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T regulatory cells in B-cell malignancy – tumour support or kiss of death?

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Summary

It is well established that T regulatory (Treg) cells counteract tumour immunity. However, conflicting results describing the role of Treg cells in haematological tumours warrant further investigations to clarify the interactions between Treg cells and the tumour. B-cell malignancy derives from different stages of B-cell development and differentiation in which T cells play a profound role. The transformed B cell may still be in need of T-cell help to thrive but simultaneously they may be recognized and destroyed by cytotoxic lymphocytes. Recent reports demonstrate that Treg cells can suppress and even kill B cells as part of their normal function to rescue the body from autoimmunity. An emerging body of evidence points out that Treg cells not only inhibit tumour-specific T cells but may also have a role in suppressing the progression of the B-cell tumour. In this review, we discuss the origin and function of Treg cells and their role in patients with B-cell tumours.

Keywords: B-cell leukaemia; B-cell lymphoma; cytotoxic regulatory cells; immune regulation; T regulatory cells

Introduction

The immune system plays an important role in tumour transformation and progression as chronic inflammation generates reactive oxygen species (ROS), which increase DNA damage and thereby cause genomic instability and accumulation of mutations.[1] However, the adaptive responses may instead detect and destroy tumour cells because of their aberrant expression of various tumour-associated, or tumour-specific, proteins. Indeed, there is a positive correlation between the number of infiltrating T cells and patient survival in many solid cancers.[2] Nevertheless, immune cells in the tumour area are often anergized by the presence of immunosuppressive cells and cytokines. In haematological cancers, the tumour cell itself is an immune cell that complicates the cell interactions in the tumour microenvironment. The T regulatory (Treg) cells, for example, restrain unwanted immune responses (autoimmunity, post-infection inflammation). These cells increase during cancer progression and have been correlated to a worse prognosis in many malignancies.[3–5] However, the role of Treg cells in haematological tumours is debated. Contradicting results in B-cell malignancies demonstrate that Treg cells can be associated with both poor prognosis and increased survival.[6–13] As immunosuppressors, Treg cells may act against the attacking effector lymphocytes as well as against the B-cell-derived tumour. In this review, we will discuss Treg cells and their complex role in haematological tumours.

The birth of T regulatory cells

The suppressive capacity of lymphocytes has been known to man for decades. As early as the 1970s, Gershon and Kondo[14,15] discovered that T cells pre-treated with high doses of antigen became tolerant and that tolerance could be passed on to surrounding T and B cells. Subsequent studies aimed at characterizing these cells were undertaken but no specific markers were available and the existence of the cells was hard to prove.[16] It was not until 1995, when Sakaguchi *et al.*[17] suggested that murine suppressive T cells could be identified by their high expression of CD25 [interleukin-2 receptor α (IL-2Rα)], that the interest in this subset resurfaced. They demonstrated that mice developed autoimmune diseases upon depletion of $CD25^+$ cells. Further, these mice reacted strongly to skin transplants and this reaction could be hampered by the reinfusion of $CD4^+$ $CD25^+$ cells.[17] During the following years suppressive T cells were rebranded as regulatory T cells.[16]

Treg cell-associated markers

Discrepancies in the results from published studies evaluating Treg cells in B-cell malignancies as well as in other studies may be the result of the difficulty of finding one good marker for identification of the cells. $CD4^+$ $CD25^+$ cells were identified as Treg cells in humans a couple of years after Sakaguchi's milestone paper.[17–21] However, as CD25 is also up-regulated on activated T cells, it is not a specific marker.[22] Even so, because of the lack of a more unique cell surface marker, CD25 has been used extensively to sort Treg cells for experimental evaluation. Most Treg cells express CD25 (70–80%[23]) but the existence of CD25-negative Treg cells has been reported by us and others.[24–26] Other markers are needed to encompass all or certain populations of Treg cells. Additional markers expressed by Treg cells are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4),[27,28] glucocorticoid-induced tumour necrosis factor receptor[29] and lymphocyte-activation gene 3.[30] These markers are constitutively or highly expressed on Treg cells but suffer the same fate as CD25 by being up-regulated on conventional T cells upon activation.[26,27]

The transcription factor Forkhead box P3 (FoxP3) is considered the most specific marker for Treg cells.[31–33] FoxP3 acts both as a repressor and an activator of gene transcription and binds over 700 genes.[34] It is known to repress the gene expression of IL-2, CD127 (IL7R), tumour necrosis factor-α and interferon-γ, and to enhance the expression of CD25 and CTLA-4.[31–33,35–38] Given the fact that CD127 is a cell surface marker it can be used to distinguish the $CD25^{high}$ $CD127^{low}$ Treg cells from the $CD25^{high}$ $CD127^{high}$ activated T cells.[39] This may be crucial because some studies have shown that FoxP3 can be transiently up-regulated in human effector T cells that do not exhibit regulatory functions.[40–43] However, as several of these studies were performed with an antibody of questioned specificity (PCH101, eBiosciences, San Diego, CA) it is hard to interpret these results.[40,42–44] Whether the transient FoxP3 expression confers transient regulatory functions on T cells is still a matter of debate and may be difficult to investigate because of the short timespan of FoxP3 expression.

Treg cell development and subtypes

Natural Treg cells originate in the thymus but Treg cells can be induced from naive T cells in the periphery as well. These two cell populations have been hard to distinguish from one another because they are very similar, both phenotypically and functionally. However, recent studies identified Helios, a zinc finger transcription factor, as being highly expressed in the natural Treg cells but not in peripherally induced Treg cells.[45,46] Natural Treg cells are selected in the thymus in the same way as conventional T cells. They can be either $CD4^+$ or $CD8^+$ but the $CD4^+$ subclass dominates. Their T-cell receptors have high affinity for self antigens, meaning that these are in the border of thymic elimination.[47] Because they are reactive with autopeptides they are likely to have a role in preventing autoimmunity. Nevertheless, considering the thymic involution and the fact that Treg cells do not decrease with age, inducible Treg cells must also be an important source of Treg cells in adults. Although natural Treg cells are important in protection against autoimmunity, inducible Treg cells are thought to protect the surrounding tissues in an ongoing immune response. If the B-cell tumour is producing antibodies that react to autoantigens, natural Treg cells may play an important role in hampering tumour cell progression whereas both natural and inducible Treg cells may restrain tumour-reactive T cells. Considering that the malignant B cells have an aberrant behaviour compared with normal B cells (regarding proliferation and apoptosis resistance), it is possible that the malignant B cells are also targets for the inducible Treg cells.

Inducible Treg cells, also known as adaptive Treg cells, are induced from naive T cells by antigenic stimulation in combination with factors that are not optimal for effector T-cell generation, such as high levels of IL-10, IL-2 and transforming growth factor-β (TGF-β).[48] Retinoic acid has been shown to induce Treg cells even in the presence of inflammatory cytokines.[49] Several subsets of inducible Treg cells have been described, including $CD4^+$ Tr1 and Th3 Treg cells, as well as $CD8^+$ Treg cells. Recently, T follicular regulatory cells were described in mice, and these Treg cells were shown to regulate germinal centre reactions.[50–52] This $CXCR5^+$ subset is, however, most likely induced from natural Treg cells.[50,51] The environment defines the Treg cell phenotype because it has been demonstrated that transcriptional regulators associated with different types of T helper cell responses (Th1, Th2, Th17) shape the Treg cell response.[53] Further, Treg cell status is not at a differentiation endpoint because studies have shown that these cells can transform into effector cells, lose their regulatory function and produce IL-17 or interferon-γ.[54,55] Hence, the plasticity of these cells allows for the multitask functions needed to balance between immune regulation and activation. However, these characteristics makes it difficult to pinpoint certain Treg cell subtypes and their intermediate phases, and it complicates the role of these cells in haematological malignancies because their status may either regulate or stimulate the tumour cell. The environment in B-cell tumours will, hence, determine the subtypes of Treg cells present in the tumour.

Treg cell effector functions

Treg cells are able to suppress a wide range of immune cells. The suppression can either be direct or mediated through secondary immune cells. To exert their function Treg cells need to be activated in an antigen-dependent manner but are then able to suppress nearby immune

cells by antigen-dependent mechanisms or, more commonly, independently of specificity. Hence, an immune effector cell does not need to share the same specificity as the Treg cell in order to be suppressed. Treg cells are able to suppress target cells by the release of inhibitory cytokines such as IL-10, TGF-β and IL-35.[56] Interleukin-10 induces a long-lasting anergy in both $CD4^+$ and $CD8^+$ T cells and down-regulates the expression of co-stimulatory molecules, adhesion molecules and MHC class II on antigen-presenting cells.[57–60] The TGF-β is able to inhibit T-cell proliferation by disturbing IL-2 production and can block differentiation of naive T cells.[61] Interleukin-35 suppresses T-cell proliferation[62] and can stimulate Treg cells to proliferate and produce high levels of IL-10.[63]

Treg cells are also able to inhibit T cells by interfering with their metabolism. By expressing the ectozymes CD39 and CD73, Treg cells generate adenosine, which suppresses effector T cells by binding to the adenosine receptor 2A.[64,65] Further, cAMP can be transported from Treg cells to T cells through gap junctions where they inhibit proliferation and IL-2 production.[66,67] Prostaglandin E_2, which is generated by cyclooxygenase-2, can be secreted by Treg cells[68] and mediates its suppressive function by increasing the level of cAMP, which further suppresses the T cells.[69] Recently, we suggested that Treg cells are able to release CD25 to further deprive its microenvironment of IL-2 and by this means inhibit the proliferation of conventional T cells.[70] Treg cells can also deprive T cells of thiols, such as cysteine, that are provided to T cells in the dendritic cell vicinity.[71] The Treg cells are able to induce indoleamine 2,3-dioxygenase production by dendritic cells in a CTLA-4-dependent manner.[72] Indoleamine 2,3-dioxygenase is an enzyme that catabolizes tryptophan into the toxic kynurenine metabolites 3-HAA (3-Hydroxyanthranilic acid) and QUIN (Quinolinic acid), which induce apoptosis in Th1[73] and Th2 cells.[74]

Treg cells are able to suppress a wide range of immune cells by induction of apoptosis. This killing is generally mediated by Fas–Fas ligand interaction[75,76] or through the release of perforins/granzymes.[77–82] Grossman *et al.*[78] demonstrated that natural Treg cells preferentially express granzyme A upon activation, in contrast to inducible Treg cells, which express granzyme B.[78] Treg cells can also kill T cells by using the TRAIL–DR5 pathway.[83] Also, galectin-1, which can induce apoptosis in target cells, is up-regulated on Treg cells.[84] By killing their target, Treg cells can control $CD4^{+}$[78] and $CD8^{+}$[75,78,80] T cells, monocytes,[78] dendritic cells,[78] B cells[76,79,81] and natural killer cells.[80]

Treg cells in B-cell-derived tumours

The role of Treg cells is more complex in haematological cancers compared with non-haematological cancers because the tumour is derived from the immune system. The normal behaviour of antigen-presenting cells, such as the B cell, in lymph nodes or at sites of inflammation is to interact with T cells including Treg cells. It is possible that Treg cells have a dual role in patients with leukaemia or lymphoma. On one hand, they may suppress anti-tumour immune responses mediated by T cells but on the other hand they may regulate the malignant immune cell, either directly or by interfering with T-cell help (Fig. 1). Some studies have investigated the relationship between the number of Treg cells and patient outcome in B-cell lymphoma. Several of these showed that patients with a high number of tumour infiltrating $FoxP3^+$ cells (Treg cells) have a better survival than patients with few $Foxp3^+$ cells.[7–13]

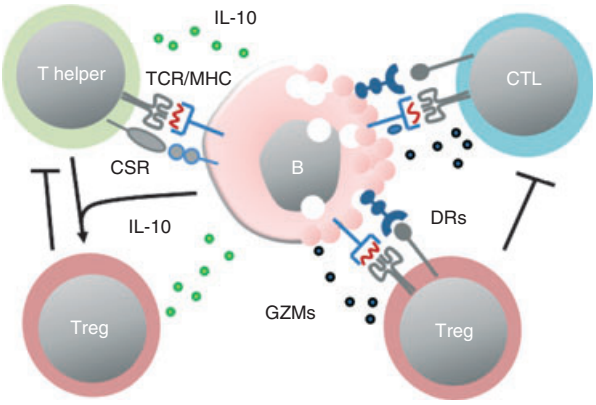

Figure 1. Interactions between T cells and malignant B cells. The malignant B cells may require T helper cells for sustained growth but the helper cells may also be part of the control of malignant cells by direct cytotoxicity or by stimulating anti-tumour immunity. Helper cells can interact via T-cell receptor (TCR)/MHC-II and via co-stimulatory receptors (CSR). The malignant B cells can release substances [such as interleukin-10 (IL-10)] that promote the conversion of T helper cells into regulatory T (Treg) cells that suppress both T helper cells and cytotoxic T cells (CTL). The Treg cells can also recognize MHC-II on the B cells. If cytotoxic, they may interact via death receptors (DRs) to induce B-cell apoptosis. The CTLs recognize MHC-I on malignant B cells and induce apoptosis via DRs. GZMs, granzymes.

The tumour microenvironment is beneficial for the maintenance and expansion of Treg cells because of the presence of IL-10, TGF-β and immature dendritic cells,[85,86] and these factors are present in both non-haematological and haematological cancers. Although, IL-10 is an immunosuppressive molecule for effector T cells, it is stimulatory for B cells. Hence, IL-10 may drive both Treg cells and the B-cell tumour. In B-cell lymphoma it was shown that patients with elevated levels of both IL-10 and tumour necrosis factor-α were less likely to respond well to treatment.[87] In follicular lymphoma, the tumour B cells were shown to convert nearby $CD4^+$ T cells into $FoxP3^+$ Treg cells in an antigen-specific manner.[88]

During different steps of B-cell development, T cells control the fate of the B cell by either killing it[89] or promoting its survival by up-regulating anti-apoptotic mole-

cules as well as inducing its proliferation. B-cell malignancies arise during different stages of B-cell maturation[90] and several of them originate from germinal centre-derived B cells. The B-cell tumour in patients with chronic lymphocytic leukaemia (CLL) dies rapidly *in vitro*, which highlights the importance of the tumour micro-milieu in tumour survival. It has been shown that CLL cell survival *in vitro* can be supported by stromal accessory cells.[91] As a result of the close contact of B cells and T helper cells it is likely that T-cell help is an important feature of tumour progression. Hence, by suppressing T helper cells in the tumour vicinity through the effector mechanisms discussed above, Treg cells may block tumour cell progression. Correspondingly, studies have shown that Treg cells are able to regulate B cells by interfering with their need for T-cell help in germinal centres.[92,93] However, a study on Hodgkin's lymphoma demonstrated that many Treg cells in combination with few Th2 cells correlated with increased risk of relapse.[6]

Treg cells are also able to regulate B cells directly by induction of apoptosis.[76,79,81] In a study recently published by our group, we demonstrated that FoxP3$^+$ Treg cells in patients with B-cell leukaemia or lymphoma, expressed cytolytic markers and were able to kill malignant B cells *in vitro*.[94] The same phenomenon has been noted in patients with systemic lupus erythematosus. In that study, Treg cells were able to regulate malignant autoantibody producing B cells.[95] In CLL, at least half of the patients have tumour cells with somatically mutated immunoglobulin heavy chain variable genes and more than 20% express homologous stereotyped B-cell receptors. These findings indicate that a certain antigen may have caused the disease onset.[96] It is not clear if this agent (or agents) still drives the disease. Some antigens suggested are present on apoptotic cells, or bacteria.[97,98] It has been proposed that CLL is driven by autoantigens and CLL cells were shown to produce autoantibodies.[99,100] Since then, CLL has been connected to several different autoimmune conditions.[101] By controlling CLL cells, Treg cells may exert their natural function as suppressors of autoimmunity. As an interesting parallel; studies have shown that several autoimmune diseases associated with autoantibody production have Treg cells at a decreased level of function.[102–106] Treg cells controlling B cells may suppress the B cells in an antigen-specific manner (T-cell receptor–MHC-II-restricted) because malignant B cells express MHC-II and killing via death receptor ligands or granzyme release is commonly regulated via T-cell antigen recognition. However, other mechanisms exerted by the Treg cells may be used.

Even if several studies show a positive correlation between FoxP3 and survival in B-cell malignancy, there are also studies demonstrating that Treg cells are associated with a worse outcome in these patients.[6] The discrepancy may lay in methods of Treg cell detection. For example, the PCH101 antibody can mistakenly also stain activated T cells.[41] Hence, some of the detected FoxP3$^+$ cells may have been activated T cells which at least in other cancers have been consistently shown to be beneficial. As a result of the promiscuous phenotype of Treg cells, these cells may also represent an intermediate phenotype on their way to transform into effector T cells. Indeed, FoxP3$^-$ T cells in patients with leukaemia or lymphoma also displayed markers of cytolysis[94] demonstrating the active participation of the immune system to combat the malignant B cell. Clearly, further investigations are needed to elucidate the role of Treg cells, and T cells in general, in patients with haematological tumours such as B-cell malignancy.

Conclusion

Treg cells exist as many subtypes changing their wardrobe depending on the ongoing immunological scenario. The role of Treg cells in solid non-haematopoietic cancers is to suppress tumour immunity probably through their importance in inhibiting immune activity to self cells. In haematological tumours the role of Treg cells may be more complex because the Treg cells on the one hand create a tumour-supporting environment by blocking ongoing immune attacks in the tumour milieu, and on the other hand may kill the tumour by recognizing tumour antigens on MHC-II on the tumour cell leading to the traditional 'kiss of death'. Understanding the basic interactions between T cells, Treg cells and normal B cells will give new insights into the various immune responses occurring in patients with B-cell-derived tumours.

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Disclosures

The authors declare no conflict of interest.

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