Human Genome Center

Division of Metagenome Medicine
メタゲノム医学分野

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Abnormal compositions of intestinal microbiota have been reported to be associated with various diseases. We analyze intestinal bacteriome and virome in various diseases and search for "pathobiont" that causes the diseases. By making use of bioinformatics, we are constructing an analysis pipeline for intestinal microbiome, conducting comprehensive metagenomic analysis, and developing phage therapy for the specific control of pathobionts.

1. Analysis of skin microbiota in axillary osmidrosis.

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About 10% of the Japanese population is said to have axillary odor. The odor is caused by apocrine gland secretions contained in axillary sweat. They are odorless immediately after secretion, but are transformed into malodorous metabolites when metabolized by indigenous bacteria in the skin. Each axillary odor has its own characteristics, and about 90% of people can be divided into, in order of prevalence, milk-like odor (Type M), acid-like odor (Type A), and curry-spice-like odor (Type C). In a joint study with Mandom Corporation, we collected body fluid samples extracted from the axillae of 20 healthy adult males and classified them into 11 C-type and 9 M-type individuals based on the judgment of odor judges. Analysis of metabolites in the samples showed an increase in precursors of odor-causing metabolites in the C group. Next, shotgun metagenomic analysis of axillary skin flora showed that *Streptococcus hominis*, which is involved in the production of odorant precursors in type C, was significantly increased, indicating that it plays an important role in the production of odorant substances. Furthermore, we searched for a specific bacteriolysis enzyme for *S. hominis* using metagenomic data and succeeded in obtaining a new bacteriolysis enzyme sequence that could be purified. We also confirmed that this bacteriolysis enzyme has no bacteriolytic effect on typical skin-dwelling bacteria other than the targeted *S. hominis*. The results of this study may be a useful tool for specifically lysing *S. hominis*, which is involved in the production of odor substance. In the future, we plan to develop phage deodorants for axillary odors.

2. The development of a new therapeutic agent for GVHD

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In recent years, it has become clear that "dysbiosis" is found in a variety of diseases, due to improved genome analysis technology. In organ transplantation, immune cells attack the transplanted organ as a foreign body, resulting in rejection. In hematopoietic stem cell transplantation for the treatment of leukemia and other diseases, immune cells derived from the transplanted hematopoietic stem cells may develop graft-versus-host disease (GVHD), in which immune cells attack the transplant patient's organ as if it were a foreign body. Previous studies have reported that GVHD is exacerbated when the balance of the intestinal microflora is disturbed during the treatment process of hematopoietic stem cell transplantation and *Enterococcus spp*. increase. We performed a metagenomic analysis of fecal samples from 46 hematopoietic stem cell transplant (allogeneic transplant) patients at Osaka Metropolitan University Hospital and found not only an increase in *Enterococcus spp.* in 30 of the 46 patients, but also the presence of highly toxic *Enterococcus faecalis*, involved in the development of GVHD. During the treatment of hematopoietic stem cell transplantation, antimicrobial agents are used to protect against infection, and it was thought that this highly toxic *E. faecalis* escaped from the antimicrobial agents by forming biofilms in the intestinal tract, thereby proliferating. In addition, it was found that GVHD worsened in the gnotobiotic mice in which highly toxic *E. faecalis* was established. Therefore, we tried to couduct a metagenomic analysis of *E. faecalis* to search for a bacteriolysis enzyme that specifically acts on *E. faecalis*. As a result, we successfully identified the sequence of a novel bacteriolysis enzyme, endolysin and synthesized the enzyme according to the sequence. This enzyme nicely lysed *E. faecalis*. Furthermore, the endolysin destroyed biofilme of *E. faecalis*, *in vitro* and *in vivo*. When this bacteriolysis enzyme was administered to GVHD model mice in which highly toxic *E. faecalis* was established, we confirmed that it inhibited the worsening of GVHD and significantly improved the mortality rate. The phage-derived bacteriolysis enzyme obtained in this study is expected to lead to the development of new therapeutic agents for GVHD in the future.

3. Development of a microbiome digital twin to predict disease states based on metagenome analysis of intestinal microflora

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We are developing a digital twin that predicts disease states using metagenomic data of the intestinal microbiota and gene pathway analysis data as teaching data in collaboration with Fujitsu Limited. For this purpose, we collected fecal samples from 10 Crohn's disease patients and 18 Parkinson's disease patients and performed metagenomic analysis. We compared these data with metagenomic data of 100 healthy subjects and have performed machine learning and deep learning. We are tuning to further develop it into an XAI.

4. Development of next-generation mucosal vaccine against infectious diseases

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A next-generation vaccine strategy capable of inducing both systemic and mucosal immunity is awaited. We showed that intramuscular vaccination with a combination of CpG oligodeoxynucleotides and curdlan as adjuvants systemically induced antigen-specific IgA and IgG production in mice. After priming, markedly high titers and long-lasting antigen-specific IgA and helper T-cell responses including Th1 and Th17 responses in the mucosa were acquired by antigen boosting of the target organs. This immunization effectively regulated *Streptococcus pneumoniae* infection in mice. The patent of this new vaccine strategy was granted in 2019 in Japan, in 2020 in US and in 2021 in Europe. We have conducted monkey experiments for formulation in human by using PspA, a universal Ag of *S. pneumoniae*. Although vaccination is recommended for protection against invasive pneumococcal disease, the frequency of pneumococcal pneumonia is still high worldwide. In fact, no vaccines are effective for all pneumococcal serotypes. Fusion pneumococcal surface protein A (PspA) has been shown to induce a broad range of cross-reactivity with clinical isolates and afford cross-protection against pneumococcal challenge in mice. Furthermore, we developed prime-boost-type mucosal vaccines that induce both antigen-specific IgG in serum and antigen-specific IgA in targeted mucosal organs in previous studies. We investigated whether our prime-boost-type immunization with a fusion PspA was effective against pneumococcal infection in mice and cynomolgus macaques. C57BL/6 mice were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan. Six

weeks later, PspA was administered intranasally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some mice were given intranasal *Streptococcus pneumoniae* and the severity of infection was analyzed. Macaques were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan at week 0 and week 4. Then, 13 or 41 weeks later, PspA was administered intratracheally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some macaques were intranasally administered *S. pneumoniae* and analyzed for the severity of pneumonia. Serum samples from mice and macaques injected with antigens in combination with CpG oligodeoxynucleotides and/or curdlan contained antigen-specific IgG. Bronchial samples contained antigen-specific IgA after the fusion PspA boosting. This immunization regimen effectively prevented *S. pneumoniae* infection. Prime-boost-type immunization with a fusion PspA prevented *S. pneumoniae* infection in mice and macaques. Unlike mice, primates were able to induce sIgA sufficiently with emulsion and curdlan, and we would like to create a new emulsion that compensates for the action of CpG DNA and consider using it as a new vaccine adjuvant.

Publications

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