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Nutrigenomic Study on Immunomodulatory Function of *Cordyceps* Mycelium Extract (*Paecilomyces hepiali*) in Mitomycin C–Treated Mice

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Abstract

***Cordyceps* (CS) is a Chinese herb that produces various effects including immune modulation, and now CS culture product is interested in the use as a functional food. We prepared CS mycelium culture extract (*Paecilomyces hepiali*, CBG-CS-2), CS extract, for functional foods. This study aimed to deduce the molecular mechanism of immunomodulatory effect of CS extract in Peyer's patches (PPs), a main gut immune site, in mice that treated with mitomycin C (MMC), an immunosuppressing antibiotics. Nutrigenomics give us invaluable molecular information about both of foods and nutrition to improve or maintain good health. Here we performed nutrigenomics using DNA microarray to investigate the effect of CS extract on gene expression altered in PPs of the immunosuppressed mice. Interestingly, CS extract protected from the MMC-mediated downregulation of 22 genes, which are associated with IgA production and other immune response in PPs. These suggested that CS extract alleviated the downregulated expression of immune related genes in the gut immune site in an immunosuppressed state. Thus CS extract appears to be practical functional food for immunodepression and/or its related hypofunction.**

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Keywords

DNA Microarray, Functional Food, Gene Expression, Immunosuppression

1. Introduction

Nutrigenomics is a genomic study on the effects of foods and food constituents on the level of gene expression. It gives us high-throughput methods and tools to look for disease preventing and health promoting foods, functional foods [1] [2]. From this point of view, nutrigenomics was introduced in recent paper of this Journal [3]. DNA microarray is powerful nutrigenomic tool to investigate global gene expression events in target tissues or cells and provides invaluable information to elucidate the molecular mechanism of the effects of functional foods.

Cordyceps (*Cordyceps sinensis*, CS) has been used as a medicine in China for over 300 years and was featured in the book, "Ben-Cao-Bei-Yao", which was edited by Wang Ang in 1694. CS has several effects including immunomodulation [4]-[6]. CS-derived natural products are comprised of complicated components, cordycepin derivatives, cordycepic acid, ergosterol, polysaccharides, and nucleosides [7] [8]. Cordycepin and cordycepic acid are thought to be the main active ingredients in CS; however, this is still debated [8]. Recently people have interested in CS and its culture products to use as a functional food [9]-[11]. CS mycelium culture techniques were developed to yield alternative CS products with a constant composition [12] [13]. However, there is little information regarding the molecular mechanisms underlying function of CS culture product.

In the current study, we performed nutrigenomis to elucidate the *in vivo* immunomodulatory function of CS mycelium culture extract (*Paecilomyces hepiali*, CBG-CS-2) on gut immunity. Peyer's patches (PPs) are main gut immune site and participate in essential gut immune responses against orally invading infectious microorganisms [14] [15] and IgA immunity [16]-[18]. We examined the effect of CS extract on gene expression in PPs of an immunosuppressed mice treated with mitomycin C (MMC). MMC is an apoptosis-inducing antibiotic that inhibits DNA replication, and it is utilized for cancer therapy and organ transplantation due to its immunosuppressive action [19]-[21]. This antibiotics was also applied to induce immunodepression in animals to study the effect of herbal medicines in a previous paper [22]. For these reasons we used MMC to prepare immunosuppressed mice in this study.

Here we performed nutrigenomic study and revealed previously unknown functional mechanism of CS extract to improve downregulated expression of immune-related genes in PPs of MMC-treated mice.

2. Material and Methods

2.1. Preparation of *Cordyceps* Mycelium Culture Extract (*Paecilomyces hepiali*, CBG-CS-2)

Paecilomyces hepiali, a pure strain from the culture collection of Chebigen Inc., was initially grown on potato-dextrose agar medium slants in Petri dishes and then transferred into a seed culture medium composed of 4% Dextrose, 1% yeast extract, and 1% peptone (DYP). The seed culture was grown in a 250 mL flask containing a nutritive medium (100 mL of 2% potato-dextrose broth) at 25˚C in a shaking incubator (150 rpm for 5 - 7 days). The obtained seed culture was transferred into a stirred tank fermentor containing DYP medium with 10% (v/v) working volume, and the culture was fermented at 25˚C, 150 rpm, for 5 days. The fermented product was concentrated and extracted with a vacuum concentrator and extractor at 80˚C to 10% of the initial volume. The extracts were freeze dried and used as CS mycelium culture extract (*Paecilomyces hepiali*, CBG-CS-2) in this study.

The main components of the mycelium culture extract were as follows: 32% cordyceps polysaccharide, 7.3% cordycepic acid, 0.13% adenosine, and 0.001% cordycepin.

2.2. Animals and Administration of *Cordyceps* Mycelium Culture Extract and Mitomycin C (MMC)

All procedures were carried out according to the Institutional Animal Care and Committee Guide of Intelligence and Technology Lab. Male BALB/c mice (9-week-old; Japan SLC, Inc., Hamamatsu, Japan) were used for this

study. Four to five mice per cage were housed under a specific pathogen-free condition (controlled temperature of 20˚C - 26˚C and humidity of 40% - 60%) and fed standard laboratory food (FR-2; Funabashi Firm, Inc., Funabashi, Japan) *ad libitum*.

CS mycelium culture extract (*Paecilomyces hepiali*, CBG-CS-2) was prepared by Chebigen Inc. (Jeonju, S. Korea). The CS mycelium extract solution was dissolved in sterile water, and it was orally administered to mice for 28 days at 120 or 400 mg/kg/day. Mitomycin C (MMC; Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan) solution (3 mg/kg) was dissolved in saline and intraperitoneally administered to mice at day 21, day 24, and day 27. The mice were divided into 6 groups: 1. control group (Cont; n = 9), 2. CS group with 120 mg/kg (CS120; n = 9), 3. CS group with 400 mg/kg (CS400; n = 9), 4. MMC group without CS (MMC; n = 9), 5. MMC group with 120 mg/kg CS (MMC + CS120; n = 9), and 6. MMC group with 400 mg/kg CS (MMC + CS400; n = 9). At 28 days, all mice were anesthetized with isoflurane for blood collection, followed by sacrifice to harvest PPs.

2.3. RNA Preparation

RNA was prepared as described previously [23]. Briefly, total RNA was extracted from the harvested PPs using Isogen (Nippon gene, Tokyo, Japan) and DNase-treated in the aqueous phase using the RNase-Free DNase Set (Qiagen, Valencia, CA). The extracted RNA was further purified using an RNeasy MinElute Cleanup Kit (Qiagen), and the quantity and purity were evaluated photometrically at 260 nm, 280 nm and 320 nm using an Ultrospec 2000 (Pharmacia Biotech/GE Healthcare, Uppsala, Sweden).

2.4. DNA Microarray Analysis

Two-hundred nanograms of total RNA pooled from each group was used to generate cDNA and Cy3-labeled cRNA using the Low Input Quick-Amp Labeling Kit (Agilent Technologies, Santa Clara, CA), according to the manufacturer's instructions. The labeled cRNA was photometrically examined to determine the quantity and dye-incorporation ratio using an Ultrospec 2000, and it was hybridized to a Mouse Gene Expression 4 × 44K v2 Microarray (Agilent Technologies). The array was scanned with GenePix 6000B (Molecular Devices, Sunnyvale, CA), and the obtained image was processed using GenePix Pro 6.0 Software (Molecular Devices). Features were manually examined, and poor quality spot were flagged and filtered out.

Signal data for the features (spots of array) were then imported to GeneSpring 12.6 (Agilent Technologies, Inc.), and further analysis was performed using this software. Signal data from the arrays was normalized using the 75th percentile method [24], and baseline transformation was performed using the median of the control samples. Quality control was performed to filter out signal data with a standard error over 0.2.

To analyze differentially expressed genes, the fold change (FC) vs. control samples was calculated, and genes with a FC greater than 1.5 were extracted for the CS-administered and MMC-injected groups. To further analyze the alleviative effects of CS on MMC treatment, genes shifted toward control levels in MMC + CS groups in comparison with MMC groups were extracted with the criteria set as 25% upregulation or downregulation induced by MMC treatment. Gene Ontology analysis was performed on the extracted genes using software of GeneSpring 12.6, and gene sets with a corrected p-value < 0.1 were selected as significant gene groups.

2.5. Assay of Plasma IgG

Plasma samples were prepared from collected blood by centrifugation, and the plasma level of total IgG was determined using a sandwich ELISA with a capture antibody (goat anti-mouse IgG (ab97031, Abcam com., Cambridge, MA, USA)), a detection antibody (goat anti-mouse IgG-HRP (ab98717, Abcam)) and a substrate (TMB (3,3',5,5'-Tetramethylbenzidine, T8665, Sigma-Aldrich Corporation, Saint Louis, MO, USA)). The optical density at 450 nm, with a reference at 630 nm, was measured using a Well Reader SK601 (Seikagaku Corporation, Tokyo, Japan).

3. Results

3.1. Preliminary Results on Mitomycin C (MMC)-Treatment

Plasma IgG levels markedly decreased from 18.3 ± 1.9 mg/mL of normal to 10.3 ± 2.9 mg/mL of MMC-treated mice (data not shown). The wet weight of spleen also markedly decreased from 89.4 ± 1.7 mg of normal to 53.6

± 3.2 mg of MMC-treated mice (data not shown). These indicated that some immunosuppressive state occurred in MMC-administrated mice.

3.2. Nutrigenomic Analysis

MMC treatment altered gene expression in PPs. Pretreatment with 120 or 400 mg/kg CS extract protected against MMC-induced alterations in gene expression. **Table 1** and **Table 2** list 84 MMC-mediated downregulated genes that were protected by CS extract, as determined by ontology analysis. Interestingly, 22 genes involved in the immune system (Aicda, Ms4a1, Bcl6, Pik3cd, Slamf1, Vav1, Syk, Bcl11a, Cxcr4, Gcsam, Scimp, Lyn, Btk, Igh-Vj558, Nuggc, Endou, Tnfrsf13c, Klhl6, Sh2b2, H2Ob, Map4k2, and Nos2) represent 26% of the total CS-alleviated genes (**Table 1**). Other protected genes include 22 genes (26%) associated with signal transduction or stimulus response and 11 genes (13%) associated with localization and DNA replication or transcription (**Table 2**).

Table 3 and **Table 4** list 55 MMC-mediated upregulated genes that are alleviated by the CS extract. Four

Table 1. Genes downregulated by MMC and alleviated by CS. A gene set categorized as "Immune system" by Gene Ontology analysis.

Function	Gene symbol	Gene name	Fold change		
			MMC/Control	MMC + CS120/MMC	MMC + CS400/MMC
Immune system	Aicda	activation-induced cytidine deaminase	−3.17	1.10	1.48
	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	−1.67	−1.15	1.45
	Bcl6	B cell leukemia/lymphoma 6	−1.60	1.02	1.35
	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	−1.63	1.17	1.35
	Slamf1	signaling lymphocytic activation molecule family member 1	−1.75	−1.05	1.34
	Vav1	vav 1 oncogene	−1.54	1.09	1.33
	Syk	spleen tyrosine kinase	−1.52	1.07	1.31
	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	−1.61	1.12	1.28
	Cxcr4	chemokine (C-X-C motif) receptor 4	−1.51	−1.21	1.25
	Gcsam	germinal center associated, signaling and motility	−2.64	1.10	1.58
	Scimp	SLP adaptor and CSK interacting membrane protein	−2.28	1.04	1.47
	Lyn	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	−1.62	1.10	1.26
	Btk	Bruton agammaglobulinemia tyrosine kinase	−1.69	−1.09	1.26
	Igh-VJ558	immunoglobulin heavy chain (J558 family)	−1.81	1.28	−1.18
	Nuggc	nuclear GTPase, germinal center associated	−3.62	−1.03	1.72
	Endou	endonuclease, polyU-specific	−2.07	1.27	1.71
	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	−1.56	−1.17	1.46
	Klhl6	kelch-like 6	−1.63	1.28	1.46
	Sh2b2	SH2B adaptor protein 2	−1.97	−1.02	1.43
	H2-Ob	histocompatibility 2, O region beta locus	−1.55	−1.07	1.40
	Map4k2	mitogen-activated protein kinase kinase kinase kinase 2	−1.52	1.09	1.34
	Nos2	nitric oxide synthase 2, inducible	−1.88	1.43	−1.24

To analyze the alleviative effects of CS extract on MMC treatment, genes shifted toward control levels in MMC + CS groups in comparison with MMC groups were extracted with the criteria set as 25% upregulation or downregulation induced by MMC treatment.

Table 2. Genes downregulated by MMC and alleviated by CS. Gene sets categorized as other than "Immune system" by Gene Ontology analysis.

Function: (Gene symbols)
Signal transduction, Response to stimulus: (Bfsp2, Cacna1s, Csprs, Cyp1a1, Eaf2, Foxo6, Gng2, Hbegf, Il1rl1, Il21r, Itga10, Lnpep, Lrrk2, Mc3r, Msh5, Mum1, Olfr1466, Parp1, Rasgrp4, Rgs13, Stac2, Tbx6)
Localization: (Atg16l2, Crym, Slc9a3, Sorl1, Ttyh1)
DNA replication, Transcription: (Atf3, Bach2, Baz1a, Cpsf2, Fgd3, Lbh)
Ubiquitination, Apoptosis, Cell division: (Cdc123, Chpf2, Fam72a, Hmces, Lama3, Mal, Napsa, Otub2)
Protein binding: (Myl4, Myo1g, Tppp)
Others: (Fam13c, Gm8995, Gsdmc3, Hist2h2bb, Megf6, Rs5-8s1, Rsph1, Srpk3, St8sia4, Xkrx, Zpbp2, 2010109I03Rik, 6330509M05Rik, 9130230L23Rik, 9430015G10Rik, A630033H20Rik, D13Ertd608e, D9Ertd720e)

To analyze the alleviative effects of CS extract on MMC treatment, genes shifted toward control levels in MMC+CS groups in comparison with MMC groups were extracted with the criteria set as 25% upregulation or downregulation induced by MMC treatment.

Table 3. Genes upregulated by MMC and alleviated by CS. A gene set categorized as "Immune system" by Gene Ontology analysis.

Function	Gene symbol	Gene name	Fold change		
			MMC/Control	MMC + CS120/MMC	MMC + CS400/MMC
Immune system	Igkv9-129	immunoglobulin kappa variable 9-129	1.64	−1.33	−1.63
	Sectm1b	secreted and transmembrane 1B	1.68	1.03	−1.58
	Tnfsf15	tumor necrosis factor (ligand) superfamily, member 15	1.69	−1.91	−1.48
	Oas3	2'-5' oligoadenylate synthetase 3	1.62	1.11	−1.32

To analyze the alleviative effects of CS extract on MMC treatment, genes shifted toward control levels in MMC + CS groups in comparison with MMC groups were extracted with the criteria set as 25% upregulation or downregulation induced by MMC treatment.

Table 4. Genes upregulated by MMC and alleviated by CS. Gene sets categorized as other than "Immune system" by Gene Ontology analysis.

Function: (Gene symbols)
Organic substrate metabolic process: (Abcb4, Abcc6, Agmat, Cyp2j6, Dcxr, Dnph1, Dpep1, Hgfac, Hopx, Hunk, Ick, Idh1, L2hgdh, Mboat1, Pcx, Pi4k2b, Prss8, Ptprr, Rasd2, Rbm3, Tfdp2)
Response to stimulus: (Bex1, Ghrl, Nqo1, Pros1, Slc19a1)
Catalytic activity: (Cyp2c67, Cyp3a57, Dynll2, Neu1, Tst, Uap1l1)
Structure, Adhesion, Localization: (Cdhr2, Krt23, Lrrc26, Mfsd2a, Osbpl10, Slc44a1)
Cell division, Apoptosis: (Macc1, Tmem97)
Binding: (Nts, Ppl, Sst)
Others: (R3hdml, Rundc3b, Sh3d21, Sowahc, Tm4sf20, Tmem144, Tnks1bp1, B930041F14Rik)

To analyze the alleviative effects of CS extract on MMC treatment, genes shifted toward control levels in MMC + CS groups in comparison with MMC groups were extracted with the criteria set as 25% upregulation or downregulation induced by MMC treatment.

genes (Igkv9-129, Sectm1b, Tnfsf15, Oas3) were categorized in the immune system, which represents only 7 % of the total ameliorated genes.

4. Discussion

Cordyceps (CS) is a traditional Chinese herb that displays numerous effects, including immune modulation [25]

[26]. Now people have concern to develop its culture products as a functional food [9]-[11]. However, little is known about functional mechanism of CS culture products. Both of natural CS and its culture product are orally used, and the gut immune system would be its first site of action. Peyer's patches (PPs) are essential gut lymphoid tissues associated with immune response against orally invading infectious microorganisms [17] [18] and NK cells, which induce target cell apoptosis through the perforin/granzyme pathway [27] [28]. Thus, we examined the molecular events that occurred in PPs of mice after treatment with CS, immunosuppressive antibiotic, mitomycin C (MMC), or both of CS and MMC.

In the current study, we performed nutrigenomic study using DNA microarray to deduce the mechanism of action of CS mycelium culture extract (*Paecilomyces hepiali*, CBG-CS-2) in mouse intestinal immune tissue (PPs). CS modulates immune responses [29] [30], and as a result, its culture product is expected to be an effective functional food for immune-related diseases. This study confirmed the immune effect of the CS extract using functional genomics and DNA microarray analyses of PPs from MMC-treated mice. MMC is a potent immunosuppressive antibiotic, and it downregulated or upregulated the expression of various genes. Impressively, pretreatment with the CS extract protected against MMC-elicited downregulation of expression of 22 genes participating in the immune system, which represented 26% of the 84 total CS extract-alleviated genes (**Table 1**). PPs are central sites for intestinal immunity because they produce IgA [16]-[18]. Among the immune related genes expressed in PPs, Aicda, Tnfrsf13c, and Nos2 participate in B cell differentiation into IgA(+) B cells [31], activating B cell for IgA class switching [32], and regulating IgA class switch recombination of plasma cells [33], respectively. Slamf1 is involved in NK, T, and B cell development [34]. Cxcr4 modulates lymphocyte trafficking into PPs and lymph nodes [35], and Sh2b2/APS [36] and Klh16 [37] regulate linkage to the B cell antigen receptor. H2-Ob is selectively expressed in B cells, and it downregulates presentation of exogenous antigen to T cells [38]. CS extract also alleviated MMC-mediated upregulation of expression of four immune related genes (**Table 3**).

5. Conclusion

In conclusion, these results indicate that CS extract has an action to moderate the gut immunosuppressive state by modulating the expression of immune response genes in PPs. These suggest that CS extract is a possible functional food to be expected to prevent or improve malfunction in immune response or related immunity.

Conflict of Interests

The authors declare that they have no competing interests.

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References

[1] Afman, L. and Müller, M. (2006) Nutrigenomics: From Molecular Nutrition to Prevention of Disease. *Journal of the American Dietetic Association*, **106**, 569-576. http://dx.doi.org/10.1016/j.jada.2006.01.001

[2] Liu, B. and Qian, S.B. (2011) Translational Regulation in Nutrigenomics. *Advances in Nutrition*, **2**, 511-519. http://dx.doi.org/10.3945/an.111.001057

[3] Thunders, M., Mangai, S. and Cooper, R. (2013) Nutrigenetics, Nutrigenomics, and the Future of Dietary Advice. *Food and Nutrition Sciences*, **4**, 999-1003. http://dx.doi.org/10.4236/fns.2013.410129

[4] Paterson, R.R. (2008) *Cordyceps*: A Traditional Chinese Medicine and Another Fungal Therapeutic Biofactory? *Phytochemistry*, **69**, 1469-1495. http://dx.doi.org/10.1016/j.phytochem.2008.01.027

[5] Zhou, X., Gong, Z., Su, Y., Lin, J. and Tang, K. (2009) *Cordyceps* Fungi: Natural Products, Pharmacological Functions and Developmental Products. *The Journal of Pharmacy and Pharmacology*, **61**, 279-291. http://dx.doi.org/10.1211/jpp.61.03.0002

[6] Shin, S., Kwon, J., Lee, S., Kong, H., Lee, S., Lee, C.K., *et al.* (2010) Immunostimulatory Effects of *Cordyceps militaris* on Macrophages through the Enhanced Production of Cytokines via the Activation of NF-κB. *Immune Network*, **10**, 55-63. http://dx.doi.org/10.4110/in.2010.10.2.55

[7] Li, S.P., Yang, F.Q. and Tsim, K.W.K. (2006) Quality Control of *Cordyceps sinensis*, a Valued Traditional Chinese Medicine. *Journal of Pharmaceutical and Biomedical Analysis*, **41**, 1571-1584. http://dx.doi.org/10.1016/j.jpba.2006.01.046

[8] Yue, K., Ye, M., Zhou, Z., Sun, W. and Lin, X. (2013) The Genus *Cordyceps*: A Chemical and Pharmacological Review. *The Journal of Pharmacy and Pharmacology*, **65**, 474-493. http://dx.doi.org/10.1111/j.2042-7158.2012.01601.x

[9] Lo, H.C., Hsu, T.H., Tu, S.T. and Lin, K.C. (2006) Anti-Hyperglycemic Activity of Natural and Fermented *Cordyceps sinensis* in Rats with Diabetes Induced by Nicotinamide and Streptozotocin. *The American Journal of Chinese Medicine*, **34**, 819-832. http://dx.doi.org/10.1142/S0192415X06004314

[10] Xiao, J.H., Li, Y., Xiao, Y. and Zhong, J.J. (2013) Advance and Prospect of Studies on Bioactivity and Mechanism of *Cordyceps* Fungi. *Zhongguo Zhong Yao Za Zhi*, **38**, 640-647.

[11] Yan, W., Li, T., Lao, J., Song, B. and Shen, Y.H. (2013) Anti-Fatigue Property of *Cordyceps guangdongensis* and the Underlying Mechanisms. *Pharmaceutical Biology*, **51**, 614-620. http://dx.doi.org/10.3109/13880209.2012.760103

[12] Kim, H.O. and Yun, J.W. (2005) A Comparative Study on the Production of Exopolysaccharides between Two Entomopathogenic Fungi *Cordyceps militaris* and *Cordyceps sinensis* in Submerged Mycelial Cultures. *Journal of Applied Microbiology*, **99**, 728-738. http://dx.doi.org/10.1111/j.1365-2672.2005.02682.x

[13] Xu, C.P., Sinha, J., Bae, J.T., Kim, S.W. and Yun, J.W. (2006) Optimization of Physical Parameters for Exo-Biopolymer Production in Submerged Mycelial Cultures of Two Entomopathogenic Fungi *Paecilomyces japonica* and *Paecilomyces tenuipes*. *Letters in Applied Microbiology*, **42**, 501-506. http://dx.doi.org/10.1111/j.1472-765X.2006.01884.x

[14] Doe, W.F. (1989) The Intestinal Immune System. *Gut*, **30**, 1679-1685. http://dx.doi.org/10.1136/gut.30.12.1679

[15] Walker, R.L. and Owen, R.L. (1990) Intestinal Barriers to Bacteria and Their Toxins. *Annual Review of Medicine*, **41**, 393-400. http://dx.doi.org/10.1146/annurev.me.41.020190.002141

[16] Lycke, N.Y. and Bemark, M. (2012) The Role of Peyer's Patches in Synchronizing Gut IgA Responses. *Frontiers in Immunology*, **3**, 329. http://dx.doi.org/10.3389/fimmu.2012.00329

[17] Sato, A. and Iwasaki, A. (2005) Intestinal Epithelial Barrier and Mucosal Immunity. *Cellular and Molecular Life Sciences*, **62**, 1333-1338. http://dx.doi.org/10.1007/s00018-005-5037-z

[18] Hashizume, T., Togawa, A., Nochi, T., Igarashi, O., Kweon, M.N., Kiyono, H. and Yamamoto, M. (2008) Peyer's Patches Are Required for Intestinal Immunoglobulin A Responses to *Salmonella* spp. *Infection and Immunity*, **76**, 927-934. http://dx.doi.org/10.1128/IAI.01145-07

[19] Yamaguchi, Y., Mori, K. and Bollinger, R.R. (1990) Suppression of Hepatic Allograft Rejection in the Rat by Mitomycin C-Treated Donor Splenocytes: Analysis of the Immune Status. *Journal of Clinical & Laboratory Immunology*, **32**, 59-66.

[20] Micheau, O., Solary, E., Hammann, A., Martin, F. and Dimanche-Boitrel, M.T. (1997) Sensitization of Cancer Cells Treated with Cytotoxic Drugs to Fas-Mediated Cytotoxicity. *Journal of the National Cancer Institute*, **89**, 783-789. http://dx.doi.org/10.1093/jnci/89.11.783

[21] Volpato, M. and Phillips, R.M. (2007) Tailoring Targeted Therapy to Individual Patients: Lessons to Be Learnt from the Development of Mitomycin C. *Cancer Genomics & Proteomics*, **4**, 175-186.

[22] Li, A.L., Komatsu, Y., Ono, Y., Nakatani, F., Nakashima, K. and Yamaguchi, N. (1996) The Effect of Herbal Medicines on the Immunodeficient Animals by Injecting Cancer Chemotherapeutic Agent-Special Reference to Age Related Recovery of the Function. *Kansenshogaku Zasshi*, **70**, 717-726.

[23] Jeong, A.R., Nakamura, S. and Mitsunaga, F. (2008) Gene Expression Profile of Th1 and Th2 Cytokines and Their Receptors in Human and Nonhuman Primates. *Journal of Medical Primatology*, **37**, 290-296.

[24] Shippy, R., Fulmer-Smentek, S., Jensen, R.V., Jones, W.D., Wolber, P.K., Johnson, C.D., *et al*. (2006) Using RNA Sample Titrations to Assess Microarray Platform Performance and Normalization Techniques. *Nature Biotechnology*, **24**, 1123-1131. http://dx.doi.org/10.1038/nbt1241

[25] Li, C.Y., Chiang, C.S., Tsai, M.L., Hseu, R.S., Shu, W.Y., Chuang, C.Y., *et al*. (2009) Two-Sided Effect of *Cordyceps sinensis* on Dendritic Cells in Different Physiological Stages. *Journal of Leukocyte Biology*, **85**, 987-995. http://dx.doi.org/10.1189/jlb.0908573

[26] Lin, B.Q. and Li, S.P. (2011) Chapter 5. *Cordyceps* as an Herbal Drug. In: Benzie, F.F. and Wachtel-Galor, S., Eds., *Herbal Medicine*: *Biomolecular and Clinical Aspects*, 2nd Edition, CRC Press, Boca Raton, 73-105. http://dx.doi.org/10.1201/b10787-6

[27] Trapani, J.A. and Smyth, M.J. (2002) Functional Significance of the Perforin/Granzyme Cell Death Pathway. *Nature Reviews Immunology*, **2**, 735-747. http://dx.doi.org/10.1038/nri911

[28] Cupedo, T. (2011) Human Lymph Node Development: An Inflammatory Interaction. *Immunology Letters*, **138**, 4-6. http://dx.doi.org/10.1016/j.imlet.2011.02.008

[29] Yang, L.Y., Chen, A., Kuo, Y.C. and Lin, C.Y. (1999) Efficacy of a Pure Compound H1-A Extracted from *Cordyceps sinensis* on Autoimmune Disease of MRL lpr/lpr Mice. *The Journal of Laboratory and Clinical Medicine*, **134**, 492-500. http://dx.doi.org/10.1016/S0022-2143(99)90171-3

[30] Kuo, Y.C., Tsai, W.J., Wang, J.Y., Chang, S.C., Lin, C.Y. and Shiao, M.S. (2001) Regulation of Bronchoalveolar Lavage Fluids Cell Function by the Immunomodulatory Agents from *Cordyceps sinensis*. *Life Sciences*, **68**, 1067-1082. http://dx.doi.org/10.1016/S0024-3205(00)01011-0

[31] Yanagibashi, T., Hosono, A., Oyama, A., Tsuda, M., Hachimura, S., Takahashi, Y., *et al*. (2009) *Bacteroides* Induce Higher IgA Production than *Lactobacillus* by Increasing Activation-Induced Cytidine Deaminase Expression in B Cells in Murine Peyer's Patches. *Bioscience, Biotechnology, and Biochemistry*, **73**, 372-377. http://dx.doi.org/10.1271/bbb.80612

[32] Maeda, S., Ohno, K., Fujiwara-Igarashi, A., Tomiyasu, H., Fujino, Y. and Tsujimoto, H. (2014) Methylation of TNFRSF13B and TNFRSF13C in Duodenal Mucosa in Canine Inflammatory Bowel Disease and Its Association with Decreased Mucosal IgA Expression. *Veterinary Immunology and Immunopathology*, **160**, 97-106. http://dx.doi.org/10.1016/j.vetimm.2014.04.005

[33] Tezuka, H., Abe, Y., Iwata, M., Takeuchi, H., Ishikawa, H., Matsushita, M., *et al*. (2007) Regulation of IgA Production by Naturally Occurring TNF/iNOS-Producing Dendritic Cells. *Nature*, **448**, 929-933. http://dx.doi.org/10.1038/nature06033

[34] De Calisto, J., Wang, N., Wang, G., Yigit, B., Engel, P. and Terhorst, C. (2014) SAP-Dependent and -Independent Regulation of Innate T Cell Development Involving SLAMF Receptors. *Frontiers in Immunology*, **5**, 186. http://dx.doi.org/10.3389/fimmu.2014.00186

[35] Bai, Z., Hayasaka, H., Kobayashi, M., Li, W., Guo, Z., Jang, M.H., *et al*. (2009) CXC Chemokine Ligand 12 Promotes CCR7-Dependent Naive T Cell Trafficking to Lymph Nodes and Peyer's Patches. *Journal of Immunology*, **182**, 1287-1295. http://dx.doi.org/10.4049/jimmunol.182.3.1287

[36] Yokouchi, M., Suzuki, R., Masuhara, M., Komiya, S., Inoue, A. and Yoshimura, A. (1997) Cloning and Characterization of APS, an Adaptor Molecule Containing PH and SH2 Domains that Is Tyrosine Phosphorylated upon B-Cell Receptor Stimulation. *Oncogene*, **15**, 7-15. http://dx.doi.org/10.1038/sj.onc.1201163

[37] Kroll, J., Shi, X., Caprioli, A., Liu, H.H., Waskow, C., Lin, K.M., *et al*. (2005) The BTB-Kelch Protein KLHL6 is Involved in B-Lymphocyte Antigen Receptor Signaling and Germinal Center Formation. *Molecular and Cellular Biology*, **25**, 8531-8540. http://dx.doi.org/10.1128/MCB.25.19.8531-8540.2005

[38] Walter, W., Scheuer, C., Lingnau, K., Reichert, T.E., Schmitt, E., Loos, M. and Maeurer, M.J. (2000) H2-M, a Facilitator of MHC Class II Peptide Loading, and Its Negative Modulator H2-O Are Differentially Expressed in Response to Proinflammatory Cytokines. *Immunogenetics*, **51**, 794-804. http://dx.doi.org/10.1007/s002510000210

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