TLR2-independent Activity of SMP-105 (BCG-CWS) on BC-1, a Mouse Immature Dendritic Cell Line

Yoshikazu Yanagi,[1] Shuichi Kishimoto,[2] and Shoji Fukushima[2]

[1]MBR Co., Ltd., and [2]Facalty of Pharm. Sci., Kobe Gakuin Univ.

Introduction

The cell wall skeleton of *Mycobacterium bovis* BCG (BCG-CWS) has been used as an effective adjuvant for immunotherapy of a variety of cancer patients.

SMP-105 is a highly purified BCG-CWS.[1] It activated Toll-like receptor 2 (TLR2) in various cells.[2] On the other hand, the *in vivo* antitumor effect of SMP-105 mostly remained in TLR2 KO mice, although its effect was completely cancelled in MyD88 KO mice.[2] Thus the mechanisms of action of SMP-105 are controversial.

It has been reported that SM-105 activated BC-1, a mouse immature dendritic cell line, in a manner different from Pam3CSK4, another TLR2 ligand.[3] These data suggest that the effect of SMP-105 on BC-1 is not TLR2-mediated one. The present study was carried out to confirm this hypothesis.

SMP-105 is very slightly soluble in water. The present study was carried out using a new formulation of SMP-105, which is an aqueous suspension of a single particle of SMP-105, in order to obtain the quantitative and reproducible data *in vitro*.

Materials & Methods

SMP-105 (BCG-CWS): SM-105 was prepared in Dainippon Sumitomo Pharm Co., Ltd. as described previously.[1] SMP-105 aqueous suspension (Fig. 1) was prepared in MBR Co., Ltd.

Reagents: Pam3CSK4 was purchased from Calbiochem, Latex beads (0.8 μm) from Sigma-Aldrich Japan, human IgG from Oriental Yeast Co., Ltd., and monoclonal antibody to mouse TLR2 from Hycult Biotech.

Cells: BC-1, a mouse immature dendtritic cell line,[4] was kindly provided from Dr. Yoshiki Yanagawa in Health Science University of Hokkaido. BC-1 cells were maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal calf serum (FCS), 30% NIH/3T3 supernatant, 10 ng/mL mouse recombinant GM-CSF, 4 mM L-glutamine, 50 μg/mL streptomycin, 50 U /mL penicillin, and 50 μM 2-mercaptoethanol.
HEK-Blue™-2 cells were purchased from InvivoGen, which were designed for studying the stimulation of TLR2.

***In vitro* experiments:** BC-1 cells were cultured with SMP-105 and Pam3CSK4 overnight. The production of IL-12/IL-23 p40 were determined by enzyme-linked immunosorbent assay (ELISA)(R & D Systems, Inc.).

Monoclonal antibody to mouse TLR2 were added to BC-1 cells at 2 μg/mL simultaneously with SMP-105 and Pam3CSK4.

3.73×10^8 Particles of Latex beads were incubated with 25 μg/mL IgG in 50 μL PBS at 37°C for 30 min., centrifuged, and resuspended in 1 mL culture medium. Latex beads were added to BC-1 cells at 1.9×10^8, 5.6×10^7, and 1.9×10^7 particles/ml simultaneously with SMP-105.

HEK-Blue™-2 cells were incubated with SMP-105 overnight.

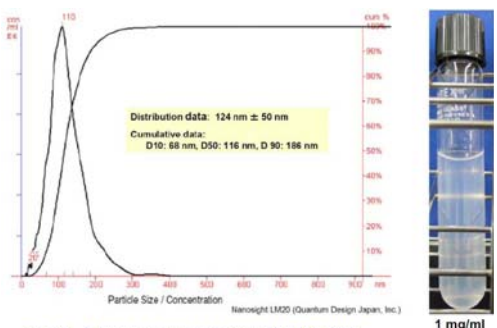

Fig. 1. Aqueous suspension of SMP-105

The particle size of SMP-105 aqueous suspension was 124 nm ± 50 nm.

Results

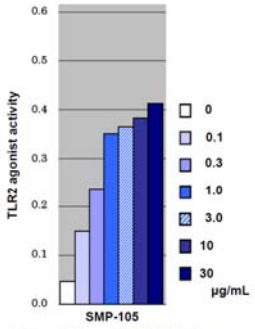

Fig. 2. TLR2 agonist activity of SMP-105

SMP-105 activated TLR2 concentration-dependently in the range between 0.1 to 1.0 μg/ml. Its activity plateaued at more than 1.0 μg/ml.

TLR2 agonist activity was assayed in HEK-Blue™-2 cells (InvivoGen).

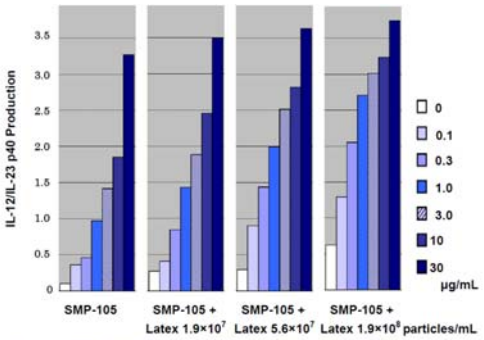

Fig. 3. SMP-105 induced IL-12/IL-23 p40 in BC-1 cells and its effect was enhanced by Latex beads.

SMP-105 produced IL-12/IL-23 p40 concentration-dependently in the range between 0.1 to 30 μg/ml in BC-1 cells. Its production was markedly enhanced depending on the concentration of latex beads. Latex beads were added to BC-1 at the concentrations of 1.9×10^7, 5.6×10^7, and 1.9×10^8 particles/ml.

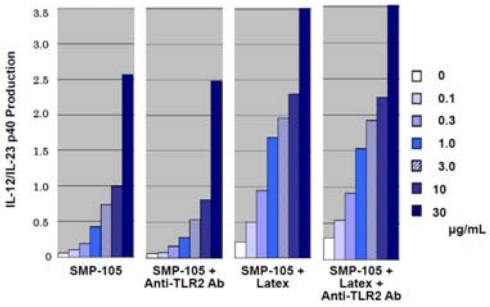

Fig. 4. Effect of anti-TLR2 antibody on IL-12/L-23 p40 production induced by SMP-105 in BC-1 cells.

Monoclonal antibody to mouse TLR2 was added to BC-1 cells at 2 μg/ml.

The IL-12/IL-23 p40 production induced by SMP-105 and its enhancement by latex beads were not almost inhibited by anti-TLR2 antibody.

The data suggest that SMP-105 induces IL-12/IL-23 p40 production without mediating TLR2 in BC-1 cells.

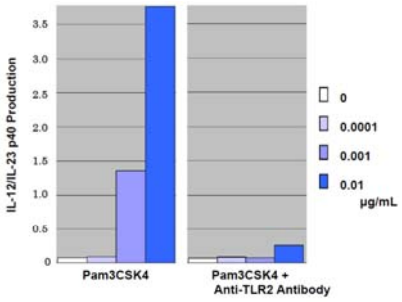

Fig. 5. Effect of anti-TLR2 antibody on IL-12/L-23 p40 production induced by Pam3CSK4 in BC-1 cells.

Monoclonal antibody to mouse TLR2 was added to BC-1 cells at 2 μg/ml.

The IL-12/IL-23 p40 production induced by Pam3CSK4 was inhibited by anti-TLR2 antibody.

The data shows that Pam3CSK4 induces IL-12/IL-23 p40 production through TLR2 in BC-1 cells.

Conclusions

1. SMP-105 activated TLR2 in HEK-Blue™-2 cells (InvivoGen).
2. SMP-105 induced IL-12/IL-23 p40 production in BC-1 cells.
3. IL-12/IL-23 p40 production induced by SMP-105 in BC-1 cells was markedly enhanced by Latex beads.
4. SMP-105-induced IL-12/Il-23 p40 production in BC-1 cells was not inhibited by the monoclonal antibody to mouse TLR2 (Hycult Biotech), although the IL-12/IL-23 p40 production induced by Pam3CSK4, another TLR2 ligand, was inhibited.
5. The data suggest that SMP-105 induces the maturation of BC-1 cells without mediating TLR2.

References

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Contact Information

Yoshikazu Yanagi, Ph.D.

E-mail address: yanagiy276@mbresearch.co.jp
Tel: 072-646-7765 Fax: 072-646-7745

MBR Co., Ltd.
210, Saito-Bioincubator,
7-7-15, Saito-asagi, Ibaraki-city, Osaka, 567-0085, JAPAN
URL: http://www.mbresarch.co.jp

COI Disclosure Information

Yoshikazu Yanagi

I have the following financial relationships to disclose.

Leadership position/advisory role for: MBR Co., Ltd.

Stockholder in: MBR Co., Ltd.