Exploring the mechanism behind the differentiation of immune cells

December 19 2011

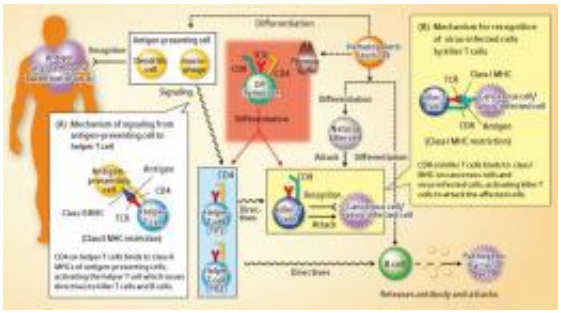

Figure 1: The immune system and the process of T-cell differentiation Pathogenic bacteria and viruses are sensed by antigen-presenting cells, such as dendritic cells, and the signal is transmitted to helper T (Th) cells. Th1 cells direct killer T cells to attack virus-infected cells and cancerous cells, while Th2 cells direct B cells to produce antibodies to attack pathogens. Most immune cells differentiate from hematopoietic stem cells in the bone marrow. T cells, however, differentiate in the thymus, where T-cell progenitor cells from the bone marrow differentiate into DP thymocytes, which further differentiate into either helper or killer T cells. CD4, CD8 and TCR, are expressed on the surfaces of DP thymocytes; CD8 disappears with differentiation into helper T cells and CD4 disappears with differentiation into killer T cells. Credit: RIKEN

Ichiro Taniuchi at the RIKEN Research Center for Allergy and Immunology, Japan, is carrying out research to understand the mysteries of organism evolution by investigating the mechanism responsible for the differentiation of T cells.

When a virus or bacterium enters the body, the foreign 'antigen' is detected and attacked by the body's immune system—a remarkable defense mechanism that has evolved on all living organisms. Immunity is mediated by helper T cells, which serve as the lynchpins in signaling and regulatory functions, and also by killer T cells, which directly attack antigen-infected cells. Ichiro Taniuchi, team leader of the Laboratory for Transcriptional Regulation at the RIKEN Research Center for Allergy and Immunology, is carrying out research to understand the mysteries of organism evolution by investigating the mechanism behind the differentiation of these immune cells.

Roles of T cells in the immune system

All living organisms possess an immune system that senses the entry of antigens, such as viruses and bacteria, and attacks these foreign invaders. This immune response is acted out by immune cells in leukocytes, commonly known as white blood cells. Immune cells are diverse, ranging from dendritic cells to macrophages, T cells (T lymphocytes) and B cells (B lymphocytes). These cells circulate throughout the body in the blood and lymph systems, and upon detection of an antigen, act cooperatively in defense (Fig. 1).

"Lymphocytes are the major player in the immune system in higher animals such as humans," says Taniuchi. "According to their roles, lymphocytes can be classified into B cells and T cells, and T cells can be further classified into helper T cells and killer T cells. Upon entry of pathogenic bacteria or a virus into the body, cells that sense antigens, such as dendritic cells and macrophages, first work to transmit signals about the foreign matter to helper T cells. On receiving the signal, the helper T cells issue directives to killer T cells and B cells. The killer T cells attack virus-infected or cancerous cells directly, whereas B cells release antibodies to attack pathogenic bacteria. The immune system functions are realized through the various roles of the different types of

immune cells."

Th-POK, the master transcription factor for helper T cells

Although they have varied functions, all types of immune cells have their origins in hematopoietic stem cells in the bone marrow. However, while most immune cells are produced in the bone marrow, T cells are produced in the thymus, an organ located near the heart. Migrating from the bone marrow to the thymus, T-cell progenitor cells become involved in the processes of forming diverse T-cell clones that can recognize a wide variety of antigens and distinguish the body's own cells from the invaders. Following this course of 'positive' selection, double positive (DP) thymocytes are prompted to determine their own fate and differentiate into either helper or killer T cells.

"I am working to elucidate the mechanism by which T cells determine their own fate in differentiating into helpers or killers," says Taniuchi. "To understand the mechanism behind the determination of T-cell fate and to differentiate into the two types with different roles is very important for controlling graft rejection in organ transplantation, as well as having a role in allergies, autoimmune diseases and cancerous cells. Artificially creating helper T cells and killer T cells could lead to applications for regenerative medicine and immune therapy."

The two types of T cells are easily distinguishable by the expression pattern for the glycoproteins CD4 and CD8 on the cells' surfaces. Helper T cells only express CD4, whereas killer T cells only express CD8. DP thymocytes, the undifferentiated precursor of these two cell types, express both CD4 and CD8 at the same time.

Most cells in the body contain an antigen-presenting molecule called the

'major histocompatibility complex' (MHC), which allows cells to present fragments of foreign invaders on the cell surface. When a T cell encounters an antigen-presenting MHC, the 'T- cell antigen receptor' (TCR) on the T cell's surface reacts with it and antigen information is passed from the MHC to the TCR.

This is not the full story, however: there are two types of MHCs, referred to as class I and class II. Class I MHCs are possessed by most cells, including virus-infected cells, whereas class II MHCs are possessed only by specific antigen-presenting cells. The TCR on T cells is only reactive with one of these two types of MHC, a phenomenon known as 'MHC restriction.' CD4 in helper T cells only binds to class II MHCs and aids the reaction with the TCR, whereas CD8, only expressed in killer T cells, binds only to class I MHCs. The differentiation of T cells into killers and helpers is therefore directly coupled with this MHC restriction. "It was presumed that signaling from the TCR by MHC restriction may be involved in determining the fate of T cells as helper versus killer T cells, but the precise mechanism remains elusive," says Taniuchi.

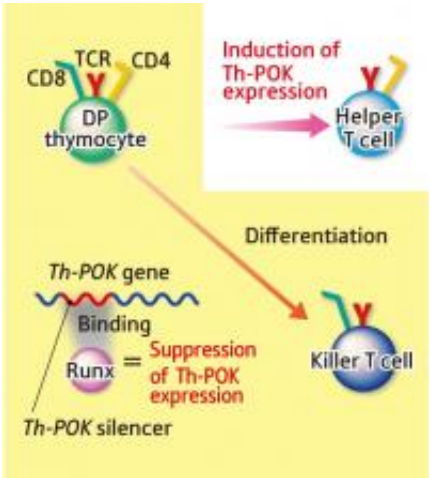

Figure 2: Suppression of Th-POK expression by Runx For the differentiation of DP thymocytes into killer T cells, it is essential that Runx binds to the Th-POK

silencer to suppress the expression of Th-POK. Without normal suppression of Th-POK expression, DP thymocytes do not differentiate into killer T cells. Differentiation into helper T cells requires the mechanism behind the suppression of Th-POK expression to be cancelled. Credit: RIKEN

To solve this mystery, research at the gene level is needed. “In multicellular organisms, all the somatic cells of the same individual carry exactly the same genome,” says Taniuchi. “For example, somatic cells cannot differentiate into skin cells unless only the gene that is necessary for differentiation into skin cells is transcribed from all the genetic information embedded on the DNA. Transcription of genes is regulated by proteins known as transcription factors, which bind to the regulatory regions in the genes. Among these transcription factors, the one that turns on the switch of the developmental program for a particular type of cell is called the ‘master transcription factor.’”

In 2005, a groundbreaking achievement concerning the determination of the differentiation fate of DP thymocytes was published by two research groups from the US. They discovered the master transcription factor for helper T cells, called Th-POK. It was found that in mice bearing artificially expressed Th-POK, all DP thymocytes differentiated into helper T cells, and that conversely in mice deprived of the Th-POK function, all DP thymocytes differentiated into killer T cells. “Hence, the key to lineage fate determination for DP thymocytes depends on whether Th-POK is expressed or not. The next problem to solve is how the helper-lineage specific expression of the Th-POK gene is regulated.”

Runx, the transcription factor for killer T-cell differentiation

In February 2008, Taniuchi and his colleagues succeeded in clarifying

the mechanism behind the expression of Th-POK. Taking note of the transcription factor Runx, which had been assumed to play a key role in the differentiation of immune cells, he identified Runx to be a factor that binds to a 'transcriptional silencer' on the Th-POK gene to suppress its expression (Fig. 2). In the DNA sequence of a gene, there is a regulatory domain for controlling the gene's expression, in addition to a domain where the structure of the protein generated on the basis of the genetic information is written. A transcriptional silencer is a regulatory region that suppresses the expression of a gene when a particular transcription factor binds to it. "We discovered in 2002 that the transcription factor that binds to the silencer of the CD4 gene is Runx. We then thought that Runx may also play some roles in determining the developmental fate of DP thymocytes."

Taniuchi and his colleagues then bred mice lacking the Runx function in DP thymocytes, and carried out some experiments. "First, we examined the mice and found, surprisingly, that almost all killer T cells had disappeared. Due to the lack of Runx, even the cells that were otherwise destined to differentiate into killer T cells differentiated into helper T cells." Next, they examined whether the non-Runx mice exhibited any abnormality in the expression of Th-POK. They found that Th-POK, which would normally have negligible expression in DP thymocytes, was expressed in amounts more than 40 times greater in the Runx-deficient DP thymocytes. "This finding demonstrates that the expression of Th-POK is aggressively suppressed in DP thymocytes by Runx."

While working to elucidate the mechanism behind suppressing the expression of Th-POK by Runx, Taniuchi discovered a silencer region where Runx binds to the Th-POK gene. This Th-POK silencer proved to be indispensable for the differentiation of killer T cells because it suppresses the expression of Th-POK, the master transcription factor for helper T- cell differentiation.

“We bred mice deprived of the Th-POK silencer and found that Th-POK was abnormally expressed and all DP thymocytes differentiated into helper T cells. These experiments revealed the mechanism by which Runx binds to the Th-POK silencer to suppress the expression of Th-POK. Hence, Runx is essential for the differentiation of DP thymocytes into killer cells.”

Increased expression of Th-POK gene essential for helper T-cell differentiation

After announcing their achievement that Runx suppresses the expression of the Th-POK gene, Taniuchi and his colleagues proceeded to elucidate the mechanism behind control of the expression of the Th-POK gene in further detail. In September 2008, they clarified the molecular mechanism by which Th-POK binds directly to the silencer of the Th-POK gene to increase its expression (Fig. 3).

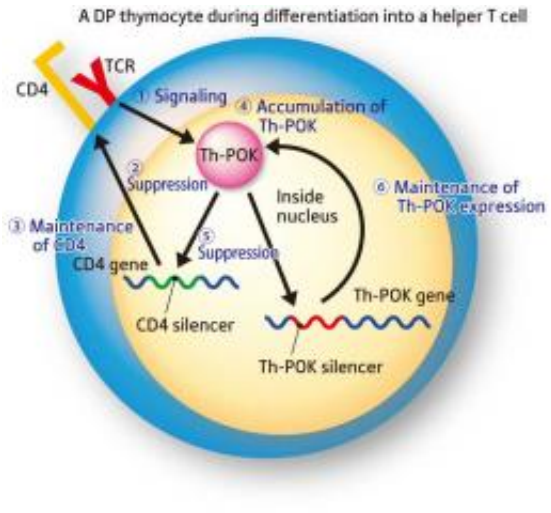

Figure 3: Mechanism by which silencer functioning is stopped to promote differentiation into helper T cells. Credit: RIKEN

“The first step we took was to extensively examine the level to which the Th-POK gene was expressed in the thymus. We found that the Th-POK gene, which is essential for differentiation into helper T cells, was expressed in the process of the initial differentiation of a small but measurable number of killer T cells. This means that even cells expressing the Th-POK gene retain their potential for differentiating into killer T cells,” says Taniuchi. Next, they examined the differentiation potential of a population of cells that had a moderate reduction in the expression of CD8 in the midst of their differentiation process of DP thymocytes. “A reduction in the expression level of CD8 should be indicative of an ongoing process for differentiation into helper T cells. It was known, however, that the potential for differentiating into killer T cells still persists at the stage of a moderate reduction in the expression level. We conducted further experiments to quantify the actual residual potential of these cells for differentiating into killer T cells. As a result, about half were found to retain the potential for differentiating into killer T cells. This suggests that a further increase in the expression of the Th-POK gene is necessary for differentiation into helper T cells.”

Next, Taniuchi bred mice deprived of one of two enhancer regions in the Th-POK gene. An enhancer region is a control region that promotes the expression of a gene—effectively the opposite number to the silencer region. “We discovered two enhancer regions in the Th-POK gene. We had presumed that the enhancer region located downstream was important for maintaining as well as amplifying the expression of the Th-POK gene, and when we bred mice actually deprived of this enhancer region, the enhancer region was found to be necessary for the elevation and maintenance of the expression of the Th-POK gene.”

Further experimentation revealed that Th-POK promotes differentiation into helper T cells and cancels the potential for differentiation into killer T cells. Also, it appears that a sufficient elevation of the expression of

the Th-POK gene is critical for the complete differentiation of helper T cells.

Mechanism behind the elevated expression of Th-POK

“Our investigations have shown that in the process of differentiation into killer T cells, the expressions of the CD4 and Th-POK genes are repressed by Runx as it binds to the respective silencers, that is, as the silencers work. Therefore, conversely, it can be considered that in the process of differentiation into helper T cells, CD4 and Th-POK can be expressed because these silencers are not working. We examined this mechanism using mice and found that the expression of CD4 was maintained as Th-POK suppressed the functioning of the CD4 silencer. It was also found that the expression of the Th-POK gene was maintained as Th-POK binds directly to the Th-POK silencer within its own gene to suppress the function of the silencer.”

Figure 3 shows the mechanism that determines the differentiation of helper T cells, deduced from these experimental results. When a DP thymocyte receives an initial external signal for differentiation into a helper T cell via TCR, expression of the Th-POK gene is induced. The expressed Th-POK binds to the CD4 silencer to make the silencer no longer functional, allowing CD4 to be expressed on the cell surface. Signaling from the TCR continues and expression of the Th-POK gene persists, leading to the accumulation of Th-POK. Meanwhile, Th-POK binds not only to the CD4 silencer but also to the Th-POK silencer in its own gene to inactivate the silencer, which allows the expression of the Th-POK gene to continue and Th-POK to accumulate further. At this stage, the expression of Th-POK is maintained, even without signaling from the TCR, so that helper T cells are able to remain as they are even after leaving the thymus and entering the body.

"The process of differentiation into helper T cells is now understood up to this point," says Taniuchi. "However, how the expression of Th-POK is induced initially remains a mystery. I think probably the point resides in the cancellation of the function of the Th-POK silencer due to signaling from the TCR bound to class II MHC, although the mechanism is still unclear. I feel, however, that the main players are at last lined up as research has progressed with a focus on Th-POK."

Discussing biological evolution from the viewpoint of immunity

"The traces of biological evolution can be found in the mechanism behind the differentiation of immune cells," states Taniuchi. Although killer T cells work as attackers, their defensive measures are rudimentary. Meanwhile, helper T cells are highly functional in that they fight alongside the B cells as a team. "Hence, we have hypothesized that T cells initially emerged as killer T cells, and that helper T cells evolved later. If this is true, we can conclude that the immune system originally existed as an assembly of attackers, such as killer T cells and phagocytes, but later became unable to cope with the invaders merely through such one-on-one battles and hence created helper T cells to build an organized system for immunity."

The human body is cohabited by a diverse range of organisms, including enteric bacteria. The immune system ignores these, but selectively attacks those that must be eliminated. The immune system can therefore be described as the only 'sensory organ' for distinguishing among organisms in the body. "Clarifying the processes of emergence and evolution of the immune system will lead to an understanding of the biological strategies that have been developed by organisms to enable them to cohabit with the wide variety of other organisms on Earth," concludes Taniuchi.

Provided by RIKEN