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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Elucidation of the cause of allergy by identifying somatic mutations in human IgE+ memory B cells.

研究課題名（英文）Elucidation of the cause of allergy by identifying somatic mutations in human IgE+ memory B cells.

研究代表者

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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）：Starting with blood B cells of hay fever allergic donors, we successfully obtained almost pure population of IgE+ B cells with this procedure in combination with cell sorting. Exome analysis of purified IgE+ B cells from allergic donors revealed various somatic mutations.

研究成果の学術的意義や社会的意義
Membrane IgE endocytosis is defective in allergic donor-derived B cells, leading to high IgE expression, possibly due to somatic mutation of endocytosis-related genes. New IgE isoform facilitated spontaneous plasma-cell differentiation and apoptosis of IgE+ B cells more than conventional IgE.

研究成果の概要（英文）：I have developed a system to selectively expand IgE+ B cells by a four-step B cell culture procedure. Starting with blood B cells of hay fever allergic donors, we successfully obtained almost pure population of IgE+ B cells with this procedure in combination with cell sorting. Exome analysis of the patients' IgE+ B cells revealed various somatic mutations, which may be the cause of membrane IgE endocytosis defect of the patients' IgE+ B cells. We have also identified 2 species of mRNA of membrane-bound εH chain: one encoding the conventional εH chain with an extracellular membrane-proximal domain (EMPD) and transmembrane domains, and the other lacking these domains but containing C-terminal peptide with a shifted reading frame, termed IgE-NET. The IgE-NET facilitated spontaneous plasma-cell differentiation and apoptosis of IgE+ B cells more than conventional IgE, and thus may contribute to the regulation of IgE+ B cells and of IgE synthesis in allergic disease.

研究分野：Immunology

キーワード：Allergy　B cells　IgE

１．研究開始当初の背景

One hundred and ten years ago, Clemens von Pirquet, an Austrian scientist and pediatrician, introduced the term “allergy” to distinguish immune responses that are harmful to the host from a physiological state of protective immunity. The most common form of allergy - IgE-mediated hypersensitivity - affects more than 25% of the population in developed countries. Especially, Japan is one of the most exceptionally vulnerable countries to allergy. By 2008, hay fever alone accounted for more than 33.8 million Japanese allergic patients (26.5% population), in which high blood IgE caused by pollen from *Cryptomeria japonica*. The half-life of human serum IgE antibody is less than 2 days and IgE expression is tightly regulated in healthy individuals, but serum IgE levels often remain high for months or years in the patients suffering from allergic diseases. It has been largely unknown how IgE production is regulated, because of the low frequency of IgE-switched B cells and technical limitations in identifying them, especially in humans. Previous reports about long-term helminth-specific IgE (Mitre and Nutman, *J Allergy Clin Immunol* 2006, 117: 939) or the cases demonstrating that bone marrow transplantation from allergic patients to non-allergic recipients resulted in the transfer of the disease due to bone-marrow plasma cells (PC) (Bellou et al., *Ann Allergy Asthma Immunol* 1997, 78:513; Hallstrand et al., *Blood* 2004, 104:3086) indicated a significant contribution of IgE^+ LLPCs to allergic diseases in humans. In addition, in some other diseases such as hay fever or food allergies, blood IgE can be induced from undetectable to high levels by re-exposure with even a low amount of allergen, indicating a role of IgE B_m cells. However, the mechanism for generation of such IgE B-cell memory, namely IgE^+ LLPCs and B_m cells, is unknown.

Recently, it was found that membrane-bound IgE (mIgE) autonomously triggers quick PC differentiation and apoptosis of GC B cells through mutually independent CD19-PI3K-Akt-IRF4 and BLNK-Jnk/p38 pathways (Haniuda et al., *Nat Immunol* 2016, 17:1109). Moreover, in $BLNK^{-/-}$ mice after immunization, $mIgE^+$ GC B and B_m cells, and IgE-producing LLPCs are formed, the blood IgE antibody is maintained for a long time, and anaphylaxis occurs upon re-immunization. Even in CD19 heterozygous mutant mice, the similar abnormalities were observed. These data indicate the autonomous mIgE prevents GC B cells from generating IgE^+ LLPCs and B_m cells. Thus, defects in the spontaneous mIgE signaling pathway may cause allergic diseases in humans. Such gene defects could be induced somatically in GC where somatic hypermutation can be generated in non-immunoglobulin genes (Liu et al., *Nature* 2008, 451:841).

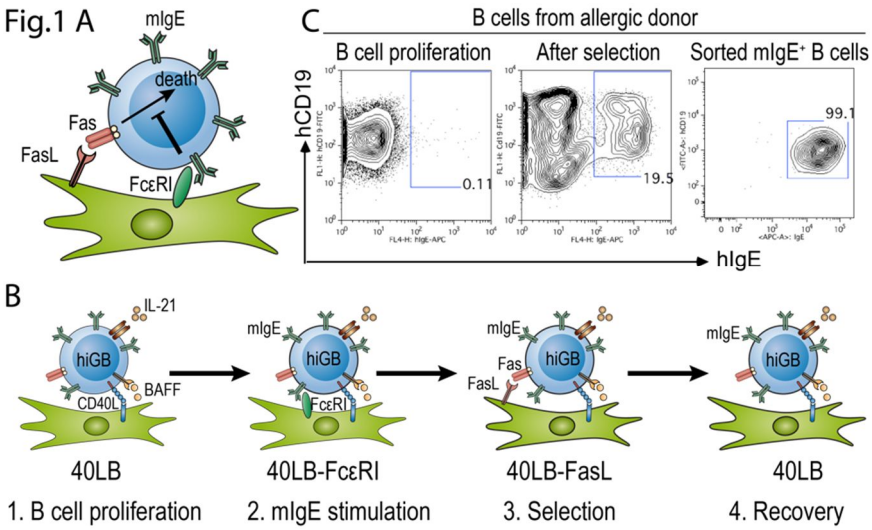
２．研究の目的

The purpose of this research was elucidation of the mechanism for the IgE B-cell memory generation. In this research, I was searching for causal somatic gene abnormalities in allergic patients that enable $mIgE^+$ B cells to survive and to become memory B (B_m) cells or long-lived plasma cells (LLPCs), and verified the functions of such abnormal genes in the autonomous mIgE signaling that forces IgE^+ germinal center (GC) B cells to be short-lived plasma cells, and in pathogenesis of allergy. In addition, I elucidated the function of a novel IgE εH chain isoform, namely IgE-NET, an alternative splicing variant that I have identified.

３．研究の方法

To verify such genetic mechanisms in human mIgE+ B cells, I have developed a system to selectively expand rare mIgE+ B cells from blood by a novel cell culture system consisting of four consecutive stages (Fig. 1A, B): (1) B cells are cultured with IL-21 on feeder cells transfected with CD40L and BAFF (40LB), where B cells significantly proliferate, become germinal center-

like B cells (termed iGB cells) and express the apoptosis-inducing receptor Fas. (2) Next, these iGB cells are transferred onto 40LB cells expressing FcεR1, a high-affinity ligand of mIgE, to stimulate mIgE+ iGB cells. (3) Then, the iGB cells are transferred onto 40LB cells expressing Fas-ligand (FasL), where these cells undergo apoptosis, except mIgE+ cells having been stimulated through mIgE. (4) The survived cells are recovered during the following culture on 40LB cells. It was easy to purify by cell sorter when the frequency of mIgE+ iGB cells becomes more than a few percent (Fig. 1C).



I searched for such mutations in genomic genes of mIgE$^+$ B$_m$ cells by comparing with non-B leukocytes from the same patients of various allergic diseases, using whole exome sequencing and RNA-Seq. Further, by using RNA from purified mIgE$^+$ B$_m$ cells, I will confirm mRNA sequences and expression levels of candidates.

４．研究成果

Starting with blood B cells of hay fever allergic donors, we successfully obtained almost pure population of IgE+ B cells with this procedure in combination with cell sorting. Exome analysis of the patient's IgE+ B cells revealed various somatic mutations (Table), which may be the cause of membrane IgE endocytosis defect of the patient's IgE+ B cells.

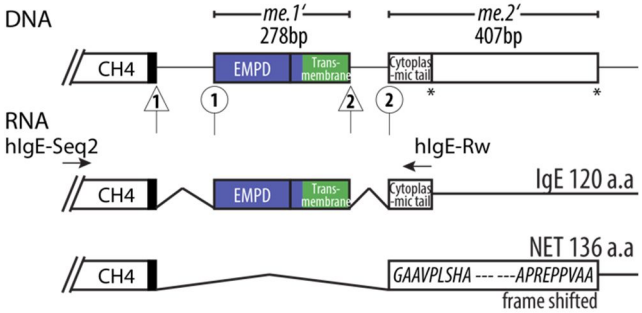
Effect	Genes	Location	Function
High	30	8 membrane, 2 Endoplasmic reticulum	High and moderate: cell-cell interaction, ER stress, apoptosis, proliferation, endocytosis ubiquitination
Moderate	128	39 membrane, 4 Endoplasmic reticulum	
Modifier	239		
Low	410		

High: exon lost, frame shifted, stop gained, start lost, splice donor and acceptor variant;
Moderate: Disruptive inframe insertion, missense variant, inframe insertion;
Modifier: non-coding exon, 3'-UTR, 5'-UTR, up/down-stream genes;
Low: synonyms, slice region.

Gene	Full name
LRP6	LDL receptor related protein 6
MICALL1	MICAL like 1
EPS15	Epidermal growth factor receptor pathway substrate 15
ACK1(TNK2)	activated Cdc42-associated kinase 1 (Tyrosine kinase non receptor 2)

We have also identified 2 species of mRNA of membrane-bound εH chain: one encoding the conventional εH chain with an extracellular membrane-proximal domain (EMPD) and transmembrane domains, and the other lacking these domains but containing C-terminal peptide with

a shifted reading frame, termed IgE-NET. The IgE-NET facilitated spontaneous plasma-cell differentiation and apoptosis of IgE+ B cells more than conventional IgE, and thus may contribute to the regulation of IgE+ B cells and of IgE synthesis in allergic disease.



In summary, the exome analysis of purified IgE+ B cells from allergic donors revealed various somatic mutations. Membrane IgE endocytosis is defective in allergic donor-derived B cells, leading to high IgE expression, possibly due to somatic mutation of endocytosis genes.

Human IgE-NET isoform facilitated spontaneous plasma-cell differentiation and apoptosis of IgE+ B cells more than conventional IgE, and may play a role in IgE B cell survival and differentiation mechanism.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1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 Al-Huseini Isehaq、Harada Masayuki、Nishi Kiyoto、Nguyen-Tien Dat、Kimura Takeshi、Ashida Noboru	４．巻 9
２．論文標題 Improvement of insulin signalling rescues inflammatory cardiac dysfunction	５．発行年 2019年
３．雑誌名 Scientific Reports	６．最初と最後の頁 1-13
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1038/s41598-019-51304-8	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

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　4件）

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３．学会等名 Keystone Symposia Fibrosis and Tissue Repair: From Molecules and Mechanics to Therapeutic Approaches（国際学会）
４．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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