## 第 13 回日本化学療法学会西日本支部支部長賞受賞論文概要

**タイトル**：Syk 依存性経路を介したカスポファンギンによるサイトカインおよびケモカイン産生修飾作用

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**概要**：

【背景】カンジダ症の重症化にサイトカイン産生過剰の関与が示唆され[1~4]，重症化の抑止にサイトカイン制御の観点も重要と考えられる。近年，宿主免疫細胞の真菌感染症に対する免疫応答に spleen tyrosine kinase（Syk）の関与を示す報告があるが[5~7]，抗真菌薬におけるサイトカイン制御作用については十分に解明されていない。

【目的】抗真菌薬によるサイトカイン制御とその過程における Syk の関与について検証することを目的とする。

【方法】単球系 THP-1 細胞を酵母真菌壁成分 zymosan で刺激し，キャンディン系およびアゾール系抗真菌薬を添加し，サイトカイン・ケモカイン［tumor necrosis factor alpha（TNF-α），interleukin（IL）-6，interferon-gamma inducible protein（IP）-10 等］の産生を enzyme linked immunosorbent assay（ELISA）および multiplex assay により調べた。また，Syk 依存性経路における作用を Western blot により検証した。

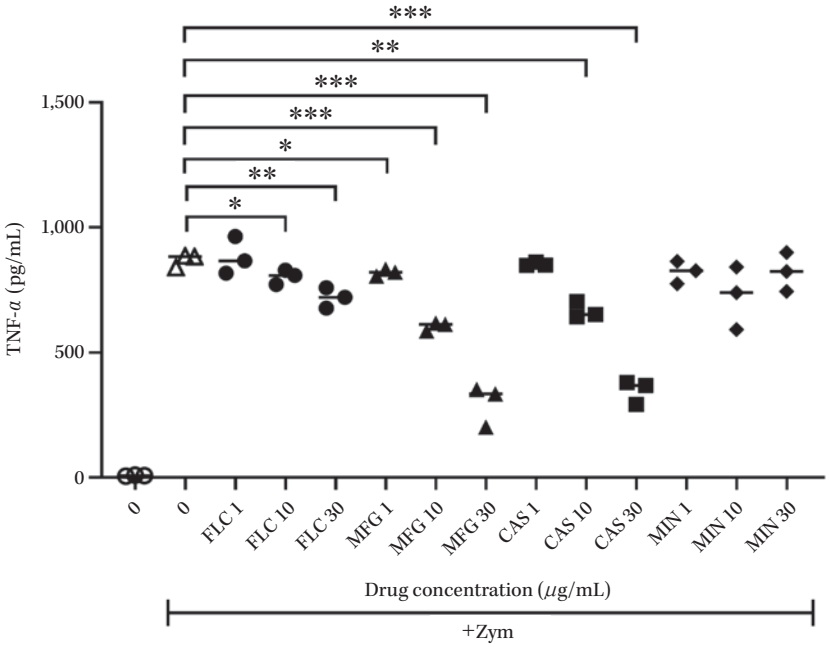


Fig. 1.　Effects of antimicrobial agents on TNF-α production in supernatants of zymosan-stimulated THP-1 cells.
THP-1 cells were incubated with zymosan (10 μg/mL) alone as a control. Zymosan-stimulated THP-1 cells were treated with 1-30 μg/mL of antimicrobial agents (FLC, MFG, CAS and MIN) for 4 h. The TNF-α levels in the supernatants of THP-1 cells were determined by ELISA. Horizontal bars are the median of three independent experiments. Unpaired two tailed t-test was used to evaluate whether differences between incubation with zymosan and antimicrobial agent versus zymosan only were statistically significant. *$P$＜.05; **$P$＜.01; ***$P$＜.001; antimicrobial agent-treated versus untreated control. Zym, zymosan; FLC, fluconazole; MFG, micafungin; CAS, caspofungin; MIN, minocycline.

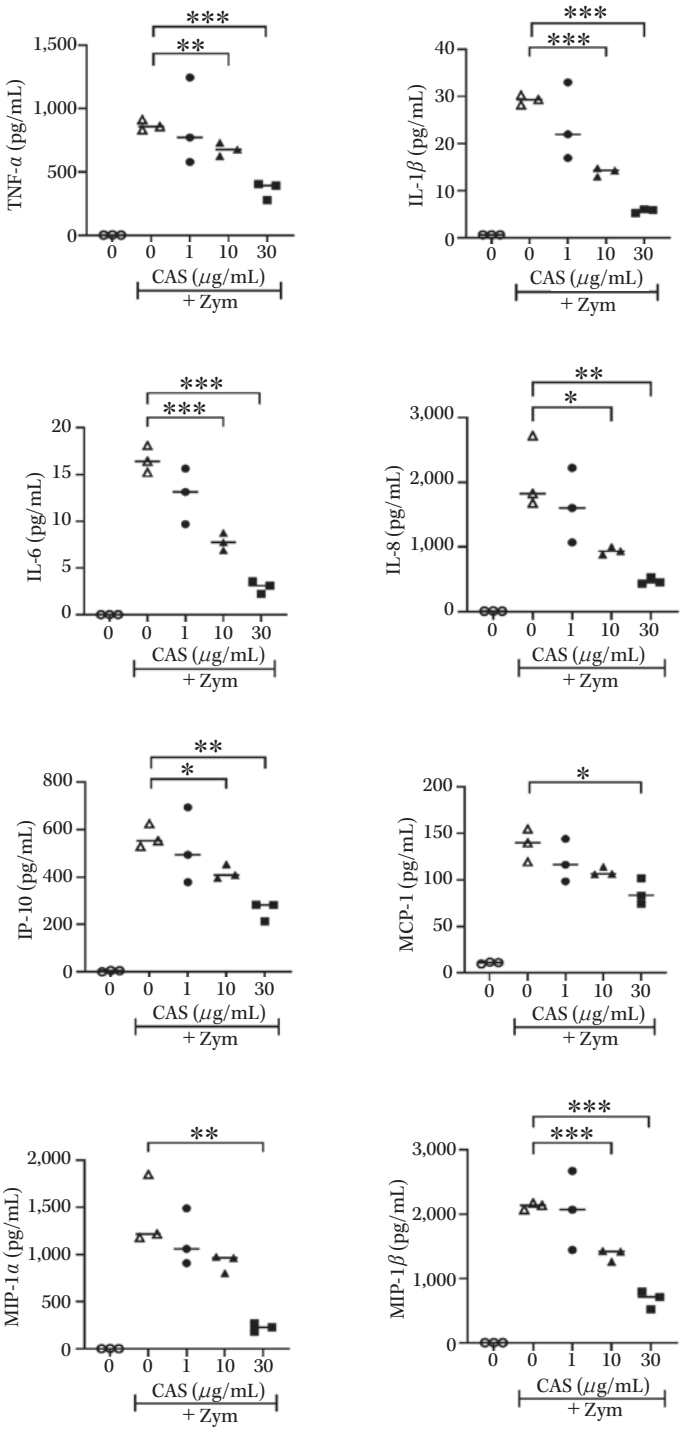


Fig. 2. Effects of CAS on cytokine and chemokine release in zymosan-stimulated THP-1 cells.

THP-1 cells were incubated with or without zymosan (10 μg/mL). Zymosan-stimulated THP-1 cells were then treated with 1-30 μg/mL CAS for 4 h. Cytokine and chemokine levels including TNF-α, IL-1β, IL-6, IL-8, IP-10, MCP-1, MIP-1α, and MIP-1β in the supernatants from zymosan-stimulated THP-1 cells were determined simultaneously using multiplex bead immunoassays. Horizontal bars are the median of three independent experiments. *$P<.05$; **$P<.01$; ***$P<.001$; CAS-treated versus untreated control. Zym, zymosan.

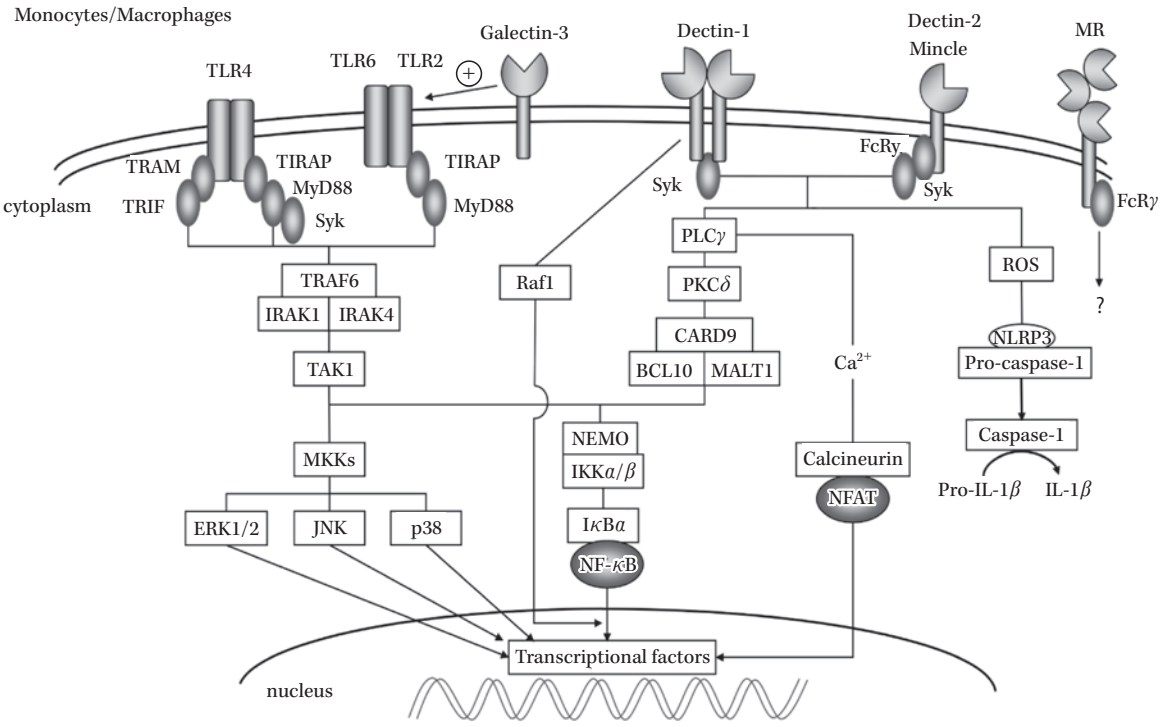


Fig. 3. Signaling pathways in zymosan recognition.
Zymosan is recognized by PRRs. Membrane-bound PRRs include TLRs and CLRs [TLRs: TLR2, TLR4, and TLR6; CLRs: dectin-1, dectin-2, Mincle, mannose receptor (MR), and galectin-3]. Binding to fungal pathogen-associated molecular patterns including $\beta$-glucans, mannans, and chitins, such as zymosan, activates intracellular signaling pathways.

【結果】Zymosan 10 $\mu$g/mL で刺激した THP-1 細胞に，フルコナゾール（FLC），ミカファンギン（MFG）およびカスポファンギン（CAS）を添加したところ，濃度依存的に TNF-$\alpha$ 産生抑制を認めた（Fig. 1）。各抗真菌薬 30 $\mu$g/mL 添加時にコントロールと比較し，TNF-$\alpha$ 中央値がそれぞれ CAS 42%，MFG 38%，FLC 82% まで低下し，アゾール系よりキャンディン系抗真菌薬において，TNF-$\alpha$ 抑制効果が強かった。IL-6，IP-10 等もキャンディン系抗真菌薬は濃度依存的な抑制効果を示した（Fig. 2）。Western blot にて細胞内シグナル伝達経路を解析した。Zymosan による刺激は，細胞膜の Toll-like receptors（TLR）や C-type lectin receptors（CLR）を介し，Syk が活性化され細胞内シグナルを下流に伝達する[8~11]（Fig. 3）。CAS は CLR 系では Syk およびその下流の蛋白として，p-38，extracellular signal-regulated kinase，c-Jun N-terminal kinase，inhibitor of nuclear factor-kappa-B $\alpha$，nuclear factor of activated T-cells 1 のリン酸化および caspase-1 の活性化を抑制し，TLR 系では下流分子である IL-1 receptor-associated kinase（IRAK）1，IRAK4，transforming growth factor-beta-activated kinase 1 のリン酸化を抑制した（Fig. 4）。さらに zymosan 刺激による THP-1 細胞における TNF-$\alpha$ 産生および Syk とその下流分子の活性化は Syk 阻害薬によっても抑制されることを確認した（data not shown）。

【考察】キャンディン系抗真菌薬はアゾール系抗真菌薬よりもサイトカイン産生抑制効果が強く，カンジダ感染の重症化における高サイトカイン状態をより効率的に抑制できる可能性がある。CAS は Syk 依存性経路抑制によるサイトカイン・ケモカイン過剰産生抑止に貢献することが示された。Zymosan 投与による非感染性炎症の動物モデルにおいて，Syk 阻害薬が炎症性サイトカインを抑制し低血圧や頻脈などショックに伴う状態を改善させることが報告されている[12]。真菌感染症の重症化に関して，CAS は抗真菌作用に加え，Syk 依存性経路の活性化抑制作用が加わることで，より有効に働く可能性があることが示された。

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利益相反自己申告：申告すべきものなし。

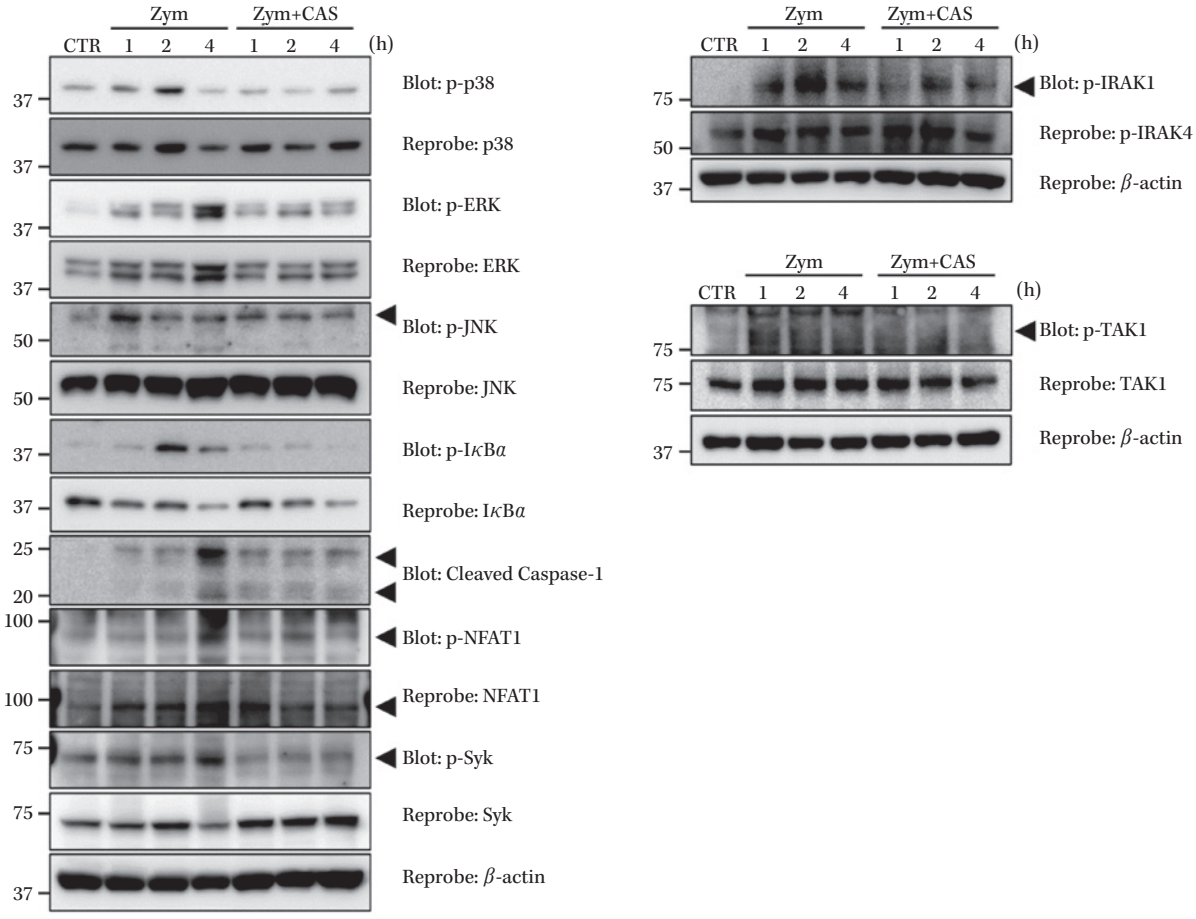


Fig. 4. Effects of CAS Syk signaling pathways in zymosan-activated THP-1 cells.
THP-1 cells were incubated with or without zymosan (10 μg/mL) in the presence or absence of CAS (30 μg/mL) for 0, 1, 2, and 4 h. Detergent-soluble lysates were analyzed by immunoblotting using the indicated antibodies. Molecular size markers are indicated at the left of the figure in kDa. The results are representative of three independent experiments. CTR, control; p-ERK, phosphorylated ERK; Zym, zymosan.

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## Modulation of cytokine and chemokine production by caspofungin through inhibition of the spleen tyrosine kinase-dependent pathway

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*Candida* sepsis is considered to contribute to hypercytokinemia in both patients with severe infection and immunocompromised condition. Past research has demonstrated that antibiotics and antifungals not only have antimicrobial efficacy but also affect the immune system. We previously examined whether immune cells were modulated by antibiotics such as tetracyclines or macrolides. The modulation of cytokine/chemokine release from lipopolysaccharide-stimulated cells by those agents was elucidated. However, few reports about the modulation of the immune system by antifungal agents were found. In this study, the production of pro-inflammatory cytokines and chemokines and signaling pathways involved were investigated in zymosan-activated THP-1 cells. The effects were examined using antifungal drugs such as caspofungin (CAS), micafungin and fluconazole. Pro-inflammatory cytokine and chemokine levels were determined using enzyme linked immunosorbent assay (ELISA) and multiplex assay. Protein phosphorylation was evaluated by western blot analysis. CAS significantly decreased zymosan-induced pro-inflammatory cytokine and chemokine release in THP-1 cells, as shown by ELISA and multiplex assay. In western blot analysis, inhibitor of nuclear factor-kappa-B alpha, p38, c-Jun N-terminal kinase, extracellular signal-regulated kinase, and nuclear factor of activated T-cells phosphorylation and activation of caspase-1 that are found downstream of C-type lectin receptors and interleukin-1 receptor-associated kinase 1/4 and transforming growth factor-beta-activated kinase 1 that are downstream molecules of Toll-like receptors and spleen tyrosine kinase (Syk) were downregulated. The major underlying mechanism of pro-inflammatory cytokine and chemokine suppression by CAS is to inhibit activation of Syk and its downstream signaling molecules. Based on the results, it can be concluded that CAS activity possibly involves Syk signaling pathways and has potential to prevent hypercytokinemia in fungal sepsis.