Original article

Expression of Transient Receptor Potential Vanilloid 1 (TRPV1) mRNA in Human Immunocompetent Cells

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ABSTRACT The transient receptor potential (TRP) channel family, known as the calcium-permeable non-selective cation channel, is mainly distributed in the nervous system. TRPV1, which belongs to the TRP channel family, is recognized and activated by capsaicin, heat and acid. We investigated which cells in human immunocompetent cells are positive for the expression of TRPV1 by using reverse-transcription/real-time quantitative polymerase chain reaction (RT/RQ-PCR). α β T cells, γ δ T cells, B cells, NK cells, monocytes, myeloid DCs and plasmacytoid DCs were investigated for the expression of TRPVI. PCR products amplified by TRPV1 specific primers were detected in all of the investigated immunocompetent cells. Quantitative real-time PCR indicated that T cells and plasmacytoid DCs tended to express more strongly than other immunocompetent cells. These data demonstrated that TRPV1 was expressed in not only the nervous system but also in immunocompetent cells, and suggested an intimate relationship between the expression of TRPV1 and immune reactions.

KeyWords: Vanilloid receptor VR1; Mononuclear leukocyte; PCR

It has been demonstrated that ion channel receptors, which are expressed on the nervous system, are related to noxious stimulation. The transient receptor potential (TRP) channels are one of the main families of molecules in these ion channels. TRP channels are classified into seven main subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NOMPC: no mechanoreceptor potential C) (1-3). These TRP channels are non-selective cation channels, and induce cell excitation by cation (calcium and sodium) influx from extracellular to intracellular upon specific stimulation (4).

TRP channels function as temperature receptors. There are eight temperature receptors belonging to the TRP ion channel superfamily. TRPV1, a capsaicin receptor, is the temperature receptor in which molecular entity became clear for the first time. TRPV1 is activated by various noxious stimuli (capsaicin, acid (pH < 6) and heat (≥ 43℃)) and induces cell excitation by cation influx (5-7). Fever affects the immune system, however, the details have not been elucidated. In human, it has been known that fever causes rapid neutrophil migration, secretion of antibacterial chemicals and T cell proliferation (8-12). Although many studies have reported that TRPV1 involved in TRP ion channel family is expressed in not only neuronal cells but also non-neuronal cells such as lung epithe lial cells, prostate, bladder smooth muscle, kidney and liver cells (13-14), only a few studies showed the expression of TRPV1 in peripheral blood mononuclear cells (PBMNCs) (15). Furthermore, as the relationship between TRPV1 and multiple chemical sensitivity (MCS) syndrome has been reported, it is important to clarify which immunocompetent cells express TRPV1 in normal PB cells in association with the pathophysiology of MCS. Currently, it is poorly understood which cell fractions in PBMNCs express TRPV1. Here, we performed quantitative analysis of TRPV1 mRNA in immunocompetent cells, which were isolated from PBMNCs by FACSAria, by using real-time quantitative polymerase chain reaction.

Materials and methods

Cell preparation

PBMNCs were separated from buffy coat (provided by Niigata Red Cross Blood Center) by density gradient centrifugation using Lymphoprep (Axis-Shield Poc AS, Oslo, Norway). For cell sorting, PBMNCs were labeled with fluorescein-conjugated monoclonal antibodies (mAbs) for 30 min at 4℃ in the dark. The following mAbs were used: anti-CD3-fluorescein isothiocyanate (FITC), anti-CD4-FITC, anti-α β TCR-FITC, anti-γ δ TCR-FITC

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(BD Biosciences, San Jose, CA), anti-BDCA1-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany), anti-CD3-phycoerythrin (PE), anti-CD14-PE, anti-CD19-PE, anti-CD56-PE (BD Biosciences) and anti-BDCA4-PE (Miltenyi Biotec). Dead cells were excluded by being stained with 7-aminoactinomycin D (7AAD; Sigma Chemical Co., St. Louis, MO). After washing, cells were resuspended with RPMI-1640 medium (Kohjin-Bio, Saitama, Japan) containing 10% fetal bovine serum (FBS; Terumo, Tokyo, Japan), 100 U/ml penicillin, 100μg/ml streptomycin (Bio Whittaker, Walkersville, MD) and 0.5 mM EDTA (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Each immunocompetent cells were sorted by the following definitions of cell surface markers using FACSAria (BD Biosciences), NK cells, $CD56^+CD3^-$; γδ T cells, γδ TCR^+; B cells, $CD19^+$;αβ T cells,αβ TCR^+; Monocyte, $CD14^+$; myeloid DCs, $BDCA1^+CD3^-CD14^-CD19^-CD56^-$; plasmacytoid DCs, $CD4^+BDCA4^+$. Purity of the sorted cells was more than 99%.

RNA extraction

Total RNA was purified from individual immunocompetent cells by using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. After isopropanol precipitation, the RNA pellet was hydrated in 10 μL of UltraPure DNase/RNase Free Distilled Water (Invitrogen, Carlsbad, CA), incubated at 55℃ for 10 min, and samples were stored at -80℃ until use.

Reverse transcription

Reverse transcription of mRNA from immunocompetent cells was performed by the random primer method using TaKaRa RNA PCR™ Kit (AMV) Ver. 3.0 (TAKARA BIO INC, Shiga, Japan). An aliquot of 4.75 μL of RNA suspension was mixed with the random 9mer primer and incubated at 70℃ for 10 min to change the higher-order structure of the RNA. Then, $MgCl_2$, 10 × RT buffer, dNTP Mixture, RNase Inhibitor and Avian myeloblastosis virus (AMV) reverse transcriptase XL were added to the first mixtures. Reverse transcription was performed at 30℃ for 10 min, 60℃ for 30 min and 95℃ for 5 min. Reverse transcription products were stored at -20℃ until use and used as a template for real-time PCR.

Real-time quantitative polymerase chain reaction (real-time Q-PCR)

Quantification of TRPV1 mRNA was performed by real-time Q-PCR using $SYBR^R$ Green. Real-time Q-PCR was performed with StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA), and Fast $SYBR^R$ Green Master Mix (Applied Biosystems) was used as the quantification regent. Primer pairs were designed online, Primer 3 or Roche Applied Science, Universal ProbeLibrary Assay Design Center. Primer sequences are indicated below: human TRPV1 (GenBank accession no NM_080704.3) forward primer 5'- AGC CAC CTC AAG GAG TAT GTG -3', reverse primer 5'- CCC GAA CAA GAA GAC GAT G -3' (product size: 177 bp) (synthesized by NIHON GENE RESEARCH LABORATORIES Inc., Sendai, Japan), human β-actin (GenBank accession no NM_001101.3) forward primer 5'- AGA GCT ACG AGC TGC CTG AC -3', reverse primer 5'- GGA TGC CAC AGG ACT CCA -3' (product size: 111 bp) (synthesized by NIHON GENE RESEARCH LABORATORIES Inc.). Using the StepOne™ Real-Time PCR System, the real-time Q-PCR program consisted of one cycle of the first denaturation step (95℃ for 20 seconds), 45 cycles of the PCR step (denaturation at 95℃ for 3 seconds and annealing/elongation at 60℃ for 30 seconds) and a melting curve step (60-95℃ at a heating rate of 0.3℃ per second and continuous fluorescence measurement).

Data analysis

Acquired data was analyzed by StepOne™ software v2.0.2 (Applied Biosystems). The relative quantification value was calculated by using the threshold cycle (Ct) of TRPV1 and β-actin. The calculating formula is described below: △Ct = (Ct of TRPV1) - (Ct of β-actin), relative expression to β-actin = $2^{-\triangle Ct}$. Data is represented as mean and standard error of the mean (S.E.M.).

Results

Separation of immunocompetent cells from PBMNCs from the healthy donor

In order to elucidate what type of cells express TRPV1, first of all, we sorted peripheral blood immunocompetent cells from PBMNCs by using FACSAria. We chose NK cells and γδ T cells as cells involved in innate immunity, B cells and αβ T cells as cells involved in acquired immunity, and monocytes, myeloid DCs and plasmacytoid DCs as antigen presenting cells linking innate and acquired immunity in this assay. Each immunocompetent cell was sorted according to surface phenotypes described above. Reanalysis of sorted cells revealed that the purity was more than 99% and there was almost no contamination of other cells (Figure 1).

TRPV1 mRNA expression in peripheral blood immunocompetent cells

Quantitative analysis of TRPV1 mRNA was performed by real-time Q-PCR. cDNA was synthesized by reverse transcription of total RNA which was extracted from immunocompetent cells isolated with a high purity from normal PBMNCs indicated in Figure 1. Five samples were investigated. As shown in Figure 2, in seven types of investigated immunocompetent cells, higher levels of TRPV1 mRNA expression were confirmed in γδ T cells ($32.21 \pm 11.99 \times 10^{-5}$ fold expression compared to β-actin expression) and

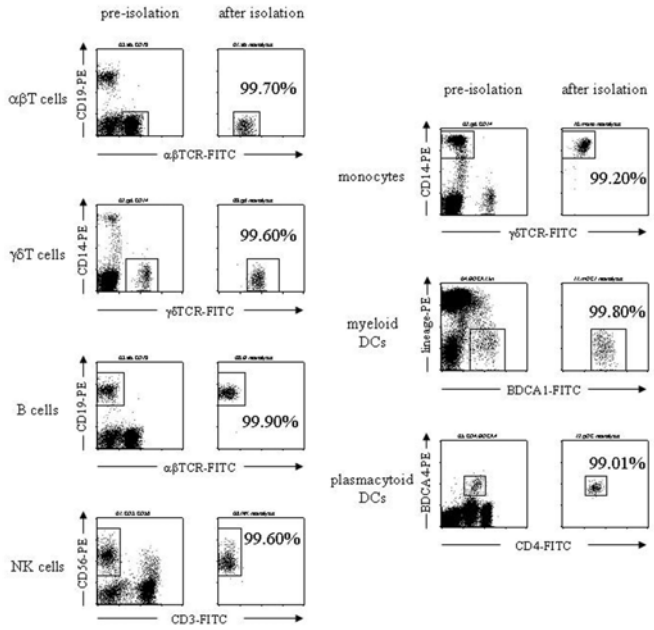

Fig.1 Purity of individual immunocompetent cells sorted from PBMNCs. Isolation of immunocompetent cells was performed by using FACSAria. Mononuclear cells were separated from buffy coat of a healthy donor, and labeled with fluorescein–conjugated mAbs and 7AAD, then isolated by using FACSAria. Left and right columns indicate pre–isolated and post–isolated data, respectively. The number indicates the percentage of gated cells at post–isolation. Purity of recovered cells was more than 99%. Representative data from 5 independent experiments are shown.

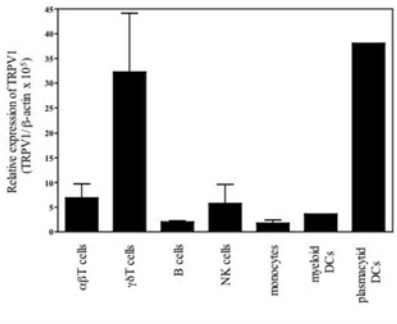

Fig.2 Quantitative analysis of TRPV1 mRNA in individual immunocompetent cells. Real –time Q –PCR was performed by using SYBR? Green. Quantitative values were calculated by using calculating formula previously described (see Materials and Methods). Data are represented as mean ± S.E.M. (n=5).

plasmacytoid DCs (38.04 x 10^{-5} fold expression compared to β -actin expression) than in other immunocompetent cells. On the other hand, B cells (1.99 ± 0.22 x 10^{-5} fold expression compared to β -actin expression), monocytes (1.73 ± 0.67 x 10^{-5} fold expression compared to β -actin expression) and myeloid DCs (3.604 x 10^{-5} fold expression compared to β -actin expression) showed lower levels of TRPV1 expression.

Discussion

Although, as previously mentioned, capsaicin, acid and heat are representative ligands for TRPV1, various compounds and chemicals including toluene, xylene, benzene, alcohols, ketones, ethers, aldehydes, formaldehydes, isocyanates and chlorine also stimulate TRPV1 (16-17). These various chemicals have been thought to cause MCS, including sick building syndrome (SBS). SBS is separated mainly into two types, one of which is associated with volatile organic solvents derived from various materials and the other is associated with hypersensitivity to surrounding molds (18-21). Several different fungi produce hydrophobic dialdehydes which stimulate TRPV1 (22-23). In addition, it is known that the mold Stachybotrys is able to produce similar hydrophobic dialdehyde toxins (24), though it has not been clarified whether these toxins stimulate TRPV1 or not. Furthermore, mold can produce various organic metabolites including alcohols, terpenes, ketones, aldehydes, esters and aromatic compounds. The types of chemicals produced by mold depends on the species of the mold and the environment, however, the ability of these microbial volatile organic compounds (MVOC) to induce sensory irritation has been investigated in experiment using mouse models (25).

In this study, the expression of TRPV1 mRNA in all the human PB-immunocompetent cells examined (α β T cells, γ δ T cells, B cells, NK cells, monocytes, mDCs and pDCs) was con-

firmed. Basu S et al. demonstrated that immature DC differentiated to mature DC by stimulation with capsaicin, the ligand of TRPV1, in experiments using mice (26). However, the physiological function of TRPV1 expressed in immunocompetent cells in vivo has not been elucidated. It is known that pDCs, which express high levels of TRPV1 mRNA (Figure 2), can initiate immune responses rapidly by recognizing virus and microbial antigens via toll-like receptors and secreting a large quantity of type I interferon (27-28). Functions of TRPV1 expressed in pDCs have not been reported and it remains unclear how pDCs, taking a role in the front line of innate immunity, react to various compounds and chemicals thought to cause SBS and MCS.

Our data suggested the presence of some type of relationship between immunocompetent cells and SBS/MCS, because TRPV1, a receptor for substances responsible for SBS and MCS, was confirmed to be expressed in human PB-immunocompetent cells.

Our data suggested the presence of some type of relationship between immunocompetent cells and SBS/MCS, because TRPV1, a receptor for substances responsible for SBS and MCS, was confirmed to be expressed in human PB-immunocompetent cells. Furthermore, as pDCs were demonstrated to express TRPV1 mRNA in a higher level than other investigated immunocompetent cells, pDC were presumed to take a pivotal role in pathophysiology of SBS/MCS.

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