

Brief Communication



Heat-Killed *Saccharomyces cerevisiae*, A Dectin-1 Agonist, Selectively Induces IgG4 Production by Human B Cells

Ha-Yan Park^{1†}, Hee-Kyung Yoon^{1†}, Jong-Yeup Kim^{2†}, Seok-Rae Park^{1,3,*}

¹Department of Microbiology, College of Medicine, Konyang University, Daejeon 35365, Korea

²Department of Otorhinolaryngology-Head and Neck Surgery, College of Medicine, Konyang University, Daejeon 35365, Korea

³Priority Research Center, Myunggok Medical Research Institute, College of Medicine, Konyang University, Daejeon 35365, Korea



Received: Sep 30, 2018

Revised: Dec 2, 2018

Accepted: Dec 14, 2018

*Correspondence to

Seok-Rae Park

Department of Microbiology, College of Medicine, Konyang University, 158 Gwanjeodong-ro, Seo-gu, Daejeon 35365, Korea.

E-mail: srpark@konyang.ac.kr

[†]These authors contributed equally to this work.

Copyright © 2018. The Korean Association of Immunologists

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Seok-Rae Park

<https://orcid.org/0000-0002-1407-4495>

Conflicts of Interest

The authors declare no potential conflicts of interest.

Abbreviations

AID, activation-induced cytidine deaminase; CSR, class switch recombination; CTLD, C-type lectin-like domain; GLT, germline transcripts; HKSC, heat-killed *Saccharomyces cerevisiae*; IgG4-RD, IgG4-related disease

ABSTRACT

Dectin-1 is a major receptor that recognizes fungal cell wall β -glucan. We previously reported that heat-killed *Saccharomyces cerevisiae* (HKSC), a Dectin-1 agonist, selectively induces IgG1 class switching in mouse B cells. Dectin-1 is also expressed on human B cells; however, Dectin-1 function in human B cells remains unknown. This study aimed to investigate the direct effect of *in vitro* stimulation using HKSC on Ig class switching in human B cells. HKSC selectively induced the expression of germline γ 4 transcripts (GLT γ 4) by human B cell line 2E2, and HKSC significantly augmented GLT γ 4 promoter activity. Moreover, HKSC selectively enhanced GLT γ 4 expression and IgG4 production by anti-CD40-activated human tonsillar resting B cells. Thus, these results suggest that Dectin-1 maybe involved in selective IgG4 class switching by human B cells.

Keywords: Heat-killed *Saccharomyces cerevisiae*; Human B cells; Dectin-1; Germline γ 4 transcripts; IgG4

INTRODUCTION

Dectin-1 is a C-type lectin receptor expressed on myeloid dendritic cells, macrophages/monocytes, T cells, and B cells. The Dectin-1 recognizes β -glucan of fungal cell wall particles, such as heat-killed *Saccharomyces cerevisiae* (HKSC), heat-killed *Candida albicans*, and zymosan, to protect fungal infection (1-8). Thus, recognizing β -glucan by Dectin-1 induces numerous cellular responses, including phagocytosis, respiratory burst, arachidonic acid metabolite production, and cytokine and chemokine induction, for promoting antifungal immunity. Antifungal antibodies are essential for the protection of hosts from pathogenic fungi (9-12).

Ig class switching is a process in which B cells shift from production of IgM to IgG3, IgG1, IgG2b, IgG2a, IgE, or IgA in mice or to IgG3, IgG1, IgA1, IgG2, IgG4, IgE, or IgA2 in humans (13). LPS, CD40 ligand, and various cytokines directly activate B cells and induce Ig class switching. In humans, IL-4 and IL-13 drive B cell switching to IgG4, IgE (14-16), IgG3, and IgG1 (17), IL-10 to IgG3 and IgG1 (18), IL-13 to IgG4 and IgE (14), and TGF- β to

Author Contributions

Conceptualization: Park SR, Kim JY; Data curation: Park HY, Yoon HK, Park SR; Formal analysis: Park HY, Park SR; Funding acquisition: Park SR; Investigation: Park HY, Yoon HK, Kim JY, Park SR; Resources: Park SR, Kim JY; Supervision: Park SR; Writing-original draft: Park HY, Yoon HK, Park SR.

IgA (13,19). Moreover, IL-10, IL-12, IL-21, and vascular endothelial growth factor have been reported to skew class switching toward IgG4 (20-24). This class switching is mediated by the class switch recombination (CSR) of the Ig heavy chain gene. The transcription of germline transcripts (GLT) on each switch region of the Ig heavy chain DNA in mature B cells is a prerequisite for each Ig CSR process (16). For instance, selective induction of GLT transcription initiates IgE class switching by increasing the accessibility of activation-induced cytidine deaminase (AID), which is an essential enzyme for the Ig CSR process (25), to the non-transcribed DNA strand of the switch region.

We reported recently that Dectin-1 stimulation with its agonists (i.e., HKSC and depleted zymosan) selectively induces IgG1 class switching resulting in an increase of IgG1 production by mouse B cells (26,27). Dectin-1 is also expressed on human B cells (2). However, the role of Dectin-1 in human B cells has not been determined. Here we found that direct Dectin-1 stimulation with the Dectin-1 agonist HKSC selectively induces GLT γ 4 expression and IgG4 production by human B cells.

MATERIALS AND METHODS**Human B cell line and isolation of human tonsillar resting B cells**

The mature human B cell line 2E2 (surface IgM⁺ and IgD⁺) (28) was provided by Dr. P. Casali (University of Texas Long School of Medicine, San Antonio, TX, USA). Fresh human tonsil tissues were obtained from tonsillectomies performed at the Department of Otorhinolaryngology–Head and Neck Surgery (Konyang University Hospital, Daejeon, Korea). The tonsil tissues were cut with sterilized scissors and homogenized by a homogenizer with HBSS (WelGENE, Daegu, Korea) containing 1% penicillin/streptomycin (Gibco, Invitrogen, Carlsbad, CA, USA). The suspended tonsillar cells were passed through a 70- μ m cell strainer (BD Falcon, San Jose, CA, USA) to separate single cells. Tonsillar cells were prepared by a Ficoll/Histopaque-1077 (Sigma Aldrich, Saint Louis, MO, USA) density gradient method and further isolated by MACS negative selection using anti-CD43 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) to obtain untouched resting B cells. The purity of tonsillar resting B cells (CD43⁻CD19⁺, $\geq 98\%$) was assessed by flow cytometry using a FACSCalibur (BD Biosciences, San Jose, CA, USA), following staining of the cells with anti-human CD43 FITC (eBioscience, San Diego, CA, USA) and anti-human CD19 PE (BioLegend, San Diego, CA, USA). The Institutional Review Board of Konyang University Hospital approved this study (approval No. KYUH 2015-05-007-003).

Cell culture and reagents

Cells were cultured at 37°C in a humidified CO₂ incubator (Forma Scientific, Marietta, OH, USA) in RPMI-1640 medium (WelGENE) supplemented with 10% fetal bovine serum (PAA Laboratories, Etobicoke, ON, Canada). Cells were stimulated using HKSC (1 \times 10⁷ cells/ml, InvivoGen, San Diego, CA, USA). Anti-human CD40 Ab was purchased from eBioscience and rhIL-4 was obtained from R&D Systems (Minneapolis, MN, USA). The Dectin-1 antagonist laminarin was purchased from InvivoGen.

RT-PCR

RNA preparation and RT-PCR were performed as previously described (27). PCR primers (Supplementary Table 1) were synthesized by Bioneer (Daejeon, Korea). The PCR for β -actin was simultaneously performed to normalize cDNA concentrations within each sample set. PCR products were resolved using electrophoresis on 2% agarose gels.

Reporter plasmid, transfection, and luciferase assays

The human GLT γ 4 promoter DNA fragment (-1076 to +100) was amplified from human tonsil genomic DNA using PCR. PCR primers (**Supplementary Table 1**) were derived from previously reported human GLT γ 4 promoter nucleotide sequences (29,30). The GLT γ 4 promoter fragment was subcloned into the pGL3-basic vector (Promega, Madison, WI, USA), and the reporter plasmid was named pGL3-h γ 4[-1076/+100]. Transfection was performed by electroporation using a Gene Pulser II electroporation system (Bio-Rad, Hercules, CA, USA) as described previously (31). The reporter plasmid was co-transfected with pCMV- β -gal (Stratagene, La Jolla, CA, USA), and luciferase and β -gal assays were performed as described previously (31).

Isotype-specific ELISA

Abs produced in B cell cultures were detected using isotype-specific ELISAs as described previously (27).

Cell viability assay

Cell viability was determined using EZ-Cytox cell viability assay kits (Daeil Lab Service Co., Seoul, Korea) as described previously (32). Briefly, 20 μ l of EZ-Cytox kit reagent was added to each cell-cultured well of a 96-well microplate and then incubated at 37°C in a humidified CO₂ incubator for 3 h. After incubation, OD was measured at a wavelength of 450 nm using an absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis

Statistical differences between experimental groups were determined by analysis of variance. All p-values were calculated using unpaired 2-tailed Student's *t*-tests.

RESULTS AND DISCUSSION

Effect of HKSC on GLT expression in a human B cell line

First, we examined the effect of the Dectin-1 agonist HKSC on the expression of GLTs by the human B cell line 2E2. The 2E2 cells (IgD⁺IgM⁺) can undergo Ig CSR through initiation of GLT transcription after *in vitro* exposure to appropriate stimuli (28,33). As shown in **Fig. 1A**, HKSC selectively induced GLT γ 4 expression. AID mRNA expression was not affected by HKSC stimulation. Human Dectin-1 is alternatively spliced, resulting in several isoforms (splice variants: Dectin-1 [full length], Dectin-1b, and Dectin-1c) in peripheral blood mononuclear cells and immature monocyte-derived dendritic cells (34-36). The expression of these isoforms is cell and activation specific, and Dectin-1b is mainly expressed in human dendritic cells and macrophages (2,34,36,37). The differences underlying specific isoform expression and function are unclear, although there are evidences for specific isoform-related functions in mice (5,38,39). We observed that 2E2 B cells express Dectin-1b but not full-length Dectin-1 or Dectin-1c under basal conditions (**Fig. 1A**, lower left panel). Interestingly, Dectin-1c expression was dramatically induced by HKSC, and this expression is correlated with GLT γ 4, whereas the basal expression of Dectin-1b was decreased. Hence, we speculate that HKSC-induced Dectin-1c mRNA expression has a certain critical role in inducing GLT γ 4 transcription by human B cells. However, in a study with dendritic cells, Hermanz-Falc3n et al. (34) described that Dectin-1c lacks a complete C-type lectin-like domain (CTLN); therefore, this spliced variant is unlikely to encode a functional lectin. The major function of Dectin-1 is likely carried out by Dectin-1 (full length) and Dectin-1b, both bearing a complete CTLN. Thus, the various Dectin-1 isoforms

may serve specific roles in each immune cell type and differential isoform usage may represent a mechanism of regulating cellular responses to its ligand in the immune system (2,35,38). Nonetheless, it will be important to address the function of the Dectin-1c isoform and the significance of the positive correlation between Dectin-1c and GLT γ 4 expression in HKSC-stimulated B cells. On the other hand, the Dectin-1 antagonist laminarin abrogated HKSC-induced GLT γ 4 and Dectin-1c expression (Fig. 1A, lower center panel). Next, we constructed a GLT γ 4 promoter reporter and then determined whether HKSC activates the promoter activity. HKSC significantly enhanced GLT γ 4 promoter activity (Fig. 1B). IL-4 treatment was used as a positive control for GLT γ 4 induction (Fig. 1A, lower center panel and Fig. 1B). These results suggest that B cell Dectin-1 stimulation selectively induces GLT γ 4 transcription through the regulation of Dectin-1b and Dectin-1c expression.

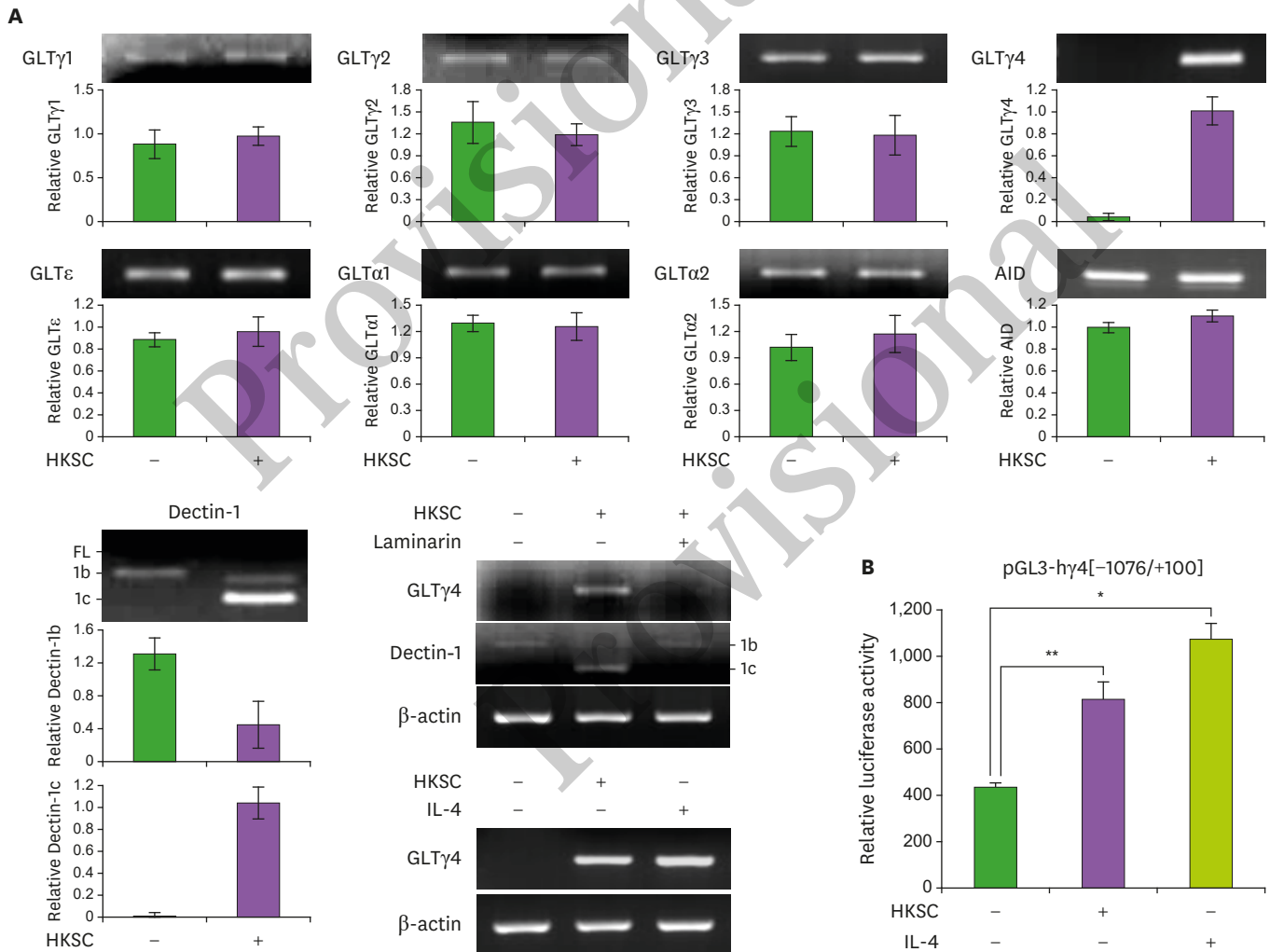


Figure 1. Dectin-1 agonist HKSC selectively induces GLT γ 4 expression in human B cell line 2E2. (A) 2E2 cells were stimulated with HKSC (1×10^7 cells/ml), laminarin ($10 \mu\text{g/ml}$), and IL-4 (10 ng/ml). After 2 days of culture, mRNAs were isolated, and GLTs, AID, and Dectin-1 mRNA levels were measured by RT-PCR. Graphs indicate relative cDNA levels that are normalized to β -actin cDNA expression using ImageJ (National Institutes of Health, Bethesda, MD, USA) analysis. Densitometric data are averages of 2 independent experiments with ranges (bars). FL, full length. (B) 2E2 cells were transfected with the indicated human GLT γ 4 promoter reporter (pGL3-hy4[-1076/+100], $10 \mu\text{g}$) and then stimulated with HKSC (1×10^7 cells/ml) and IL-4 (10 ng/ml). After 16 h, luciferase activities were analyzed. Data are presented as means \pm SEM of 3 independent transfections. * $p < 0.05$; ** $p < 0.01$.

Effect of HKSC on GLT expression and Ig production by primary human B cells

We used untouched tonsillar resting B cells (CD43⁻CD19⁺) purified from human tonsils to investigate the direct effect of the Dectin-1 agonist HKSC on GLT expression and Ig production by primary human B cells. The purity of resting B cells assessed by flow cytometric analysis was higher than 98% (Fig. 2A). HKSC enhanced GLT γ 4 and Dectin-1c expression by anti-CD40-stimulated B cells, and IL-4 also induced GLT γ 4 (Fig. 2B). However, other GLTs were not induced by HKSC (data not shown). Furthermore, HKSC increased IgG4 production and

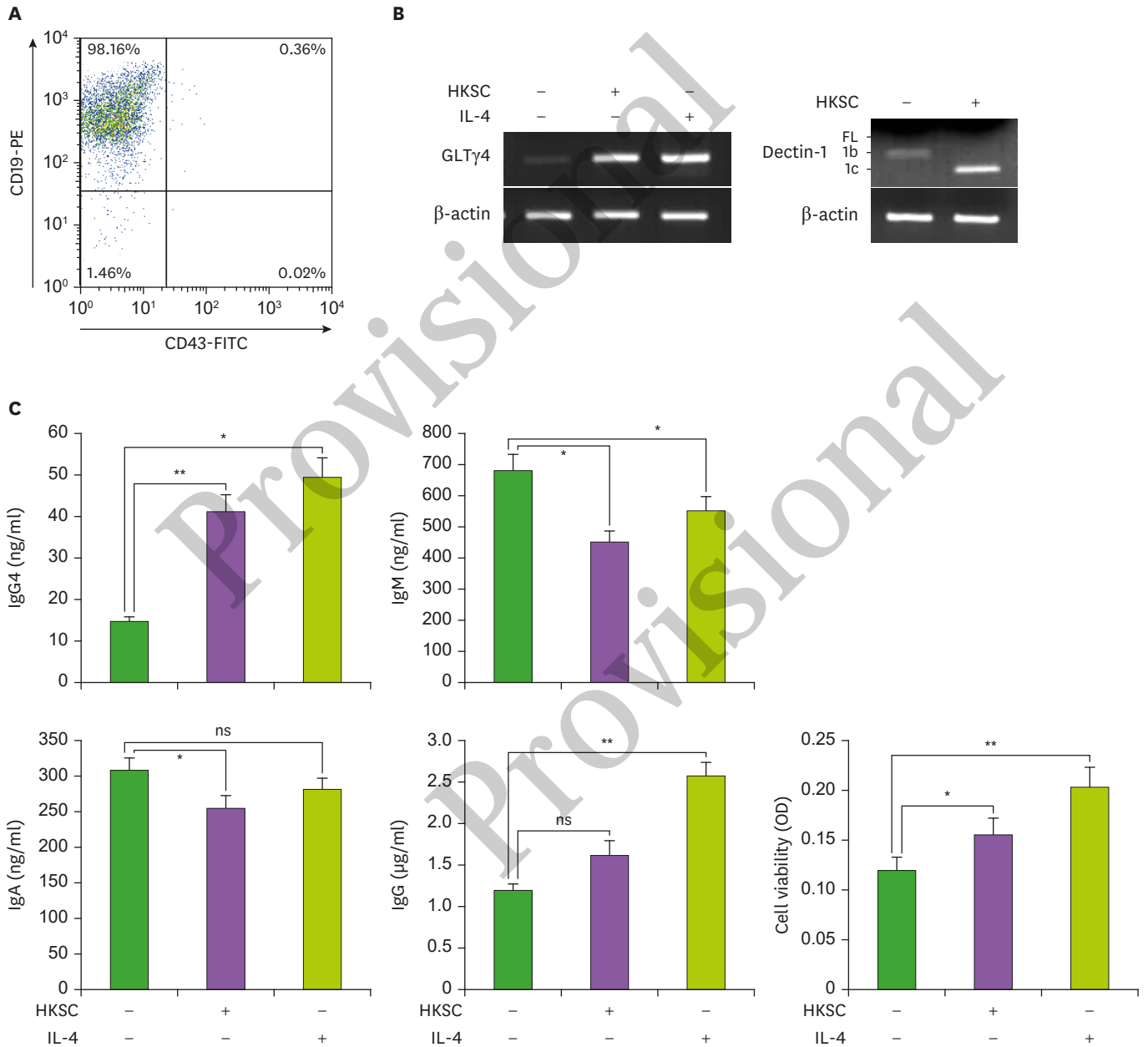


Figure 2. HKSC selectively enhances GLT γ 4 expression and IgG4 production by human B cells. (A) The purity of isolated human tonsillar resting B cells (CD43⁻CD19⁺) was assessed using flow cytometric analysis. Resting B cells were stimulated with anti-CD40 Ab (5 μ g/ml), HKSC (1 \times 10⁷ cells/ml), and IL-4 (10 ng/ml). After 4 days of culture, RNAs were isolated, and GLT γ 4 and Dectin-1 mRNA levels were measured by RT-PCR (B). After 9.5 days of culture, supernatants were harvested, and Ig production levels were determined by isotype-specific ELISA (C). After 2.5 days of culture, cell viability (OD) was measured using the EZ-Cytox assay kit (C, lower right panel). Data are presented as means \pm SEM of 3 independent experiments. *p<0.05; **p<0.01; ns: not significant.

decreased IgM and IgA production (**Fig. 2C**). Total IgG also tended to increase, but this seems to be due to an increase in cell viability by HKSC stimulation (**Fig. 2C**, lower right panel). These results indicate that HKSC selectively increases IgG4 production through the induction of GLT γ 4 by human B cells. HKSC, a Dectin-1 agonist, may contain additional pathogen-associated molecular patterns that affect activation of other pattern recognition receptors of human B cells because it is a whole microorganism. Thus, the specific action of HKSC on Dectin-1 need to be further clarified.

IgG4 is the least abundantly found subclass of human IgG in normal serum. Many reports have demonstrated that IgG4 has unique structural and functional properties, such as anti-allergic (40-46) and anti-inflammatory (47-49). IgG4 has the highest affinity for the inhibitory receptor Fc γ RIIB (48), which can have implications for inhibiting immune cells including B cells. Moreover, IgG4 plays a major role in the IgG Ab response in fungal infectious onychomycosis caused by *Trichophyton* (50). Thus, IgG4 can be used as a therapeutic Ab for allergic and inflammatory diseases and fungal infections (51-54). IgG4 is not clearly understood in human pathology, but elevated IgG4 levels are triggered in response to a chronic antigenic stimulus and inflammation. IgG4-related disease (IgG4-RD) is a chronic fibroinflammatory condition characterized by elevated serum IgG4 concentrations and tissue infiltration of IgG4-positive plasma cells that affects many organs (55,56). IgG4-RD includes patients with autoimmune pancreatitis, hypophysitis, inflammatory aorticaneurysm, inflammatory pseudo-tumor, interstitial nephritis, interstitial pneumonitis, lymphadenopathy, Mikulicz's disease, prostatitis, retroperitoneal fibrosis, and Riedel thyroiditis. The immunopathogenesis of IgG4-RD has not been completely elucidated, and the role of IgG4 itself in disease pathogenesis remains unclear. A therapeutic agent used to treat IgG4-RD is rituximab, an anti-CD20 antibody, which depletes B cells. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-RD patients who do not respond to glucocorticoids, conventional steroid-sparing agents, or both (57).

As previously mentioned, we have reported that HKSC selectively induces GLT γ 1 transcription, IgG1 class switching, and IgG1 production by mouse B cells (26,27). In the present study, we found that HKSC selectively induces GLT γ 4 transcription and IgG4 production by human B cells. The functional characteristics of mouse IgG1 are very similar to human IgG4. Yet they, the 'inactive' isotypes, cannot activate complement by the classical pathway (i.e., they bind C1q very weakly and are also poor complement activators), bind more avidly to an inhibitory than to stimulatory FcRs, suppress immune complex deposition, and have limited ability to aggregate pathogens (48,58-63). Furthermore, we compared the sequences between human GLT γ 4 and mouse GLT γ 1 promoter. We found that there are highly conserved sequences (**Supplementary Fig. 1**). The nucleotide sequences of the 2 promoters showed an identity of 77.8%. The highly conserved sequences contain 3 previously identified NF- κ B binding sites (29,30,64) (underlined in **Supplementary Fig. 1**) and 2 putative C-Ets-1 binding sites. Together, these data raise the intriguing possibility that HKSC stimulation regulates NF- κ B and C-Ets-1 signaling to activate transcription of both mouse GLT γ 1 and human GLT γ 4 by B cells. However, the underlying mechanisms need to be clarified.

In summary, our present study demonstrates for the first time the possibility that Dectin-1 can be involved in selective IgG4 class switching and IgG4 production by human B cells. Dectin-1 agonists including HKSC can be used as B cell adjuvants to augment IgG4 responses to control allergic, inflammatory, and fungal diseases. Moreover, a clear understanding of the

mechanisms of Dectin-1 agonist HKSC-induced selective IgG4 production would contribute to the development of new therapeutic agents to modulate IgG4-RD.

ACKNOWLEDGEMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (MEST) (NRF-2016R1D1A1B04935588) and the Priority Research Centers Program through the NRF funded by the MEST (NRF-2017R1A6A1A03015713). This work was also supported in part by Konyang University Myunggok Research Fund of 2016.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

RT-PCR and cloning primers

[Click here to view](#)

Supplementary Figure 1

Highly conserved sequences of huGLT γ 4 and moGLT γ 1 promoter. Alignment of promoter sequences of huGLT γ 4 and moGLT γ 1. Gray shading indicates conserved homologous sequences between the GLTs. Three NF- κ B sites and 2 putative C-Ets-1 binding sites are boxed and indicated above the sequences. The putative C-Ets-1 binding sites were identified using the MATCH™ public version 1.0 (BIOBASE GmbH, Wolfenbüttel, Germany). The underlined sequences indicate previously reported NF- κ B binding sites (29,30,64).

[Click here to view](#)

REFERENCES

1. Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. *Nature* 2001;413:36-37.
[PUBMED](#) | [CROSSREF](#)
2. Willment JA, Marshall AS, Reid DM, Williams DL, Wong SY, Gordon S, Brown GD. The human beta-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells. *Eur J Immunol* 2005;35:1539-1547.
[PUBMED](#) | [CROSSREF](#)
3. Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S, et al. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat Immunol* 2007;8:31-38.
[PUBMED](#) | [CROSSREF](#)
4. Kimberg M, Brown GD. Dectin-1 and its role in antifungal immunity. *Med Mycol* 2008;46:631-636.
[PUBMED](#) | [CROSSREF](#)
5. Drummond RA, Brown GD. The role of Dectin-1 in the host defence against fungal infections. *Curr Opin Microbiol* 2011;14:392-399.
[PUBMED](#) | [CROSSREF](#)
6. Romani L. Immunity to fungal infections. *Nat Rev Immunol* 2011;11:275-288.
[PUBMED](#) | [CROSSREF](#)
7. Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. *Nat Immunol* 2012;13:817-822.
[PUBMED](#) | [CROSSREF](#)

8. Wüthrich M, Deepe GS Jr, Klein B. Adaptive immunity to fungi. *Annu Rev Immunol* 2012;30:115-148.
[PUBMED](#) | [CROSSREF](#)
9. Casadevall A, Pirofski LA. A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. *Adv Immunol* 2006;91:1-44.
[PUBMED](#) | [CROSSREF](#)
10. McClelland EE, Nicola AM, Prados-Rosales R, Casadevall A. Ab binding alters gene expression in *Cryptococcus neoformans* and directly modulates fungal metabolism. *J Clin Invest* 2010;120:1355-1361.
[PUBMED](#) | [CROSSREF](#)
11. Casadevall A, Pirofski LA. Immunoglobulins in defense, pathogenesis, and therapy of fungal diseases. *Cell Host Microbe* 2012;11:447-456.
[PUBMED](#) | [CROSSREF](#)
12. Elluru SR, Kaveri SV, Bayry J. The protective role of immunoglobulins in fungal infections and inflammation. *Semin Immunopathol* 2015;37:187-197.
[PUBMED](#) | [CROSSREF](#)
13. Zhang K. Accessibility control and machinery of immunoglobulin class switch recombination. *J Leukoc Biol* 2003;73:323-332.
[PUBMED](#) | [CROSSREF](#)
14. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, de Waal Malefyt R, de Vries JE. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-3734.
[PUBMED](#) | [CROSSREF](#)
15. Snapper CM, Marcu KB, Zelazowski P. The immunoglobulin class switch: beyond "accessibility". *Immunity* 1997;6:217-223.
[PUBMED](#) | [CROSSREF](#)
16. Stavnezer J. Molecular processes that regulate class switching. *Curr Top Microbiol Immunol* 2000;245:127-168.
[PUBMED](#) | [CROSSREF](#)
17. Fujieda S, Zhang K, Saxon A. IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but not gamma 2. *J Immunol* 1995;155:2318-2328.
[PUBMED](#)
18. Defrance T, Vanbervliet B, Brière F, Durand I, Rousset F, Banchereau J. Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naïve human B cells to secrete immunoglobulin A. *J Exp Med* 1992;175:671-682.
[PUBMED](#) | [CROSSREF](#)
19. van Vlasselaer P, Punnonen J, de Vries JE. Transforming growth factor-beta directs IgA switching in human B cells. *J Immunol* 1992;148:2062-2067.
[PUBMED](#)
20. de Boer BA, Kruize YC, Rotmans PJ, Yazdanbakhsh M. Interleukin-12 suppresses immunoglobulin E production but enhances immunoglobulin G4 production by human peripheral blood mononuclear cells. *Infect Immun* 1997;65:1122-1125.
[PUBMED](#)
21. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998;160:3555-3561.
[PUBMED](#)
22. Satoguina JS, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 2005;174:4718-4726.
[PUBMED](#) | [CROSSREF](#)
23. Maehara T, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Tanaka A, Shinozaki S, Kubo Y, Nakamura S. Interleukin-21 contributes to germinal centre formation and immunoglobulin G4 production in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. *Ann Rheum Dis* 2012;71:2011-2019.
[PUBMED](#) | [CROSSREF](#)
24. Akdis CA, Akdis M. Mechanisms of immune tolerance to allergens: role of IL-10 and Tregs. *J Clin Invest* 2014;124:4678-4680.
[PUBMED](#) | [CROSSREF](#)
25. Park SR. Activation-induced cytidine deaminase in B cell immunity and cancers. *Immune Netw* 2012;12:230-239.
[PUBMED](#) | [CROSSREF](#)
26. Seo BS, Lee SH, Lee JE, Yoo YC, Lee J, Park SR. Dectin-1 stimulation selectively reinforces LPS-driven IgG1 production by mouse B cells. *Immune Netw* 2013;13:205-212.
[PUBMED](#) | [CROSSREF](#)

27. Seo BS, Park HY, Yoon HK, Yoo YC, Lee J, Park SR. Dectin-1 agonist selectively induces IgG1 class switching by LPS-activated mouse B cells. *Immunol Lett* 2016;178:114-121.
[PUBMED](#) | [CROSSREF](#)
28. He B, Qiao X, Cerutti A. CpG DNA induces IgG class switch DNA recombination by activating human B cells through an innate pathway that requires TLR9 and cooperates with IL-10. *J Immunol* 2004;173:4479-4491.
[PUBMED](#) | [CROSSREF](#)
29. Agresti A, Vercelli D. c-Rel is a selective activator of a novel IL-4/CD40 responsive element in the human Ig gamma4 germline promoter. *Mol Immunol* 2002;38:849-859.
[PUBMED](#) | [CROSSREF](#)
30. Sinquett FL, Dryer RL, Marcelli V, Batheja A, Covey LR. Single nucleotide changes in the human Iggamma1 and Iggamma4 promoters underlie different transcriptional responses to CD40. *J Immunol* 2009;182:2185-2193.
[PUBMED](#) | [CROSSREF](#)
31. Park SR, Lee JH, Kim PH. Smad3 and Smad4 mediate transforming growth factor-beta1-induced IgA expression in murine B lymphocytes. *Eur J Immunol* 2001;31:1706-1715.
[PUBMED](#) | [CROSSREF](#)
32. Lee SH, Park SR. Toll-like receptor 1/2 agonist Pam3CSK4 suppresses lipopolysaccharide-driven IgG1 production while enhancing IgG2a production by B cells. *Immune Netw* 2018;18:e10.
[PUBMED](#) | [CROSSREF](#)
33. Schaffer A, Cerutti A, Shah S, Zan H, Casali P. The evolutionarily conserved sequence upstream of the human Ig heavy chain S gamma 3 region is an inducible promoter: synergistic activation by CD40 ligand and IL-4 via cooperative NF-kappa B and STAT-6 binding sites. *J Immunol* 1999;162:5327-5336.
[PUBMED](#)
34. Hermanz-Falcón P, Arce I, Roda-Navarro P, Fernández-Ruiz E. Cloning of human DECTIN-1, a novel C-type lectin-like receptor gene expressed on dendritic cells. *Immunogenetics* 2001;53:288-295.
[PUBMED](#) | [CROSSREF](#)
35. Willment JA, Gordon S, Brown GD. Characterization of the human beta -glucan receptor and its alternatively spliced isoforms. *J Biol Chem* 2001;276:43818-43823.
[PUBMED](#) | [CROSSREF](#)
36. Grünebach F, Weck MM, Reichert J, Brossart P. Molecular and functional characterization of human Dectin-1. *Exp Hematol* 2002;30:1309-1315.
[PUBMED](#) | [CROSSREF](#)
37. Brown GD. Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol* 2006;6:33-43.
[PUBMED](#) | [CROSSREF](#)
38. Heinsbroek SE, Taylor PR, Rosas M, Willment JA, Williams DL, Gordon S, Brown GD. Expression of functionally different dectin-1 isoforms by murine macrophages. *J Immunol* 2006;176:5513-5518.
[PUBMED](#) | [CROSSREF](#)
39. del Pilar Jiménez-A M, Viriyakosol S, Walls L, Datta SK, Kirkland T, Heinsbroek SE, Brown G, Fierer J. Susceptibility to *Coccidioides* species in C57BL/6 mice is associated with expression of a truncated splice variant of Dectin-1 (Clec7a). *Genes Immun* 2008;9:338-348.
[PUBMED](#) | [CROSSREF](#)
40. Ishizaka A, Sakiyama Y, Nakanishi M, Tomizawa K, Oshika E, Kojima K, Taguchi Y, Kandil E, Matsumoto S. The inductive effect of interleukin-4 on IgG4 and IgE synthesis in human peripheral blood lymphocytes. *Clin Exp Immunol* 1990;79:392-396.
[PUBMED](#) | [CROSSREF](#)
41. Ando H, Movérare R, Kondo Y, Tsuge I, Tanaka A, Borres MP, Urisu A. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol* 2008;122:583-588.
[PUBMED](#) | [CROSSREF](#)
42. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, Kleine-Tebbe J; EAACI Task Force. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. *Allergy* 2008;63:793-796.
[PUBMED](#) | [CROSSREF](#)
43. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009;39:469-477.
[PUBMED](#) | [CROSSREF](#)
44. James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, Jacobson MR, Kimber I, Till SJ, Durham SR. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 2011;127:509-516.e501-e505.
[PUBMED](#) | [CROSSREF](#)

45. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, Houghton S, Clark AT, Stephens A, Turcanu V, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol* 2015;135:1249-1256.
[PUBMED](#) | [CROSSREF](#)
46. Davies AM, Sutton BJ. Human IgG4: a structural perspective. *Immunol Rev* 2015;268:139-159.
[PUBMED](#) | [CROSSREF](#)
47. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002;105:9-19.
[PUBMED](#) | [CROSSREF](#)
48. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daëron M. Specificity and affinity of human Fcγ4 receptors and their polymorphic variants for human IgG subclasses. *Blood* 2009;113:3716-3725.
[PUBMED](#) | [CROSSREF](#)
49. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med* 2012;18:736-749.
[PUBMED](#) | [CROSSREF](#)
50. Summerbell RC. Epidemiology and ecology of onychomycosis. *Dermatology* 1997;194 Suppl 1:32-36.
[PUBMED](#) | [CROSSREF](#)
51. Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. *Pediatr Allergy Immunol* 2006;17:408-415.
[PUBMED](#) | [CROSSREF](#)
52. van Helden PM, van den Berg HM, Gouw SC, Kaijen PH, Zuurveld MG, Mauser-Bunschoten EP, Aalberse RC, Vidarsson G, Voorberg J. IgG subclasses of anti-FVIII antibodies during immune tolerance induction in patients with hemophilia A. *Br J Haematol* 2008;142:644-652.
[PUBMED](#) | [CROSSREF](#)
53. van Schouwenburg PA, Kriekaert CL, Nurmohamed M, Hart M, Rispen T, Aarden L, Wouters D, Wolbink GJ. IgG4 production against adalimumab during long term treatment of RA patients. *J Clin Immunol* 2012;32:1000-1006.
[PUBMED](#) | [CROSSREF](#)
54. Crescioli S, Correa I, Karagiannis P, Davies AM, Sutton BJ, Nestle FO, Karagiannis SN. IgG4 characteristics and functions in cancer immunity. *Curr Allergy Asthma Rep* 2016;16:7.
[PUBMED](#) | [CROSSREF](#)
55. Mahajan VS, Mattoo H, Deshpande V, Pillai SS, Stone JH. IgG4-related disease. *Annu Rev Pathol* 2014;9:315-347.
[PUBMED](#) | [CROSSREF](#)
56. Bozzalla Cassione E, Stone JH. IgG4-related disease. *Curr Opin Rheumatol* 2017;29:223-227.
[PUBMED](#) | [CROSSREF](#)
57. Khosroshahi A, Bloch DB, Deshpande V, Stone JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. *Arthritis Rheum* 2010;62:1755-1762.
[PUBMED](#) | [CROSSREF](#)
58. Strait RT, Posgai MT, Mahler A, Barasa N, Jacob CO, Köhl J, Ehlers M, Stringer K, Shanmukhappa SK, Witte D, et al. IgG1 protects against renal disease in a mouse model of cryoglobulinaemia. *Nature* 2015;517:501-504.
[PUBMED](#) | [CROSSREF](#)
59. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. *Nat Rev Immunol* 2008;8:34-47.
[PUBMED](#) | [CROSSREF](#)
60. Barrington R, Zhang M, Fischer M, Carroll MC. The role of complement in inflammation and adaptive immunity. *Immunol Rev* 2001;180:5-15.
[PUBMED](#) | [CROSSREF](#)
61. Dangi JL, Wensel TG, Morrison SL, Stryer L, Herzenberg LA, Oi VT. Segmental flexibility and complement fixation of genetically engineered chimeric human, rabbit and mouse antibodies. *EMBO J* 1988;7:1989-1994.
[PUBMED](#) | [CROSSREF](#)
62. van der Neut Kofschoten M, Schuurman J, Losen M, Bleeker WK, Martínez-Martínez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science* 2007;317:1554-1557.
[PUBMED](#) | [CROSSREF](#)

63. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, McDonald JU, Orr SJ, Berger M, Petzold D, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of Fc γ RIIB and dectin-1. *Nat Med* 2012;18:1401-1406.

[PUBMED](#) | [CROSSREF](#)

64. Lin SC, Stavnezer J. Activation of NF-kappaB/Rel by CD40 engagement induces the mouse germ line immunoglobulin Cgamma1 promoter. *Mol Cell Biol* 1996;16:4591-4603.

[PUBMED](#) | [CROSSREF](#)

Provisional
Provisional