Press Release

Keio University

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Keio University School of Medicine

Immune System Found to Regulate Cholesterol to Resolve Inflammation

Research opens new doors for treating inflammatory diseases

A research team at Keio University / National Institutes of Health has discovered a new anti-inflammatory mechanism related to cholesterol metabolism. The international joint research team was led by Dr. Hayato Takahashi and Dr. Masayuki Amagai (Department of Dermatology, Keio University School of Medicine, Japan) together with Dr. Yuka Kanno and Dr. John O'Shea (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, USA).

Until now, the immune and metabolic systems have been investigated independently, and their roles have been understood separately. Through this study, the research team has uncovered a mechanism by which the immune system uses lipid metabolism to constrain inflammation. The results of this basic research may lead to the development of new therapies for diseases associated with inflammation.

All cells, including immune cells, use cholesterol, a type of lipid, for cell activity. When cells lack cholesterol, they will actively synthesize it to maintain cholesterol concentration (See Fig. 1). Cholesterol, together with oxysterol[1)], which is formed when cholesterol is oxidized, play important roles in this mechanism, and it is thought that the same mechanism exists within immune cells.

In this study, the team found that $CD4^+$ T cells[2)], a type of immune cell, secrete 25-hydroxycholesterol[3)] (25OHC), a type of oxysterol. The secreted 25OHC acts on surrounding immune cells, weakening their ability to synthesize cholesterol via a mechanism that regulates cholesterol concentrations in cells, resulting in cholesterol deprivation in the cells. Consequently, the inflammatory immune cells lack the cholesterol needed to maintain their activities, leading to cell death and resolution of the inflammation mediated by the immune cells (See Fig. 2).

The results of this research were published online in the international journal *Science Immunology* on October 8, 2021.

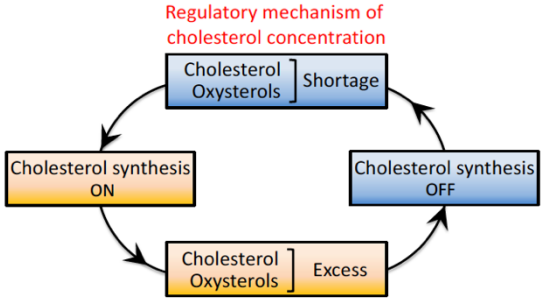

Figure 1

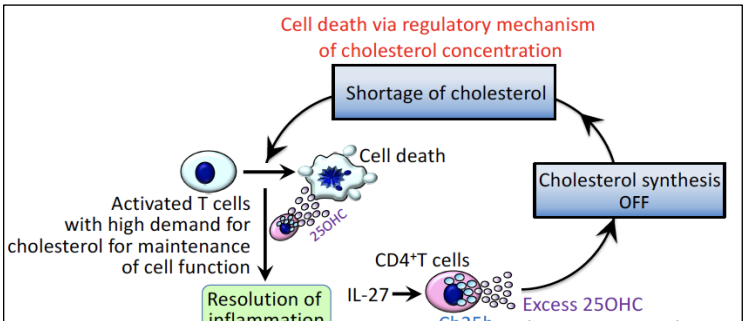

Figure 2

1. Research Background

Cellular activity requires cholesterol, which enzymes in the body metabolize into oxysterol. When the amounts of cholesterol and oxysterols in cells increase, cholesterol synthesis is suspended, thereby maintaining cholesterol concentrations in the cells at an appropriate level (See Fig. 1).

Inflammation in the body can be triggered and resolved without causing excessive tissue damage via various mechanisms. For example, in infectious diseases, inflammation eliminates pathogens such as viruses and bacteria. When the pathogens have been eliminated, the mechanisms that resolve inflammation reduce the inflammation, and the tissues return to their normal state. Interleukin-27[4] (IL-27) is a cytokine that is important for attenuating inflammation. Mice lacking IL-27 suffer unnecessary tissue damage during infections because they cannot control inflammation appropriately. However, the mechanism by which IL-27 suppresses inflammation is not fully understood.

2. Research Results

In this study, we first found that when $CD4^+$ T cells were stimulated with IL-27, the T cells expressed cholesterol 25-hydroxylase (Ch25h) and secreted its metabolite 25-hydroxylated cholesterol (25OHC, a type of oxysterol) (See Fig. 2). Next, we found that activated $CD4^+$ T cells (theoretically associated with inflammation) died in the presence of 25OHC, whereas non-activated $CD4^+$ T cells (theoretically not associated with inflammation) did not (See Fig. 3).

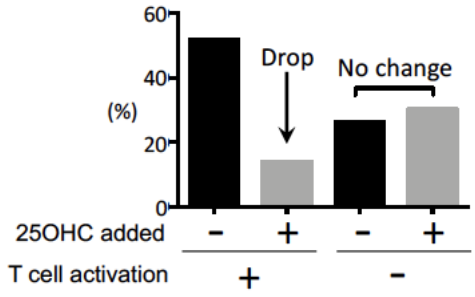

Figure 3. 25OHC-induced cell death was observed in activated T cells, but not in non-activated T cells. $CD4^+$ T cell viability was measured after culture for 2 days under various conditions. 25OHC reduced the viability of activated T cells (14.0% *vs.* 51.9%), but had no effect on non-activated T cells (30.4% *vs.* 26.4%).

We examined the gene-expression profiles of the T cells killed by 25OHC and found that their ability to synthesize cholesterol was significantly impaired. Exogenous supplementation of activated-T-cell cultures with cholesterol prevented 25OHC-induced cell death, indicating that it was a lack of cholesterol in cells that caused the 25OHC-induced cell death (See Fig. 4).

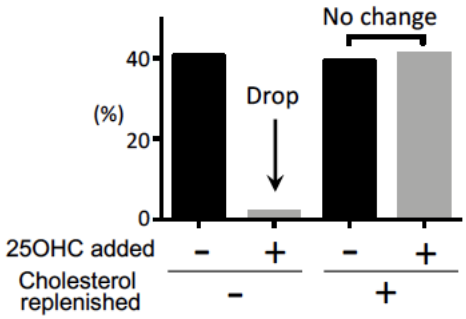

Figure 4. Exogenous supplementation of cultures with cholesterol prevented 25OHC-induced cell death. In the absence of cholesterol supplementation, 25OHC added to the culture decreased T cell viability (1.9% *vs*. 40.7%). With cholesterol supplementation, 25OHC did not decrease viability (41.2% *vs*. 39.3%).

We then examined the function of Ch25h in vivo using an animal model in which autoreactive $CD4^+$ T cells attack epidermal cells in the skin, causing dermatitis. We found that Ch25h was expressed in skin-infiltrating T cells at the inflamed site but not in the T cells in skin-draining lymph nodes. Furthermore, in mice lacking the IL-27 receptor, Ch25h expression in skin-infiltrating T cells was reduced (See Fig. 5), and dermatitis worsened. Simply deleting the Ch25h gene in the disease-inducing T cells also exacerbated dermatitis (See Fig. 6).

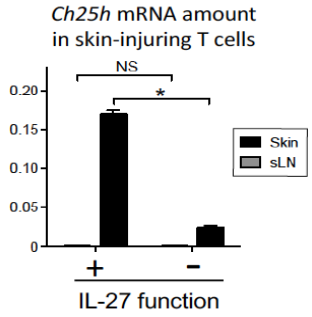

Figure 5. *Ch25h* expression in skin-infiltrating T cells was reduced in a dermatitis model using mice lacking the IL-27 receptor. T cells that can attack the skin were isolated from the skin (black bar) and skin-draining lymph nodes (sLNs, gray bar) of the dermatitis model, and their *Ch25h* expression was measured. *Ch25h* expression was observed in T cells from inflamed skin, but not in those from sLNs, and was reduced in the absence of IL-27.

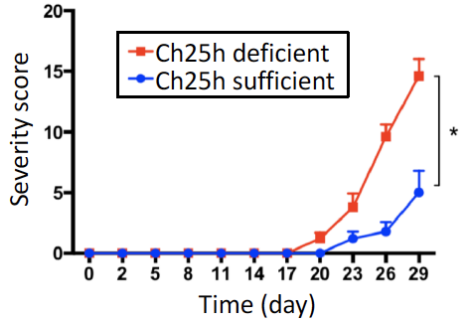

Figure 6. Skin inflammation was exacerbated in the skin-disease model with *Ch25h*-deficient $CD4^+$ T cells. The severity of skin inflammation was evaluated over time in the skin-inflammatory-disease model. Severity scores were significantly higher when disease-inducing $CD4^+$ T cells were *Ch25h* deficient (red line) compared with *Ch25h* sufficient (blue line).

In an animal contact-dermatitis model in which a chemical is applied to the skin of mice to induce skin inflammation, recovery from dermatitis was delayed in Ch25h-deficient mice.

These results imply the existence of an immunoregulatory mechanism linked to lipid metabolism: IL-27 induces Ch25h-expressing $CD4^+$ T cells to secrete 25OHC at local inflammation sites. The 25OHC kills surrounding inflammatory immune cells by inducing cholesterol depletion, ultimately resolving the tissue inflammation (See Fig. 2).

3. Research Significance and Future Developments

Until now, the immune and metabolic systems have been investigated independently, and their roles understood separately. The mechanism that regulates intracellular cholesterol concentrations via oxysterols has long been known to regulate lipid metabolism in many cells. However, its other functions were unknown until now. This research is considered a major step forward in understanding body mechanisms, having established a new concept: immune function uses a cholesterol regulatory mechanism to control inflammation. It has also clarified the interface between the immune and metabolic systems.

The cytotoxic effect of 25OHC is observed only in activated T cells because of the active rate of division and state of high cholesterol demand needed in order to maintain their cellular functions. When cells are unable to produce cholesterol by themselves due to the action of 25OHC, it is thought that the cells cannot maintain their functions and die because they are unable to meet this high demand. However, T cells that are not activated do not have a high demand for cholesterol, so they are less affected by 25OHC and are less likely to die.

Immune cells that are directly involved in the pathogenesis of inflammation are considered activated. If the mechanism discovered here can be used successfully, it may be possible to develop a therapy that kills only disease-causing immune cells. Many conventional therapies that act on the immune system have various side effects because they also act on cells unrelated to the disease. Therapy with fewer side effects could be developed by taking advantage of the results from this study.

4. Research Paper

Title：Cholesterol 25-hydroxylase is a metabolic switch to constrain T cell-mediated inflammation in the skin
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6. Notes

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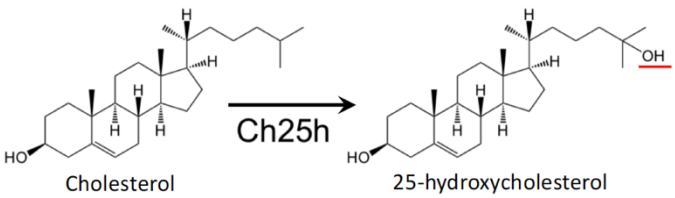
[Glossary]

1) Oxysterol: A general term for compounds produced when cholesterol is oxidized with an OH group.

2)$CD4^+$ T cells: Immune cells expressing CD4 molecules on their surface that play a central role in immune function. $CD4^+$ T cells help B cells produce antibodies and release a variety of cytokines and bioactive substances, which are involved in various pathological conditions such as infection and autoimmune diseases.

3) 25-hydroxycholesterol: A type of oxysterol. The OH group underlined in red in the figure below is added to the carbon at position 25 of cholesterol.



4) Interleukin-27 (IL-27): A type of cytokine. Cytokines are soluble proteins secreted mainly by immune cells; after their release from a cell, they act on other cells, triggering various physiological activities.

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