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Proceedings Mini Review

# CCL1 and IL-1 induce damaged human colon epithelial cells to produce IL-22 for autonomous cure

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## Abstract

We have established a damage and recovery model of human colon epithelial cells. Human colon precancerous FPCK-1-1 cells are prepared from a patient with familial adenomatous polyposis. We have reported that *Spirulina* complex polysaccharides ameliorated the colon epithelial cell damages. Treatment with *Spirulina* polysaccharides recovered the damages caused by THP-1 cells stimulated with peptidoglycan (PGN) prepared from *Staphylococcus aureus*. In the previous study, FPCK-1-1 cells can produce IL-22 that is necessary to cure damages in response to either *Spirulina* complex polysaccharides or IL-1 + IL-6 + IL-23 in the presence of conditioned medium from PGN-stimulated THP-1 cells. In this study, we examined if IL-1, IL-6, and IL-23 are induced in PGN-stimulated THP-1 cells by analyzing the mRNA expressed in these cells. We have also analyzed mRNAs of FPCK-1-1 cells recovered in response to *Spirulina* complex polysaccharides. In conclusion, we have found that large amounts of CCL1, IL-1, and IL-23 mRNAs in PGN-stimulated THP-1 cells. However, we could not detect IL-6 mRNA, suggesting that IL-6 is not required for FPCK-1-1 cells to produce IL-22. We found that CCL1 and IL-1 are required for the production of IL-22 in FPCK-1-1 cells in the presence of conditioned medium from PGN-stimulated THP-1 cells. IL-23 did not enhance the level of IL-22 in this condition. We also found that mRNAs of the cell structure proteins such as keratin associated molecules and ankyrin-repeat containing molecules were increased when damaged FPCK-1-1 cell are recovered in response to *Siprulina* complex polysaccharides. Furthermore, we found that mRNAs for taste receptors and olfactory receptors are induced. It is suggested that the expression of these receptors for sense organs might be a criterion of the recovery stage of colon epithelial cells.

## Introduction

We have shown the evidence of autonomous cure of colon epithelial cells by producing IL-22 (Tominaga *et al*., 2013). In the experiment, we established a damage and recovery model of human colon epithelial cells. We used human colon precancerous FPCK-1-1 cells that are prepared from a patient with familial adenomatous polyposis (Kawaguchi *et al*., 1991). Treatment with *Spirulina* polysaccharides recovered the damages caused by THP-1 cells stimulated with peptidoglycan (PGN) prepared from *Staphylococcus aureus*.

In this review, we will focus on the condition to induce FPCK-1-1 cells to produce IL-22 that recovers the level of transepithelial resistance (TER). At first, we will briefly introduce two damage model of FPCK-1-1 cells. One is the early phase damage model, the curative reagents such as *Spirulina* complex polysaccharides (CPS) are added at the time of starting co-culture between FPCK-1-1 cells and macrophage-like cell line, THP-1 cells. The other is the late phase damage model, in which curative reagents are added three days after the co-culture when TER of FPCK-1-1 cells were decreased.

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In the previous paper, we reported that in the early phase damage, the level of claudin-2 is decreased and recovered by the addition of *Spirulina* CPS. In the late phase damage, we could not observe the rapid recovery of claudin-2. Instead, we found the production of IL-22 and the polysaccharides from FPCK-1-1 cells. This is probably the transient repair process before recovering the tight junction. We also reported that simultaneous addition of IL-1, IL-6, and IL-23 have equivalent ability with that of *Spirulina* CPS to recover the reduced level of TER of FPCK-1-1 cells to the normal level. I this review, we will introduce you our gene expression study focused on the level of cytokine mRNAs. We have found that there was no IL-6 mRNA in PGN-stimulated THP-1 cells. Instead, we found extremely high level of mRNA of CCL1 chemokine. We found that CCL1 and IL-1 can induce FPCK-1-1 cells to produce high level of IL-22 that is induced by *Spirulina* CPS. We will also introduce you the mRNAs that are induced in FPCK-1-1 cells in response to *Spirulina* CPS.

## Materials and methods

## Early phase damage model (preventive model)

FPCK-1-1 cells (2 X $10^5$) were cultured on the collagen-coated Teflon membrane with the pore of 0.4 $\mu$m (3493, Corning, Ithaca, NY) for five or six days before starting the co-culture. THP-1 cells (1 x $10^5$) were cultured in a 12 well culture plate (3513, Corning, Ithaca, NY) (Fig. 1). PGN was added to THP-1 cells one day before starting a co-culture. Curative reagents were added at the time of starting the co-culture. All cultures were performed as described previously (Tominaga *et al*., 2011; Tominaga *et al*., 2013).

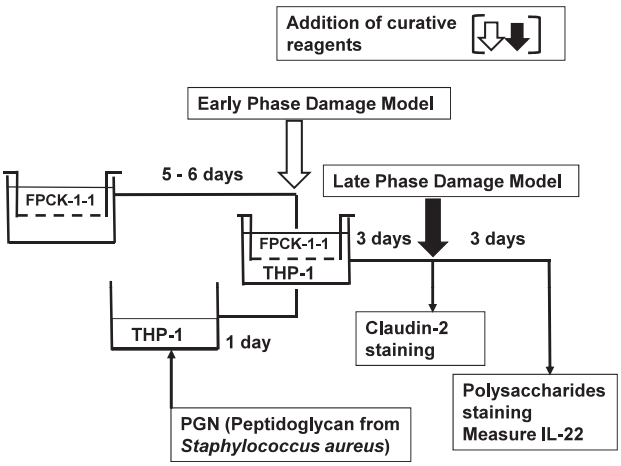


**Fig. 1. Time Schedule of co-culture showing both early phase damage model and late phase damage model.**

## Late phase damage model (curative model)

FPCK-1-1 cells (2 X $10^5$) were co-cultured with PGN-stimulated THP-1 cells as described in early phase damage model. Curative reagents were added three days after starting the cu-culture of FPCK-1-1 cells with PGN-stimulated THP-1 cells.

## Transcriptome analysis

RNA was extracted using RNA isolation kit, nucleoSpin RNA XS ( Macherey-Nagel GmbH & Co. KG, Düren, Germany). To analyze the gene expression, Human Transcriptome Array 2.1 was used (Affymetrix, Santa Clara, CA). In this Array, 44,699 protein coding genes and 22,829 non-coding genes were detected by analyzing 245,349 protein coding transcripts and 40914 non-coding transcripts. RNA expression analysis was performed according to the manufacturer's protocol. Expression Console and Transcriptome Analysis Console were used to analyze the gene expression (Affymetrix, Santa Clara, CA).

## Results and Discussion

IL-22 is engaged in the tissue regeneration and in the host defense at the barrier surface not only in intestines, but also in skin, lung, thymus, liver, kidney, and pancreas (Dudakov *et al*., 2015). It is reported that IL-22 is produced in $\alpha\beta$T cells, $\gamma\delta$T cells, innate lymphoid cells (ILCs), NKT cells, neutrophils, fibroblasts, and alveolar macrophages (Ikeuchi *et al*., 2005, Hansson *et al*., 2013, Dudakov *et al.*, 2015). Wolk *et al.* reported that IL-22 activated STAT3 and the transcription of $\beta$-Defensin 2 and 3 in keratinocytes (Wolk *et al*., 2004).

In a mouse model of ulcerative colitis, Sugimoto *et al.* reported that IL-22 stimulates mucus production and goblet cell recovery from the intestinal damage caused by intestinal inflammation (Sugimoto *et al*., 2008). They also showed that IL-22 can induce colon epithelial cells to produce mucin through STAT3 signaling pathway.

In our previous reports, we showed the ability of FPCK-1-1 colon epithelial cells to produce IL-22 in response to *Spirulina pacifica* complex polysaccharides (*Spirulina* CPS: Spi CPS) and the co-culture with peptidoglycan-stimulated THP-1 cells, and IL-22 acts autonomously to recover the TER of FPCK-1-1 cells by inducing the production of polysaccharides. We have found that less than 1 % FPCK-1-1 cells secrete IL-22, suggesting that special subpopulation of this cell line can secrete IL-22 in response to peptidoglycan-stimulated THP-1 cells. Lindemans *et al*. reported that IL-22 contributes intestinal stem cell niche to restore the epithelium after tissue injury (Lindemans *et al.*, 2015). Although FPCK-

1-1 cells are cloned, this cell line may contain both IL-22 secreting cells and intestinal stem cells resulting in the autonomous recovery by producing IL-22.

As we reported, damage of the colon epithelial cells was induced by co-culturing FPCK-1-1 cells with peptidoglycan (PGN prepared from *Staphylococcus aureus*)-stimulated THP-1 macrophage like cells (Fig. 1). The damage of colon epithelial cells was monitored by the decrease of TER of FPCK-1-1 monolayer cells. This decrease of TER was recovered by adding *Spirulina* CPS but not *Escherichia coli* lipopolysaccharides (*E. coli* LPS) (Fig. 2A). Both of these bacterial species are Gram negative and both of these polysaccharides are extracted using a hot phenol method developed by Westphal *et al* (Westphal *et al*., 1965).

Two damage models of colon epithelial cells were designed by changing the timing of the addition of curative reagent such as *Spirulina* CPS. In the early damage model, curative reagent was added at the time of starting the co-culture of FPCK-1-1 cells and PGN-stimulated THP-1 cells. In the late damage model, *Spirulina* CPS was added after TER was decreased by co-culturing FPCK-1-1 cells with PGN-stimulated THP-1 cells for three days (Fig. 1).

In the early damage model, FITC-coupled dextran passed the FPCK-1-1 monolayer cells from the upper chamber (apical side) to the lower chamber (basolateral side) (Fig. 2B). By adding the *Spirulina* CPS, leakage of FITC-coupled dextran through FPCK-1-1 monolayer cells decreased to the normal level (Fig. 2B). To clearly examine the tight junction, claudin-2 was stained with rabbit anti-claudin-2 antibodies followed by the goat anti-rabbit antibodies coupled with Alexa-fluor 488 (Fig. 2C). In normal condition, claudin-2 was observed clearly between the cells. By co-culturing with PGN-stimulated THP-1 cells, junctions between FPCK-1-1 cells became loose and claudin-2 remained in the cytoplasm. Moreover, there are spaces between FPCK-1-1 cells, suggesting that some of the FPCK-1-1 cells were lysed. When *Spirulina* CPS added, claudin-2 re-accumulated around the cell borders but not yet reassembled at the site of tight junction (Fig. 2C).

In the late phase damage model, although TER of FPCK-1-1 monolayer cells was recovered significantly, level of claudin-2 did not recover within three days after adding the curative reagents such as *Spirulina* CPS or IL-22 as we reported (Tominaga *et al*., 2013). When FPCK-1-1 cells were damaged by the three days co-culture, significant amounts of FITC-coupled dextran passed the FPCK-1-1 monolayer cells from the apical side to the basolateral side (Fig. 3B). The recovery of TER of FPCK-1-1 cells by the addition of *Spirulina* CPS depends on the production of IL-22 by FPCK-1-1 cells (Tominaga *et al*., 2013). By adding *Spirulina* CPS or

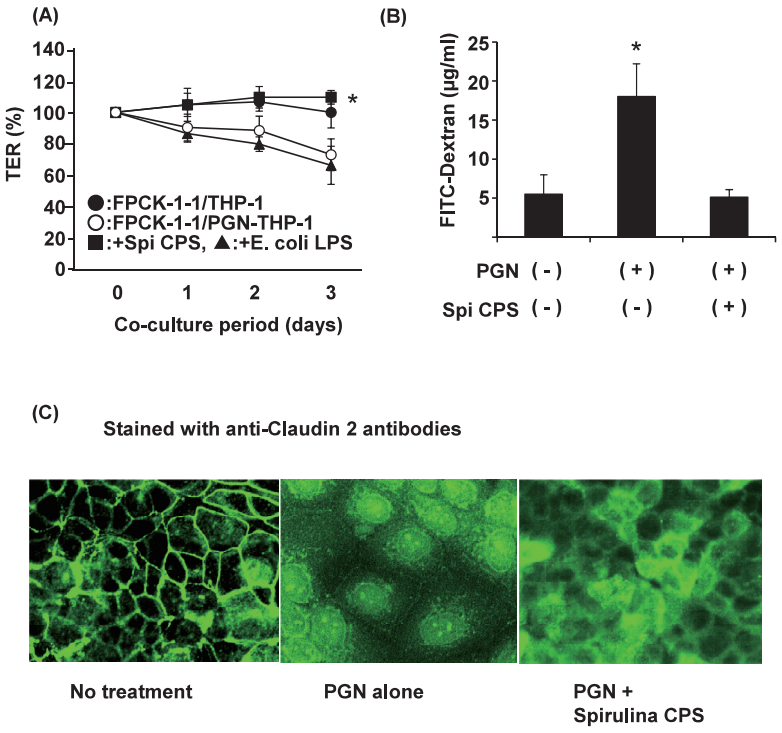


**Fig. 2. Characteristics of early phase damage model.** (A): Decline of TER (transepithelial resistance) after co-culturing FPCK-1-1 cells with PGN-stimulated THP-1 cells. (B): Lekage of FITC-dextran through FPCK-1-1 monolayer cells. (C): Expression of claudin 2 in FPCK-1-1 cells. Stained with rabbit anti-claudin 2 antibodies followed by goat anti-rabbit antibodies coupled with Alexa-fluor 488. Observed by laser scanning microscope. Magnification X 400. Modified from Tominaga *et al*., 2013.

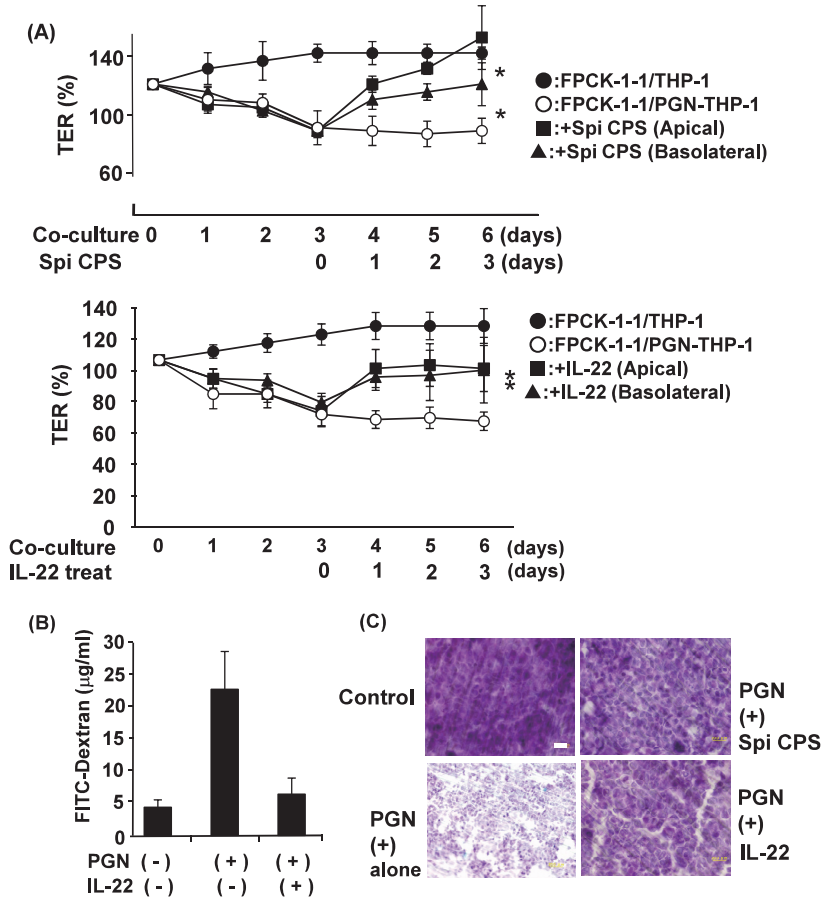


**Fig. 3.Characteristics late phase damage model.** (A): Addition of either *Spirulina* CPS (Spi CPS) or IL-22 to FPCK-1-1 cells could recover the TER. Addition of curative reagents to either apical or basolateral side worked well. (B): FITC-coupled dextran's leakage from apical side to the basolateral side was blocked by the addition of IL-22. (C): Polysaccharides production by FPCK-1-1 cell in response to Spi CPS or IL-22. Polysaccharides were stained by Alcian blue-PAS staining. Bars in photographs represent 20 $\mu$m. Modified from Tominaga *et al*., 2013.

IL-22, leakage of FITC-coupled dextran through FPCK-1-1 monolayer cells decreased to the normal revel (Fig. 3B). In case of the late phase damage model, the recovery of TER probably depends on the production of polysaccharides by FPCK-1-1 cells at the first (Fig. 3C). The recovery of the claudin-2 level probably follows this production of polysaccharides.

We have reported that the equivalent level of IL-22 was produced by FPCK-1-1 cells in response to *Spirulina* CPS was observed by the stimulation of IL-1$\beta$, IL-6, IL-23 (Fig. 4A). Higher level of production of IL-22 was not observed even *Spirulina* CPS was added in addition to IL-1$\beta$, IL-6, IL-23 (Fig. 4A). Addition of the supernatants of PGN-THP-1 cells increased the level of IL-22 production more than twice compared to that of *Spirulina* CPS alone (Fig. 4B).

To clarify the expression level of cytokines, we have analyzed the level of mRNAs of THP-1 cells in response to PGN by Affimetrix. As shown in Fig. 5, we found that level of CCL-1 is 348-fold increased. Levels of IL1B, IL-23 A, and IL-1A mRNAs are also increased 232-, 183-, and 109-fold, respectively. However, we could not found higher level of IL-6 mRNA in THP-1 cells in response to PGN.

Then, we measured the level of IL-22 produced by FPCK-1-1 cells in response to CCL-1 and IL-1. We found the high level of IL-22 in the group of conditioned medium of PGN-stimulated THP-1 cells (CM) and IL-1 (Fig. 6). We also found that IL-22 level of CM + CCL1 + IL-1 is equivalent to that of CM + CCL1 + IL-1 + IL-23 and IL-22 level of CM + CCL1 + IL-1 is higher than that of CM + CCL1 + IL-23 (Fig. 6). So, we concluded that CCL1 and IL-1 are the major stimuli to induce FPCK-1-1 cells to produce IL-22.

CCL1 is a chemokine mainly produced by activated T lymphocytes and attracts NK cells, B cells, monocytes and dendritic cells (Lira *et al*., 2012). The receptor for CCL1 is CCR8. It is suggested that CCR8 positive cells are engaged in an amplification of the innate immune response and are

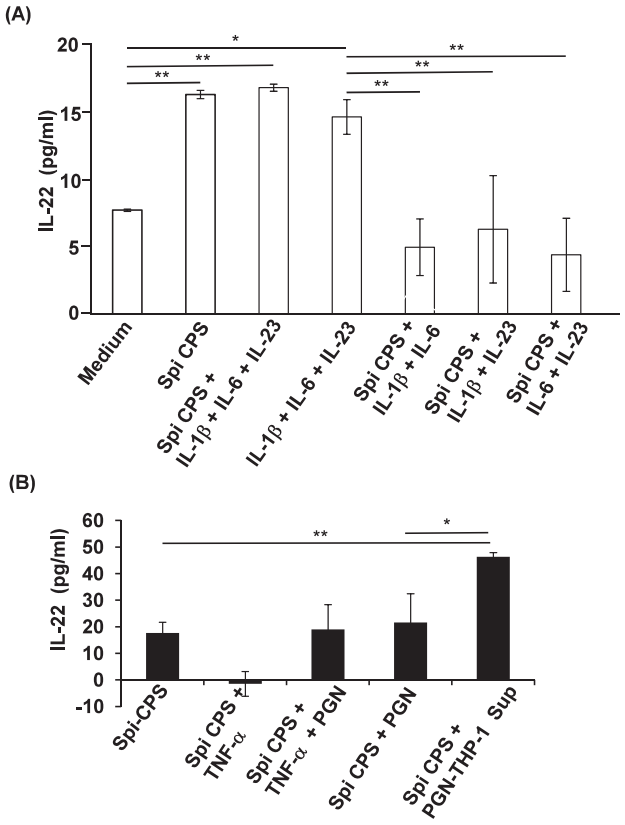


**Fig. 4. Conditions necessary for the production of IL-22 from FPCK-1-1 cells.** (A): Either IL-1$\beta$ + IL-6 + IL-23 or Spi CPS induced a similar level of IL-22 in FPCK-1-1 cells. (B): Supernatants of peptidoglycan from *Staphylococcus aureus*(PGN)-stimulated THP-1 cells enhanced the production of IL-22 in FPCK-1-1 cells. * $p < 0.05$; Tukay-Kramer. ** $p < 0.01$; Tukay-Kramer.

regulators of Th2 cell homing that triggers IL-5 induced allergic inflammation (Lira *et al*., 2012; Qu *et al*., 2004)

We also analyzed the level of increased mRNAs in FPCK-1-1 cells in response to *Spirulina* CPS when co-cultured with PGN-stimulated THP-1 cells (Fig. 7). Besides mRNAs for molecules that support the structure of cells such as keratin associated protein 5-8, 2-4, ankyrin repeat-containing proteins, heat shock 70 kDa protein 9, and oculomedin, taste receptor type 2 member 20 and 40, olfactory receptor, family 1, subfamily E, member 2 were induced. It is suggested that mRNA expression of taste receptors and olfactory receptor can be the sign of the recovery of intestinal epithelial cells.

We have also reported that the effects of *Spirulina* CPS on FPCK-1-1 cells to induce the production of IL-22 was blocked by adding either anti-TLR2 or anti-TLR4 antibodies.

We have a hypothesis that *Spirulina* CPS may induce a complex formation of TLR2-TLR2/TLR4-TLR4 to induce

| Fold Change (Linear) (PGN vs. Control) THP-1 mRNA | Gene Symbol | Upper chamber: FPCK-1-1 Lower chamber: THP-1 |
|---|---|---|
| 348.49 | CCL1: Chemokine (C-C motif) ligand 1 | |
| 232.33 | IL-1B : Interleukin 1, beta | |
| 194.53 | TFPI2 : Tissue factor pathway inhibitor 2 | |
| 183.08 | IL-23A: Interleukin 23, alpha subunit p19 | |
| 163.75 | MMP1: Matrix metallopeptidase (interstitial collagenase) | |
| 127.52 | PLA2G7: Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) | |
| 120.88 | PTGS2: Prostaglandin-endoperoxide synthase 2 (Prostaglandin G/H synthase and cyclooxygenese) | |
| 109.67 | IL-1A: Interleukin 1, alpha | |
| 102.91 | EBI3: Epstein-Barr virus induced 3 | |
| 96.62 | TNFAIP6: Tumor necrosis factor, alpha-induced protein 6 | |
| 95.42 | BCL2A1: BCL2-related protein A1 | |
| 94.38 | TNFSF15: Tumor necrosis factor (ligand) superfamily, member 15 | |
| 86.7 | MMP9: Matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase) | |

**Fig. 5. Induced mRNAs in THP-1 cells in response to peptidoglycan of *Staphylococcus aureus*.** mRNAs were extracted from PGN-stimulated THP-1 cells from the lower chamber. In the upper chamber, non-stimulated FPCK-1-1 cells were cultured.

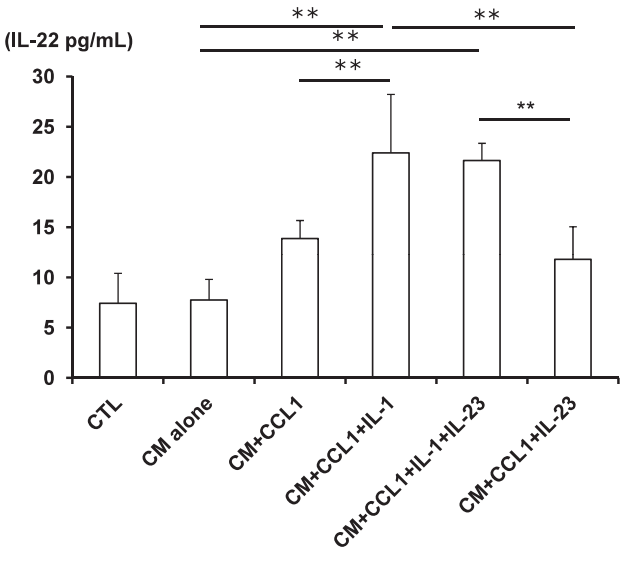


**Fig. 6. Requirement of CCL1 for the production of IL-22 by FPCK-1-1 cells.** Supernatants of PGN-stimulated THP-1 cells + CCL1 + IL-1 could induce a high level production of IL-22 in FPCK-1-1 cells without the addition of IL-6. IL-23 had no effects to enhance the production of IL-22 by FPCK-1-1 cells.
** $p < 0.01$; Tukay-Kramer.

| Fold Change (Linear) (Spi CPS vs. Control) FPCK-1-1 mRNA | Gene Symbol | Upper chamber: FPCK-1-1 Lower chamber: PGN-THP-1 |
|---|---|---|
| 3.72 | LOC642838: Ig kappa chain V-I region Walker-like | |
| 3.54 | TAS2R20: Taste receptor, type 2, member 20 | |
| 2.64 | KRTAP5-8: Keratin associated protein 5-8 | |
| 2.6 | TAS2R40: Taste receptor, type 2, member 40 | |
| 2.56 | ASB9: Ankyrin repeat and SOCS box containing 9 | |
| 2.35 | OR1E2: Olfactory receptor, family 1, subfamily E, member 2 | |
| 2.27 | KRTAP2-4: Keratin associated protein 2-4, keratin associated protein 2-4-like | |
| 2.26 | ANKRD36: Ankyrin repeat domain 36, ankyrin repeat domain 36C | |
| 2.17 | OCLM: Oculomedin | |
| 2.15 | TRAJ43: T cell receptor alpha joining 43 | |
| 2.12 | HSPA9: Heat shock 70 kDa protein 9 (mortalin) | |

**Fig. 7. Induced mRNAs in FPCK-1-1 cells in response to *Spirulina* Complex polysaccharides.** FPCK-1-1 cells were cultured in the upper chamber, while PGN-stimulated THP-1 cells were cultured in the lower chamber.

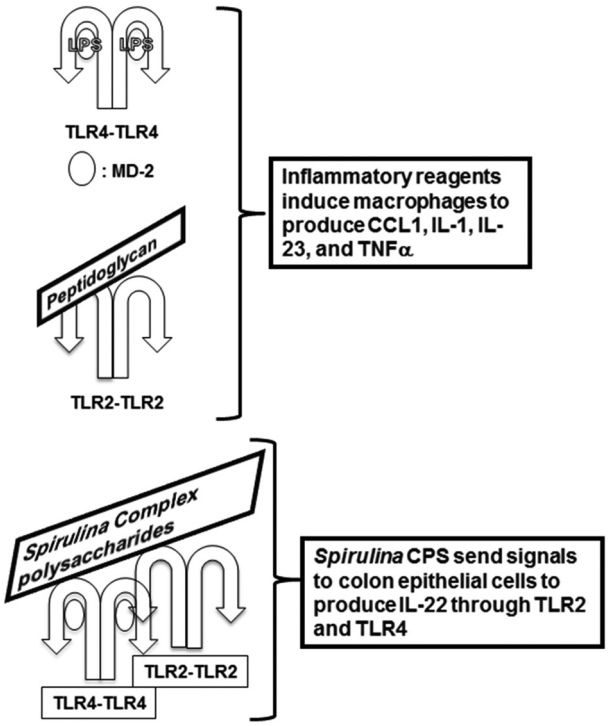


**Fig.8. A hypothesis how lipopolysaccharides and peptidoglycan induce inflammatory cytokines and how *Spirulina* complex polysaccharides induce IL-22.** *Spirulina* complex polysaccharides (CPS) may induce FPCK-1-1 cells to produce IL-22 by making a complex TLR2-TLR2/TLR4-TLR4.

colon epithelial FPCK-1-1 cells to produce IL-22 (Fig. 8).

We have also reported that *Petalonia binghamiae* polysaccharides but not fucoidan suppressed the delayed-type hypersensitivity against picryl chloride (2,4,6-trinitro-1-chlorobenzene). This suppression depends on TLR4, because this suppression was observed in wild type C3H/HeN mouse but not in TLR4 mutant C3H/HeJ mouse (Tominaga *et al*., 2010; Tominaga *et al*., 2011). These findings suggest that polysaccharides regulate the signal through TLRs. We think it is possible that polysaccharides can be used to manipulate the immune response in human, because edible algae polysaccharides suppress the delayed-type hypersensitivity by oral administration in mice (Tominaga *et al*., 2011).

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