

Self-reactive T cells: suppressing the suppressors

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Abstract The immune system is a tightly regulated and complex system. An important part of this immune regulation is the assurance of tolerance toward self-antigens to maintain immune homeostasis. However, in recent years, antigen-specific cellular immune responses toward several normal self-proteins expressed in regulatory immune cells have been reported, especially in patients with cancer. The seemingly lack of tolerance toward such proteins is interesting, as it suggests a regulatory function of self-reactive T (srT) cells, which may be important for the fine tuning of the immune system. In particular, surprising has been the description of cytotoxic srT cells that are able to eliminate normal regulatory immune cells. Such srT cells may be important as effector cells that suppress regulatory suppressor cells. The current knowledge of the nature and function of srT cells is still limited. Still, the therapeutic targeting of srT cells offers a novel approach to harness immune-regulatory networks in cancer.

Keywords Regulatory T cells · PD-L1 · IDO · Foxp3 · CD8 T cells

Abbreviations

APC Antigen presenting cells
CTL Cytotoxic T lymphocytes
CTLA-4 Cytotoxic T-lymphocyte antigen-4

DC Dendritic cells
Foxp3 Forkhead box P3
FoxO3 Forkhead box O3
IDO Indoleamine 2,3-dioxygenase
MDSC Myeloid derived suppressor cells
Tregs Regulatory T cells
srT cells Self-reactive T cells
TCR T-cell receptor
TDO Tryptophan 2,3-dioxygenase

Introduction

The immune system is tightly controlled to avoid the occurrence of autoimmunity when it is responding to various pathogens [30]. Thus, the adaptive immune system is characterized by the ability to respond to *foreign antigens*, while being tolerant to *self-antigens* due to central (i.e., thymic selection) and peripheral tolerance. This enantioselectivity, allowing at the same time destruction and self-tolerance, is guarded by several feedback mechanisms. Unfortunately, some of the mechanisms preventing autoimmunity are hijacked by cancers to attain immune escape [17]. Indeed, in the recently updated version of ‘The Hallmarks of cancer’ by Hanahan and Weinberg, evasion of immune destruction is now listed as an emerging hallmark [14]. This evasion of immune destruction is based on several mechanisms. Solid tumors are composed of the cancerous cells themselves as well as the stroma that not only provides a supportive framework but also is involved with immune evasion. For this purpose, tumors attract and/or convert immune cells that procreate and sustain an immune permissive microenvironment. These immune cells, which are normally involved in the elaborate network of central and peripheral tolerance mechanisms maintaining immune

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homeostasis, include factor forkhead box P3 (Foxp3) positive regulatory T cells (Tregs), NKT cells, dendritic cell subtypes, M2 (tumor-associated) macrophages, myeloid derived suppressor cells (MDSC), as well as granulocytes [32]. Additionally, cancerous cells express a plethora of membrane-bound as well as soluble molecules that educate infiltrating immune cells to become functional as part of a tumor-permissive rather than a tumor-suppressive microenvironment. Furthermore, other cell types that are abundant in the tumor microenvironment, e.g., fibroblasts, adipocytes, and endothelial cells, secrete various factors that impact directly on cells present in the microenvironment or act as attractants for other cells; these factors include IL-6, Foxo3, TGF- β , COX-2, VEGF, SDF-1, CXCL1/2, and IL-1 β among others.

However, over the last couple of years, it has been described that the immune system is counteracting these immune evasion mechanisms. Hence, srT cells which specifically recognize epitopes derived from the above-described immune-suppressive proteins are gaining increasing attention [19, 22–25, 27, 37, 43–45] (Table 1).

Circulating CD34⁺ hematopoietic stem cells reside in the bone marrow and produce precursors of T cells, which seed the thymus. The fate of these progenitors expressing clonally distinct $\alpha\beta$ TCRs depends on how their TCRs react against self-peptides presented by HLA. Cells without a

TCR or cells that are expressing a TCR which is not able to react with respective complexes are neglected and die. Cells expressing TCRs of low affinity toward HLA/peptide undergo positive selection and develop into ‘normal’ CD4⁺ or CD8⁺ T cells. In contrast, cells harboring TCRs of high affinity are clonally deleted for the preservation of tolerance toward self [28]. However, in recent years, several distinct subpopulations of self-reactive lymphocytes have been described, which are positively selected [46]. These cells are increasingly apportioned to immune regulation and immune homeostasis. Such self-reactive lymphocytes include natural Tregs (nTregs), natural T helper 17 (nTh17) cells, invariant natural killer T cells (iNKT cells), and natural CD8 $\alpha\alpha$ ⁺ intraepithelial T cells (nIEL). srT cells may similarly be positive selected in the thymus. In the present review, we summarize the recent findings on srT cells.

Cytotoxic srT cells recognizing forkhead box (fox) proteins

Among the T cells recruited to solid tumors are CD4⁺ CD25^{high} Tregs [29]. Tregs, characterized by expression of Foxp3, are critical for maintenance of immune homeostasis, prevention of autoimmunity by regulating immune responses to foreign, and self-antigens [15]. Tregs accumulate in tumors and the peripheral blood of cancer patients, and an increased frequency of Tregs is in most cases a marker of poor prognosis, which is presumably due to suppression mediated by Tregs of anti-tumor immunity. Thus, several immune therapies for cancer involve the ablation or modulation of Foxp3⁺ Tregs [6, 18, 20]. To this end, Gilboa and colleagues in an animal model stimulated strong Foxp3-specific cytotoxic T-cell (CTL) responses by the use of dendritic cells (DC) transfected with Foxp3 mRNA. These measures reduced the number of Foxp3⁺ Tregs in the animals [27]. Furthermore, when vaccinating simultaneously against TRP-2 and Foxp3, they observed a superior protection against B16 melanoma in comparison with vaccinating against TRP-2 alone. Notably, Foxp3-specific CTL responses are not restricted to mice. We recently demonstrated that Foxp3-reactive T cells were present among the peripheral blood mononuclear cells (PBMCs) of cancer patients and healthy volunteers [19]. These specific CD8⁺, cytotoxic T cells recognized Foxp3-derived peptide in an HLA-A2-restricted manner. We showed that the recognition of target cells indeed was Foxp3-dependent, as DC were only recognized after internalization and cross-presentation of recombinant Foxp3 protein. In addition, Foxp3-specific CD8⁺ T cells were able to lyse malignant Foxp3⁺ T cells. More significant was the ability of Foxp3-specific T cells to recognize Tregs. Hence, Foxp3-specific CTL may impact immune regulation by suppressing Tregs

Table 1 Self-reactive T cells

Target protein	CD8 and/or CD4 responses	Target cells	References
Foxp3	CD8	Cancer cells Tregs	[19, 27]
Foxo3	CD8	Cancer cells TADC	Unpublished
IDO	CD8 and CD4	Cancer cells Stroma cells MDSC TADC	[25, 43, 44]
IDO2	CD8	Cancer cells Stroma cells Liver cells? DC?	[45]
TDO	CD8 and CD4	Cancer cells Liver cells? MDSC?	Unpublished
PD-L1	CD8 and CD4	Cancer cells Stroma cells MDSC TADC Resting lymphocytes? DC and macrophages	[22–24]

in vivo. Consequently, Foxp3-based immunotherapy may be synergistic with additional anti-cancer immune therapy. It should be noted that cyclophosphamide has been used to boost adaptive T-cell responses to cancer due to its ability to deplete Tregs. However, cyclophosphamide may at the same time increase the presence and activity of MDSC in tumors depending of the microenvironment [41].

FoxO3 is another Forkhead box transcription factor. It is a member of the class-O family and was originally described to be a tumor suppressor gene [33]. Recently, pioneering work by Hurwitz and colleagues showed that increased FoxO3 expression was associated with or even responsible for tumor-associated DCs (TADCs)-induced T-cell tolerance [49]. We recently detected the natural presence of FoxO3-specific, cytotoxic CD8 T cells among PMBC of cancer patients, whereas we did not detect reactivity toward FoxO3 in healthy individuals (unpublished data). As FoxO3 programs TADCs to become tolerogenic and thereby comprises a significant immune-suppressive mechanism, the targeting of FoxO3 may not only lead to

the killing of cancer cells but in addition to diminished immune suppression mediated by FoxO3-expressing TADC.

Cytotoxic srT cells recognizing metabolic enzymes

An important immune-suppressive characteristic of the tumor microenvironment is an altered metabolism, which results in the depletion of essential amino acids and is leading to an increase in suppressive metabolites. The immune-regulatory enzyme IDO is involved in the suppression of T cells and inducing tolerance in different pathological settings including allergic inflammation, chronic infection and cancer. IDO depletes the amino acid tryptophan thereby inducing T-cell apoptosis and anergy and creating an immune permissive microenvironment [26]. As IDO is upregulated by inflammatory cytokines such as type I and II interferon's, it is thought to be an important counter-regulatory enzyme, which controls disproportionate immune responses. In cancer patients, IDO is highly expressed in both the tumor-draining lymph nodes as well as in the tumor [21].

IDO is an important player in the creation of T-cell tolerance mediated by tolerogenic DC [16, 34, 50]. Recently, it was further shown that IDO is a crucial player for MDSC-mediated suppression of anti-tumor immune responses [42, 52]. Hence, IDO expression was upregulated in MDSC isolated from fresh cancer tissue. Furthermore, IDO was responsible for MDSC-mediated suppression of T-cell immunity. Given the central and multifarious functions of IDO in immune modulatory processes, targeting IDO-expressing cells would provide a powerful feedback mechanism. Indeed, we recently identified spontaneously occurring CTL responses to IDO-derived T-cell epitopes in both patients with cancer [43] and healthy persons [44].

The IDO-specific CTL were functional effector cells that in an HLA-restricted manner killed target cells that expressed IDO, e.g., in vitro-generated DC as well as tumor cells. We further examined the function of IDO-specific T cells on the adaptive immune response [44]. Hence, the addition of such cells to PBMC cultures stimulated with either tumor-associated or viral antigens effectively boosted the specific immunity toward such antigens. Thus, by directly targeting IDO-expressing cells, IDO-specific CTL diminished immune suppression. In addition, the presence of IDO-specific CTL decreased the number of Tregs and in contrast increased both IL-17 and IL-6 production in the cultures.

Additionally, we recently identified CD8⁺ CTL that specifically recognized IDO2, which is an IDO analogue enzyme [45]. These IDO2-reactive CD8⁺ T cells were cytotoxic effector T cells, which were able to recognize

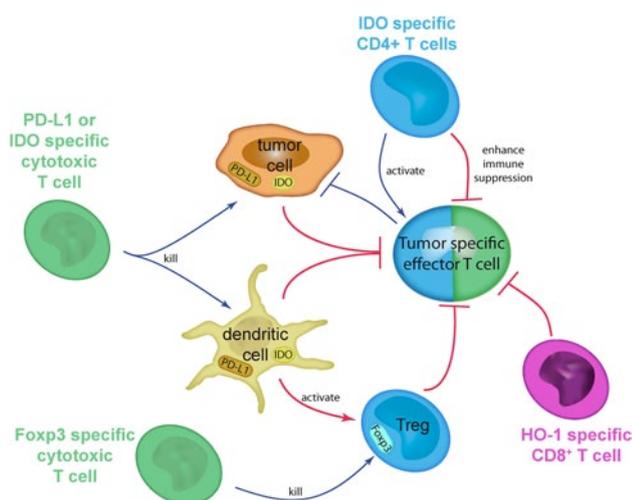


Fig. 1 The different roles of srT cells in tumor immunity. Cytotoxic srT cells (e.g., PD-L1, IDO, and FoxP3-specific) are able to eliminate regulatory immune cells, thereby suppressing the suppressors. In addition, they can eliminate cancer cells expressing these molecules and thus reduce their immune-suppressive effect. It must be assumed that cytotoxic srT cells themselves are hampered by the suppressive effects of their targets; however, in vitro-generated PD-L1-specific CTL do not express PD-1, suggesting a selection of PD-1 negative cells. Likewise, CD4⁺ srT cells specific for IDO or PD-L1 have been described to release pro-inflammatory cytokines and to suppress IL-10 production. Such helper srT cells may participate as counter-response cells in immune-regulatory networks by postponing the immune-suppressive effects of their targets. In that respect, the expression of both IDO and PD-L1 are early events during inflammation. However, some CD4⁺ srT cells may have an immune-suppressive phenotype. Similarly, immune-suppressive CD8⁺ srT cells specific for heme oxygenase-1 have been described in patients with cancer. Obviously, immune-suppressive srT cells may augment the target-mediated immune suppression

IDO2-expressing cancer cells, i.e., breast and colon cancer cells. However, preliminary data suggest that IDO2-specific T cells are not able to boost additional immune reactions in a similar manner as IDO-specific T cells (Andersen, unpublished data).

Cancer cells can employ yet another enzyme for the degradation of the amino acid tryptophan, thereby resisting immune rejection; tryptophan 2,3-dioxygenase (TDO). TDO is located in the cytosol and is encoded by the TDO2 gene. In normal tissue, it is almost exclusively expressed at high amounts in the liver. Recently, it was described that tumors of different origin express TDO, especially melanoma, bladder cancer, hepatocarcinoma as well as human glioblastomas [31]. It promotes tumor progression through depletion of tryptophan and production of kynurenine, which results in reduced anti-tumor immune responses. We were recently able to demonstrate that specific T cells react against TDO in cancer patients (unpublished data). Thus, TDO may be yet another metabolic enzyme that is recognized by srT cells.

srT cells specific for the immunological checkpoint PD-L1

The rearrangement of cell components to form the distinctive immunological synapse occurs when immune cells polarize in response to the recognition of an antigen presented by an HLA molecule [2]. The CD28 family comprises key elements of the immunological synapse [10]. The family consists of several members including CD28, PD-1, ICOS, and CTLA-4, which upon binding with their matching ligands are able to produce powerful co-stimulatory or inhibitory signals in T cells. The expression of ligands for the respective inhibitory receptors has been repeatedly described on tumor cells [8]. PD-L1 (B7-H1, CD274) is an important example [5, 8]. It binds PD-1, which is an inhibitory receptor expressed on activated T cells, thereby contributing to the functional exhaustion of T cells [11]. The PD1 pathway is playing a vital role in the regulation of the effector phase of an immune response by down-regulating the activity of T cells in order to prevent tissue damage [35]. Correspondingly, PD-1 expressing lymphocytes infiltrating the tumor become repressed by PD-L1 present in the tumor microenvironment. Antibody blockade of both PD-L1 and PD-1 results in clinical responses in cancer patients [4, 48]. However, it seems that the immune system itself has established a respective counteractive mechanism, i.e., PD-L1-specific CD8⁺ cytotoxic srT cells [22, 24]. PD-L1-reactive srT cells were readily isolated from PBMC of cancer patients (melanoma) and less frequently from blood of healthy donors. These PD-L1-specific CTL lysed melanoma and cutaneous T-cell lymphoma cells, as well as DC.

Importantly, the killing of the target cells was dependent on PD-L1 expression. Interestingly, the in vitro-generated PD-L1-specific CTL did not express surface PD-1, proposing a selection of PD-1 negative CTL. Furthermore, long peptides derived from PD-L1 were rapidly cross-presented by HLA-A2 on the cell surface by APC, which is interesting since soluble PD-L1 has been detected in the sera from cancer patients [12].

The induction of PD-L1-specific srT cells may boost immunity by killing immune-suppressive tumor cells as well as PD-L1 expressing stroma cells. It should be noted, however, that PD-L1 is also expressed on normal immune cells, e.g., resting lymphocytes, DC, and macrophages [51], and is further up-regulated upon activation in response to interferons [3, 9]. The overall biological role of PD-L1-specific srT cells may thus vary depending on the microenvironment and the state of the immune response [2].

Additional srT cells

In addition to the above-described CD8⁺ cytotoxic srT, recently both IDO- and PD-L1-specific CD4⁺ T cells were described in the PBMC of cancer patients and healthy donors [23, 25]. These CD4⁺ srT cells released INF γ , TNF α , and IL-17. Furthermore, in some donors, these CD4⁺ T cells suppressed IL-10 production. Hence, such CD4⁺ srT cells are likely to contribute to immune regulation by antagonizing the immune-suppressive actions of the targeted proteins. CMV is probably the most immune-dominant antigen in the human immune system, since a considerable fraction of human T cells in most individuals recognize CMV epitopes [47]. CMV infection of monocytes induces expression of IDO. IDO expression in CMV-infected monocytes is thus believed to help virus to escape anti-CMV T-cell responses [13]. Notably, we were able to correlate the number of IDO-specific CD4⁺ srT cells with CMV-specific T cells [25]. Thus, IDO-specific srT-cell responses may develop as support for the constitutive anti-CMV T-cell responses in the donors. The present data, however, do not exclude that some of the CD4⁺ srT cells may represent immune-suppressive Tregs. In this regard, it should be mentioned that we have previously described HLA-A2-restricted CD8⁺ T cells that recognized heme oxygenase-1 (HO-1). HO-1 is an anti-inflammatory molecule stress molecule [37]. Interestingly, the specific CD8⁺ T cells were not classical effector CTL but exerted a very strong immune-suppressive effect [1]. The connection of cellular stress and the regulation of adaptive immune responses add a new dimension to the potential function of srT cells. The importance of TCR-mediated signals for the function of Tregs is still unknown. However, the presence of antigens is thought to play a role in the generation and

maintenance of Tregs [39]. The signals within the thymus during T-cell differentiation that lead to lineage specificity are still debated, yet it is established that nTregs arise in the thymus [38]. Thus, CD4⁺ srT cells specifically recognizing peptides derived from self-proteins like IDO or PD-L1 may represent antigen-specific Tregs. Moreover, the CD8⁺ compartment of T cells, as exemplified for HO-1-reactive CD8⁺ immune-suppressive srT cells, is likely to contain regulatory T cells (Fig. 1).

Summary and perspective

srT cells may be important for the fine tuning of immune responses by suppressing or supporting the function of immune modulating cells. The substantial numbers of srT cells that are readily detectable in healthy individuals suggest that these T cells presumably contribute to immune homeostasis.

However, it is still an open question how and when srT cells are induced or become activated and to what extent they influence ongoing immune responses. The recognized antigens may be useful for immunotherapy of cancer, where immune-suppressive mechanisms antagonize the desired effects. Counter-regulatory feedback mechanisms such as interferon-induced IDO and/or PD-L1 expression [3, 9, 36, 40] are important to prevent immune responses in becoming so powerful that they could become dangerous to the host. However, when it comes to tumor immunotherapy, this immune evasion is detrimental to the host. By meaning, all immune therapeutic strategies aim at activating the immune system to destroy the tumor. Since immune-suppressive cells antagonize this therapeutic goal, targeting these cells by vaccination is an attractive option to boost immunotherapy. Notably, the preclinical proof-of-concept of this approach has already been provided by the work of Gilboa and colleagues [27].

The induction of srT cells represents a new and intriguing concept for immune therapeutic interventions, in which the specific depletion of undesired cells is not limited to targeting functional proteins present on the cell surface, which already is very successful beyond expectation [35]. An important difference between therapeutically induced srT cells and antibody blockade is that the former not only reduce the target protein-mediated suppression, but also additional immune-suppressive effects mediated by the target cells.

The significance of srT in vivo remains to be determined; still, the data summarized in this review suggest that such cells are common in patients with cancer. In general, it is a well-preserved regulatory mechanism to eliminate unwanted cells, as this is commonly happening during the development and homeostasis of healthy organisms [7];

thus, the notion that CTL, i.e., cytotoxic srT cells, are functioning as regulatory cells is maybe not so surprising after all.

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Conflict of interest None of the authors have conflicts of interests to declare. Mads Hald Andersen and Per thor Straten previously have filed a patent application based on the use of IDO for vaccination. In addition, Mads Hald Andersen has previously filed a patent application based on the use of PD-L1 for vaccination. The rights of both patent applications have been transferred to the University Hospital at Herlev according to Danish Law of Public Inventions at Public Research Institutions.

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