Eur J Nutr (2011) 50:421–426
DOI 10.1007/s00394-010-0153-4

ORIGINAL CONTRIBUTION

Dietary deoxynucleic acid induces type 2 T-helper immune response through toll-like receptor 9 in mice

Mariko Nakamoto · Emi Shuto · Toshio Hosaka · Tohru Sakai

Received: 8 June 2010 / Accepted: 18 November 2010 / Published online: 4 December 2010

Abstract
Background It has been shown that dietary nucleotides modulate immune response. Due to their unique properties in immune responses, nucleotides are used as immunonutrition in the field of clinical nutrition.
Aim of the study In this study, we examined the effect of dietary deoxynucleic acid (DNA) on antigen (Ag)-specific immune response in ovalbumin (OVA)-immunized BALB/c mice and determined the mechanism using toll-like receptor 9 (TLR9) knock-out (KO) mice.
Methods BALB/c or TLR9 KO mice were fed control and 1% DNA diets and immunized with OVA. Spleen cells from OVA-immunized mice were stimulated with OVA in vitro, and the contents of IFN-γ and IL-4 in supernatants were measured by an ex vivo system. $CD11c^+$ dendritic cells were purified, and ability of cytokine induction to $CD4^+$ cells was examined.
Results The level of OVA-specific IL-4 production in the DNA group was significantly higher than that in the control group. In contrast, the level of OVA-specific IFN-γ production in the DNA group was lower than that in the control group. The DNA diet decreased Ag-specific IL-4 production and enhanced Ag-specific IFN-γ production in TLR9 KO mice. $CD11c^+$ DCs from mice fed the DNA diet had a greater ability than $CD11c^+$ DCs from mice fed the control diet to induce the production of IL-4 from DO11.10 $CD4^+$ T cells.
Conclusions Dietary DNA increases Ag-specific IL-4 production and decreases IFN-γ production through a TLR9-dependent pathway. $CD11c^+$ dendritic cells are target cells in dietary DNA-induced immune regulation.

Keywords Nucleic acid · T-helper · Dendritic cell · IL-4 · $CD4^+$ T cell

M. Nakamoto · E. Shuto · T. Hosaka · T. Sakai (✉)
Department of Public Health and Applied Nutrition,
Institute for Health Biosciences, The University of Tokushima,
Tokushima 770-8503, Japan
e-mail: tohrus@nutr.med.tokushima-u.ac.jp

Introduction

Nucleotides and nucleosides have an indispensable role in the structure of deoxynucleic acid (DNA) and ribonucleic acid (RNA). Various forms of DNA and RNA found in cells and biological fluids are required intermediaries in energy metabolism, glycoconjugate synthesis, and signal transduction among other functions. Additionally, nucleotides have been identified as conditionally essential nutrients, since their de novo presence in rapidly growing tissues is both a functional and structural requirement for proper cell function [1]. In clinical studies, nucleotides have been shown to have effects on some immunological parameters, and these effects have become known as immunonutrition. In studies on infant formulas, it has been shown that dietary RNA enhances infant immune status by increasing poliovirus type 1 neutralizing antibody (Ab) responses against infant vaccine immunization [2–4]. In clinical approach, an enteral formula supplemented with RNA, arginine, and fish oil reduces hospital length of stay along with a significant reduction in the frequency of acquired infections [5]. Furthermore, it has been reported that perioperative administration of an enteral formula supplemented with RNA, arginine, and fish oil ameliorated host defense mechanisms, controlled inflammatory responses, and improved the synthesis of short half-life constitutive proteins [6].

As the first step before conducting human clinical studies, animal studies were carried out to elucidate the mechanisms

underlying the immune regulatory effects of nucleotides. The impact of nutrients on the immune system has been demonstrated in numerous investigations [7]. Dietary sources of RNA and mononucleotides are known to influence optimal growth and functions of metabolically active cells such as lymphocytes and macrophages [8] and thus affect host immune function. Previous studies have shown that nucleotide-supplemented diets prevent a decrease in T-cell-dependent humoral immunity, modulate antigen (Ag)-specific T-cell response, and increase interferon (IFN)-γ production in C57BL/6 mice [9–12]. Similar effects of dietary nucleotides have also been reported in type 2 T-helper (Th2)-prone BALB/cJ mice [13]. It has also been reported that RNA-supplemented diets modulate Th-cell responses against specific antigens in BALB/c mice [14–16].

In the innate immune system, toll-like receptors (TLRs) have been revealed to recognize specific patterns of microbial components. So far, 13 members of the TLR family have been identified. Recognition of bacterial components by TLRs initiates signal transduction pathways, triggering the expression of genes whose products control innate immune responses and further instruct development of antigen (Ag)-specific acquired immunity. Toll/IL-1 receptor domain-control adaptors, such as myeloid differentiation primary response gene 88, Toll/IL-1 receptor domain-containing adaptor protein, Toll/IL-1 receptor domain-containing adaptor-inducing interferon-β, and Toll/IL-1 receptor domain-containing adaptor-inducing interferon-β-related adaptor molecule, play pivotal roles in TLR signaling pathways. Differential utilization of these TIR domain-containing adaptors provides specificity of individual TLR-mediated signaling pathways [17]. Among TLRs, TLR9 is known to recognize the sequence of bacterial unmethylated deoxycytidine-deoxyguanosine DNA (CpG DNA).

Effects of nucleosides, nucleotides, and ribonucleic acid on immune function have been extensively studied. However, it has not been determined whether actions of the immune function of DNA are the same as those of nucleotides and whether DNA exerts its effects through TLR9 signaling despite contribution of TLR9 in dietary DNA-mediated immune regulation was speculated [13]. In this study, we examined the effects of DNA on the induction of Th1 and Th2 response in ovalbumin (OVA)-immunized BALB/c mice and determined their mechanism using TLR9 knock-out (KO) mice.

Materials and methods

Mice and diets

Eight-weeks-old female BALB/c mice (Japan SLC, Shizuoka, Japan), DO11.10 mice on BALB/c background (The Jackson Laboratory, Bar Harbor, ME, USA), and TLR9 KO mice on BALB/c background (Oriental Yeast Co. Ltd., Osaka, Japan) were maintained under pathogen-free conditions with a 12-h light:dark cycle at 25 ± 2 °C and 55 ± 10% relative humidity. All studies were performed in accordance with the ethical guidelines for animal experimentation by the Institution of Health Bioscience, The University of Tokushima, Japan, and were approved by the institution review board of the animal ethics committee.

The mice were maintained on either a control diet or DNA diet for 35 days. The composition of the control diet was 20% casein, 44.5% α-starch (Oriental Yeast Co. Ltd., Chiba, Japan), 22% sucrose (Mitsui Sugar Co. Ltd., Osaka, Japan), 5% corn oil (Wako, Osaka, Japan), 2% cellulose, 5% mineral mixture, 1% vitamin mixture (Oriental Yeast Co. Ltd.), 0.3% *DL*-methionine, and 0.2% cholin bitartrate (Wako) as used previously [18]. The DNA diet contained purified salmon milt DNA (Biochem Co. Ltd., Saitama, Japan) at a concentration 1% in the control diet. Electrophoresis data showed that the molecular weight of DNA used in this study were between 100 and 1,000 bp. Contents of DNA, protein, and water were over 91%, less than 1%, and 3.6%, respectively.

Immunization

Mice were immunized intraperitoneally with 500 μL of OVA solution containing 10 μg of OVA (Sigma Chemical Co., St. Louis, MO, USA) absorbed in 2 mg of aluminum hydroxide gel adjuvant (HCI Biosector, Frederikssund, Denmark) at 1 and 3 weeks after starting to give the test food.

Proliferation assay

To prepare single cell suspensions, the spleen was squeezed with two slide glasses in RPMI-1640 medium (Sigma Chemical Co.) and filtered through mesh. To remove red blood cells, the cells were treated with 0.84% NH_4Cl (pH 7.5) at 37 °C for 10 min. Then, the cells were washed two times with RPMI-1640 medium and suspended in RPMI-1640 medium supplemented with 10% fetal bovine serum, 50 mmol/L 2-mercaptoethanol, 100 μg/mL streptomycin, and 100 U/mL penicillin. Five × 10^5 splenocytes were stimulated with 200 μg/mL OVA or with plate-bound anti-CD3 mAb (coated overnight at 1 μg/mL) in a 96-well flat-bottom plate at 37 °C under 5% CO_2 for 72 h. For the last 20 h of culture, 37 KBq of [^{3}H] thymidine deoxyribose (TdR) was added to the wells, and the amount of [^{3}H]TdR incorporated was measured by a scintillation counter (Aloka, Tokyo, Japan).

Cytokine production

Splenocytes (5×10^6 cell/mL) were stimulated with 200 μg/mL OVA or plate-bound anti-CD3 monoclonal (m)Ab (coated overnight at 1 μg/mL) in a 48-well flat-bottom plate at 37 °C under 5% CO_2 for 72 h. After the culture, culture supernatants were collected and stored at −40 °C until used. Interferon (IFN)-γ and interleukin (IL)-4 in the supernatants were determined using a mouse IFN-γ or IL-4 ELISA kit (eBioscience, CA, USA) according to the manufacturer's instructions.

Dendritic cell (DC) function

$CD11c^+$ cells were purified from spleens of 3 or 4 mice that had been maintained on either the control diet or DNA diet using CD11c microbeads and a MACS LS column (Milteryi Biotec Inc, Auburn, CA, USA) according to the manufacturer's instructions. $CD4^+$ T cells were purified from DO11.10 transgenic mice, which express OVA-specific T-cell receptor, using a $CD4^+$ T cell isolation kit (Milteryi Biotec Inc). $CD4^+$ T cells (4×10^5 cells) from DO11.10 mouse spleens were cultured in a 48-well plate with $CD11^+c$ cells (1×10^5 cells) in the presence of 400 μg/mL OVA for 72 h at 37 °C/5% CO_2. After the culture, culture supernatants were collected and stored at −40 °C until used.

Statistical analysis

All data are shown as means ± SD. Effects of DNA diet and TLR9 KO were examined using 2-way ANOVA. Following determination of the DNA diet and TLR9 KO phenotype effects using 2-way ANOVA, Tukey's post hoc test was used to compare individual means among treatment groups. For comparison between the control group and DNA group, statistical analysis was carried out using two-tailed Student's *t* test. Differences with $P < 0.05$ were considered significant. All statistical analyses were performed using SPSS version 16.0 J (SPSS Inc. USA).

Results

Effects of NA on body weight and spleen weight

There were no differences between the groups in mean body weight, weight gain, and spleen weight in BALB/c mice. The body weight in control and DNA groups was 21.1 ± 0.7 and 21.5 ± 0.9 g, respectively. The overall body weight gains in control and DNA groups were 2.1 ± 0.4 and 1.8 ± 0.3 g, respectively. The spleen weight in control and DNA groups was 432 ± 21 mg/100 g body weight and 425 ± 19 mg/100 g body weight, respectively. Also in TLR9 KO mice, no differences were found between the control and DNA groups in mean body weight, weight gain, and spleen weight (data not shown).

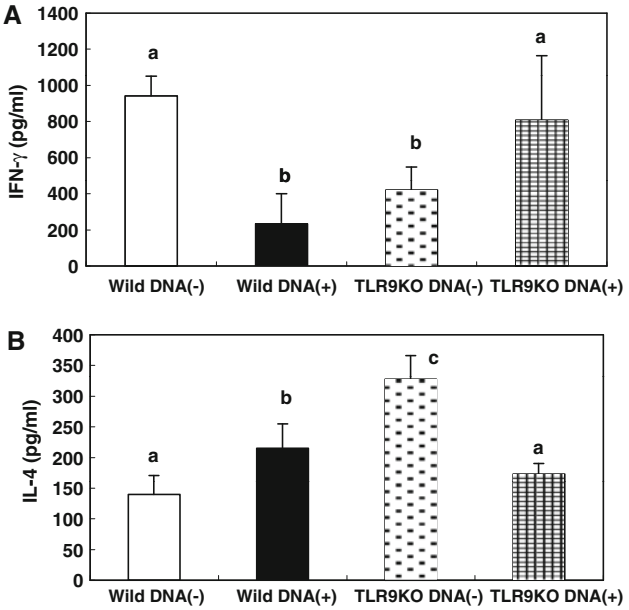

Fig. 1 Effects of DNA on ex vivo OVA-specific IFN-γ and IL-4 production in OVA-immunized BALB/c and TLR9 KO mice. BALB/c mice were fed test food and immunized with OVA twice. Splenocytes from DNA diet-fed mice were cultured with OVA for 72 h. Levels of IFN-γ (**a**) and IL-4 (**b**) upon stimulation with OVA were measured by ELISA. Each value is the mean ± SD of 6 to 8 mice/group. The interaction between DNA diet and TLR9 KO phenotype was <0.001. Means at a concentration without a common letter differ ($P < 0.05$)

DNA increases OVA-specific IL-4 production but decreases OVA-specific IFN-γ production in BALB/c mice but not in TLR9 KO mice

At 35 days after starting to give the test food, we examined the in vitro proliferative responses of splenocytes by co-culture with OVA. A significant difference in proliferative response was not observed between the control and DNA groups in either BALB/c or TLR9 KO mice (data not shown). We also determined the levels of IL-4 and IFN-γ production by an ex vivo system. When the splenocytes were stimulated with OVA, the level of IL-4 production was higher in the DNA diet group than in the control diet group (Fig. 1b). In addition, the level of IFN-γ production was significantly lower in the DNA diet group than in the control diet group (Fig. 1a). In contrast to BALB/c mice, DNA significantly decreased the level of IL-4 production (Fig. 1b) and, moreover, significantly increased the level of IFN-γ production in TLR9 KO mice (Fig. 1a).

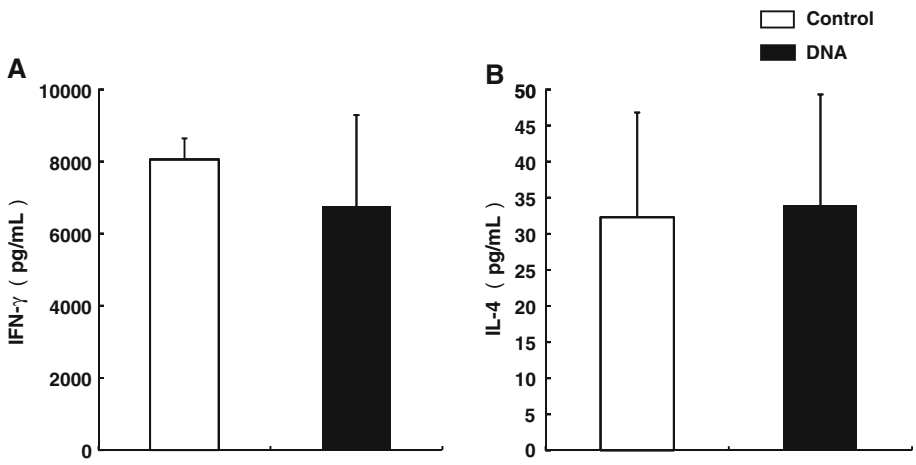

Fig. 2 Effects of DNA on IFN-γ and IL-4 production in DO11.10 mice. DO11.10 mice were given test food for 28 days. Splenocytes from DNA diet-fed mice were cultured with OVA for 48 h. Levels of IFN-γ (**a**) and IL-4 (**b**) upon OVA stimulation in DO11.10 mice were measured by ELISA. Each value is the mean ± SD of 6 to 8 mice/group

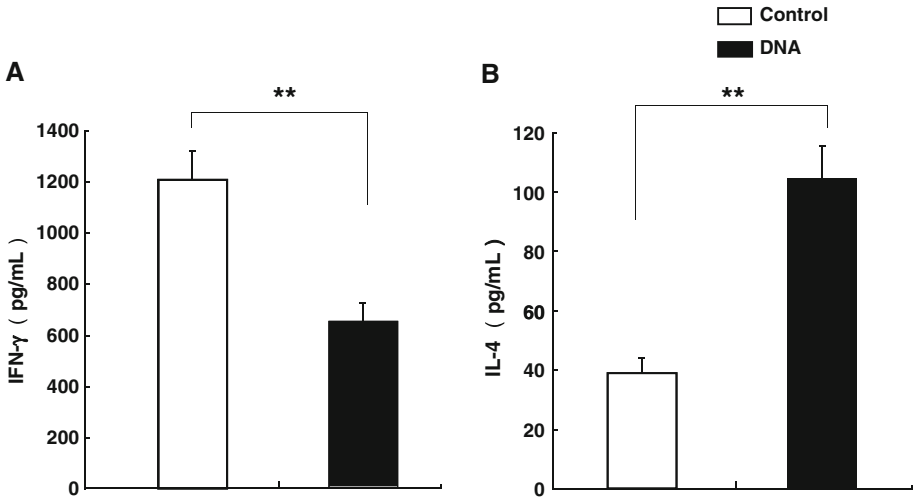

Fig. 3 Effect of DNA on function of $CD11c^+$ dendritic cells. $CD11c^+$ dendritic cells were purified from control and DNA diet-fed mice as described in the "Materials and methods" section. $CD11c^+$ cells were co-cultured with DO11.10 mouse $CD4^+$ T cells in the presence of OVA. Levels of IFN-γ (**a**) and IL-4 (**b**) production when $CD4^+$ T cells were cultured without DCs were less than 2 and 10 pg/mL, respectively

DNA does not change the cytokine production in DO11.10 mice

Since a regulatory effect of DNA on cytokine production was observed in OVA-immunized mice, we examined the effect of DNA on cytokine production profile in DO11.10 mice to determine whether the effect is a direct effect on T cells. DO11.10 spleen cells responded to OVA and produced IL-4 and IFN-γ, but a significant difference was not found between the groups (Fig. 2).

DNA affects the function of $CD11c^+$ DCs

To address the target cell populations that affect the Ag-specific immune responses in DNA-fed mice, we investigated the function of dendritic cells, which are major Ag-presenting cells. We purified $CD11c^+$ dendritic cells from control and DNA-fed mouse splenocytes and co-cultured the cells with $CD4^+$ T cells from OVA-specific TCR transgenic mice. As shown in Fig. 3, $CD11c^+$ dendritic cells from DNA-fed mice induced more IL-4 production than did $CD11c^+$ dendritic cells from control mice. In contrast, the level of IFN-γ production in the DNA diet group was lower than that in the control diet group.

Discussion

The identification of a functional polarization of mouse $CD4^+$ Th-cell clones based on their cytokine secretion profiles has provided a molecular insight into the regulation of immune responses. Th1 cells producing IL-2, IFN-γ, and LTβ represent a T-cell subset that is important for enhancing cellular immune responses, while Th2 cells, characterized by their secretion of IL-4, IL-5, IL-6, IL-10, and IL-13, evoke the production of IgE and accumulation of eosinophils [19, 20]. In this study, we showed that dietary DNA modulates Ag-specific cytokine production. Previous studies have suggested that dietary nucleotides and RNA up-regulate Ag-specific Th1 cytokine production

and modulate Th1/Th2 balance toward Th1 responses [12–14]. In contrast to the results obtained for nucleotides and RNA, the high molecular weight of DNA preferentially induces Ag-specific IL-4-producing cells in OVA-immunized BALB/c mice (Fig. 1). To our knowledge, this is the first study showing the DNA induces Th2 response in vivo.

To examine whether the effect of DNA on $CD4^+$ T-cell function is direct, $CD4^+$ T cells from DO11.10 transgenic mice fed the DNA diet were stimulated with OVA and cytokine production was determined in vitro. Levels of IFN-γ and IL-4 production in the DNA diet group were similar to those in the control diet group (Fig. 2), suggesting that DNA does not affect cytokine production from $CD4^+$ T cells directly. Furthermore, a difference in IFN-γ and IL-4 production from $CD4^+$ T cells was not observed between the control and DNA diet groups when these cells were stimulated with anti-CD3 mAb (data not shown). Naïve $CD4^+$ T cells recognize antigenic peptide/MHC class II complex on Ag-presenting cells and differentiate into either Th1 or Th2. To address the possibility that DNA influences the function of dendritic cells, which are Ag-presenting cells, DO11.10 mouse $CD4^+$ cells were co-cultured with splenic DCs from mice fed control and DNA diets. As shown in Fig. 3, DCs from DNA-fed mice had the ability to induce more production of IL-4 from DO11.10 mouse $CD4^+$ cells than did DCs from control mice. Therefore, the effect of DNA on the induction of Th2 is related to DC function.

Immune regulatory effects of nucleotides have been shown by many studies. However, there has been no study in which the role of TLR9 in those immune regulatory effects was examined. To identify this issue, we used TLR9 KO mice and investigated the effect of DNA on cytokine production in these mice. Interestingly, induction of Th2 by DNA was cancelled in TLR9 KO mice (Fig. 1). CpG DNA is known to be a ligand for TLR9 and to induce Th1-dominant immune response [21]. Vollmer et al. found that some non-CpG DDNA induces Th2-biased immune response and in a TLR9-dependent manner. The study defines a new efficient immune stimulatory motif aside from CpG dinucleotide that consists of a 5′-TC dinucleotide in a thymidine-rich background [22]. In another study, as a vaccine adjuvant in mice, non-CpG DDNA induced Th2-dominated immune response, in contrast to the Th1-biased effects seen with CpG DNA [23–25]. In this study, dietary DNA affected the cytokine production profile differently in BALB/c and TLR9KO BALB/c mice; a Th2-dominant immune response was induced in BALB/c mice, whereas a Th1-dominant immune response was induced in TLR9KO BALB/c mice. The exact mechanism was not determined in this study. It has been shown that nucleoside and/or nucleotide preferentially induce a Th1 immune response [21] and that non-CpG motif DNA induces a Th2 immune response [22]. Considering these facts, we speculate that the effect of non-CpG motif DNA exceeds the effect of nucleoside and/or nucleotide in BALB/c mice, whereas the effect of nucleoside and/or nucleotide exceeds the effect of non-CpG motif DNA in TLR9KO mice. Further study is needed to elucidate the mechanism of dietary DNA-mediated immune regulation and to determine whether this motif exists in the DNA used in this study.

Although immune modulation by dietary nucleoside, nucleotide, and ribonucleotide has been extensively studied, studies on the effect of high molecular weight DNA have been limited. In this study, we used salmon milt DNA because it is a commercially available product and has been used in animal studies and is supplemented with the enteral alimentation formula for humans. In animal studies, salmon milt DNA has been shown to enhance lymphocyte function and antibody response [26, 27].

To examine the possibility that DNA influences immune function by changing the composition of microflora in the intestine, intestinal microflora in the control and DNA diet groups was compared by the terminal restriction fragment length polymorphism assay. When the PCR product of 16S ribosome RNA gene was digested with *HhaI*, 250- to 270-bp peak areas were decreased in DNA-fed mice compared to those in control mice. Furthermore, reduced 550- to 570-bp peak areas were observed in the DNA diet group when the PCR product was digested with *MspI*. Reduced peak areas correspond to *Lactobacillus delbrueckii, Lactobacillus animalis*, or *Enterococcus faecalis*. The terminal restriction fragment length polymorphism assay is useful for analyzing most of the bacterial species in the intestine, but the results obtained are not quantitative. Although we determined the number of *Lactobacillus* bacteria in the intestine by the selected culture plate method, a significant difference was not observed between the control and DNA groups (data not shown). Further study is needed to determine whether dietary DNA alters the composition of microflora in the intestine.

In this study, we showed that dietary DNA induces Th2 immune response in OVA-immunized BALB/c mice. Th2 responses might have therapeutic applications for a number of diseases such as Crohn's disease or organ-specific autoimmune disorders. Furthermore, it has recently been reported that T cells crucially contribute to the development of type 2 diabetes and other elements of metabolic syndrome [28–30]. A study by Winer et al. [28] suggested that smaller Th2-cell proportions in diet-induced obesity-associated visceral adipose tissue represent a candidate mechanism for worsening metabolic control in progressively obese mice and strategies that increase Th2-cell content might be of therapeutic benefit.

Acknowledgments This work was partly supported by Grant-in-Aid for Scientific Research. We thank Drs. Nemoto H. and Kataoka K. for technical analyses.

Conflict of interest The authors of this research disclose any potential conflict of interest.

References

1. Aggett P, Leach JL, Rueda R, MacLean WC Jr (2003) Innovation in infant formula development: a reassessment of ribonucleotides in 2002. Nutrition 19:375–384
2. Pickering LK, Granoff DM, Erickson JR, Masor ML, Cordle CT, Schaller JP, Winship TR, Paule CL, Hilty MD (1998) Modulation of the immune system by human milk and infant formula containing nucleotides. Pediatrics 101:242–249
3. Schaller JP, Kuchan MJ, Thomas DL, Cordle CT, Winship TR, Buck RH, Baggs GE, Wheeler JG (2004) Effect of dietary ribonucleotides on infant immune status. Part 1: humoral responses. Pediatr Res 56:883–890
4. Buck RH, Thomas DL, Winship TR, Cordle CT, Kuchan MJ, Baggs GE, Schaller JP, Wheeler JG (2004) Effect of dietary ribonucleotides on infant immune status. Part 2: immune cell development. Pediatr Res 56:891–900
5. Bower RH, Cerra FB, Bershadsky B, Licari JJ, Hoyt DB, Jensen GL, Van Buren CT, Rothkopf MM, Daly JM, Adelsberg BR (1995) Early enteral administration of a formula (impact) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial. Crit Care Med 23:436–449
6. Braga M, Gianotti L, Vignali A, Di Carlo V (1998) Immunonutrition in gastric cancer surgical patients. Nutrition 14:864–865
7. Kubo C, Johnson BC, Gajjar A, Good RA (1987) Crucial dietary factors in maximizing life span and longevity in autoimmune-prone mice. J Nutr 117:1129–1135
8. Carver JD (1994) Dietary nucleotides: cellular immune, intestinal and hepatic system effect. J Nutr 124:S144–S148
9. Yamauchi K, Adjei AA, Ameho CK, Chan YC, Kulkarni AD, Sato S, Okamoto K, Yamamoto S (1996) A nucleoside-nucleotide mixture and its components increase lymphoproliferative and delayed hypersensitivity responses in mice. J Nutr 126:1571–1577
10. Jyonouchi H, Sun S (1996) An orally supplemented mononucleotide mixture prevents the decrease in T cell-dependent humoral immunity in C57BL/6 mice fed a nucleotide-free diet. J Nutr 126:1586–1593
11. Jyonouchi H, Sun S, Sato S (1996) Nucleotide-free diet suppresses antigen-driven cytokine production by primed T cells: effects of supplemental nucleotides and dietary fatty acids. Nutrition 12:608–615
12. Jyonouchi H, Sun S, Abiru T, Winship T, Kuchan MJ (2000) Dietary nucleotides modulate antigen-specific type 1 and type 2 T-cell responses in young C57BL/6 mice. Nutrition 16:442–446
13. Nagafuchi S, Hachimura S, Totsuka M, Takahashi T, Goto M, Yajima T, Kuwata T, Habu S, Kaminogawa S (2000) Dietary nucleotides can up-regulate antigen-specific Th1 immune responses and suppress antigen-specific IgE responses in mice. Int Arch Allergy Immunol 122:33–341
14. Sudo Naiba Y, Takaki A, Tanaka K, Yu XN, Oyama N, Koga Y, Kubo C (2000) Dietary nucleic acids promote a shift in Th1/Th2 balance toward Th1-dominant immunity. Clin Exp Allergy 30:979–987
15. Jyonouchi H, Sun S, Winship T, Kuchan MJ (2001) Dietary ribonucleotides modulate type 1 and type 2 T-helper cell responses against ovalbumin in young BALB/cJ mice. J Nutr 131:1165–1170
16. Jyonouchi H, Sun S, Winship T, Kuchan MJ (2003) Dietary ribonucleotides increase antigen-specific type 1 T-helper cells in the regional draining lymph nodes in young BALB/cJ mice. Nutrition 19:41–46
17. Takaoka A, Hayakawa S, Yanai H, Stoiber D, Negishi H, Kikuchi H, Sasaki S, Imai K, Shibue T, Honda K, Taniguchi T (2003) Integration of interferon alpha/beta signaling to p53 responses in tumor suppression and antiviral defense. Nature 424:516–523
18. Kogiso M, Sakai T, Mitsuya K, Komatsu T, Yamamoto S (2006) Genistein suppresses antigen-specific immune responses through competition with 17β-estradiol for estrogen receptors in ovalbumin-immunized BALB/c mice. Nutrition 22:802–809
19. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
20. Mosmann TR, Coffman RL (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145–173
21. Wagner H (1999) Bacterial CpG DNA activates immune cells to signal infectious danger. Adv Immunol 73:329–368
22. Vollmer J, Weeratna RD, Jurk M, Samulowitz U, McCluskie MJ, Payette P, Davis HL, Schetter C, Krieg AM (2004) Oligodeoxynucleotides lacking CpG dinucleotides mediate Toll-like receptor 9 dependent T helper type 2 biased immune stimulation. Immunology 113:212–223
23. McCluskie MJ, Weeratna RD, Davis HL (2001) The potential of oligodeoxynucleotides as mucosal and parenteral adjuvants. Vaccine 19:2657–2660
24. McCluskie MJ, Davis HL (2000) Oral, intrarectal and intranasal immunizations using CpG and non-CpG oligodeoxynucleotides as adjuvants. Vaccine 19:413–422
25. Sano K, Shirota H, Terui T, Hattori T, Tamura G (2003) Oligodeoxynucleotides without CpG motifs work as adjuvant for the induction of Th2 differentiation in a sequence-independent manner. J Immunol 170:2367–2373
26. Fedianina LN, Zaitseva EA, Epshtein LM, Besednova NN, Somov GP (2005) Experimental study on impact of salmon milt DNA on T-cellular immunity. Antibiot Khimioter 50:14–17
27. Besednova NN, Kasianenko I, Epshtein LM, Gazha AK (1999) The immunotropic properties of the deoxyribonucleic acid from Salmonidase milt. Antibiot Khimioter 44:13–15
28. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM (2009) Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 15:921–929
29. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15:930–939
30. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R (2009) $CD8^+$ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15:940–945