様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）An approach to increase the gut-derived maternal immunoglobulin A (IgA) production using dietary tryptophan via aryl hydrocarbon receptor

研究課題名（英文）An approach to increase the gut-derived maternal immunoglobulin A (IgA) production using dietary tryptophan via aryl hydrocarbon receptor

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：0.5% Trp添加と無添加の基礎飼料を与えたマウスの糞便を採取し、糞便中のIgA濃度を測定した。また、給餌開始から6週間後に大腸を採取し、IgA産生形質細胞数を計測した。さらには、給餌開始から6～8週間後の母マウスから生まれた仔マウスを用い、糞便と乳汁（胃内容物）を採取しIgA濃度を測定した。糞便中のIgA濃度は、給餌開始4週間後にTrp群で有意に増加したが、給餌開始6週間後の大腸の形質細胞数には有意な差はなかった。一方、妊娠初期～後期にかけての糞便中IgA濃度は、Trp群で有意に上昇した。これらの結果から、Trp供与により、特に妊娠期の腸管における免疫機能を向上可能であることが示唆された。

研究成果の学術的意義や社会的意義
Most of the changes in the amount of intestinal IgA and its role during the perinatal period is still unknown. This study for the first time investigated the intestinal IgA production and its role in strengthening immunity during the perinatal period through tryptophan (Trp) supplementation.

研究成果の概要（英文）：Feces of mice fed with 0.5% Trp-added basal feed or basal feed over time were collected and the IgA concentration in the feces was measured. Six weeks after the start of feeding, the large intestine was collected to address the number of plasma cells. In addition, using mice born 6 to 8 weeks after the start of feeding, feces and milk were collected to measure IgA concentration. The large intestine and mammary gland were collected in the second week of breastfeeding, to investigate the number of plasma cells. Fecal IgA levels increased significantly in the Trp group after 4 weeks of feeding bottom. However, there was no significant difference in the number of plasma cells in the large intestine 6 weeks after the start of feeding. Fecal IgA levels also increased significantly in the Trp group during early and late pregnancy. Trp is highly demanding, suggesting that Trp can enhance immunity in the intestinal tract.

研究分野：粘膜免疫学、栄養学

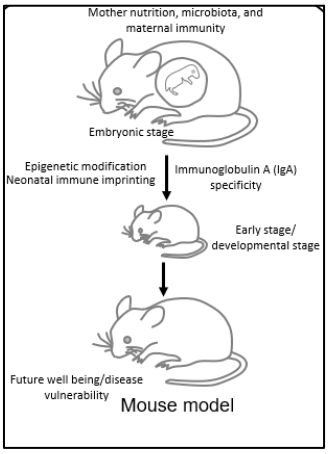
キーワード：IgA　腸管　トリプトファン

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The first few days/weeks of life represent a window of extreme vulnerability and a virtual immune “black box” due to our limited understanding of the active antipathogen immune functions, the mechanisms of antibody transfer, and the influence of early infections on lifelong health. Immunoglobulin A (IgA), a potential maternal antibody transferring to offspring is responsible for improving neonatal health and wellbeing, not only for human infants but in fact all mammals. It is vital to investigate how tryptophan diet changed the microbial composition during early and late stage pregnancy's and overall immunoglobulin A (IgA) secretion in the mother and its impact on neo-born IgA production and its disease immunity.

２．研究の目的



In recent decades, the rapid expansion of antibiotic use in both animal and human population, as well as intake of high-calorie, low-fiber diets in human have contributed to disturbances in the maternal gut microbial community, even during pregnancy. In that cases, the produced IgA with low specificity transferring to offspring, which may responsible for an altered metabolic and immune imprinting in the neonatal. Thus, a large proportion of neonates are affected in numerous diseases or even deaths from infectious diseases occur in the first month of life due to compromised immune function. Regarding this, it is vital to explore that do IgA antibodies in a mother’s milk help to select for a healthy bacterial repertoire or are colonizing microbiota imprinting an IgA memory repertoire that, when established, maintains homeostasis in the neonatal. Alternatively, how the epigenetic modification linked to maternal or neonatal microbiome and nutrition critically linked to the function of polyreactive IgA, is still not fully clarified. My purpose of this study is to introduce the IgA production in the mother during normal, pregnancy and after birth. Finally, how the maternal IgA impacts on neonatal IgA production.

３．研究の方法

Flow cytometry: Mononuclear cells isolated from mammary glands, Peyer’s patches (PP), small intestine (one-third in the middle), and large intestine and then stained with appropriate combinations of antibodies: Flow cytometry analysis was performed using either an Attune™ NxT Acoustic Focusing Cytometer (Thermo Fisher Science) or an Accuri™ C6 flow cytometer (BD Bioscience).

ELISA: The level of IgA in feces or milk collected from the stomach contents of mouse pups was measured by ELISA. The titer of milk IgA to intestinal microorganisms or certain bacterium was described as the OD450.

Metagenomics analysis: Genomic DNA obtained from directly extracted from feces using a QIAamp DNA Stool Mini Kit was subjected to metagenomics analysis. Specifically, the V3 and V4

regions of the bacterial 16S ribosomal RNA gene were amplified by PCR using PrimeSTAR HS DNA polymerase. All PCR products were then sequenced using the MiSeq platform (Illumina) with the MiSeq reagent kit v2 (500 cycles).

Quantitative RT-PCR: Total RNA was extracted from the mammary glands, small and large intestine using a ReliaPrep RNA Tissue Miniprep System, and cDNA was synthesized by reverse transcription using the PrimeScript RT reagent kit with oligo (dT) primers and random hexamers. All primers were designed using the Takara perfect real-time support system.

Histochemistry: Samples of small intestine udies were fixed in 4% (w/v) paraformaldehyde and embedded in paraffin. For histopathological analysis, the tissue sections were stained with hematoxylin and eosin. The images were obtained using either BZ-9000 (Keyence) or BX63 (Olympus).

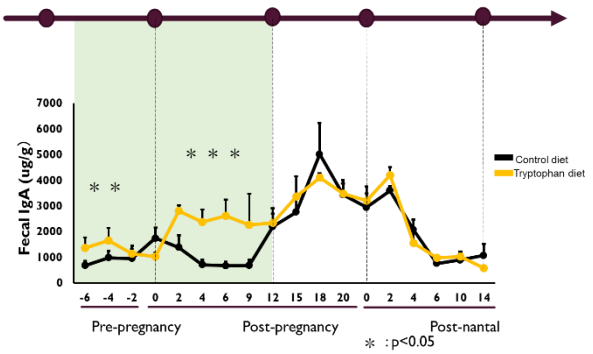
４．研究成果

Results 1: Tryptophan oral gavage changes microbial composition

In our first study, oral gavage of tryptophan (o.5%Trp 1ml/day ×2weeks) is found to increase the mature B cell and plasma cell number compare to control. Comparing the 16s rRNA amplicon sequencing, tryptophan oral gavage is found to change the alpha diversity index especially Shannon index. However, there is no changes observed in beta diversity. Comparing the qPCR reuslts, it is found that tryptophan also increase the aryl hydrocarbon receptor (AHR) expression in mammary gland and large intestine after two weeks. Along with this, tryptophan is also found to increase the fecal IgA level significantly.

Results 2: Tryptophan supplemented with diets increased IgA level in the early pregnancy

We have found that the migration of mammary gland plasma cells from the intestinal tract during lactation significantly increases IgA production in the mammary gland after parturition. Under normal conditions before pregnancy, the intestinal IgA concentration remained at 500 μg / g. In the latter half of pregnancy, the IgA concentration increased remarkably up to 5000 μg / g. In addition, it has been clarified that the morphology of the intestinal tract, which is the source of migration, also fluctuates. Taking the large intestine as an example, its size becomes very large during the perinatal period. It has also been reported that not only the size but also the internal villi and other structures change. From postpartum to lactation, plasma cells proliferated in late pregnancy migrate to the mammary gland, resulting in a decrease in IgA production in the intestinal tract. Ingested tryptophan is metabolized mainly in the stomach and small intestine by the kynurenine pathway and the serotonin pathway. However, it has been reported that some of them are also metabolized by the intestinal flora, and the metabolites bind to a receptor called AHR to induce IgA production. In the first trimester of pregnancy, a significant increase in intestinal IgA concentration was observed in the Tryptophan feed group compared to the basal feed group. From this, it was clarified that



IgA production can be controlled by tryptophan metabolites from pre-pregnancy to early pregnancy.

From this experiment it is found that tryptophan is highly demanding, suggesting that Tryptophan can enhance immunity in the intestinal tract, especially in the early pregnancy stage, which may responsible for occurring a significant difference in the plasma cell and milk IgA concentrations in the large intestine and mammary gland at the second week of lactation. Finally from this study, the following major impacts may be established:

1) Identifying the physiological mechanisms to enhance the IgA production in the pregnancy period.
2) Developing the neo-natal vaccine and maternal probiotic's development during pregnancy.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Usami Katsuki, Niimi Kanae, Matsuo Ayumi, Suyama Yoshihisa, Sakai Yoshifumi、Sato Shintaro、Fujihashi Kohtaro、Kiyono Hiroshi、Uchino Saeka, Furukawa Mutsumi, Islam Jahidul, Ito Kaori, Moriya Taiki, Kusumoto Yutaka、Tomura Michio、Hovey Russell C., Sugawara Junichi, Yoneyama Hiroshi, Kitazawa Haruki, et al	４．巻 36
２．論文標題 The gut microbiota induces Peyer’s-patch-dependent secretion of maternal IgA into milk	５．発行年 2021年
３．雑誌名 Cell Reports	６．最初と最後の頁 109655～109655
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.celrep.2021.109655	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Islam Jahidul、Agista Afifah Zahra、Watanabe Kouichi、Nochi Tomonori、Aso Hisashi、Ohsaki Yusuke、Koseki Takuya、Komai Michio、Shirakawa Hitoshi	４．巻 99
２．論文標題 Fermented rice bran supplementation attenuates chronic colitis-associated extraintestinal manifestations in female C57BL/6N mice	５．発行年 2022年
３．雑誌名 The Journal of Nutritional Biochemistry	６．最初と最後の頁 108855～108855
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jnutbio.2021.108855	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Islam Jahidul、Tanimizu Masae、Shimizu Yu、Goto Yoshiaki、Ohtani Natsuki、Sugiyama Kentaro、Tatezaki Eriko、Sato Masumi、Makino Eiji、Shimada Toru、Ueda Chise、Matsuo Ayumi、Suyama Yoshihisa、Sakai Yoshifumi、Furukawa Mutsumi、Usami Katsuki、Yoneyama Hiroshi、Aso Hisashi、Tanaka Hidekazu、Nochi Tomonori	４．巻 10
２．論文標題 Development of a rational framework for the therapeutic efficacy of fecal microbiota transplantation for calf diarrhea treatment	５．発行年 2022年
３．雑誌名 Microbiome	６．最初と最後の頁 31
掲載論文のDOI（デジタルオブジェクト識別子） 10.1186/s40168-021-01217-4	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計0件

〔図書〕　計0件

〔出願〕　計1件

産業財産権の名称 下痢治療剤および牛の下痢治療方法	発明者 野地智法、Jahidul Islam、田中秀和	権利者 同左
産業財産権の種類、番号 特許、特願2021-196413	出願年 2022年	国内・外国の別 国内

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関