Press Release

Keio University

Japan Agency for Medical Research and Development

November 2, 2020

Keio University School of Medicine
Japan Agency for Medical Research and Development

Novel Receptor Protein for Short-Chain Fatty Acids Helps Elucidate Defense Mechanism Against Salmonella Infection

A research team at Keio University has successfully identified apoptosis-associated speck-like protein (ASC), a novel receptor protein for short-chain fatty acids (SCFAs) that are produced by gastrointestinal bacteria, and discovered that ASC mediates enhancement of innate immunity to prevent Salmonella infection leading to food poisoning. The team was led by Assistant Professor Hitoshi Tsugawa, Associate Professor Yasuaki Kabe, and Professor Makoto Suematsu of the Department of Biochemistry, Keio University School of Medicine.

It is known that SCFAs are produced in large quantities through the breakdown of dietary fiber by gastrointestinal bacteria and are involved in the regulation of the immune system, but their molecular mechanism of action has remained unknown. In this study, the research team succeeded in identifying a human-derived receptor apoptosis-associated speck-like (ASC) protein that specifically binds to SCFAs using a proprietary drug receptor detection technology. ASC is also known to be a key adaptor protein[1] in the activation of the inflammasome[2]. The research team has clarified that SCFAs enhance the natural defenses of macrophages, a type of immunocompetent cells, by activating the inflammasome-forming ability of ASC. The team also found that this immunostimulatory effect contributes to protection against Salmonella bacteria, which causes food poisoning. The team observed that the survival of mouse models of Salmonella infection was significantly prolonged through the ingestion of water-soluble dietary fibers, which are broken down in the intestine to produce large amounts of SCFAs. These findings reveal a new regulatory mechanism by which SCFAs suppress Salmonella infection through inflammasome activation. They are expected to lead to the development of new treatments and prevention methods for defending against pathogen infection through the ingestion of food components such as the dietary fiber that produce SCFAs.

The results of this research were published on September 29, 2020 (PDT), in the online version of the scientific journal *PLoS Biology*.

1. Research Background

Propionate and butyrate, which have very short carbon chains, are known as short-chain fatty acids (SCFAs). These acids are produced in large quantities in the intestine through the fermentation of dietary fiber by gastrointestinal bacteria. SCFAs produced in the intestine are known to contribute to the regulation of various immune responses, but until now the precise molecular mechanisms of these acids remained unknown. In this study, we detected receptor proteins of SCFAs from macrophages and other effector cells and demonstrated how they enhance innate immune response.

2. Research Details

The research team first created immobilized beads bearing the structures of propionate and butyrate using high-performance affinity nanobeads[3] (See Fig. 1A) and extracted proteins from the human U937 monoblastic leukemia cell line, which is known to exhibit the morphology and characteristics of mature macrophages. The team then purified a group of proteins that specifically bind to SCFAs from the protein extract. (See Fig. 1B) As a result, we identified a protein with an apparent molecular mass of 25 kDa that selectively binds propionate and butyrate-immobilized beads. When analyzed by mass spectroscopy, it was identified as the inflammasome adaptor protein ASC, which is responsible for activating an immune response to foreign substances. The inflammasome complex responds to external stimuli such as foreign substances in immunocompetent cells. A complex is formed by binding to the PYRIN domain of the ASC and activating caspase-1. This is known to increase the production of pro-inflammatory cytokines such as 1ß (IL-1ß) and induce cell death. Upon examining the effect of SCFAs on ASC, it became clear that the binding of ASC and NLRP3, which are constituent proteins of the inflammasome complex, is significantly enhanced by the addition of propionate or butyrate. (See Fig. 1C) In addition, we examined the amount of IL-1ß produced when the inflammasome was treated with endotoxin (LPS) stimulation and ATP. We found that treatment with lactate, which does not bind to ASC, had no effect, but treatment with propionate and butyrate significantly increased IL-1ß production. (See Fig. 1D) From these results, we clarified that the binding of SCFAs to ASC promotes the formation of the inflammasome complex and contributes to the activation of innate immunity, including enhancing IL-1ß production.

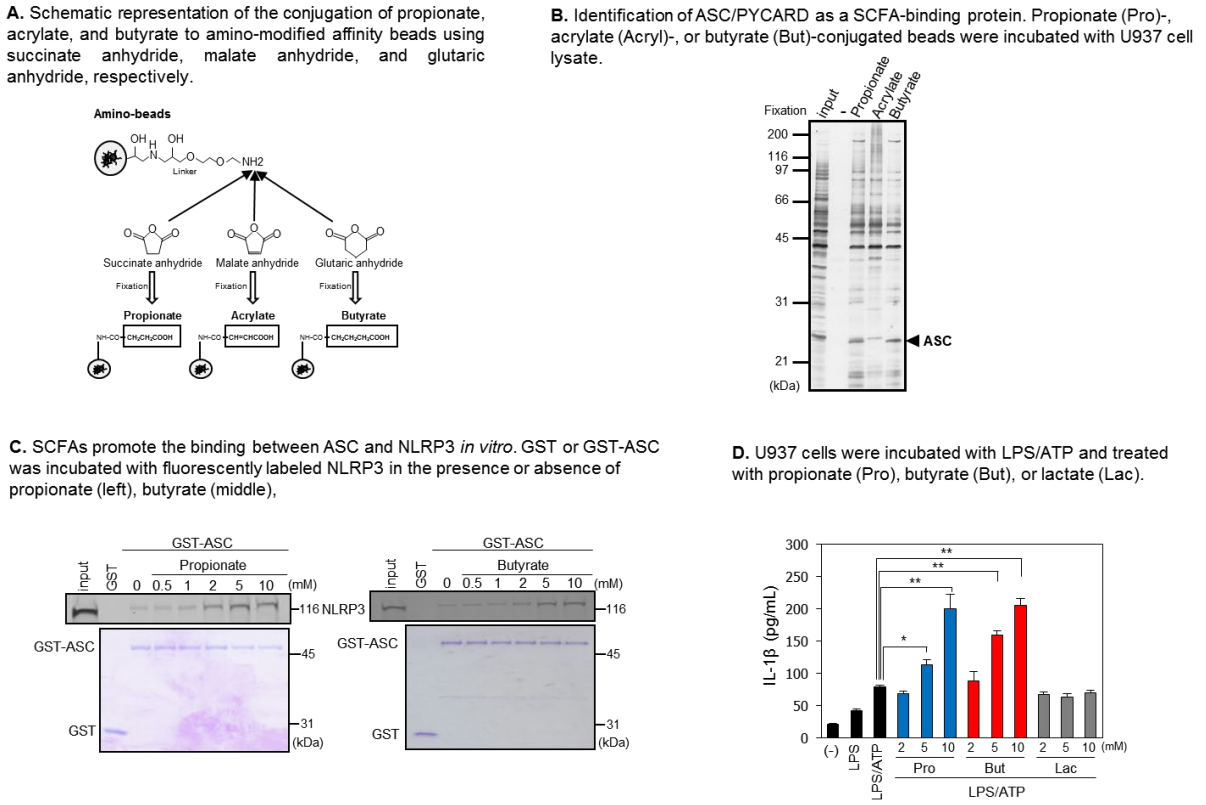
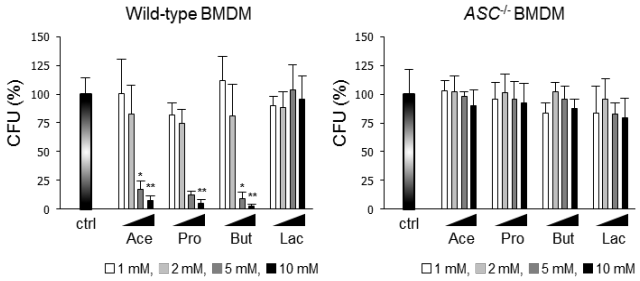


Fig. 1: Identification of ASC as a novel SCFA-binding protein to activate inflammasome complex

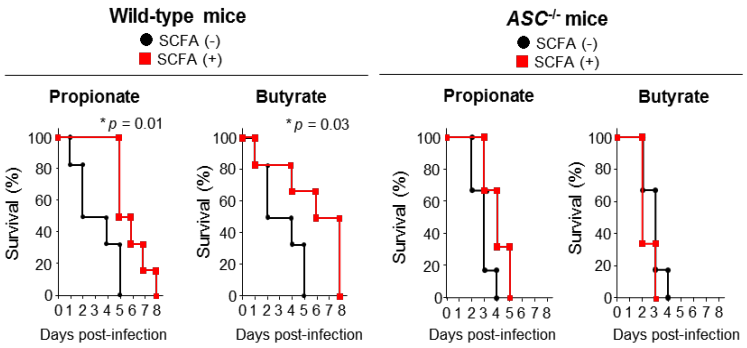
A. Schematic representation of the conjugation of SCFAs to high-performance affinity nanobeads
B. Purification and identification of novel SCFA-binding proteins using SCFA-conjugated beads
C. SCFAs promote binding between ASC and NLRP3 by enhancing oligomerization of the inflammasome complex
D. SCFAs increase IL-1ß production (increased inflammasome activity)

Salmonella, a well-known cause of food poisoning, infects macrophages in the intestine, breaks through the intestinal barrier, and enters the bloodstream, causing serious symptoms such as septic shock. Since activation of the inflammasome complex is known to suppress bacterial infections such as Salmonella, we examined the effect of SCFAs on infection suppression. As shown in Fig. 2A, infection and growth of Salmonella in primary mouse macrophages were not affected by the addition of lactate, but it was clearly suppressed by the addition of propionate or butyrate. Furthermore, this inhibitory effect was not observed in ASC-deficient macrophages, suggesting that SCFAs enhance Salmonella elimination in macrophages by ASC-mediated inflammasome activation. In addition, in a mouse model for Salmonella, administration of SCFAs significantly improved suppression of Salmonella survival, and no such effect was observed in ASC-deficient mice. (See Fig. 2B) Water-soluble dietary fiber is known to ferment in the intestine to produce a large amount of SCFAs. Therefore, in the Salmonella infection model, administration of partially hydrolyzed guar gum[4] (PHGG, obtained from Taiyo Kagaku), which is a type of dietary fiber, was found to significantly improve suppression of Salmonella survival. (See Fig. 2C)

A. SCFAs enhance the *S.* Typhimurium elimination inside murine bone marrow-derived macrophages (BMDMs) through ASC-dependent mechanisms.



B. Administration of propionate or butyrate significantly prolonged the survival of *S.* Typhimurium-infected wild-type mice through ASC-dependent mechanisms.



C. Administration of Indigestible dietary fibers also prolonged the survival of *S.* Typhimurium-infected mice.

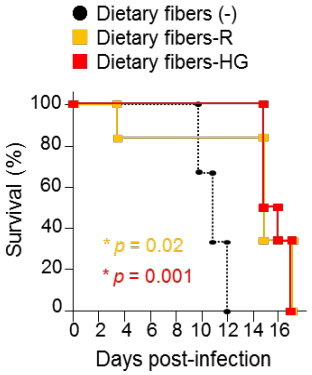


Fig. 2: SCFAs enhance host defense against Salmonella infection

A. SCFAs suppress survival of Salmonella in macrophages
B. Administration of SCFAs prolong the survival of Salmonella–infected mice through ASC-mediated inflammasome activation
C. Effect of dietary fiber on the survival of Salmonella-infected mice

From the above results, we clarified that SCFAs bind to and activate ASC and activate innate immunity by promoting the formation of inflammasome complexes. Inflammasome activation induces programmed death (pyroptosis)[5] in macrophages infected with Salmonella and eliminates Salmonella invading macrophages, suggesting that by enhancing the production of cytokines such as IL-1ß and IL-18, neutrophils are recruited and contribute to the elimination of Salmonella. (See Fig. 3)

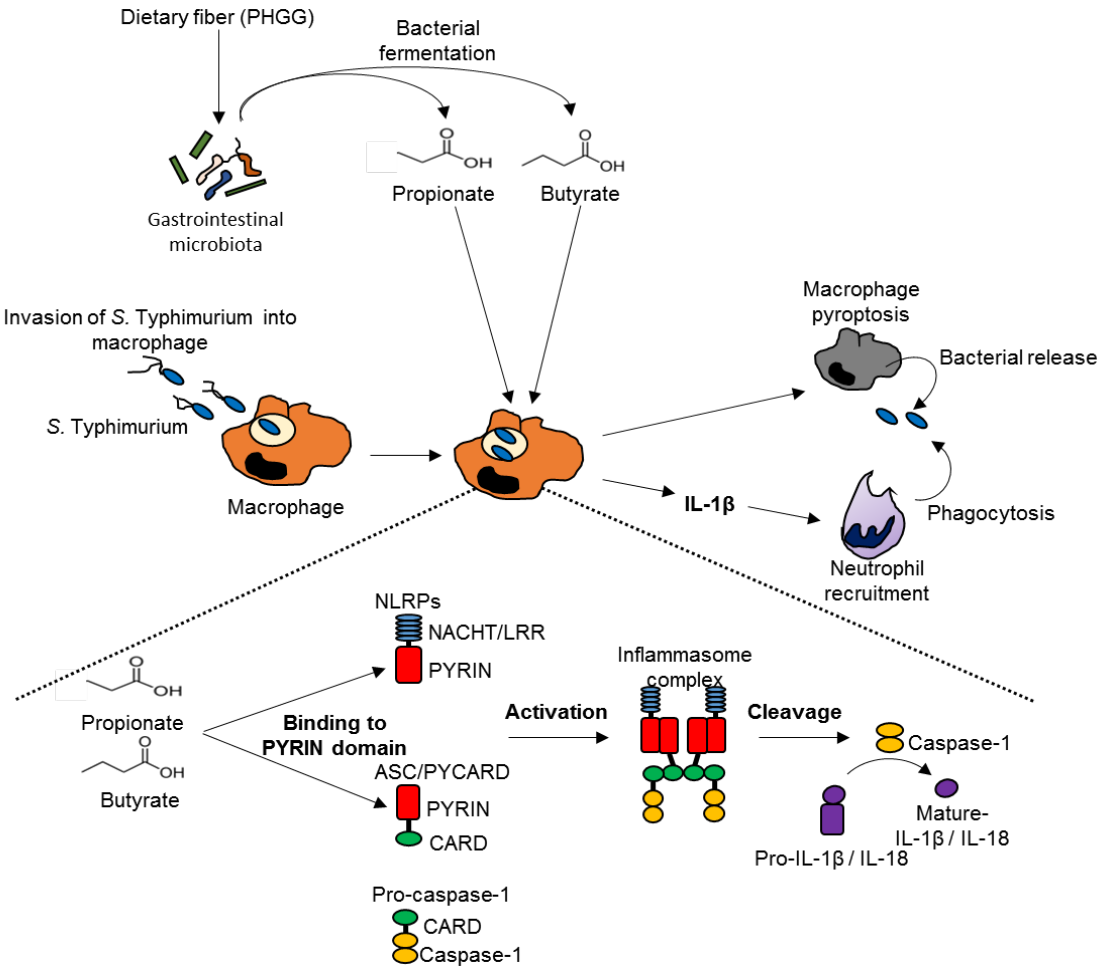


Fig. 3: SCFA-induced inflammasome activation and defense against Salmonella

SCFAs, which are produced in the intestine through the ingestion of dietary fiber, promote ASC-mediated inflammasome activity in macrophages infected with Salmonella and enhance the release of IL-1ß and IL-18. By inducing cell death (pyroptosis), they promote the removal and sterilization of Salmonella and contribute to infection suppression.

3. Research Significance and Future Development

The findings of this study clarified the molecular mechanism of immunostimulatory activity in SCFAs, which was previously unknown. These findings are expected to inform the creation of a system for new treatments and prevention methods for defending against pathogen infection through the ingestion of food components such as dietary fiber that produce SCFAs.

4. Notes

This research was supported by the Project for the Promotion of Partnerships in Medicine, Welfare, Food and Agriculture of the Ministry of Agriculture, Forestry and Fisheries, the SUEMATSU Gas Biology Project of the JST Exploratory Research for Advanced Technology (ERATO) Research Funding Program as well as the following programs of the Japan Agency for Medical Research and Development (AMED):-Advanced Research & Development Programs for Medical Innovation (AMED-CREST): "Translational Research for Controlling Diseases through Mining Key Molecules Regulating Metabolic Systems" (Principal Investigator: Yasuaki Kabe) (Grant Number: JP19gm0710010) Professor Suematsu has not received any funding from AMED for this research.

5. Research Paper

English Title: Short-chain fatty acids bind to apoptosis-associated speck-like protein to activate inflammasome complex to prevent Salmonella infection
Japanese Title: 短鎖脂肪酸類は ASC と結合してインフラマソーム複合体を活性化することにより サルモネラ菌感染を防御する
Authors: Hitoshi Tsugawa, Yasuaki Kabe, Ayaka Kanai, Yuki Sugiura, Shigeaki Hida, Shunichiro Taniguchi, Toshio Takahashi, Hidenori Matsui, Zenta Yasukawa, Hiroyuki Itou, Keiyo Takubo, Hidekaz Suzuki, Kenya Honda, Hiroshi Handa, Makoto Suematsu
Publication: *PLoS Biology* (online edition)
DOI: 10.1371 / journal.pbio.3000813

[Glossary]

[1] Adaptor Proteins
Adaptor proteins are a type of protein involved in signal transduction, and while they lack functions such as enzyme activity, they contribute to activation by binding to other proteins.

[2] Inflammasome
The inflammasome is a complex that detects foreign substances such as intracellular pathogens via NOD-like receptors (NLRs), including NLRP1, NLRP3, and NLRP6. The complex is formed via the signal transduction molecule apoptosis-associated speck-like protein containing a CARD (ASC), converts inactive procaspase-1 to active procaspase-1, and aids in removing foreign matter through the activation of cytokines IL-1β and IL-18.

[3] Affinity Nanobeads
Affinity nanobeads are small, uniform particles 100 nanometers in size, which have an extremely low non-specific absorption rate of proteins and other compounds. By immobilizing a target ligand such as a chemical (drug) on a particle's surface, these beads can use their "affinity" to purify receptor proteins that selectively bind to the drug.

[4] Guar Gum
Guar gum is a polysaccharide derived from guar beans. It contains a unique polysaccharide that contains mannose.

[5] Pyroptosis
Pyroptosis is a type of programmed cell death. In cells infected with Salmonella or similar bacteria, caspase-1 is activated, causing induction of inflammatory cytokines such as IL-1β and IL-18, or cell death.

*Please direct any requests or inquiries for coverage to the contact information provided below in advance.

[Inquirie regarding this press release]
Keio University School of Medicine
Department of Biochemistry
Associate Professor Yasuaki Kabe
Tel: 03-5363-3753　Fax: 03-5363-3466　E-mail: ykabe@z3.keio.jp

[Inquiries regarding AMED]
Japan Agency for Medical Research and Development
Division of Innovative Research and Development
Department of Innovation and Clinical Research Center
Tel: ＋81-3-6870-2224　Fax: ＋81-3-6870-2246　E-mail: kenkyuk-ask@amed.go.jp

[Source of this release]
Keio University
Shinanomachi Campus
Office of General Affairs: Yamasaki / Iizuka
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582
Tel: 03-5427-1541　Fax: 03-5441-7640　E-mail: med-koho@adst.keio.ac.jp
http://www.med.keio.ac.jp/en/

*A color version of this press release is available. Please contact the above address for more information.