Regulatory T cells and immunotherapy

203 Misako Kuga

Summary

This paper aims to clarify the mechanism of how regulatory T cells are controlled. Tregs suppressive function is used for some immunotherapy, but those treatments are not the ones which work to inactivate or reduce Tregs. To develop more safe treatment, it may be efficient to think about Tregs from the different viewpoints, not from the point of its suppressive function. For this purpose, a specialist of Cambridge University was interviewed some questions. Through this interview, I had gotten some information about how Tregs starts their suppressive function or how immune check points therapy work. Then, considering the results of the interview and research, I have learned that Tregs are controlled is mainly Fas/FasL pathway. This pathway is concerned with various cells, so it is difficult to use for treatments. However, some possibility that there is a mechanism to control Tregs' function selectively will still exist.

Introduction

Currently, the research on human immune system has been improved, not only the development of antibody medicine, but also the new types of immunotheraphy like CAR-T cell therapy (One of the treatments for leukemia. T cells are removed from a patient and applied to gene manipulation to strengthen them to attack cancer cells. These T cells which are called CAR-T cells are multiplied and administered to the patient himself.) (Article of The Chunichi Shinbun, 2019.3.2) are well known. Also, it is clear that CD25＋CD4＋T cells have the effect on the development of autoimmunity (Sakaguchi et al,.2008). This suppressive T cells were named Regulatory T Cells (Tregs) and they work to regulate immune responses. The research question in this paper is "What is the mechanism of inactivating or decreasing Tregs?". This question is meant to develop the new treatment for cancer by weakening Tregs' function and maintaining Cytotoxic T cells' (TCLs') function for cancer cells. The representable cancer immunotheraphy involved with Tregs which is available is anti-CTLA-4 mAb (Ipilimumab). Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is a negative regulator of T cells' function (Peggs et al., 2009). Human anti-CTLA-4 mAbs is mainly used for melanoma. However, this antibidy works to inhibit the conection between Tregs and TCLs, so it does not work to reduce the number of Tregs. Besides, this treatlent has a problem of side effects. Activation of the immune system can also lead to immune responces to normal tissue and consequently to autoimmune side effects (Kahler et al.,2016). It may be able to reduce the risk of side effects by developing new immunotheraphy.

Fundamental

1. T cells

T cells are immune cells which mature in the thymus. Immuture T cells often called preT cells are produced in bones, and they move to tymus. After that, preT cells differentiate into various T cells. They are classified into CO4＋T cells and CD8＋T cells. CD4＋T cells are connected with Major Hiscompatibility Complex Ⅱ (MHCⅡ), and classified into more detailed groups (Th1,Th2,Th17,Treg). Th cells (T helper cells) are presented antigen by antigen presenting cells, and they stimulate other cells like TCL, macrophage (MΦ), and B cells to activate immune responces. On the other hands, Tregs works to suppress other CD4＋T cells. CD8＋T cells are connected with MHC Ⅰ, which is expressed by normal cells, and lead infected cells apotosis (programmed cell death). Activated CD8＋T cells are called TCLs.

2. Centrality Immune Tolerance

T cells express TCR (T cell receptor) to recognize each antigen. PreT cells differentiate into CD4＋CD8＋T cells in a thymus, and express TCR. These T cells meet thymic cortical epithelial cells, which express

MHC, and T cells which cannot recognize MHC die, then survived T cells lose either CD4 or CD8. After that, they meet medullary epithelial cells presenting self-antigen. In this process, self-reactive T cells are excluded (clonal delition) or replace self-reactive TCR with nonreactive ones (receptor editing.) (Morita, 2017.Sakaguchi, 2008). However, Tregs are weakly recognizing self- antigen (Ono and Tanaka, 2017).

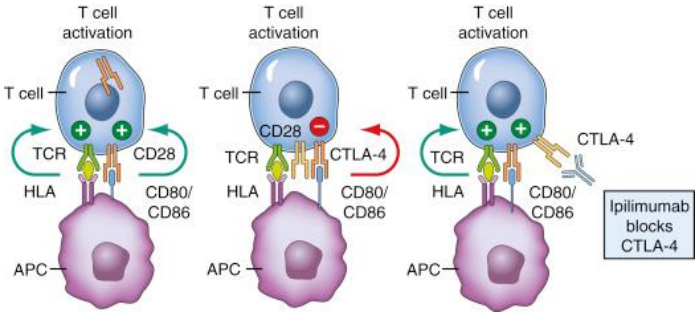
3.Treg

Tregs are often recognized as FoxP3＋CD25＋CD4＋T cells. The transcription factor FoxP3 (forkhead box P3) is critical for T cells to differentiate to Tregs. CD25 constitute IL-2 (interleukin 2) receptor and express on Tregs. Tregs occupy about 10% of CD4＋T cells. They have some ways to regulate other T cells. First, they express CTLA-4 and block the connection between CD80/CD86 (expressed on antigen presenting cells) and CD28 (on TCLs). These inhibit the activation of TCLs. Second, CD25 on the T cells are connected with IL-2, and prevent them from being activated. Third, Tregs produce suppressive cytokine, IL-10. These are the examples. Tregs have a lot of complicated routes to control immune systems (Taniguchi et al., 2013).

4. Immune Checkpoint Inhibiting Therapy

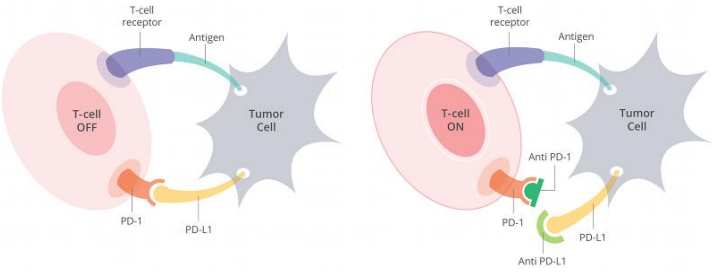
Ⅰ, Anti CTLA-4 mAb

CTLA-4 is one of the inhibitory costimulatory molecules. Activated T cells will express CTLA-4 after sone work. T cells are activated by a connection between CD28 and CD80/ CD86. However, CTLA-4 combine with CD80/CD86 more prior, and this connection leads the T cell anergy (functionally inactivation) (Saito,2018, P112). In addition, CTLA-4 rob antigen presenting cell (APC) of CD80/CD86 (Kubo, Hirohashi and Torigoe, 2016). Tregs are involved in this relation. Tregs also express CTLA-4 and inhibit T cells' activation. Anti CTLA-4 mAb not only inhibits the suppressive function of Tregs, but also enhances T cells by preventing them from being inactivated. Anti CTLA-4 mAb is used as a medicine for some kinds of cancer. It is called Ipilimumab, and it started to be used in Japan in July 2015 (Kubo, Hirohashi and Torigoe, 2016).



↑ The mechanism of CTLA-4

Ⅱ,Anti PD-1 Antibody



↑ The mechanism of PD-1

Programmed cell death-1(PD-1) is expressed in activated T cells. Their ligands are PDL-1 and PDL-2. PDL-1 is expressed in normal cells. Otherwise, PDL-2 is expressed in a dendritic cell (DC), MΦ and B cell. Importantly, some cancer cells also express PDL-1, so T cells are inactivated by cancer cells (Saito, 2018, Kubo, Hirohashi and Torigoe, 2016). Anti PD-1 antibody (Nivolumab) connects with PD-1 and maintains the T cells' activation, and is used for cancer immunotherapy.

Ipilimumab and Nivolumab are known as immune checkpoint inhibiting therapy.

Methodology

In this paper, I show one main research question and three more subsidiory questions to deepen the understand this field more deeply.

The main question,

1) In the end of the inflammation, how Treg cells are controlled in vivo? Is there something which workes as the factor?

The subsidery questiins

2) How Treg cells decide when to start their suppressive function?
3) What is the reason that self reactive T cells become Treg cells? Is there any benefit to use those cells?
4) How can we reduce the side effect of anti CTLA-4 mAb? Is it effective that inhibit the Tregs function directory?

Hypothesis for these questions

1) ‣Something like special substance or cytokine control their function.
‣The connection between receptors cause suppressive signal.
‣The cell death caused by the life span.
2) A substance produced by antigen presenting cells stimulate Treg cells.
3) Treg cells aim to protect self-cells, so by recognizing self-cells, Treg cells can understand what they have to protect.
4) Something work for inhibiting Tregs directly can be used for the treatment.

↑ Dr Nick Holmes
Department of Pathology

. To ask these questions, I met with Dr Nick Holmes, Division of Immunology Department of Pathology University of Cambridge, on July 30, 2019 at the Department of Pathology.

Results

1) In the end of the inflammation, how Treg cells are controlled?

A(Answer): Effector T cells need to be controlled by the counter-balance of regulatory T cells. It is an important thought of regulatory process, but that is not the only thing. It is not important “at the end of the inflammation” actually. It is more important early on. Thus, it is normally when if we have a successful immune response. This means that the immune response eliminate antigen, and antigen eliminate the stimulus. That is driving immune response, so the level of antigen went down, the number of T cells also went down. Basically, the infection is taught with simple models. It starts in a small number of organisms and they replicate over time, so the amount of the antigen goes up. Then, that starts triggering immune responses. Immune response starts the replication to platoon to become more static and then gradually the immune response starts to stop the pathogens during the infection. When it comes to the point where the stimulus is illustrated, it does not really need. Any other factor to stop the inflammation no longer stimulate or lead the interaction of other response on itself. The fluctuation of the number of T cells had the same that antigen went down. Probably, it is not very dependent on Tregs. The things that very dependent on Tregs is in the peek. (Figure,3)

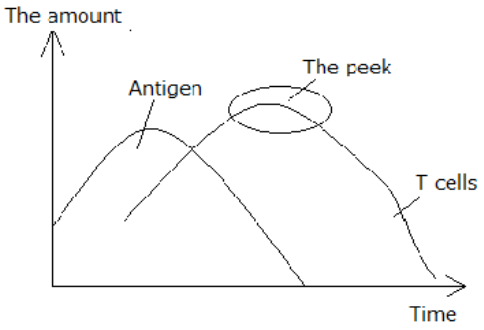


Figure3, Fluctuation of antigen and T cells.

2) Why Tregs strongly effect to effector T cells at the proper timing?

A: The clearly one of the reasons is likely to be the Treg cells they have the different shape before they expand. It depends on what type of Treg cells talking about. Now talking about Tregs that prevent the response of the

immune system to self-tissues what we call natural Tregs or in Tregs. Tregs regulate responses to something from the outside of the body like a virus, those Tregs part of that does not present before a virus infection or prevent a very small number. There is a “lag”. Effector cells expand different shapes faster than the Tregs control them. This is because, T cells respond their sort of responding to the expansionary effector cells. They Tregs all of this point, when people have a virus that they have never seen before, all of the T cells what called are naïve T cells start out. They differentiate to different types of T cells. The question is the differentiation between these processes. They are not only differentiating, but expanding, dividing, and increasing the number. The question is whether the process of differentiation of two types of T cells. Effector T cells are differentiating more quickly.

Additional question 1) If Tregs increase faster than effector T cells completely attack to pathogens, what will happen? CAn this situation happen?

A: It is impossible.

It depends on the antigen. I think it could only happen they virus which contains antigens very similar to self-antigens. In that case, in the Tregs might present very higher numbers before versus virus. If they start at the higher number, that could happen. In this situation, these Tregs work for frequent before effector naïve cells can respond.

Additional question 2) T cells are controlled by cytokines?

A: Yes.

Additional question 3) Then what cytokine control?

A: That is very complicated. Innate cells like macrophages and dendritic cells are illuminated influence cells. These guides contribute some of the cytokines particularly in early days of response, and then you have the cytokines that are made by the T cells and B cells which are responding to the infection. There is a complex interaction. That interaction changes over times because there are very few antigens pacific cells at the very early time. Most of the cytokines coming from the innate cells. Cells have specific antigen receptors co-stimulated by DNA or LPS (lipopolysaccharide). By a some bacterial LPS whatever fragile in the bacterial cell membrane or RNA from the replicating virus or whatever. These are pattern recognition molecules. You have receptors that respond to these pattern recognition molecules. For example, there is a sense in the cytoplasmic cells look out for these things because they know that they indicate infection. The same things are true of different types of DNA as well, so you can sense the presence of these pathogens in whole variety of different ways of some of the macromolecules they made for this surface. If it is not a pattern recognition molecule, receptors respond all kinds of different things inside the cells, because you have to be able to detect different types of infections. You need a wide variety of mechanisms and triggering these innate cells through the receptors are stimulated cytokine production. It depends on different cytokines which will be stimulated by different triggers.

3) I read some report say self-reactive naïve T cells change into Treg cells, is it true?

A: T cells develop in the thymus, thymus is the organ where cells come from the Born narrow, and those cells come from a born marrow do not have antigen receptors. During the thymus, they expand very much, and they develop their antigen receptors. During this process, these antigen receptors get tested against self. Cells that that have a very low reactive, these cells die during the thymic diorama, so we require cells which have some affinity to self, and what they are recognizing is MHC molecules with peptide bund. However, they have too much affinity then they are eliminated in a different way. The guys of the middle of them go out to become the T cells or naïve T cells. Some of the T cells in the thymus differentiate into Tregs in the thymus that is called natural Tregs or tTregs. You can get very few T cells differentiate in Tregs at the area of low reactivity, their profit is a little more bit like that tends to have slowly high affinity. It is a probability thing. These type of naïve T cells sometimes become Tregs and sometimes not. If we eliminate all the self-reactive cells, then it would be too easy for pathogens to evolve to be unrecognized. In human immune system, we have about 3 hundred million cells, and about 30% of those 90 million cells are the CD4 cells. Besides, 15% of those 90 million CD4 cells become Tregs. In this case, the people get about 15 million Tregs. By this experimental thought you can easily kill all of these cells in two or three days. What happens if you do that? You basically get autoimmunity, because T cells except for Tregs are no longer controlled by Tregs. This shows these cells do have self-reactivity not all the 90 million cells (CD4 cells) but some of them. It shows that those cells are kept in check by some of the Tregs.

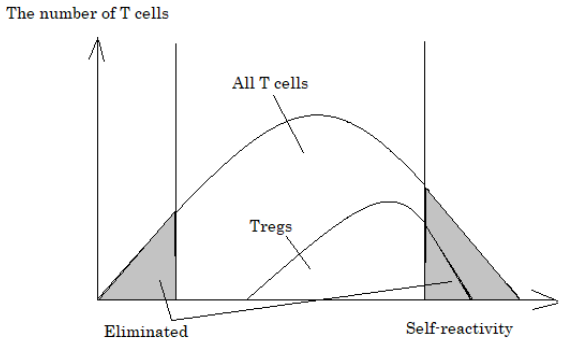


Figure4, Fluctuation of the number of T cells and Tregs. Concern between self-reactivity and the amount of Tcells.

Additional question 2) Why the peek of the Treg cells come to the point (a)(Figure3)? Not like (b)?

A: We do not know really why that is. You know basically if you think in individual cell. When the T cell receptors interacting with the antigen presenting cells, then you get the series of biochemical reactions occur inside the T cell. The cell behaves differently depending on how much of the signal being received. The more signal was received, the more cells die. If you receive too small a signal, the cell dies. Somewhere in the middle has to make it decision does they differentiate into a naïve T cells or into Tregs. Besides, that decision is led by the amount of signal being received, so the more signals you are receiving, the more likely you ought to be a Treg but exactly how the process is controlled we do not understand.

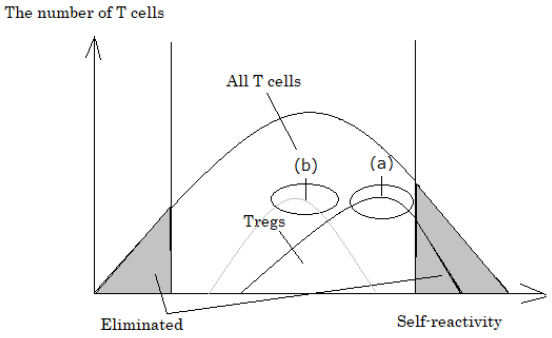


Figure5,

4.1) I would like to know immunotherapy. I read that some immunotherapy like anti CTLA-4 mAb cause serious side effects. Then how to reduce the side effects?

A: CTLA-4 is an interesting molecule. It appears on Tregs and it is expressed at a low level on all naïve T cells. However, it is not expressed on the surface but it is expressed inside the cells when the naïve T cells become activated. Some of its traffic to the cell surface is not as much as Treg cells. If you use antibodies to CTLA-4,

then that antibodies could potentially work in various ways. We think that in the majority case that work by blocking interactions this CTLA-4 with target molecules. Its target molecules are the two molecules called CD80 and CD86. These guys are what we call costimulate molecules. The antigen presenting cells stimulating it have expressed CD80 or CD86. You need both these guys to activate your naïve T cells. The anti CTLA-4 antibody work by blocking the interaction with two molecules. Nevertheless, it is not hundred percent clear only that way. The side effect you guess by treating people by CTLA-4, a primary with development of autoimmune responses. Therefore, when you just treat patients with anti CTLA-4 , 10 or 15% of those who develop a disease, so they start to attack the environment, or they can develop other types of autoimmune disease like diabetes or others. That is the common observation, so that it would not be easy to explain the only way which antibodies work to prevent the activation of naïve T cells. There are also possibly inhibiting reaction of Tregs, because Treg function is very complicated. There are a lot of different ways in which they work. However, we know one of the ways they work CTLA-4 molecule responsible to control the level of these costimulatory molecules on APCs. The Tregs are capable of turning down the costimulation, but CTLA-4 is not really molecule binds CD80 or CD86 on the activated T cells. They are being bound by a different molecule called CD28. Thus, anti CTLA-4 antibody does not lock this interaction. It is a common problem you already read about. It is cancer treatment checkpoint inhibiting therapy. It stops their various and different molecule you can issue for that, but CTLA-4 is one of the early ones. Nowadays, most of the PD-1 interact with PDL-1 is used as the checkpoint inhibiting therapy.

4.2) Is it difficult to reduce this side effect?

A: Yes, it is difficult. It has been tried by treating with both, so quit off the now did I just use one or two antibodies, but it is difficult. That seems to slightly refuse a side effect with the PD-1 treatment that they will CTLA-4, so maybe by using both together you can get a bigger effect which less slightly leave side effects. However, it has not fully eliminated proper for the side effect.

4.3) Is there anything the way to use Tregs to therapy?

A: People have tried that., but it has not been terribly successful. There are a couple of reasons for that. One of them is our mention to which the cells are not necessarily there stable. It is possible to grow the Treg cells in vivo in a laboratory and put them in the patient. However, they must be put back to the same person, and this therapy is very expensive because a lot of work is needed to treat each person. Therefore, it is difficult to treat thousands of people with these kinds of therapy. You would need more scientists to treat everybody seriously an autoimmune disease with Treg therapy. This therapy is called adaptive cell therapy.

Information from afterward literature research

1, Fas receptor and Fas ligand

From the research after the interview, it was revealed that there is a cell death receptor called Fas receptor. Cells have a system of apoptosis. Tcs urge infected cells to apoptosis by stimulating their Fas receptor. Tcs get to express FasL (Fas ligand), and the connection between FasL and Fas receptor causes apoptosis. Actually, activated T cells also express Fas receptor. Activated T cell's FasL connect with Fas receptor on the other activated T cell, simultaneously, opposite FasL and Fas receptor can also be connected. Because of these

connections, activated T cells kill each other. (Saito, 2018, P113.,) Tregs are not the exception. Tregs become apoptotic throw Fas/FasL pathway. (Li, et al.,2013).

Discussion

Considering the results above

1) The T cells' fluctuation not only depends on Tregs' suppressive function, but also the fluctuation of pathogens. T cells are stimulated by pathogens, and their variation is similar to pathogens'. Tregs work in the peek of the T cells' increasing rather than at the end of the inflammation. This result leads a new question, "Why the number of T cells decrease after pathogens eliminate?". To answer this new question, I did literature research. It revealed the existence of the cell death receptor Fas.

2) Owing to the time lag between Effector T cells' differentiation and Tregs', Tregs can control effector T cells at the proper timing. Effector Tcells are differentiating more quickly. The difference of differentiation causes a time lag. Besides, basically Tregs cannot work faster than effector T cells. However, if the antigen which is very similar to self-antigen, there are some Tregs in a body in advance. In this situation, Tregs work faster than effector naïve T cells respond.

3) All T cells have some affinity to self-antigen. T cells which have high or low self-reactivity eliminated at the thymus. Nevertheless, Tregs tend to have relatively high affinity. Not all the high affinity T cells become Tregs. The decision whether naïve T cells become Tregs or effector T cells depends on the amount of the signal.

4) When the patients are treated by anti CTLA-4 antibody, 10 or 15% of them start to attack self-environment or develop other types of autoimmune diseases. To reduce the risk of side effects, both CTLA-4 mAb and PD-1 antibody are expected to be used, but it is not perfect. Now a new type of immunotherapy called adaptive cell therapy is developed. However, this still has some problems.

Conclusion

This paper was aimed to find a mechanism of controlling Tregs and a new treatment for some diseases which has low risk of side-effects. The answer the question, "How Tregs start and stop their suppressive function?" came about. Differentiation into Tregs or effector T cells have different processes, and this can cause time lag. Because of this time lag, Tregs can start working after pathogens are attacked by T cells. Judging from the two facts, the fluctuation of T cells is similar to pathogens' and there is a cell death receptor on the T cells surface, at that point, Tregs are also apoptosis because they can have much more connection with activated T cells which express FasL. To use this regulating system for treatment is difficult because Fas receptor is expressed on so many kinds of cells. However, an immune system is very complicated. There may be other mechanisms to infinite an immune response. It may be possible to find a way to control Tregs function intensively.

Acknowledgement

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Visual material

Figure1,
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/cytotoxic-t-lymphocyte-antigen-4
Figure2,
https://www.smartpatients.com/targets/pd-1
Figure3,4,5
The pictures refer to the ones Dr Holmes draw while the interview.

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