

Mini Review

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Effector and Regulatory CD4⁺ T helper lineages in cancer

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ABSTRACT

Regulatory CD4⁺T-cells have been recently classified as regulatory T helper (Th)-like cells according to the expression of specific transcription factors, cytokines and chemokine receptors that mirror effector Th lineages. In our report: "An Atlas of Human Regulatory T Helper-like Cells Reveals Features of Th2-like Tregs that Support a Tumorigenic Environment" we found that Th2-like Tregs were increased in the tumour microenvironment in comparison with Th1-like, Th17-like and Th1/Th17-like Tregs. In addition, despite similar expression of CCR4 between all Tregs subtypes, Th2-like Tregs migrated more toward CCL17 and CCL22, and expressed higher levels of CCR8 than the other Th-like Tregs. Other studies have characterised human tissue-infiltrating Tregs and demonstrated that CCR8 is the main chemokine receptor differentially expressed in Tregs isolated from malignant tissues in comparison with healthy tissues. Given that CCR8 is a marker of Th2 lineages it is possible that Th2-like Tregs and CCR8⁺ Tregs are the same population of cells involved in tumour progression. However, whether they are recruited or differentiated in situ by the tumour microenvironment is still unknown. In this mini review, we describe the role of the different Th subsets in health and cancer and we present the different hypotheses about the presence of Th2-like Tregs in the tumorigenic environments.

Human T helper CD4⁺ cells as key regulators of the immune system

CD4⁺T-cells have been classified by several research groups into different sub-types based on their genetic profile, phenotype, maturation state or function. Naïve CD4⁺T-cells undergo differentiation into specific T helper (Th) subsets via specific cytokine signals^{1,2}. The main effector CD4⁺Th lineages are the Th1, Th2 and Th17 subset, whereas the main immuno-modulatory population is called regulatory T-cells (Tregs). These CD4⁺T-cell subsets play different roles in the immunological response: Th1 cells produce high levels of IFN- γ and are protective against intracellular pathogens. The key factor necessary for their differentiation is IL-12, however a role for IFN- γ and at the late stage IL-18³ has been shown. IL-12, through STAT-4, and IFN- γ through STAT-1, promote activation of T-bet (Th1 master transcription factor), while IL-18 bolster the IFN- γ production. Th2 are induced by and confer protection against helminths, but they can also promote acute and chronic inflammatory responses against a myriad of allergens. IL-4 signalling through STAT6, is essential for Th2 activation and differentiation. Th17 cells are characterized by the capacity to

secrete IL-17 and its differentiation is induced with several cytokines such as IL-1 β , IL-6 and TGF- β , in humans. They have an important role in the clearance of extracellular bacteria and fungi, due to their capability to recruit and activate neutrophils. In addition, they have been associated with the pathogenesis of several autoimmune diseases. The expression of chemokine receptors by these subsets mediates their chemotaxis towards different tissues⁴⁻⁶. Clarifying how chemokines and their cognate receptors orchestrate T-cell trafficking and activity is essential in gaining a better understanding of their role and distribution in health and disease. Imbalance between Th-like effector cells and Tregs has been observed in several cancers⁷⁻⁹ however how Tregs, but not pro-inflammatory effectors, preferentially migrate to these malignant tissues and which subset is the main responsible for tumour maintenance remains to be fully elucidated.

Th subsets and their chemokine receptors

Th1 cells are the most abundant Th subset in peripheral blood and have been involved in pro-inflammatory immune responses. Th1 responses have been associated with the production of opsonising and complement-fixing antibodies by B cells, activation of macrophages, cell cytotoxicity and induction of cell-mediated immunity¹⁰. This subset is characterised by the expression of the transcription factor T-bet¹¹, which promotes the secretion of IFN- γ ¹² and the expression of the chemokine receptor CXCR3⁵. CXCR3 mediates migration to its ligand CXCL10, which is highly expressed in a diverse range of human diseases such as infectious diseases, inflammatory and autoimmune diseases¹³.

Th2 cells have been associated with allergy¹⁴ and asthma¹⁵. These cells are characterised by the transcription factor GATA3¹⁶, they produce IL-4, IL-5 and IL-13¹⁰ and express the chemokine receptors CCR4¹⁷ and CCR8¹⁸. CCR4 mediates the migration to its ligands CCL17 and CCL22 produced by DCs upon maturation thereby playing a key role in recruiting cells into lymphoid tissues^{19,20}.

Th17 cells mediate host defensive mechanisms to various infections, especially extracellular bacteria infections and are involved in the pathogenesis of many autoimmune diseases. The main transcription factor expressed by this subset is ROR γ ²¹. These cells produce IL-17²¹ and express the chemokine receptor CCR6²² and CCR4²³. The expression of CCL20, the ligand for CCR6, is induced by IL-17 and secreted by Th17 cells during inflammation and co-ordinates the migration of Th17 and Tregs to inflammatory sites²².

Regulatory CD4⁺ Th-like cells and their chemokine receptors

Tregs are a subset of T-cells that maintain immunological

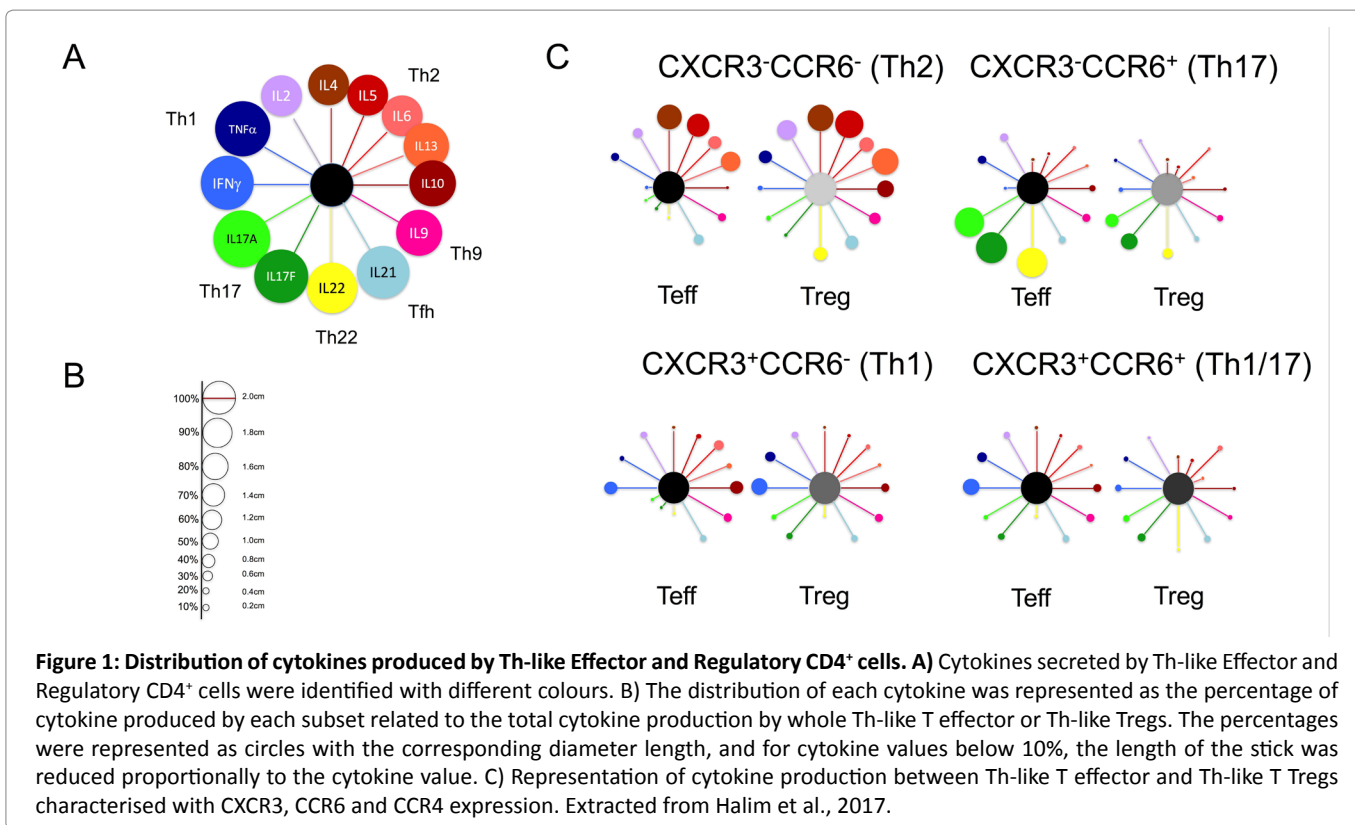
tolerance and regulate immune homeostasis^{24,25}. Human Tregs are characterised by the high expression of the IL-2 receptor α -chain (CD25), the transcription factor FoxP3 and the low expression of the IL-7 receptor α -chain (CD127). In 2012, Duhon et al., described new subpopulations of memory Tregs mirroring the classical CD4⁺Th cells. These new subpopulations, named Th-like Tregs, express chemokine receptors CXCR3, CCR6 and CCR4, typically expressed by T-bet⁺Th1, ROR γ ⁺Th17 and GATA3⁺Th2, respectively²³. The high levels of expression of CCR4 by memory Tregs, and concomitantly the presence of CCL22 in the tumour environment has been shown to induce chemotaxis of Tregs in melanoma²⁶, gastric cancer²⁷ and breast cancer¹⁹. In addition to effector cells, CXCL10 also mediates the recruitment of Tregs into chronically inflamed liver, as liver-infiltrating Tregs expressed higher levels of CXCR3 when compared to peripheral blood Tregs²⁸. Th17 cells are classically pro-inflammatory, however studies have shown that Foxp3⁺IL17⁺T-cells detected in colorectal cancer have the ability to suppress tumour-specific CD8⁺T-cells²⁹ and promote the development of cancer-initiating cells³⁰. Recently, we performed a phenotypic and functional characterization of these Th-like Tregs in periphery and we observed a similar cytokine distribution pattern than effector Th subsets (Figure 1)³¹.

Effector Th cells in cancer

Effector Th1 lineages evolved to eradicate intracellular pathogens, such as intracellular bacteria and viruses, through activation of cytotoxic T-cell (CTL) responses, as well as immunoglobulin (Ig) G2a and IgG3 class switch^{32,33}. Thus, Th1 responses are primarily responsible for activating and regulating the development and persistence of CTL, which can directly kill tumours. In addition, IFN- γ mediated Th1 responses have been shown to trigger tumouricidal activity of tumour-infiltrating macrophages and promote the secretion of CXCL9 and CXCL10, supporting the recruitment of new Th1 cells and the prevention of cancer progression³⁴. Furthermore, Th1 cytokines can promote anti-tumour responses and utilise a granzyme-perforin-dependent pathway for killing cancer cells³⁵. Th2 responses have been associated with several cancers such as pancreatic cancer^{36,37}, oesophageal and gastric cancer³⁸, melanoma³⁹ and ovarian cancer⁴⁰. The role of Th17 responses in cancer is context-dependent⁴¹. IL-17 plays a dual role in the antitumor response through a cytotoxic T-cell action and by promoting angiogenesis. In addition, they display a great degree of context-dependent plasticity making them able to acquire functional characteristics of Tregs and effector T helper cells.

Regulatory CD4⁺ Th-like helper cells in cancer

Several research groups have recently described the genetic profile of tumour-resident Tregs⁴². In 2016, Tirosh



et al., analysed single-cell expression profile of immune cell isolated from melanoma samples, including effector CD8⁺ T-cells, CD4⁺T-cells and Tregs. The main gene associated with migratory functions expressed by infiltrating-tumour Tregs in this study was CCR8⁴³. The classical ligand for CCR8 are CCL1 and CCL18, however it has been shown that CCL17 also induces migration of CCR8-expressing cells⁴⁴, which is relevant in the cancer field as CCL17 has been strongly associated with tumour progression²⁷. Same year, two independent research groups also described CCR8 as one of the main chemokine receptor in Tregs present in breast⁴⁵, colorectal and non-small-cell lung cancer⁴⁶. Whereas Plitas et al., mentioned that tumour environment has the ability to potentiate CCR8 expression in Tregs, De Simone et al., discussed that the presence of CCL18 in different tumours might be associated with the migration of CCR8⁺ cells^{45,46}. In 2017, our research group characterized the presence of Th1, Th2, Th17 and Th1/17 Tregs in peripheral blood and several tissues, such as skin, colon, thymus, spleen and liver perfusates³¹. We observed that different Treg subsets exhibited different location, cytokine profile, viability and migratory capacity. Interestingly, the presence of these subsets was significantly different between healthy and cancer tissues, obtained from patients with melanoma and colorectal cancer. Our data showed an increment of Th2-like Tregs in malignant tissues compared to the healthy counterpart; however, colorectal cancer was enriched in Th2 effector cells as well, suggesting that tumour microenvironment may preferentially select this lineage.

Before the identification of Th-like Tregs, CCR8 expression was described in CD4⁺ memory T-cells enriched for Tregs and Th2 effector cells, characterised by the production of Th2-type cytokines¹⁸. However, whether Th2-like Tregs and CCR8⁺ Tregs are the same population needs further investigation.

Altogether the evidence that Th2-like Tregs are increased in the tumour environment raised the question which is the main mechanism used by the tumour to promote the presence of Th2-like Tregs. The secretion of specific chemokines at the tumour site stimulating the migration of Th2-like cells is one possible scenario. Another possible explanation is the specific expansion of Th2-like subsets at the cancer site to reduce the anti-tumour immunological response. For example, the expression of TIGIT (T-Cell Immunoreceptor With Ig And ITIM Domains) has been found in tumour-infiltrating conventional CD4⁺T-cells compared to circulating CD4⁺T-cells⁴⁵. It has been demonstrated that TIGIT contributes to the induction of a selective Treg-cell-mediated suppression of pro-inflammatory Th1 and Th17 cells but not Th2 cell responses⁴⁷, thus it is possible that Th2 cells are not only recruited to the tumour site, but also promoted by evading TIGIT-mediated inhibition. The final explanation is that the production of IL-4, a classical Th2 cytokine, promote the differentiation of Th2 cells and inhibit IFN-g production by CD4⁺T-cells⁴⁸, therefore the secretion of this cytokine could also promote an enriched Th2 environment.

Conclusion

The increased presence of Tregs in malignant tissues plays a detrimental role in the battle between the immune system against the tumour. More recently, the presence of a specific type of Tregs (Th2-like Tregs) identified by us in melanoma and colorectal cancer could indicate that the malignant tissue is able to modify the immunological microenvironment surrounding the tumour in order to promote its development and avoid an anti-cancer response. Future studies about tumour-infiltrating Tregs or other regulatory population like tumour-associated macrophages can help us to clarify the mechanisms involved in tumour progression and to develop new alternative anti-tumour strategies.

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Conflict of interest

Estefanía Nova-Lamperti, Marco Fraga, Marco Romano and Giovanna Lombardi declare that they have no conflict of interest.

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