 DP T cell population, ~99% express the CD8$\alpha\beta$ heterodimer in human blood, indicating that they are thymically derived and not gut derived, as the latter principally expresses the CD8$\alpha\alpha$ homodimer [67].

Some CD4/CD8 DP populations have been described to lack expression of the CD3 coreceptor. For example, in a mouse model of *Chlamydia pneumoniae* infection, a population of mononuclear cells with the phenotype $CD3^-CD4^+CD8^+$ was reported to be expanded massively in the lungs of infected mice, eventually representing ~87% of all lymphocytes [70]. In addition to being $CD3^-$, this population was negative for the $\alpha\beta$ TCR [70]. The authors refer to the population as a "T cell-like lymphocyte population" as a result of the induction of cell division by Con A, a T cell mitogen. Notably, however, these cells did not produce IFN-γ. Despite major components of the TCR complex being absent, the lack of $\alpha\beta$ TCR expression may be explained by the down-regulation of the receptor in response to activation, which is a well-known phenomenon [49, 71]. On the other hand, CD4/CD8 DP cells, without expression of CD3 or TCR-$\alpha\beta$, could indicate the presence of a non-T cell population expressing both coreceptors, and CD4/CD8 NKT cells and macrophages have both been described [72–76].

FUNCTION OF PERIPHERAL CD4/CD8 DP T CELLS

With the acceptance of CD4/CD8 DP T cells as a distinct lymphocyte population, the question of functionality arises. However, little is currently known about their development or function [38]. This research area appears to be very controversial, and several conflicting papers exist. Overall, it seems that the function of CD4/CD8 DP T cells is very case specific; yet, even within similar case studies, no clear trends seem to be present. **Table 3** summarizes the reported functionalities of CD4/CD8 DP T cells in various conditions.

CYTOTOXIC CAPACITY OF CD4/CD8 DP T CELLS

The expression of CD4 on $CD8^+$ T cells has been implicated in the enhancement of cytotoxic responses during viral infections. In HIV patients, it has been shown that CD4/CD8 DP T cells are highly proliferative and active effector cells [21, 48]. Once

TABLE 2. CD4/CD8 DP T cells are found in a variety of tissues in different species

Species	Tissue	Frequency (%)	References
Human	Blood, liver, lymph nodes, skin, colonic mucosa	~3	[1, 22, 29–31, 34, 37, 51]
Mice	Small intestinal epithelium, spleen, lymph nodes	~1–10	[40, 52–54]
Rats	Peripheral lymphoid organs	~3–6	[19, 55]
Chickens	Blood, spleen, intestinal epithelium	~5–40	[44, 56]
Monkeys	Blood, lymph nodes	~5–20	[57, 58]
Swine	Blood, tonsils, Peyer's patch	~60	[2, 59–61]

Peripheral T cells expressing both CD4 and CD8 coreceptors have been observed in different species, including humans, mice, rats, chickens, monkeys, and swine. They have been found mainly circulating in the bloodstream or in the secondary lymphoid organs, such as spleen and lymph nodes. Percentages are representative of the total T cell pool.

TABLE 3. CD4/CD8 DP T cells can be suppressive or cytotoxic, depending on conditions

Condition	Functionality	References
LCMV	Cytotoxic	[49]
HIV	Cytotoxic	[48]
Cancer	Cytotoxic/suppressive	[28, 29, 31]
Systemic sclerosis	Suppressive	[1]
Inflammatory bowel disease	Suppressive	[53]
Epicutaneous immunization	Suppressive	[77]

Studies have reported the cytotoxic potential of CD4/CD8 DP T cells in various diseases, including LCMV, HIV, and cancer. On the other hand, it has also been reported that this cell population has a more regulatory role by exhibiting a suppressive phenotype in cancer, systemic sclerosis, inflammatory bowel disease, as well as in an epicutaneous immunization setting.

CD4 on the surface of $CD8^+$ T cells becomes ligated, signaling through *lck* results in higher expression of FasL and IFN-γ. Therefore, the CD4 coreceptor itself enhances expression of two highly important effector molecules of the $CD8^+$ T cell lineage [48]. A cytotoxic phenotype with elevated levels of effector molecule expression has also been reported in an in vivo study, in which CD4/CD8 DP T cells in the small intestine of mice expressed the cytotoxicity-related, MHC class I-restricted, T cell-associated molecule (CRTAM), CD244, granzyme B, perforin, and IFN-γ [16]. Moreover, these CD4/CD8 DP T cells exhibited cytotoxic capacity in vitro [16].

In addition to the up-regulation of effector molecules following ligation, CD4 expressed on $CD8^+$ T cells has been reported to function as a receptor for IL-16, hence directing migration of $CD8^+$ T cells in a chemotactic manner [3]. By allowing migration in response to IL-16 gradients, CD4 influences the distribution of $CD8^+$ T cells and directs them to the site of viral infection.

Zloza et al. [67] investigated the functionality of human CD4/CD8 DP T cells derived from $CD8^+$ T cells in vitro and reported elevated levels of IL-4 but not IFN-γ, IL-2, or IL-10 compared with $CD4^+$ T cells or $CD8^+$ T cells. In response to CMV peptide (pp65) priming, CD4/CD8 DP cells recognized the CMV pp65 tetramer, ~19-fold higher than $CD8^+$ T cells. As a result of the highly activated phenotype exhibited by these CD4/CD8 DP T cells, they could potentially be prone to apoptosis as a result of overactivation. However, a TUNEL assay revealed similar levels of TUNEL expression in CD4/CD8 DP T cells compared with $CD8^+$ T cells, indicating that CD4/CD8 DP T cells, despite being highly activated, had a similar apoptotic level to $CD8^+$ T cells [67].

In addition to the in vitro generation of CD4/CD8 DP T cells described above, coexpression of CD4 and CD8 appears to be important for CTL activity in vivo. Kitchen et al. [49] showed that $CD8^+$ T cells from CD4-knockout mice, transferred into SCID mice, displayed decreased alloreactivity or immune responsiveness to infection by LCMV compared with $CD8^+$ T cells transferred from wild-type mice, suggesting that CD4 expression on $CD8^+$ T cells plays a critical role in modulating cytotoxic T cell function in vivo.

SUPPRESSIVE POTENTIAL OF CD4/CD8 DP T CELLS

In a mouse model of inflammatory bowel disease, Das et al. [53] described CD4/CD8 DP T cells migrating into the intraepithelial layer of the intestine to play a suppressive role. The underlying mechanism of this regulatory phenotype was IL-10 dependent, although the cells also expressed IFN-γ but not IL-4. The ability of CD4/CD8 DP T cells to produce IL-10 and IFN-γ but not IL-4 has been confirmed by another group, who likewise showed that mouse splenic CD4/CD8 DP T cells can exhibit a suppressive phenotype [38] and reduce T cell proliferation when cocultured with $CD4^+$ T cells. In contrast, a study focusing on human skin biopsies from a GVHD patient reported the ability of CD4/CD8 DP T cells to produce IL-4 [37]; however, a possible regulatory function for these cells was upheld, based on their cytokine-secretion profile, which included IL-10, IFN-γ, and TGF-β. CD4/CD8 DP T cells added to a MLR decreased total $CD3^+$ T cell proliferation in a dose-dependent manner, further supporting a suppressive role for CD4/CD8 DP T cells [37].

ROLE OF CD4/CD8 DP T CELLS IN CANCER

As evidenced by the studies described above, there does not appear to be a consistent trend that can explain CD4/CD8 DP T cell function; thus, it seems likely that CD4/CD8 DP T cells are heavily influenced by as-yet-unidentified mechanisms and/or local environmental conditions. CD4/CD8 DP T cells are believed to play opposing roles in the immune response to various cancers, as described in **Table 4**. In the cancer setting, the function of these T cells appears to be unclear, perhaps as a consequence of the diverse microenvironment found between different tumor types.

In an early report, Bagot et al. [28] isolated lymphocytes from the tumor of a patient with cutaneous T cell lymphoma and described a clone with a $CD4^+CD8^{+dim}$ phenotype. This T cell clone was MHC class I restricted and cytolytic toward autologous tumor cells in vitro in a highly antigen-specific manner. In addition to the CD4/CD8 DP T cell clone, a $CD4^+$ T cell clone was isolated. In contrast to the $CD4^+$ T cell clone, which was found to be present in the blood and the tumor site, the CD4/CD8 DP T cell clone was found within the tumor site only. Interestingly, blockade of the CD4 or the CD8 coreceptor did not decrease cytotoxicity, suggesting that in this case, these two coreceptors might not be important for effector activity itself [28].

CD4/CD8 DP T cells have been described to express different cytokine biases in different cancer settings, as shown in Table 4. In a study of human breast cancer patients, pleural

TABLE 4. Cytokine profile of CD4/CD8 DP T cells in various cancer subtypes

Cancer type	Cytokine profile	References
Breast cancer	IL-2, IL-4, IL-5, IL-13	[33]
Melanoma	IL-2, IL-4, IL-5, IL-13, GM-CSF	[29]
Colorectal cancer	IL-4, IL-13	[31]

CD4/CD8 DP T cell cytokine profiles have been described in various cancer subtypes, including breast cancer, melanoma, and colorectal cancer. The cytokine profile of these cells appears to be biased toward secretion of IL-2, IL-4, IL-5, IL-13, and GM-CSF. Cytokine profiles are based on an elevated secretion level compared with conventional SP T cells.

effusion of tumors was found to contain high numbers of $CD4^{dim}CD8^{bright}$ DP T cells, expressing the $\alpha\beta$ TCR and the $\alpha\beta$ CD8 heterodimer [33]. The phenotype and cytotoxic potential of these cells was very similar to that of effector and memory $CD8^+$ T cells, as determined by positive staining for granzyme and perforin. However, and as indicated in Table 4, CD4/CD8 DP T cells, in contrast to conventional $CD8^+$ T cells, were associated with high production of IL-5 and IL-13. In addition to IL-5 and IL-13, the percentage of CD4/CD8 DP T cells secreting IL-2 and IL-4 was elevated compared with conventional $CD4^+$ and $CD8^+$ T cells [33]. In a study of melanoma conducted by the same group, a significant increase in the frequency of CD4/CD8 DP T cells was observed within tumors compared with blood [29]. Once again, CD4/CD8 DP T cells were $CD4^{dim}CD8^{bright}$ and $\alpha\beta$ TCR positive, with a cytokine production profile comprising IL-2, IL-4, IL-5, IL-13, and GM-CSF (Table 4). CD4/CD8 DP T cells also secreted elevated levels of TNF-α in response to an autologous melanoma cell line compared with $CD8^+$ T cells. The recognition of tumor cells was MHC class I restricted, and these CD4/CD8 DP T cells, with their unique cytokine profile, appeared to have anti-tumor activity in vitro [29].

On the other hand, CD4/CD8 DP T cells have been described to play a suppressive role in cancers, such as metastatic colorectal cancer [31], in which the percentage of CD4/CD8 DP T cells in the tumor-infiltrating lymphocyte pool was found to be elevated relative to SP $CD4^+$ T cells or $CD8^+$ T cells. Tumor-reactive CD4/CD8 DP T cells exhibited poor in vitro proliferation capacity and upon CD3 or cognate antigen stimulation, secreted higher levels of IL-4 and IL-13 than $CD4^+$ and $CD8^+$ T cells. Moreover, CD4/CD8 DP T cells were associated with elevated TNF-α production, and their increased frequency within metastatic colorectal cancer led the authors to suggest that these cells may favor tumor growth or metastasis and/or down-modulate immune responses to colorectal cancer [31].

Two cytokines commonly reported to be secreted by CD4/CD8 DP T cells in various cancer environments are IL-5 and IL-13. IL-5 is characteristic of Th2 responses and is usually associated with growth, maturation, and function of important immune cell types, such as eosinophils and B cells. However, with the use of an IL-5 knockout mouse model, Apostolopoulos et al. [78] also showed IL-5 to be an important cytokine for the generation of optimal tumor antigen-specific cytotoxic T cell responses. Likewise, IL-13 appears to have an important regulatory role in cancer and has been linked to the suppression of anti-tumor activities. However, IL-13 has also been reported to play a role in the immune escape of tumors in breast cancer patients [79], and one mechanism by which IL-13 may block immunosurveillance is by inducing differentiation, as well as activation of important regulatory cell populations, such as suppressive macrophages and myeloid suppressor cells [80]. NKT cells have also been reported to secrete IL-13, which has been reported to be necessary for the down-regulation of anti-tumor immune responses [81] and the enhancement of tumor growth [82].

CONCLUDING REMARKS

T cells, expressing an $\alpha\beta$ TCR, are critical adaptive immune cells, commonly divided into two subsets, based on the expression of the CD4 and CD8 coreceptors; i.e., they are classified as $CD4^+$ or $CD8^+$ T cells. During development in the thymus, thymocytes transiently express the CD4 and CD8 coreceptor on their cell surface; however, as a result of a very strict transcriptional regulation program, mediated especially by the transcription factors ThPOK and Runx3, mature peripheral T cells are believed to express only CD4 or CD8 but not both. In spite of this, CD4/CD8 DP T cells have been reported in peripheral sites of various species, including humans, mice, rats, chickens, monkeys, and swine. Initially, these cells were suggested to be thymocytes, which had prematurely escaped the thymus. More recently, however, studies have shown that CD4/CD8 DP T cells in peripheral sites express T cell maturation markers and lack thymic-stage markers, indicating that CD4/CD8 DP T cells exist as a mature population in the periphery, alongside conventional $CD4^+$ and $CD8^+$ T cells. Although associated with a multitude of human diseases and disorders, the function of CD4/CD8 DP T cells, however, remains poorly described and controversial. Taken together, CD4/CD8 DP T cells appear to play an important role at peripheral sites as potent immune suppressors or as cells with high cytotoxic potential, and there is a pressing need for a deeper examination of this population to fully elucidate their contribution to the immune response.

AUTHORSHIP

N.H.O. and J.W.W. contributed to the writing, editing, and literature search for the review. J-W.J. and R.J.S. contributed to the writing and editing of the review.

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DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

1. Parel, Y., Aurrand-Lions, M., Scheja, A., Dayer, J. M., Roosnek, E., Chizzolini, C. (2007) Presence of CD4+CD8+ double-positive T cells with very high interleukin-4 production potential in lesional skin of patients with systemic sclerosis. *Arthritis Rheum.* **56,** 3459–3467.
2. Maddon, P. J., Littman, D. R., Godfrey, M., Maddon, D. E., Chess, L., Axel, R. (1985) The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. *Cell* **42,** 93–104.
3. Kitchen, S. G., LaForge, S., Patel, V. P., Kitchen, C. M., Miceli, M. C., Zack, J. A. (2002) Activation of CD8 T cells induces expression of CD4, which functions as a chemotactic receptor. *Blood* **99,** 207–212.
4. Chang, H. C., Tan, K., Ouyang, J., Parisini, E., Liu, J. H., Le, Y., Wang, X., Reinherz, E. L., Wang, J. H. (2005) Structural and mutational analyses of a CD8alphabeta heterodimer and comparison with the CD8alphaalpha homodimer. *Immunity* **23,** 661–671.
5. Kern, P., Hussey, R. E., Spoerl, R., Reinherz, E. L., Chang, H. C. (1999) Expression, purification, and functional analysis of murine ectodomain fragments of CD8alphaalpha and CD8alphabeta dimers. *J. Biol. Chem.* **274,** 27237–27243.
6. Turner, J. M., Brodsky, M. H., Irving, B. A., Levin, S. D., Perlmutter, R. M., Littman, D. R. (1990) Interaction of the unique N-terminal region of tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. *Cell* **60,** 755–765.
7. Pecht, I., Gakamsky, D. M. (2005) Spatial coordination of CD8 and TCR molecules controls antigen recognition by CD8+ T-cells. *FEBS Lett.* **579,** 3336–3341.
8. Kaplan, D. R., Hambor, J. E., Tykocinski, M. L. (1989) An immunoregulatory function for the CD8 molecule. *Proc. Natl. Acad. Sci. USA* **86,** 8512–8515.
9. Germain, R. N. (2002) T-cell development and the CD4-CD8 lineage decision. *Nat. Rev. Immunol.* **2,** 309–322.
10. Starr, T. K., Jameson, S. C., Hogquist, K. A. (2003) Positive and negative selection of T cells. *Annu. Rev. Immunol.* **21,** 139–176.
11. Ioannidis, V., Beermann, F., Clevers, H., Held, W. (2001) The beta-catenin—TCF-1 pathway ensures CD4(+)CD8(+) thymocyte survival. *Nat. Immunol.* **2,** 691–697.
12. He, X., Park, K., Kappes, D. J. (2010) The role of ThPOK in control of CD4/CD8 lineage commitment. *Annu. Rev. Immunol.* **28,** 295–320.
13. Koch, U., Radtke, F. (2011) Mechanisms of T cell development and transformation. *Annu. Rev. Cell Dev. Biol.* **27,** 539–562.
14. Wang, L., Wildt, K. F., Castro, E., Xiong, Y., Feigenbaum, L., Tessarollo, L., Bosselut, R. (2008) The zinc finger transcription factor Zbtb7b represses CD8-lineage gene expression in peripheral CD4+ T cells. *Immunity* **29,** 876–887.
15. Egawa, T., Littman, D. R. (2008) ThPOK acts late in specification of the helper T cell lineage and suppresses Runx-mediated commitment to the cytotoxic T cell lineage. *Nat. Immunol.* **9,** 1131–1139.
16. Mucida, D., Husain, M. M., Muroi, S., van Wijk, F., Shinnakasu, R., Naoe, Y., Reis, B. S., Huang, Y., Lambolez, F., Docherty, M., Attinger, A., Shui, J. W., Kim, G., Lena, C. J., Sakaguchi, S., Miyamoto, C., Wang, P., Atarashi, K., Park, Y., Nakayama, T., Honda, K., Ellmeier, W., Kronenberg, M., Taniuchi, I., Cheroutre, H. (2013) Transcriptional reprogramming of mature CD4$^+$ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. *Nat. Immunol.* **14,** 281–289.
17. Setoguchi, R., Tachibana, M., Naoe, Y., Muroi, S., Akiyama, K., Tezuka, C., Okuda, T., Taniuchi, I. (2008) Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development. *Science* **319,** 822–825.
18. Sakaguchi, S., Hombauer, M., Bilic, I., Naoe, Y., Schebesta, A., Taniuchi, I., Ellmeier, W. (2010) The zinc-finger protein MAZR is part of the transcription factor network that controls the CD4 versus CD8 lineage fate of double-positive thymocytes. *Nat. Immunol.* **11,** 442–448.
19. Jiménez, E., Sacedón, R., Vicente, A., Hernández-López, C., Zapata, A. G., Varas, A. (2002) Rat peripheral CD4+CD8+ T lymphocytes are partially immunocompetent thymus-derived cells that undergo post-thymic maturation to become functionally mature CD4+ T lymphocytes. *J. Immunol.* **168,** 5005–5013.
20. Weiss, L., Roux, A., Garcia, S., Demouchy, C., Haeffner-Cavaillon, N., Kazatchkine, M. D., Gougeon, M. L. (1998) Persistent expansion, in a human immunodeficiency virus-infected person, of V beta-restricted CD4+CD8+ T lymphocytes that express cytotoxicity-associated molecules and are committed to produce interferon-gamma and tumor necrosis factor-alpha. *J. Infect. Dis.* **178,** 1158–1162.
21. Frahm, M. A., Picking, R. A., Kuruc, J. D., McGee, K. S., Gay, C. L., Eron, J. J., Hicks, C. B., Tomaras, G. D., Ferrari, G. (2012) CD4+CD8+ T cells represent a significant portion of the anti-HIV T cell response to acute HIV infection. *J. Immunol.* **188,** 4289–4296.
22. Nascimbeni, M., Pol, S., Saunier, B. (2011) Distinct CD4+ CD8+ double-positive T cells in the blood and liver of patients during chronic hepatitis B and C. *PLoS ONE* **6,** e20145.
23. Suni, M. A., Ghanekar, S. A., Houck, D. W., Maecker, H. T., Wormsley, S. B., Picker, L. J., Moss, R. B., Maino, V. C. (2001) CD4(+)CD8(dim) T lymphocytes exhibit enhanced cytokine expression, proliferation and cytotoxic activity in response to HCMV and HIV-1 antigens. *Eur. J. Immunol.* **31,** 2512–2520.
24. Rentenaar, R. J., Wever, P. C., van Diepen, F. N., Schellekens, P. T., Wertheim, P. M., ten Berge, I. J. (1999) CD4dullCD8bright double-positive T-lymphocytes have a phenotype of granzyme Bpos CD8pos memory T-lymphocytes. *Nephrol. Dial. Transplant.* **14,** 1430–1434.
25. Ottenhoff, T. H., Elferink, D. G., Klatser, P. R., de Vries, R. R. (1986) Cloned suppressor T cells from a lepromatous leprosy patient suppress Mycobacterium leprae reactive helper T cells. *Nature* **322,** 462–464.
26. Pérez, A. R., Morrot, A., Berbert, L. R., Terra-Granado, E., Savino, W. (2012) Extrathymic CD4+CD8+ lymphocytes in Chagas disease: possible relationship with an immunoendocrine imbalance. *Ann. N. Y. Acad. Sci.* **1262,** 27–36.
27. Giraldo, N. A., Bolaños, N. I., Cuellar, A., Guzman, F., Uribe, A. M., Bedoya, A., Olaya, N., Cucunubá, Z. M., Roa, N., Rosas, F., Velasco, V., Puerta, C. J., González, J. M. (2011) Increased CD4+/CD8+ double-positive T cells in chronic Chagasic patients. *PLoS Negl. Trop. Dis.* **5,** e1294.
28. Bagot, M., Echchakir, H., Mami-Chouaib, F., Delfau-Larue, M. H., Charue, D., Bernheim, A., Chouaib, S., Boumsell, L., Bensussan, A. (1998) Isolation of tumor-specific cytotoxic CD4+ and CD4+CD8dim+ T-cell clones infiltrating a cutaneous T-cell lymphoma. *Blood* **91,** 4331–4341.
29. Desfrançois, J., Moreau-Aubry, A., Vignard, V., Godet, Y., Khammari, A., Dréno, B., Jotereau, F., Gervois, N. (2010) Double positive CD4CD8 alphabeta T cells: a new tumor-reactive population in human melanomas. *PLoS ONE* **5,** e8437.
30. Rahemtullah, A., Reichard, K. K., Preffer, F. I., Harris, N. L., Hasserjian, R. P. (2006) A double-positive CD4+CD8+ T-cell population is commonly found in nodular lymphocyte predominant Hodgkin lymphoma. *Am. J. Clin. Pathol.* **126,** 805–814.
31. Sarrabayrouse, G., Corvaisier, M., Ouisse, L. H., Bossard, C., Le Mével, B., Potiron, L., Meurette, G., Gervois, N., Jotereau, F. (2011) Tumor-reactive CD4+ CD8αβ+ CD103+ αβT cells: a prevalent tumor-reactive T-cell subset in metastatic colorectal cancers. *Int. J. Cancer* **128,** 2923–2932.
32. Macchi, B., Graziani, G., Zhang, J., Mastino, A. (1993) Emergence of double-positive CD4/CD8 cells from adult peripheral blood mononuclear cells infected with human T cell leukemia virus type I (HTLV-I). *Cell. Immunol.* **149,** 376–389.
33. Desfrançois, J., Derré, L., Corvaisier, M., Le Mével, B., Catros, V., Jotereau, F., Gervois, N. (2009) Increased frequency of nonconventional double positive CD4CD8 alphabeta T cells in human breast pleural effusions. *Int. J. Cancer* **125,** 374–380.
34. Bang, K., Lund, M., Wu, K., Mogensen, S. C., Thestrup-Pedersen, K. (2001) CD4+ CD8+ (thymocyte-like) T lymphocytes present in blood and skin from patients with atopic dermatitis suggest immune dysregulation. *Br. J. Dermatol.* **144,** 1140–1147.
35. Quandt, D., Rothe, K., Scholz, R., Baerwald, C. W., Wagner, U. (2014) Peripheral CD4CD8 double positive T cells with a distinct helper cytokine profile are increased in rheumatoid arthritis. *PLoS ONE* **9,** e93293.
36. Sarrabayrouse, G., Bossard, C., Chauvin, J. M., Jarry, A., Meurette, G., Quévrain, E., Bridonneau, C., Preisser, L., Asehnoune, K., Labarrière, N., Altare, F., Sokol, H., Jotereau, F. (2014) CD4CD8αα lymphocytes, a novel human regulatory T cell subset induced by colonic bacteria and deficient in patients with inflammatory bowel disease. *PLoS Biol.* **12**, e1001833.
37. Eljaafari, A., Yuruker, O., Ferrand, C., Farre, A., Addey, C., Tartelin, M. L., Thomas, X., Tiberghien, P., Simpson, E., Rigal, D., Scott, D. (2013) Isolation of human CD4/CD8 double-positive, graft-versus-host disease-protective, minor histocompatibility antigen-specific regulatory T cells and of a novel HLA-DR7-restricted HY-specific CD4 clone. *J. Immunol.* **190,** 184–194.
38. Van Kaer, L., Rabacal, W. A., Scott Algood, H. M., Parekh, V. V., Olivares-Villagómez, D. (2013) In vitro induction of regulatory CD4+CD8α+ T cells by TGF-β, IL-7 and IFN-γ. *PLoS ONE* **8,** e67821.
39. Reis, B. S., Rogoz, A., Costa-Pinto, F. A., Taniuchi, I., Mucida, D. (2013) Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal CD4$^+$ T cell immunity. *Nat. Immunol.* **14,** 271–280.
40. Reimann, J., Rudolphi, A. (1995) Co-expression of CD8 alpha in CD4+ T cell receptor alpha beta + T cells migrating into the murine small intestine epithelial layer. *Eur. J. Immunol.* **25,** 1580–1588.
41. Blue, M. L., Daley, J. F., Levine, H., Craig, K. A., Schlossman, S. F. (1986) Biosynthesis and surface expression of T8 by peripheral blood T4+ cells in vitro. *J. Immunol.* **137,** 1202–1207.
42. Macchia, I., Gauduin, M. C., Kaur, A., Johnson, R. P. (2006) Expression of CD8alpha identifies a distinct subset of effector memory CD4+ T lymphocytes. *Immunology* **119,** 232–242.
43. Molteni, M., Kohn, L. D., Rossetti, C., Scrofani, S., Bonara, P., Scorza, R. (2002) Co-expression of the CD8 receptor in a human CD4+ T-cell clone influences proliferation, cytosolic Ca2+ release and cytokine production. *Immunol. Lett.* **83,** 111–117.
44. Luhtala, M., Lassila, O., Toivanen, P., Vainio, O. (1997) A novel peripheral CD4+ CD8+ T cell population: inheritance of CD8alpha expression on CD4+ T cells. *Eur. J. Immunol.* **27,** 189–193.

45. Sullivan, Y. B., Landay, A. L., Zack, J. A., Kitchen, S. G., Al-Harthi, L. (2001) Upregulation of CD4 on CD8+ T cells: CD4dimCD8bright T cells constitute an activated phenotype of CD8+ T cells. *Immunology* **103,** 270–280.
46. Kitchen, S. G., Korin, Y. D., Roth, M. D., Landay, A., Zack, J. A. (1998) Costimulation of naive CD8(+) lymphocytes induces CD4 expression and allows human immunodeficiency virus type 1 infection. *J. Virol.* **72,** 9054–9060.
47. Flamand, L., Crowley, R. W., Lusso, P., Colombini-Hatch, S., Margolis, D. M., Gallo, R. C. (1998) Activation of CD8+ T lymphocytes through the T cell receptor turns on CD4 gene expression: implications for HIV pathogenesis. *Proc. Natl. Acad. Sci. USA* **95,** 3111–3116.
48. Kitchen, S. G., Jones, N. R., LaForge, S., Whitmire, J. K., Vu, B. A., Galic, Z., Brooks, D. G., Brown, S. J., Kitchen, C. M., Zack, J. A. (2004) CD4 on CD8(+) T cells directly enhances effector function and is a target for HIV infection. *Proc. Natl. Acad. Sci. USA* **101,** 8727–8732.
49. Kitchen, S. G., Whitmire, J. K., Jones, N. R., Galic, Z., Kitchen, C. M., Ahmed, R., Zack, J. A. (2005) The CD4 molecule on CD8+ T lymphocytes directly enhances the immune response to viral and cellular antigens. *Proc. Natl. Acad. Sci. USA* **102,** 3794–3799.
50. Nascimbeni, M., Shin, E. C., Chiriboga, L., Kleiner, D. E., Rehermann, B. (2004) Peripheral CD4(+)CD8(+) T cells are differentiated effector memory cells with antiviral functions. *Blood* **104,** 478–486.
51. Blue, M. L., Daley, J. F., Levine, H., Schlossman, S. F. (1985) Coexpression of T4 and T8 on peripheral blood T cells demonstrated by two-color fluorescence flow cytometry. *J. Immunol.* **134,** 2281–2286.
52. Morrissey, P. J., Charrier, K., Horovitz, D. A., Fletcher, F. A., Watson, J. D. (1995) Analysis of the intra-epithelial lymphocyte compartment in SCID mice that received co-isogenic CD4+ T cells. Evidence that mature post-thymic CD4+ T cells can be induced to express CD8 alpha in vivo. *J. Immunol.* **154,** 2678–2686.
53. Das, G., Augustine, M. M., Das, J., Bottomly, K., Ray, P., Ray, A. (2003) An important regulatory role for CD4+CD8 alpha alpha T cells in the intestinal epithelial layer in the prevention of inflammatory bowel disease. *Proc. Natl. Acad. Sci. USA* **100,** 5324–5329.
54. Kelly, K., Pilarski, L., Shortman, K., Scollay, R. (1988) CD4+ CD8+ cells are rare among in vitro activated mouse or human T lymphocytes. *Cell. Immunol.* **117,** 414–424.
55. Kenny, E., Mason, D., Pombo, A., Ramírez, F. (2000) Phenotypic analysis of peripheral CD4+ CD8+ T cells in the rat. *Immunology* **101,** 178–184.
56. Erf, G. F., Bottje, W. G., Bersi, T. K. (1998) CD4, CD8 and TCR defined T-cell subsets in thymus and spleen of 2- and 7-week old commercial broiler chickens. *Vet. Immunol. Immunopathol.* **62,** 339–348.
57. Dean, G. A., Reubel, G. H., Pedersen, N. C. (1996) Simian immunodeficiency virus infection of CD8+ lymphocytes in vivo. *J. Virol.* **70,** 5646–5650.
58. Akari, H., Terao, K., Murayama, Y., Nam, K. H., Yoshikawa, Y. (1997) Peripheral blood CD4+CD8+ lymphocytes in cynomolgus monkeys are of resting memory T lineage. *Int. Immunol.* **9,** 591–597.
59. Saalmüller, A., Reddehase, M. J., Bühring, H. J., Jonjić, S., Koszinowski, U. H. (1987) Simultaneous expression of CD4 and CD8 antigens by a substantial proportion of resting porcine T lymphocytes. *Eur. J. Immunol.* **17,** 1297–1301.
60. Zuckermann, F. A., Gaskins, H. R. (1996) Distribution of porcine CD4/CD8 double-positive T lymphocytes in mucosa-associated lymphoid tissues. *Immunology* **87,** 493–499.
61. Zuckermann, F. A., Husmann, R. J. (1996) Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. *Immunology* **87,** 500–512.
62. Zuckermann, F. A. (1999) Extrathymic CD4/CD8 double positive T cells. *Vet. Immunol. Immunopathol.* **72,** 55–66.
63. Laux, I., Khoshnan, A., Tindell, C., Bae, D., Zhu, X., June, C. H., Effros, R. B., Nel, A. (2000) Response differences between human CD4(+) and CD8(+) T-cells during CD28 costimulation: implications for immune cell-based therapies and studies related to the expansion of double-positive T-cells during aging. *Clin. Immunol.* **96,** 187–197.
64. Lee, W. W., Nam, K. H., Terao, K., Akari, H., Yoshikawa, Y. (2003) Age-related increase of peripheral CD4+ CD8+ double-positive T lymphocytes in cynomolgus monkeys: longitudinal study in relation to thymic involution. *Immunology* **109,** 217–225.
65. Tonutti, E., Sala, P., Feruglio, C., Yin, Z., Colombatti, A. (1994) Phenotypic heterogeneity of persistent expansions of CD4+ CD8+ T cells. *Clin. Immunol. Immunopathol.* **73,** 312–320.
66. Parel, Y., Chizzolini, C. (2004) CD4+ CD8+ double positive (DP) T cells in health and disease. *Autoimmun. Rev.* **3,** 215–220.
67. Zloza, A., Sullivan, Y. B., Connick, E., Landay, A. L., Al-Harthi, L. (2003) CD8+ T cells that express CD4 on their surface (CD4dimCD8bright T cells) recognize an antigen-specific target, are detected in vivo, and can be productively infected by T-tropic HIV. *Blood* **102,** 2156–2164.
68. Zloza, A., Al-Harthi, L. (2006) Multiple populations of T lymphocytes are distinguished by the level of CD4 and CD8 coexpression and require individual consideration. *J. Leukoc. Biol.* **79,** 4–6.
69. Kenny, E., Mason, D., Saoudi, A., Pombo, A., Ramírez, F. (2004) CD8 alpha is an activation marker for a subset of peripheral CD4 T cells. *Eur. J. Immunol.* **34,** 1262–1271.
70. Penttilä, J. M., Pyhälä, R., Sarvas, M., Rautonen, N. (1998) Expansion of a novel pulmonary CD3(–) CD4(+) CD8(+) cell population in mice during Chlamydia pneumoniae infection. *Infect. Immun.* **66,** 3290–3294.
71. Boyer, C., Auphan, N., Luton, F., Malburet, J. M., Barad, M., Bizozzero, J. P., Reggio, H., Schmitt-Verhulst, A. M. (1991) T cell receptor/CD3 complex internalization following activation of a cytolytic T cell clone: evidence for a protein kinase C-independent staurosporine-sensitive step. *Eur. J. Immunol.* **21,** 1623–1634.
72. Motsinger, A., Haas, D. W., Stanic, A. K., Van Kaer, L., Joyce, S., Unutmaz, D. (2002) CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. *J. Exp. Med.* **195,** 869–879.
73. Benlagha, K., Weiss, A., Beavis, A., Teyton, L., Bendelac, A. (2000) In vivo identification of glycolipid antigen-specific T cells using fluorescent CD1d tetramers. *J. Exp. Med.* **191,** 1895–1903.
74. Lee, H., Hong, C., Shin, J., Oh, S., Jung, S., Park, Y. K., Hong, S., Lee, G. R., Park, S. H. (2009) The presence of CD8+ invariant NKT cells in mice. *Exp. Mol. Med.* **41,** 866–872.
75. Baba, T., Ishizu, A., Iwasaki, S., Suzuki, A., Tomaru, U., Ikeda, H., Yoshiki, T., Kasahara, M. (2006) CD4+/CD8+ macrophages infiltrating at inflammatory sites: a population of monocytes/macrophages with a cytotoxic phenotype. *Blood* **107,** 2004–2012.
76. Fernandez, C. S., Chan, A. C., Kyparissoudis, K., De Rose, R., Godfrey, D. I., Kent, S. J. (2009) Peripheral NKT cells in simian immunodeficiency virus-infected macaques. *J. Virol.* **83,** 1617–1624.
77. Szczepanik, M., Bryniarski, K., Tutaj, M., Ptak, M., Skrzeczynska, J., Askenase, P. W., Ptak, W. (2005) Epicutaneous immunization induces alphabeta T-cell receptor CD4 CD8 double-positive non-specific suppressor T cells that inhibit contact sensitivity via transforming growth factor-beta. *Immunology* **115,** 42–54.
78. Apostolopoulos, V., McKenzie, I. F., Lees, C., Matthaei, K. I., Young, I. G. (2000) A role for IL-5 in the induction of cytotoxic T lymphocytes in vivo. *Eur. J. Immunol.* **30,** 1733–1739.
79. Aspord, C., Pedroza-Gonzalez, A., Gallegos, M., Tindle, S., Burton, E. C., Su, D., Marches, F., Banchereau, J., Palucka, A. K. (2007) Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. *J. Exp. Med.* **204,** 1037–1047.
80. Sinha, P., Clements, V. K., Ostrand-Rosenberg, S. (2005) Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. *Cancer Res.* **65,** 11743–11751.
81. Terabe, M., Matsui, S., Noben-Trauth, N., Chen, H., Watson, C., Donaldson, D. D., Carbone, D. P., Paul, W. E., Berzofsky, J. A. (2000) NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat. Immunol.* **1,** 515–520.
82. Terabe, M., Park, J. M., Berzofsky, J. A. (2004) Role of IL-13 in regulation of anti-tumor immunity and tumor growth. *Cancer Immunol. Immunother.* **53,** 79–85.

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