Review Article
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Th17 cells: can epigenetic manipulation and artificial t cell receptors make th17 cells efficient immunotherapeutic anti-tumor effector T cells?

Abstract

Understanding T cell immunity to cancer has allowed the development of a new form of cancer therapy, which makes use of genetically engineered T cells that have chimeric antigen T cell receptors or CAR T cells. This type of cancer immunotherapy has led to an exploration of the T cell immune system for T cells that can be genetically engineered to be efficient anti-cancer immunotherapeutic effector T cells. CD8+ cytotoxic T cells were the first candidates. However, now other T cells or T cell phenotypes are sought after because of their advanced effector functions and their ability to survive during the immunotherapy process. Th17, which can be created from naïve T cells and more importantly from Tregs are able to not only survive for extended period in the body, but are also efficient effector T cells. This review will examine the epigenetic changes in the T cell genome related to the creation and derivation of the Th17/Treg cell phenotypes. Manipulation of two signature genes (ROR-gt and Fox-P3) could result in the creation of new type of immunotherapeutic effector T cell. The use of Th17 cells for the immunotherapeutic treatment of cancer will depend on the ability to alter their function, modify their T cell receptors, and arrest phenotypic differentiation.

Keywords: Th17 cells; immunotherapeutic effector T cell; T cell receptors; adaptive immune system; anti-tumor effector T cells

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Introduction

In the future cancer therapy will change because chemotherapeutic agents have become more sophisticated and will be augmented by immunotherapeutic treatments. Cancer immunologists have recently developed genetically engineered T cells that can be used as anti-tumor effectors.[1] Th17 cells, despite the fact they can induce autoimmunity, and destroy homeostasis,[2] seem to be the perfect candidates for use as immunotherapeutic tools. Two of the main reasons why Th17 cells are good candidates for this role is their plasticity (converting into other T helper phenotypes), but also their ability to survive for extended periods through proliferative self-renewal and anti-apoptosis mechanisms.[3] As far as the adaptive immune system is concerned Th17 cells, like Tregs are a conceptual T cell phenotype, which are created by the activation of specific genes (ROR-gt and Fox-P3 respectively) through detection of immune environmental signals in the form of lymphokines.[4] Continued research demonstrated that Th17 cells can be created from Tregs, but are also created in the thymus[5] in a different thymic location from Tregs and conventional ab T cell receptor (TCR) T cell phenotypes.[6] Th17 cells not only have the attributes of T cells (lymphokine homing, tissue migration, and dual T cell receptor specificities), but also have subtle and permanent T cell effector mechanisms.[7] The initial purpose of these cells was thought to be anti-pathogenic, but Th17 cells began to appear in autoimmune inflammatory responses where the cells would break immune tolerance and damage normal tissue.[8] They've been implicated in multiple autoimmune diseases (systemic lupus erythematosus or SLE, diabetes, pancreatitis, and arthritis) yet haven't been fully understood.

The problem with using these cells as genetically engineered effectors is how to control them. To achieve this goal the phenotypic plasticity of Th17 cells in terms of what types of lymphokines (INF-g) they secrete, and their ability to induce secretion of vascular endothelia growth factor (VEGF) by tumor cells would have to be controlled in order to use them to fight cancer, as well as other diseases.[9] The main advantage to having the Th17 phenotype attack cancer is two-fold. The first thing is that they do not (in general) home to a particular area of the body. They can be found in the circulation as well as the tissues. As a matter of fact, the presence of Th17 cells has diagnostic value in certain types of cancer.[10] The second and most important reason why Th17 cells are perfect for immunotherapeutic use has to do with the conversion process, which can turn protective Tregs into autoimmune anti-cancer effectors. Th17 T cells can do this, but they also can function as immune surveillance T cells. The Th17 cell effector mechanisms have been under-rated. Secretion of IL-17 by these cells can kill a pathogen, but can also neutrtrlise neoantigen-specific tumour cells. However, the incredible plasticity of Th17 cells does not always allow for this to happen.[11] Once they get to the site many of them are changed. With a TCR that recognizes tumour neoantigens many Th17 cells are converted into Tregs or Th1 cells because of the abundance of IL-2 and TGF-b and TNF-a secreted by Th1 cells in response to the initial inflammation caused by the growing tumour. The question is why would Th17 cells attack cancer? This review will offer a brief overview of the cellular dynamics (antigen-specific T cell responses/inflammation and T cell tolerance) involved with the immunology of cancer as it relates to Th17 cells and Tregs. In addition, the role and interrelationship between Tregs and the Th17 cell phenotype will be examined. This will be followed by a brief description of the intracellular translational pathways responsible for the development of these two phenotypes. Most importantly, there will be a discussion of DNA epigenetic changes that occur during the conversion process. These changes control the two-way Th17/Treg T cell conversion process. That process may ultimately control the immunologic success or failure of newly developed anti-cancer immunotherapies not only based on epigenetic manipulation, but also the effector functions of genetically engineered TCRs. Based on this information, it should be possible to examine these genetic changes and expand our knowledge of the T cell immune system.

T cell epigenetics

Mid-phase Tregs (Treg-to-Th17 cell) can promote a Th-1 response by the secretion of IL-17, but also just like their former selves (antigen-specific Tregs), they leave behind memory T cells.[12,13] These memory T cells constitute the rapid response to recurrent infections. However, the core question is: What is happening at the DNA level in these T cells, which initiate the first immunologically-based T cell response? The most important aspect of this phenomenon is to examine DNA modifications (epigenetic changes) to the target genes (ROR-gt and FOXP3) that occur in these two T cell phenotypes, which induce both autoimmunity and immune tolerance.

Recently it has been recognized that the function of the T cell immune system is governed by the modulating expression of genes, which control T cell behavior and also the phenotype of any T cell that finds itself in a particular immune environment. This is a special form of T cell differentiation; separate from thymic differentiation in that it occurs outside of the thymus. In many cases it can be reversed via a change in the immunological environment. The basis of these changes is focused on the remodeling or modification of DNA chromatin structure.[14] This chromatin-remodeling process alters DNA structure in such a way as to permit or deny accessibility of transcriptional proteins to regions (promoters, exons, etc) in order to control the T cell's phenotype and function. The rearrangement of chromatin structure and its ability to facilitate the binding of transcription proteins many times is carried out at the DNA strand level and at the chromatin level at the same time. This requires a coordinated effort by inducible transcription factors, chromatin-remodelling complexes, histone-modifying enzymes, and chromatin-associated signalling kinases.[15] This is a description of epigenetics, which is defined as gene expression other than that caused by the DNA sequence itself.[16] DNA forms nucleosomes, which are densely packed regions surrounding a core of histones. Alterations of this configuration include a series of modifications such as methylation, phosphorylation, ubiquitinylation, and acetylation.[17] Once modified, the chromatin structure takes on an epigenetic signature, which is characterized by the expression or not of specific gene products such as transcriptional proteins NF-kB, AP-1, and the genes of the STAT family.

T cell translational pathways

These transcriptional proteins mentioned above bind to promoter regions on genes such as ROR-gt and FOXP3. This signature can be rapidly written or erased within the same T cell. The focus here will be on two enzymes (histone acetyl transferases or HATs and histone deacetylases or HDACs) that are members of a large family of enzymes that take on the responsibility of modifying chromatin structure.[18] These enzymes can modify lysine residues on histones. They can also alter the expression of some the transcriptional proteins mentioned above. Acetylation of histones by HATs opens the chromatin structure allowing transcriptional proteins that activate gene expression to enter previously inaccessible regions of DNA.

Conversely, histone deacetylases (HDACs) close chromatin structure, which prevents genetic expression via promoter activation. This is one of many coordinated modifications to chromatin structure that occur during a T cell immune response. This function enables T cells with an ability to adapt to changing immunological conditions. In addition, this activity is critical to the immunological balance between Tregs and Th17 T cells.

Here we have a situation where epigenetic changes dictate the functionality of Th17 cells. It is not a leap of faith to believe modifications in DNA structure control their behavior. This fact, and the ability of this phenotype to adapt to uncertainty, makes them perfect anti-tumor T cell. However, in reality, these changes, which will create an anti-tumor Th17 T cell, can initiate an autoimmune response as a byproduct. But what about their T cell receptor? Anti-tumor Th17 T cells, which have been converted from Tregs will have a specific TCR's that recognize tumor antigens and can secrete IL-17A.[19] Th17 cells attach themselves to the target cell and kill it via cell-to-cell contact mechanisms or secrete enough IL-17 to activate Th1 T cells. These cells were initially designed to fight-off infections, but unfortunately they have participated in the destruction of normal tissue systems. After re-tasking the Th17 cell it can be an effective tool used during adoptive therapy. Once Th17 cells are 'frozen' in the effector mode, their TCR can be manipulated. The ability to 'freeze' Th17 cells once they have been differentiated into effector cells is possible. Studies involving the ROR-gt gene have revealed its ability to control IL-23 secretion and the elaboration of gINF, which can induce a Th1 CD8 effector T cell anti-tumor response. In addition, IL-23 assures Th17 cell survival as not memory T cells, but active effectors.[20]

Genetically altered T cells

Chimeric T cell receptors or CAR have been established to fight cancer.[21] CAR T cells have been successfully used to treat B-cell lymphomas and at the time there were safety issues associated with these patient-modified T cells, as well as their stability in the body.[22] The reason why Th17 cells are perfect candidates for immunotherapy is the fact that they survive in the body for extended periods.[23]

The programming of Th17 cells must to be exact. Th1 T cells from the patient must be configured to recognize tumour-specific antigens prior to Th17 conversion or tumor-specific Tregs can be converted into Th17 cells. The recognition of neoantigens by these cells makes or breaks their effectiveness. The next step is a precarious because of the conversion process. Adoptive immunotherapy using Th17 cells can result in the death of the patient unless the cells are frozen in a particular phenotypic phase, which allows them to attack the cancer and not revert into the Treg phenotype. Aberrant conversion of Th17 cells into the Treg phenotype would be a disadvantage. The idea is to have Th17 cells in the 'between phase.' Tregs can be converted into Th17 cells and Th17 cells can be converted into Tregs. During the conversion period (Treg-to-Th17 cell), there is a diminution of anti-inflammatory lymphokines (IL10 and IL35) and an increase in TH-1 supporting lymphokines (IL7, IL4, and IL-12). Cancer cells are susceptible to attack from the anti-tumour T cell immune system if they are not protected by Tregs. If the tumor site is flooded with healthy anti-tumor T cells, the Tregs are overwhelmed by a lymphokine storm initiated by a Th1 T cell response. Under these circumstances the tumor gets killed. There are a sea of T cells that can do this job. Th17 cells can do the job without haste because once in an environment that is controlled by Tregs, they can deconstruct homeostasis and attack the cancer through the conversion process by APC's secreting IL-1b, IL-23, and IL-21 during an inflammatory response. This conversion process depends on histone/protein deacetylase activity.[24]

The TCR: Chromatin Remodeling equals advanced effector mechanisms.

CAR T cell constructs have advanced. Initially, these artificial T cells, extracted from a patient, were initially designed to attack B-cell lymphomas. Some of the early patients' loss they're lives because there were problems with T cell constructs homing to the lungs.[23] The question is: How do you create an anti-cancer T cell that not only

kills the tumor, but after its death leaves a memory T cell in case of recurrence. Creation of this flux state between anti-autoimmune Treg cells and pro-inflammatory Th17 cells is the key. Using systemic inhibitors of the ROR-gt and FOXP3gene is not an option. If both or either of them were disabled the patient would die from simple pathogenic infections or GVHD. There is a phase where Treg cells start to turn into Th17 cells. This is the key to using Th17 cells as anti-cancer T cells. Producing these transitional cells in vitro or on an immunotherapeutic scale is the goal. Transcriptional proteins are the driving force behind the T cell immune system. The basis of these changes is focused on the remodeling or modification of DNA chromatin structure such as with SLE.[25] Chromatin remodeling alters DNA structure in such a way as to permit or deny accessibility of transcriptional proteins to DNA regions (promoters, exons, etc) in order to control the T cell's phenotype and function. The rearrangement of chromatin structure and its ability to facilitate the binding of transcription proteins is carried out at the DNA strand level and at the chromatin level simultaneously. This requires a coordinated effort by inducible transcription factors such as chromatin-remodelling complexes, histone-modifying enzymes, and chromatin-associated signalling kinases.[15]

As mentioned above once modified, the chromatin structure takes on an epigenetic signature, which is characterized by the expression or not of specific gene products such as transcriptional proteins NF-kB, AP-1, and the members of the STAT family, which bind to promoter regions on genes such as ROR-gt and FOXP3. This signature can be rapidly written or erased within the same T cell. The focus here will be on two enzymes (histone acetyl transferases or HATs and histone deacetylases or HDACs) that are members of a large family of enzymes[18] that take on this responsibility. These enzymes can modify lysine residues on histones and also some of the transcriptional proteins mentioned above.

Histone acetyl transferases or HATs and histone deacetylases (HDACs) can be used to alter the Th17 phenotype, but if just one Th17 cell in that transition period looks at other cells related to the tumour as an autoimmune target, game over. As therapeutic T cells, created Th17 cells must be altered by epigenetic treatment to be fully capable of the task of removing the cancer forever. The idea behind CAR Th17 cells as anti-tumor T cells does have merit,[26] but these T cells must be in total control. Controlling Th17 cell differentiation is a difficult task. A mistake in this process may result in a catastrophic autoimmune response that would most certainly result in the death of the patient. Establishment of a Th17 cell effector phenotype with patient T cells prior to engineering a CAR T cell effector is not really the problem. The major problem is disabling the expression of several genes that induce widespread autoimmunity. The expression of just one of those genes in adoptively transferred CAR Th17 cell effectors would certainly lead to a fatal autoimmune response. Expression of the IL-10 gene is another one, which would induce the creation of Tregs. Technologies to produce high quality long-lived Th17 cells, which have Th1 qualities, exist through the inclusion of ICOS gene in the CAR endodomain. However, the authors responsible for this advancement admit unpredictable effects and point toward the importance of clinical trials to answer lingering questions about the efficacy of genetically engineered anti-tumor Th17 cells.[27] Immediate removal of basic lymphokine genes from therapeutic T cells is not really a problem. Topoisomerase enzymes do this all of the time as a normal maintenance function.[28] The removal of the targeted DNA segment is an efficient process, but the insertion of the replacement represents a problem for the creation of immunotherapeutic T cells.

The DNA excision/repair function that occurs in every healthy cell is now being scrutinized. A study suggests that a robust maintenance of this system will lead to improved human health.[29] Ultimately, and after altering the Th17 cells prior to CAR transformation, advanced CAR technology[30] can be applied. These newly created Th17 anti-cancer T cells will be armed with TCRs that seem to 'think' when they encounter targeted tumour antigens and can adjust the TCR to accept another tumor antigen. In this way, autoimmunity is avoided, and recognition of the tumour is successful. Constructs such as these are based on the mathematics of the orthogonal array where numbers (1,2,3, etc.) are arranged in such a way as to predict/describe structure. These new TCR constructs are a result of the focus on synthetic biology.[31] CAR receptors in patient Th17 cells can be used to arm epigenetically altered immunotherapeutic T cells, which will be able to eradicate lethal tumours while leaving memory T cells behind.[32] This is the future of cancer treatment where immune cells from the cancer patient's body are used as a cure for their disease. In this way, tumors can be eradicated without repeated rounds of chemotherapy. This type of cancer treatment begs of an outpatient procedure where the patient comes into the clinic every 6 months for a fresh infusion of effector T cells. And after a year or so without detection of tumor antigens the patient can be classified as disease-free.

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Conflicts of interest

The authors declare there is no conflict of interests.

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