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Dietary Nucleotides Increase the Proportion of a TCR$\gamma\delta^+$ Subset of Intraepithelial Lymphocytes (IEL) and IL-7 Production by Intestinal Epithelial Cells (IEC); Implications for Modification of Cellular and Molecular Cross-talk between IEL and IEC by Dietary Nucleotides

Shinya NAGAFUCHI,[†,*,**] Mamoru TOTSUKA,[**] Satoshi HACHIMURA,[**] Masao GOTO,[**] Takeshi TAKAHASHI,[*] Takaji YAJIMA,[*] Tamotsu KUWATA,[*] and Shuichi KAMINOGAWA[**]

**Nutrition Science Institute, Meiji Milk Products Co., Ltd., 540 Naruda, Odawara, Kanagawa 250-0862, Japan*
***Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*

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We have investigated the effects of dietary nucleotides on intraepithelial lymphocytes (IEL) and intestinal epithelial cells (IEC) in weanling mice. The proportion of T-cell receptor (TCR) $\gamma\delta^+$ IEL in BALB/c mice fed a diet supplemented with nucleotides (NT(+) diet) was significantly higher than that in mice fed the nucleotide-free diet, while the proportion of TCR$\alpha\beta^+$ IEL in NT(+) diet-fed mice was significantly decreased. The change of the TCR$\alpha\beta^+$/TCR$\gamma\delta^+$ ratio was mainly observed in a CD8$\alpha\alpha^+$ subset of IEL. IEC from NT(+) diet-fed mice produced a higher level of IL-7, which is important in the development of TCR$\gamma\delta^+$ IEL, than those from control diet-fed mice. The expression levels of IL-7 and IL-2 receptors on IEL were not different between the two dietary groups. Our findings suggest that the increased population of a TCR$\gamma\delta^+$ IEL subset by feeding nucleotides may be caused by the increased production of IL-7 by IEC.

Key words: dietary nucleotides; intraepithelial lymphocytes; TCR$\gamma\delta^+$ T cells; interleukin-7; intestinal epithelial cells

Intestinal intraepithelial lymphocytes (IEL) are a population of T lymphocytes located adjacent to intestinal epithelial cells (IEC). IEL consist of $\alpha\beta$ T-cell receptor (TCR)-bearing T cells (TCR$\alpha\beta^+$ T cells) and $\gamma\delta$ TCR-bearing T cells (TCR$\gamma\delta^+$ T cells) with phenotypic and functional features distinct from those of TCR$\alpha\beta^+$ T cells in peripheral lymphoid tissues. TCR$\alpha\beta^+$ T cells among IEL can be subdivided into 5 major subsets, based on CD4 and CD8 expression: subsets with phenotypes of CD4$^+$CD8$^-$, CD4$^+$CD8$\alpha\alpha^+$, CD4$^-$CD8$\alpha\beta^+$, CD4$^-$CD8$\alpha\alpha^+$, and CD4$^-$CD8$^-$ lymphocytes, respectively. In contrast, TCR$\gamma\delta^+$ IEL consist of two subsets with phenotypes of CD4$^-$CD8$^-$ and CD4$^-$CD8$\alpha\alpha^+$ lymphocytes. The CD8$\alpha\alpha$ homodimer is unique to IEL, this homodimer not being expressed on T cells in peripheral lymphoid tissues.[1–3)]

IEL and IEC each regulate the development and/or activation of the other. It has been shown that activated TCR$\gamma\delta^+$ T cells derived from skin and intestine express keratinocyte growth factor (KGF), which promotes the growth of IEC, while intraepithelial TCR$\alpha\beta^+$ T cells, as well as all TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ T cell populations in peripheral lymphoid tissues, do not produce KGF.[4)] On the other hand, IEC express interleukin (IL-)7, which plays a critical role in the regulation of IEL.[5)] Disruption of the IL-7 receptor (IL-7R) gene or the IL-7 gene results in depletion of TCR$\gamma\delta^+$ T cells among IEL.[6,7)] Thus, cellular and molecular cross-talk between IEC and IEL, in particular TCR$\gamma\delta^+$ T cells, is considered to regulate the differentiation of IEL and IEC.

It is known that dietary nucleotides are of major importance for a normal gastrointestinal function. The addition of nucleotides increased the expression of brush border enzymes, such as sucrase, lactase, and alkaline phosphatase by an intestinal epithelial-like cell line, Caco-2 cells, when the cell culture was stressed by glutamine deprivation.[8)] *In vivo* studies demonstrated the effects of exogenous nucleotides on the proliferation and differentiation of intestinal cells.[9–11)] Dietary nucleotide deprivation in adult rats led to decreases in the contents and specific activities

† To whom correspondence should be addressed. FAX: +81-465-36-2776; E-mail: n-shinya@mx7.mesh.ne.jp

Abbreviations: IEL, intraepithelial lymphocytes; IEC, intestinal epithelial cells; TCR, T-cell receptor; ELISA, enzyme-linked immunosorbent assay

of alkaline phosphatase, leucine-aminopeptidase, maltase, sucrase and lactase in the villus tip.[9] Uauy et al.[10] found increased mucosal protein and DNA levels, villus height, and disaccharidase activities in the intestines of weanling rats fed a diet supplemented with nucleotides. Thus, dietary nucleotides play a crucial role in development of the gastrointestinal tract.[11]

Hence, it is possible that dietary nucleotides may influence the interaction between IEL and IEC. However, the influence of dietary nucleotides on IEL and IEC has not yet been discovered. In this study, we investigate the effects of dietary nucleotides on the subset composition of IEL and cytokine production by IEC.

Materials and Methods

Mice and diets. Female BALB/c mice (3 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). The mice were fed a nucleotide-free diet based on whey protein isolate (WPI) (NT(−) diet) or the NT(−) diet supplemented with nucleotides (NT(+) diet) from 3 weeks of age for 2 weeks in our animal facilities. Thereafter, the mice were killed, and then the intestines were removed from the mice and examined for IEL subsets, and *in vitro* cytokine production by IEL and IEC. The nucleotides and diet compositions are shown in Table 1. The added nucleotides and WPI were obtained from Yamasa Co. (Choshi, Japan) and Davisco International Inc. (Le Sueur, MN), respectively. The nucleotide composition had a similar proportion of nucleotides to those in human milk at 12 weeks of lactation.[12] Nucleotide 5′-diphosphates were replaced by nucleotide 5′-monophosphates. Adenosine 5′-monophosphate was also replaced by inosine 5′-monophosphate because adenosine increases the blood flow to several tissues.[13]

Table 1. Compositions of the Experimental Diets (%)

Ingredient	NT(−) diet	NT(+) diet
Whey protein isolate	22.0	22.0
Sucrose	5.0	5.0
Starch	60.0	59.6
Cellulose	3.0	3.0
Soybean oil	5.0	5.0
Vitamins[a]	1.0	1.0
Minerals[b]	4.0	4.0
Nucleotides[c]	—	0.4

[a] The composition was as follows (in mg/kg): vitamin A, 20 IU; 7-dehydrocholesterol, 2000 IU; α-tocopheryl acetate, 50; menadione, 5; choline chloride, 2000; ρ-aminobenzoic acid, 100; inositol, 100; niacin, 40; calcium pantothenate, 40; riboflavin, 8; thiamine HCl, 5; pyridoxine HCl, 5; folic acid, 2; D-biotin, 0.4; cyanocobalamine, 0.03.

[b] The composition was as follows (in mg/kg): NaCl, 5572; KI, 31.6; KH_2PO_4, 15560; $MgSO_4$, 2292; $CaCO_3$, 15256; $FeSO_4 \cdot 7H_2O$, 1080; $MnSO_4 \cdot H_2O$, 160.4; $ZnSO_4 \cdot 7H_2O$, 0.053; $CuSO_4 \cdot 5H_2O$, 19.1; $CoCl_2 \cdot 6H_2O$, 0.92.

[c] The composition was as follows (in g/kg): Cytidine monophosphate, 1.62; Guanosine monophosphate, 0.57; Inosine monophosphate, 1.1; Uridine monophosphate, 0.71.

Preparation of IEL. We isolated IEL as described.[14] In brief, small intestines were removed from mice. The small intestines free of the lumen contents were turned inside-out with the aid of polyethylene tubing. Each inverted intestine was cut into four segments and the segments were placed in a 50-ml conical tube containing 45 ml of Hanks' balanced salt solution (GIBCO, Grand Island, NY) including 5% fetal calf serum (FCS) (GIBCO). The tube was shaken at 37°C for 45 min (horizontal position on an orbital shaker at 135 rpm). The cell suspension was passed through a glass-wool column to remove sticky cells. Subsequently, the cells were suspended in 30% (wt/vol) Percoll (Amersham Pharmacia Biotech, Uppsala, Sweden) and centrifuged for 25 min at $450 \times g$. IEL were obtained using Percoll as the separation medium for density gradient centrifugation ($450 \times g$, 25 min), and IEL were recovered at the 44 and 70% Percoll interphase (>90% were TCR positive).

Preparation of IEC. IEC were isolated from mice and cultured as described by Perreault and Beaulieu.[15] In brief, small intestines were opened longitudinally, washed with PBS, and then cut into 4 segments. These segments were transferred to a 15-ml tube containing 10 ml of ice-cold MatriSperse (Collaborative Biomedical Products, Becton Dickinson Lab., Mississauga, Ontario, Canada). The intestine segments were incubated at 4°C for 8–10 hours without agitation. Then, each tube was gently shaken to separate the IEC. The suspension was washed twice in PBS (4°C, $300 \times g$, 8 min). These IEC (>94 % were CD3ε- and immunoglobulin-negative cells) were resuspended in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5×10^{-5} M), and 2 mM L-glutamine, and then plated in a 96-well plate (2×10^5 cells/well). The freshly isolated IEC were cultured at 37°C under 5% CO_2 without stimulation for 16 hours.

Flow cytometry. Two-color or three-color analysis of IEL subsets was done. The antibodies used for flow cytometry were biotin-labeled anti-mouse TCRβ (H57-597; PharMingen, San Diego, CA), biotin-labeled anti-mouse CD4 (H129.19; PharMingen), biotin-labeled anti-mouse IL-7-receptor (IL-7R) (B12-1; PharMingen), biotin-labeled anti-mouse IL-2R (CD25) (7D4; PharMingen), fluorescein isothiocyanate (FITC)-labeled anti-mouse TCRδ (GL3; Cedar Lane Lab., Ontario, Canada), FITC-labeled anti-mouse CD4 (H129.19; PharMingen), phycoerythrin (PE)-labeled anti-CD8α (53-6.7;

GIBCO), FITC-labeled anti-mouse CD8β (Y8.77; Seikagaku Co., Tokyo, Japan), and PE-labeled anti-mouse TCRβ (H57-597; PharMingen) antibodies. We did all incubations in the dark. Cells were incubated for 30 min on ice with a biotin-labeled antibody. The cells were washed in Hanks' balanced salt solution and then incubated for 30 min on ice with streptavidin-Red 670 (GIBCO), and FITC-labeled and PE-labeled antibodies. The cells were then washed by centrifugation. Stained cells were analyzed with a Becton Dickinson FACSort (San Jose, CA). The data were analyzed with Lysis II software.

Culture of IEL for the cytokine assay. We examined the cytokine production by murine IEL following CD3ε stimulation. Purified anti-CD3ε (145-2C11; Cedar Lane Lab.) monoclonal antibodies were adsorbed directly to microtiter wells overnight at 4°C and then unbound monoclonal antibodies were washed off. IEL (2.0×10^5 cells/well) were cultured at 37°C in duplicate in 0.2 ml of RPMI1640 medium on 96-well plates for 2 days under a humidified atmosphere containing 5% CO_2 and 95% air. The medium was supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5×10^{-5} M), and 2 mM L-glutamine. After incubation, culture supernatants were collected by centrifugation and stored at -25°C until used for analysis.

Cytokine assays. The concentrations of interferon-γ (IFN-γ), IL-2 and IL-7 were measured by an enzyme-linked immunosorbent assay (ELISA). The antibodies used for ELISA were rat monoclonal anti-mouse IFN-γ (6A2; PharMingen), rat monoclonal anti-mouse IL-2 (JES6-1A12; PharMingen), mouse monoclonal anti-human/mouse IL-7 (Genzyme, Cambridge, MA), biotin-conjugated rat monoclonal anti-mouse IFN-γ (XMG1.2; PharMingen), biotin-conjugated rat monoclonal anti-mouse IL-2 (JES6-5H4; PharMingen), and goat polyclonal anti-mouse IL-7 (R & D Systems, Minneapolis, MN) antibodies. A 100-μl solution of anti-mouse IFN-γ antibodies (1 μg/ml) diluted with 0.05 M Tris buffer (pH 8.9), or anti-mouse IL-2 (1 μg/ml) or anti-mouse IL-7 (2 μg/ml) antibodies diluted with 0.1 M $NaHCO_3$ (pH 8.2) was added to each well of microtitreplates (Nunc, Roskilde, Denmark), followed by incubation overnight at 4°C. The wells were then washed with phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween 20 (PBS-0.05% Tween), and blocked with PBS containing 3% polyethylene glycol 6000 (Nacalai Tesque Inc., Kyoto, Japan) and 5% FCS for 1 hour at room temperature. For ELISA of IL-7, PBS containing 3% polyethylene glycol 6000 and 10% ovalbumin (Seikagaku Co.) was used as a blocking solution. The plates were washed again, and then the culture supernatants diluted with PBS containing 3% polyethylene glycol 6000, for IL-2 and IFN-γ measurement, or PBS containing 3% polyethylene glycol 6000 and 0.1% Tween 20, for IL-7 measurement, were added, followed by incubation for 2 hours, then washing. Biotin-conjugated anti-mouse IFN-γ or biotin-conjugated rat anti-mouse IL-2 antibodies, diluted with PBS containing 3% polyethylene glycol 6000, were then added to each well, and left for 2 hours. For IL-7 measurement, the plates was incubated with 1 μg/ml of goat anti-mouse IL-7 antibodies diluted with PBS containing 3% polyethylene glycol 6000 and 0.1% Tween 20 for 2 hours, and then, after washing, biotin-conjugated anti-goat IgG antibodies, diluted with PBS containing 3% polyethylene glycol 6000 and 0.1% Tween 20, were added to each well, and then left for 15 min. The plates were washed and then alkaline phosphatase-conjugated streptavidin (Zymed Lab., South San Francisco, CA) diluted with PBS containing 3% polyethylene glycol 6000 was added to each well. After the plates had been washed, 4-nitrophenylphosphate substrate reagent (0.1% 4-nitrophenylphosphate (Tokyo Kasei Co., Tokyo, Japan) solution in 0.1 M diethanolamine buffer, pH 9.8) was added to each well. The plates were then incubated for 1 hour at room temperature and the reaction was stopped by the addition of 5 N NaOH. The absorbance was measured optically at 405 nm.

Statistics. The results were expressed as means or means with standard errors. The statistical significance of the data was evaluated by Student's t-test. A value at $p < 0.05$ was regarded as significant.

Results

Effects of dietary nucleotides on IEL subsets

We first examined the effects of dietary nucleotides on the IEL subsets in BALB/c mice, by flow cytometric measurement of TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ T cells. Among IEL, the proportion of TCR$\gamma\delta^+$ T cells in NT(+) diet fed BALB/c mice (n = 4) was significantly higher than that in control mice (n = 4) (Fig. 1). In contrast, the proportion of TCR$\alpha\beta^+$ T cells in NT(+) diet-fed BALB/c mice was significantly lower than that in control mice. The ratio of TCR$\alpha\beta^+$ T cells to TCR$\gamma\delta^+$ T cells (TCR$\alpha\beta$/TCR$\gamma\delta$) significantly decreased in mice fed the NT(+) diet compared to in those fed the NT(−) diet (Fig. 1). The total number of IEL in the NT(+) diet group ($4.88 \times 10^6 \pm 0.48 \times 10^6$ cells) was almost the same as those in the NT(−) diet group ($4.80 \times 10^6 \pm 0.67 \times 10^6$ cells).

TCR$\alpha\beta^+$ T cells among IEL are divided into five subpopulations, having phenotypes of CD4$^+$CD8$^+$, CD4$^+$CD8$^-$, CD4$^-$CD8$\alpha\beta^+$, CD4$^-$CD8$\alpha\alpha^+$, and CD4$^-$CD8$^-$ lymphocytes, respectively. TCR$\gamma\delta^+$ T cells are divided into two CD-negative subpopula-

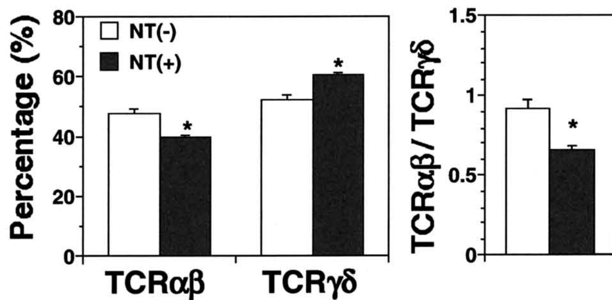

Fig. 1. Effects of Dietary Nucleotides on the Percentages of $TCR\alpha\beta^+$ T Cells and $TCR\gamma\delta^+$ T Cells in IEL of BALB/c Mice.

BALB/c mice (n=4), 3 weeks old, were fed *ad libitum* the NT(−) diet or NT(+) diet for 2 weeks. Stained cells were analyzed by flow cytometry to calculate the percentages of $TCR\alpha\beta^+$ T cells and $TCR\gamma\delta^+$ T cells, and the ratio of $TCR\alpha\beta^+$ T cells and $TCR\gamma\delta^+$ T cells among IEL of BALB/c mice at 5 weeks of age. The percentages of $TCR\alpha\beta^+$ T cells and $TCR\gamma\delta^+$ T cells were calculated on the basis of the total percentages of TCRαβ and TCRγδ-positive IEL. Results are presented as means ± SE of data for individual mice per group. An asterisk (*) indicates a significant difference at $p<0.05$ between the two groups.

tions expressing and not expressing CD8αα homodimers, respectively. We examined the effects of dietary nucleotides on the $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ IEL subsets in BALB/c mice based on CD4 and CD8 expression (Table 2). The proportion of $TCR\gamma\delta^+CD8\alpha\alpha^+$ T cells significantly increased in NT(+) diet-fed mice and that of $CD4^-CD8^-$ cells also increased slightly. On the other hand, $TCR\alpha\beta^+CD8\alpha\alpha^+$ T cells significantly decreased on oral administration of dietary nucleotides. However, there was no such difference in $TCR\alpha\beta^+CD8\alpha\beta^+$ T cells between NT(−) and NT(+) diet-fed mice. Furthermore, feeding of dietary nucleotides did not change the ratio of subsets with the phenotypes of $CD4^+CD8^-$, $CD4^+CD8^+$ and $CD4^-CD8^-$ lymphocytes. Taken together, dietary nucleotides mainly induced an increase in $TCR\gamma\delta^+CD8\alpha\alpha^+$ T cells and a decrease in $TCR\alpha\beta^+CD8\alpha\alpha^+$ T cells.

Expression of IL-2R and IL-7R

Recent studies have suggested that the signals from IL-2R[16] and IL-7R[6,7] are indispensable for $TCR\gamma\delta^+$ T cell development. IL-2R is also an important marker of the activation of T cells. Thus, we investigated the influence of dietary nucleotides on the expression of IL-2R and IL-7R in IEL (Table 3). The percentages of $IL\text{-}2R^+$ cells among $TCR\alpha\beta^+$ T cells or $TCR\gamma\delta^+$ T cells in the NT(+) diet group were almost the same as those in the NT(−) diet group. There was also no significant difference between the NT(−) and NT(+) diet groups in the $TCR\alpha\beta^+IL\text{-}7R^+$ T cells or $TCR\gamma\delta^+IL\text{-}7R^+$ T cells.

Cytokine production by IEL

To examine the effects of dietary nucleotides on the activation of IEL and the mechanism by which the oral administration of dietary nucleotides increases the proportion of the $TCR\gamma\delta^+$ T cell population in IEL, we investigated the influence of dietary nucleotides on cytokine production (IL-2 and IFN-γ) by IEL stimulated with immobilized anti-CD3ε antibodies. IL-2 has been shown to be important in the development of $TCR\gamma\delta^+$ T cells.[17] There was no significant difference between NT(+) diet-fed and control mice in IL-2 or IFN-γ production by IEL (Fig. 2). When IEL were cultured in the absence of anti-CD3ε monoclonal antibodies, the background response was under the detection limit (data not shown).

Table 2. Profiles of CD4, CD8αα, and CD8αβ Expression by $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells in IEL of BALB/c Mice Fed the NT(−) or NT(+) Diet (%)

	NT(−)	NT(+)
$TCR\alpha\beta^+$ T cell		
$CD4^+CD8^{+\,a}$	1.3±0.4	0.9±0.1
$CD4^+CD8^{-\,a}$	6.6±1.0	7.3±1.6
$CD4^-CD8\alpha\beta^{+\,b}$	14.0±1.4	13.8±0.3
$CD4^-CD8\alpha\alpha^{+\,b}$	20.6±2.2	12.5±1.8*
$CD4^-CD8^{-\,a}$	6.1±0.4	6.5±0.5
$TCR\gamma\delta^+$ T cell		
$CD4^-CD8\alpha\alpha^{+\,c}$	40.6±0.8	45.5±0.6*
$CD4^-CD8^{-\,c}$	10.0±0.8	13.2±1.0

Numbers are mean ± SE of percentages of IEL from individual mice (n=4 for each group), as measured by flow cytometry. The percentages of $CD4^+CD8^+$, $CD4^+CD8^-$, $CD8\alpha\beta^+$, $CD8\alpha\alpha^+$, and $CD4^-CD8^-$ T cells were calculated on the basis of the total percentages of TCRαβ and TCRγδ-positive IEL.

[a–c] The IEL were stained with the combinations of biotin-labeled anti-mouse TCRβ, FITC-labeled anti-mouse CD4, and PE-labeled anti-mouse CD8α antibodies (for *a*), those of biotin-labeled anti-mouse TCRβ, FITC-labeled anti-mouse CD8β, and PE-labeled anti-mouse CD8α antibodies (for *b*), and those of biotin-labeled anti-mouse CD4, FITC-labeled anti-mouse TCRδ, and PE-labeled anti-mouse CD8α antibodies (for *c*) for three-color analysis. To detect biotin-labeled antibodies, streptavidin-Red 670 was used. IEL were analyzed by flow cytometry as described in Materials and Methods.

* A significant difference at $p<0.05$ between the two groups.

Table 3. Expression of IL-2R and IL-7R in IEL of BALB/c Mice Fed the NT(−) or NT(+) Diet (%)

	NT(−)	NT(+)
$TCR\alpha\beta^+$ T cell		
$IL\text{-}2R^+$	5.8±0.8	8.4±0.8
$IL\text{-}7R^+$	5.9±0.8	7.9±1.0
$TCR\gamma\delta^+$ T cell		
$IL\text{-}2R^+$	5.4±0.7	6.6±0.5
$IL\text{-}7R^+$	4.1±0.2	5.2±0.3

Numbers are mean ± SE of percentages of IEL, as measured by flow cytometry. The percentages of $IL\text{-}2R^+$ and $IL\text{-}7R^+$ cells were calculated on the basis of the percentage of TCRαβ-positive IEL or TCRγδ-positive IEL.

IL-7 production by IEC

These findings indicate that dietary nucleotides do not influence IL-2R or IL-7R expression, or IL-2 production. To discover the mechanism underlying

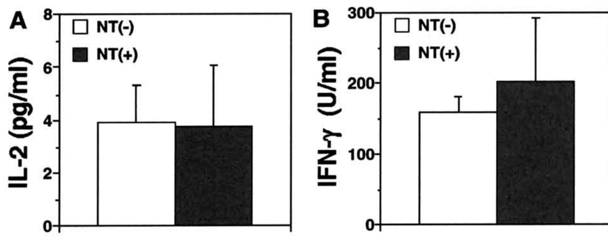

Fig. 2. Interleukin-2 (IL-2) (A) and Interferon-γ (IFN-γ) (B) Production *in Vitro* by CD3-Stimulated IEL from BALB/c Mice Fed the NT(−) or NT(+) Diet.

BALB/c mice (n = 5), 3 weeks old, were fed *ad libitum* the NT(−) diet or NT(+) diet for 2 weeks. The cell cultures were done in the presence of anti-CD3 monoclonal antibodies adsorbed directly onto microtiter wells for 2 days. The data shown are the results of a representative of two independent experiments. Each column shows the mean ± SE for individual mice per group.

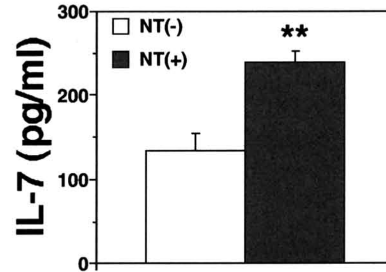

Fig. 3. Interleukin-7 (IL-7) Production *in Vitro* by IEC from BALB/c Mice Fed the NT(−) or NT(+) Diet.

BALB/c mice, 3 weeks old, were fed *ad libitum* the NT(−) diet (n = 8) or NT(+) diet (n = 9) for 2 weeks. The IL-7 levels in culture supernatants of IEC (2×10^5 cells/well) cultured for 16 hours were measured. The data shown are the results of a representative of two independent experiments. Each column shows the mean ± SE for individual mice per group. The asterisks (**) indicate a significant difference at $p < 0.01$ between the two dietary groups.

the increase in the proportion of the TCR$\gamma\delta^+$ T cell subset, we examined the effects of dietary nucleotides on IL-7 production by IEC from BALB/c mice at 5 weeks of age. Increased IL-7 production was observed in mice fed the NT(+) diet (n = 9) compared with in those fed the NT(−) diet (n = 8), the difference being significant (Fig. 3).

Discussion

We demonstrated in this study that IEL from nucleotide-fed mice contained a higher proportion of TCR$\gamma\delta^+$ T cells and a lower proportion of TCR$\alpha\beta^+$ T cells than IEL from control mice. We showed that these changes in the IEL subset populations occurred mainly in the subset of CD8$\alpha\alpha^+$ IEL, although the proportion of TCR$\gamma\delta^+$CD4$^-$CD8$^-$ T cells in nucleotide-fed mice increased slightly. Considering that there was no difference in the total number of IEL between two dietary groups, dietary nucleotides may increase TCR$\gamma\delta^+$CD8$\alpha\alpha^+$ IEL and decrease TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ IEL. Furthermore, we found that dietary nucleotides increased IL-7 production by IEC. Thus, we suggest that dietary nucleotides increased the proportion of TCR$\gamma\delta^+$ T cells in IEL through an increase in the IL-7 production by IEC. Our study demonstrated for the first time the immunomodulatory effect of dietary nucleotides on IEL and IEC.

Nucleotides have been found to stimulate IEC.[9,10,18] IEC are important in the regulation of intraepithelial TCR$\gamma\delta^+$ T cells. For example, transgenic mice expressing IL-7 showed an increased population of TCR$\gamma\delta^+$ T cells in the lymphoid organs.[19] In IL-7-deficient or IL-7R-deficient mice, TCR$\gamma\delta^+$ T cells were absent from the gut.[6,7] These findings indicate the strong relationship between the expression of IL-7 or IL-7R and the development of TCR$\gamma\delta^+$ T cells *in vivo*. Our results showed that dietary nucleotides promoted the secretion of IL-7 by IEC, while they had no effect on the percentage of IL-7R$^+$ cells among TCR$\gamma\delta^+$ T cells. Thus, the increase of IL-7 production by IEC may induce an increase in TCR$\gamma\delta^+$ T cells among IEL. Our findings support the concept that cellular and molecular cross-talk between IEL and IEC is responsible for the regulation of IEL and IEC.[5,20,21]

Studies on mice depleted of IL-2 or IL-2R also indicated that IL-2/IL-2R signaling may directly influence TCR$\gamma\delta^+$ T cell development. Reduced numbers of TCR$\gamma\delta^+$ IEL were observed in IL-2Rβ-deficient mice[16] and IL-2-deficient mice.[17] However, dietary nucleotides had no effect on the IL-2 production by CD3ε-stimulated IEL or the percentage of IL-2R$^+$ cells. Thus, IL-2/IL-2R signaling may not be involved in the mechanism by which the oral administration of dietary nucleotides increases the proportion of the TCR$\gamma\delta^+$ T cell population in IEL. In addition, dietary nucleotides may not directly influence the activation of IEL, as indicated by the unchanged levels of IL-2R$^+$ cells and cytokine (IL-2 and IFN-γ) production.

Among IEL, many of the TCR$\alpha\beta^+$ T cells express the CD8$\alpha\alpha$ homodimer and these cells are known to develop extrathymically. On the other hand, a considerable number of TCR$\alpha\beta^+$ T cells singly expressing the CD4 or CD8$\alpha\beta$ heterodimer are the progeny of thymus-dependent blasts. It was suggested that intestinal TCR$\gamma\delta^+$ T cells can also develop extrathymically. In nude mice, TCR$\gamma\delta^+$ T cells are present, although TCR$\alpha\beta^+$ T cells and CD8$\alpha\beta^+$ T cells are generally absent, suggesting that some TCR$\gamma\delta^+$ T cells are extrathymic.[1,2] Our results showed that dietary nucleotides induced an increase in TCR$\gamma\delta^+$CD8$\alpha\alpha^+$ T cells and a decrease in TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ T cells. However, dietary nucleotides had no effect on the percentages of the CD8$\alpha\beta^+$, CD4$^+$CD8$^-$, CD4$^+$CD8$^+$, and CD4$^-$CD8$^-$ subpopulations among TCR$\alpha\beta^+$ T cells. Hence, dietary

nucleotides may exert an immunomodulatory effect only on T cells that develop extrathymically.

TCR$\gamma\delta^+$ T cells, NK cells, and macrophages are involved in the regulation of innate and acquired immunity.[22] Dietary nucleotides increase NK cell and macrophage activity.[23] Adoptive transfer of small numbers of TCR$\gamma\delta^+$ T cells from mice tolerant to ovalbumin selectively suppressed IgE antibody production without affecting the parallel IgG response.[24] Therefore, one of the physiological roles of TCR$\gamma\delta^+$ T cells may be in regulation of the IgE response. In this study, we found that dietary nucleotides increased the proportion of a TCR$\gamma\delta^+$ IEL subset. Our previous finding indicates that the supplementation of diets with dietary nucleotides down-regulates the serum IgE response and induces Th1 immune responses.[25] Taken together, the induction of the Th1 immune response by dietary nucleotides may be caused by activating TCR$\gamma\delta^+$ T cells, macrophages, and NK cells.

In addition to the possible effect of TCR$\gamma\delta^+$ T cells on serum IgE, TCR$\gamma\delta^+$ T cells may play a role in regulating oral tolerance. Injection of the anti-TCRδ-chain antibody down-modulated the expression of TCR$\gamma\delta$ and inhibited the induction of oral tolerance to ovalbumin, as measured as the antibody response, and CD4$^+$ and CD8$^+$ T-cell responses.[26] This result is supported by another observation, that the oral administration of the antigen did not induce tolerance in TCR-δ knockout mice.[26] On the other hand, Fujihashi *et al.*[27] reported that the frequency of intestinal IgA plasma cells as well as the IgA levels in serum, bile, saliva, and fecal samples were markedly reduced in TCRδ-deficient mice. Considering our results regarding increased TCR$\gamma\delta^+$ IEL, it is possible that dietary nucleotides may be involved in the induction of oral tolerance and the mucosal IgA antibody response.

In conclusion, we showed that dietary nucleotides increased the proportion of a TCR$\gamma\delta^+$ IEL subset and IL-7 production by IEC. Studies on IL-7-deficient and IL-7R-deficient mice indicated that mucosal intranet occurs between IEL and IEC.[28] Our study is the first suggesting that foodstuffs may have an effect on the mucosal intranet between IEL and IEC.

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