The Effect of Oryza Sativa L. Subsp. Japonica Cultivar Yukihikari on The Immune System

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Abstract

Oryza sativa L. subsp. *japonica* cultivar Yuhikikari is proposed to alleviate atopic dermatitis, although its mechanism is unclear. To clarify this issue, we evaluated the effect of Yukihikari on the immune system *in vitro* and *in vivo* by comparison with another Japanese cultivar Kirara397. A DNA microarray analysis of mouse spleen cells cultured with either Yukihikari or Kirara397 showed that Yukihikari-added spleen cells exhibited significantly reduced expression of pro-inflammatory genes. Furthermore, another transcriptome analysis of the cultivars by RNA sequencing showed a similar result to the DNA microarray analysis. Mice fed with Yukihikari had less germinal center B cells, fewer autoantibodies, and less weight than those fed with Kirara397. These results suggest that Yukihikari has immune-regulatory functions and accounts for its allergy-ameliorating effects.

Introduction

Rice (*Oryza sativa* L.) is the most popular main staple worldwide. A large number of cultivars have been generated by breeding to improve their agronomic and economic characteristics, such as high yield and superior eating quality.

It has been shown that main staples, including rice and wheat, contain allergenic proteins. These proteins were first reported in rice by Shibasaki et al. to be in the albumin/globulin fractions of rice endosperm proteins [1]. Allergens in rice have been identified [2,3] and hypoallergenic rice has been genetically or enzymatically generated [4-6]. In addition to eggs, milk, and wheat, rice allergy is a common food allergy in Japan. In Hokkaido, Japan, the rice cultivar transition from Yukihikari to Kirara397 due to eating quality has been implicated as a reason for the increase in atopic dermatitis. Indeed, the reversal of rice consumption from Kirara397 to Yukihikari alleviated atopic dermatitis. Furthermore, it has been suggested that in an animal model Yukihikari modulated gut microbiota, and augmented gut barrier function [7]. However, there is almost no difference in the allergenic protein contents of these cultivars. Although the anti-allergic effect of Yukihikari on atopic dermatitis has been postulated, its mechanism is still unclear.

The beneficial effect of food on our health has been intensively studied. Probiotics, including lactic acid bacteria (LAB), modulate the immune system [8,9]. To analyze their functions, we established various assay systems and methodologies, including intravital imaging of the gut [8,10-15], transcriptome analyses of immune cells [9], and generation of genetically-engineered model mice [15]. Using these technologies, we recently found that LAB derived from miso-induced interleukin 22 (IL-22) production in immune cells, thereby resulting in an augmentation of the skin barrier function [8].

In this study, the function of Yukihikari on the immune system was elucidated, and we applied our established protocols to Yukihikari.lso, distinct immunological functions of Yukihikari from that of Kirara397were highlighted.

Methods

Ethics declaration

All mice were maintained in our animal facility under specific pathogen free (SPF) conditions following guidelines of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. All experimental procedures on animals were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (A2019-207C5), also all experiments were conducted under approved guidelines.

Rice

Oryza sativa L. subsp. japonica cv. Yukihikari and Kirara397 were used in this study. Seeds were sown in a greenhouse at Obihiro University of Agriculture and Veterinary Medicine on April 25, 2018. Seedlings aged 36 d were transplanted into paddy fields of Kamikawa Agricultural Experiment Station (Pippu, 43°51'N, 142°48'E) at densities of one plant per hill and a spacing of 30 × 15 cm (22.2 plants/m^2) on May 31, 2018. Plants were fertilized using 8 kg N/10a and 9.7 kg P_2O_5/10a. At maturity, the rice grains were harvested, bulked, air-dried, and dehulled. By sieving, brown rice grains smaller than 1.9 mm were removed. The remaining were polished using a rice-polishing machine (TOYO TESTER Seimaiki MC-90A, Toyo Rice Co., Ltd.) until 90% of the grains by weight were white. After which rice powder was prepared from the polished white rice.

Cells and mice

Spleen cells of C57BL/6 mice were prepared as previously described [9].

$IgA^{-/-}$ mice [21] (10-week-old) were fed modified AIN-93G (white rice powder 30.0%, a-corn sugar 22.9486%, casein Lactic 20.00%, granular sugar 10.00%, cellulose 5.00%, AIN-93 Mineral Mix 3.50%, AIN-93 Vitamin Mix 1.00%, L-cystine 0.30%, choline bitartrate 0.25%, soy oil 7.00%, and t-butylhydrochione 0.0014%) (Crea, Japan) for 4 weeks under SPF conditions.

In vitro immunological assay

A total of 1 × 10^6 spleen cells were cultured in 1-mL RPMI1640 medium containing 10% fetal calf serum (FCS) with or without 10 μg of the *rice powder* for 2 d. The activation cell surface markers—CD69 and CD86—on spleen cells were evaluated by flow cytometry.

Flow cytometry

Cells were analyzed using MACSQuant Flow Cytometer (MiltenyiBiotec) as described previously [8,9] with the following specific antibodies: VioletFluo 450-labeled anti-B220 antibodies (clone; RA3-6B2) and APC-labeled anti-CD86 antibodies (clone; GL-1), which were purchased from TONBO biosciences as well as Brilliant Violet 510 anti-mouse CD4 antibodies (clone; RM4-5) and phycoerythrin (PE)-labeled anti-CD69 antibodies (clone; H1.2F3) purchased from BioLegend. Dead cells were eliminated using propidium iodide staining. Data were analyzed using FlowJo (FLOWJO, LLC). When detecting CD19 instead of B220,

VioletFluo 450-labeled anti-CD19 antibodies (clone; 1D3, TONBO biosciences) were used. Intracellular staining of cytokines was conducted as previously described using anti-FoxP3 (clone; MF23, BD Pharmingen).

Measurement of the anti-DNA antibody levels

Immunoglobulin levels were measured as previously described [9] using enzyme-linked immunosorbent assays (ELISAs). Sonicated herring sperm DNA (Sigma-Aldrich, 10 μg/ml) was coated on ELISA plates. The following antibodies were used: alkaline phosphatase-conjugated anti-IgM and anti-IgG (Southern Biotech).

Transcriptome analyses

A total of 4×10^6 spleen cells were cultured in 4-mL RPMI1640 medium containing 10% FCS with 20 μg of rice powder for 2 d. Total RNA was prepared from spleen cells using ISOGEN II (Nippon Gene). Gene expression analysis was performed using DNA microarray and RNA sequencing.

For DNA microarray analysis, the measurement was entrusted to Macrogen Japan. Microgen provided the method as follows. Microarray results were extracted using the feature extraction software v11.0 (Agilent Technologies). Raw data for a similar gene were then summarized automatically using the Agilent feature extraction protocol to generate a raw data text file, providing expression data for each gene probed on the array. The array probes with Flag A in samples were filtered out. The selected gProcessedSignal (gpS) value was transformed logarithmically and normalized using the quantile method. Statistical significant difference of expression data was determined using fold change. For DEG set, the hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Gene-enrichment and functional annotation analysis for the significant probe list was conducted using gene ontology (www.geneontology.org/). Data analyses and visualizations of differentially expressed genes were conducted using R v.3.5.1 (www.r-project.org). The SurePrint G3 Mouse Gene Expression 8x60K microarray kit was used for DNA microarray analysis (Agilent Technologies). Finally, data were analyzed using the genetic manifested software R v.2.15.1.

For RNA sequencing analysis, total RNAs were subjected to RNA sequencing analysis (GENEWIZ). RNA-Seq library construction, next-gen sequencing and following bioinformatics analyses were conducted by GENEWIZ. Briefly, total RNA was quantified and qualified by Qubit RNA Assay (Invitrogen), and RNA ScreenTape (TapeStation; Agilent Technologies). Poly(A) mRNA was enriched using magnet beads-conjugated oligo(dT) and following library preparation for high-throughput sequencing was conducted according to the manufacturer's procedure (NEBNext Ultra II RNA Library Prep Kit for Illumina; New England BioLabs). Approximately 250 ng of total RNA was used as initial input for mRNA selection and adapter-ligated double stranded cDNA fragment was amplified 12 cycles PCR, which incorporates Illumina P5/P7 adapters and sample-specific barcode sequence. Fragment size and quantity of libraries established were confirmed by Qubit DNA Assay (Invitrogen) and DNA ScreenTape (TapeStation; Agilent Technologies). Libraries with unique sample barcodes were pooled together and loaded onto an Illumina

HiSeq/NovaSeq instrument according to manufacturer's instructions (Illumina). Sequencing was carried out using 150 bp paired-end (PE) configuration. Image analysis, base calling and demultiplex were conducted by the Illumina standard software. Approximately 20M PE reads (6 Gb output in 150 bp PE configuration) per samples were obtained. The raw sequencing reads were filtered to remove adapter and low quality reads. The resulting clean reads were used for mapping to the reference genome (Mus musculus; Ensembl/GRCm38), quantifying gene expression, studying differential gene expression and further downstream analyses.

Statistical analysis

Experimental data are presented as the mean ± standard deviations (S.D). Statistically significant differences were evaluated using a two-tailed Student's t-test for unpaired data. *P* values <0.05 were considered to be statistically significant difference was considered as $P < 0.05$.

Data availability

The data that support the findings of this study are available from the corresponding author, T. A., upon reasonable request.

Results

Yukihikari and Kirara397 did not alter cell-surface expression of T/B cell activation markers on spleen cell *in vitro*.

We established an *in vitro* screening method for probiotics based on the activation markers—CD69 on T cells and CD86 on B cells—and found some LAB possessing immune-modulatory functions [9]. To elucidate the effect of Yukihikari on the immune system, Yukihikari and Kirara397 were subjected to this screening method. The addition of these cultivars did not alter cell populations, such as B, T, and dendritic cells (Supplementary Fig. 1). Furthermore, both cultivars did not upregulate the activation markers, CD69 and CD86 on B cells as well as CD69 on T cells, suggesting that both cultivars lack salient immune-stimulatory functions.

Yukihikari induced immunoregulatory gene expression in spleen cells *in vitro*

To find these differences, we analyzed the gene expression profiles of mouse spleen cells cultured with either Yukihikari or Kirara397 using DNA microarray. Compared with Kirara397, 562 and 586 genes were significantly up- and down-regulated by Yukihikari (Supplementary Fig. 2). Among them, the immune response and inflammatory gene expression levels of Yukihikari-treated cells were significantly lower than those of Kirara397-treated cells (Table 1). Gene expressions of the pro-inflammatory cytokines—IL-1, IL-6, and TNF-a were lower in Yukihikari-treated cells than those of Kirara397, suggesting that Yukihikari possesses anti-inflammatory functions (Fig. 1).

Also, further transcriptome analysis using RNA sequencing of spleen cells cultured with cultivars Yukihikari and Kirara397 was performed (Supplementary Fig. 3). RNA-sequencing analysis of Yukihikari- and Kirara397-treated cells exhibited substantially similar results to that of the DNA microarray analysis (Table 2). Yukihikari down-regulated activation markers—CD69 and CD86—at mRNA levels although cell-surface protein levels were not altered (Figs. 2 and 3). Gene expression of anergy markers in B cells, such as ApoE and CD72 [16] were upregulated in spleen cells by Yukihikari. Furthermore, FoxP3, a master regulator of regulatory T cells, [17] was upregulated by this cultivar. Also, inhibitory receptors, such as CD22, CD72, Fc receptor for IgG2b (FcgR2b), and PIR-B [18,19] were upregulated. In contrast, pro-inflammatory cytokines, such as IL-6, IL-1b, and TNF-α [20] were down-regulated. Additionally, the GC B cell marker, B cell lymphoma 6 (Bcl-6) gene was down-regulated by Yukihikari. These results strongly suggest that Yukihikari has immune-suppressive functions.

At the protein level, the expression of FoxP3 in spleen cells cultured with rice powder for 2 d was confirmed. FoxP3 was slightly higher in cells treated with Yukihikari than those treated with Kirara397 (Fig.4). Inhibitory receptors on spleen cells were almost similar (data not shown).

"Yukihikari"-fed mice exhibited less germinal center B cells in Peyer's patches (PP) and mesenteric lymph node (MLN) than that of "Kirara397"-fed mice.

To validate the effect of Yukihikari on the immune system *in vivo*, mice were fed either Yukihikari or Kirara397 for 6 weeks and their immune cell populations in peripheral lymphatic tissues, such as the spleen, PP, and MLN were analyzed (Fig. 5). Cell surface markers, CD4, CD8, B220, and CD11c were stained as helper T cells, cytotoxic T cells, B cells, and dendritic cell markers, respectively, although other minor cell populations expressed these markers. In the spleen and PP, these populations were insignificantly different in the two groups of mice. However, the B cell ratio in MLN of the Yukihikari-fed mice decreased. Additionally, GC B cells ($GL\text{-}7^{+}B220^{+}$ cells), follicular helper T (Tfh) cells ($PD\text{-}1^{+}ICOS^{+}$ cells or $PD\text{-}1^{+}CXCR5^{+}$ cells), and regulatory T (Treg) cells ($FoxP3^{+}CD4^{+}$ cells) in these mice were analyzed. Tfh and Treg cells were almost similar in the two groups of mice, whereas GC B cells in PP and MLN were less similar in the Yukihikari-fed mice.

"Yukihikari"-fed $IgA^{-/-}$ mice exhibited less anti-DNA antibodies than that of "Kirara397"-fed mice.

We generated IgA deficient ($IgA^{-/-}$) mice, which exhibited inflammation in the small intestine [21]. First, we examined whether $IgA^{-/-}$ mice generated autoantibodies or not. Compared with the wild-type mice, $IgA^{-/-}$ mice produced anti-DNA antibodies (Fig.6a and b). As anti-DNA IgG production in $IgA^{-/-}$ mice, $IgA^{-/-}$ augmented autoimmunity. Furthermore, anti-DNA antibodies were measured after feeding in $IgA^{-/-}$ mice to evaluate the immune-suppressive effect of Yukihikari on autoimmunity. Serum anti-DNA antibodies in Yukihikari-fed mice were lower than that of Kirara397-fed mice (Fig.6c and d). These results suggest that Yukihikari negatively regulates B cell activation *in vivo*.

Also, an increase in the bodyweight of Yukihikari-fed mice was slower than that of Kirara397-fed mice (Fig.6e and f).

Discussion

To clarify the effect of *Oryza sativa* L. subsp. *japonica* cultivar Yuhikikari on the immune system, we analyzed its functions based on our protocol, which was recently established [8,9]. Transcriptome analyses using DNA microarray and RNA sequencing revealed that Yukihikari possesses immune-suppressive and anti-inflammatory functions. In contrast, Kirara397 possesses a slight immune-stimulatory function. Yukihikari elevated Treg *in vitro* and likely augmented it *in vivo*. Yukihikari-fed mice exhibited less autoantibody and less weight.

In this study, for the first time, transcriptome analyses showed that Yukihikari has a distinct immunological function from Kirara397. Yukihikari upregulated the inhibitory receptors—CD22, CD72, FcgR2b, and PIR-B—on B cells [18,19], although the upregulation of the *Cd72* gene is marginal. These receptors negatively regulated signaling of the B cell antigen receptor (BCR) [18,19]. Additionally, ApoE was upregulated by Yukihikari. Interestingly, CD72 and ApoE have been identified as molecules of the upregulated gene in anergic B cells [16], suggesting that Yukihikari negatively affected B cells. Following this notion, activation marker genes on B cells such as *Cd86, Cd69,* and *C40* were down-modulated by Yukihikari. Also, notably, FoxP3—a master regulator of Treg [17]—was upregulated by Yukihikari. Indeed, spleen cells treated with Yukihikari increased Treg cells. Moreover, Yukihikari-fed mice tend to have more Treg cells than those of Kirara397-fed mice. Alternatively, Kirara397 down-regulated CD22, PIR-B, ApoE, and FoxP3 as well as upregulated CD69 and CD86, therefore, suggesting that it slightly augments the immune system. Additionally, inflammatory cytokines—IL-1b, IL-6, TNF-a, and IFN-γ—gene expression were more decreased by Yukihikari than Kirara397, suggesting that Yukihikari has an anti-inflammatory function.

Upon activation, conventional B2 B cells generated a secondary follicle called germinal center (GC) where class-switch and hypersomatic mutation occurs [22,23]. Bcl-6—a master regulator of the GC B cells—decreased in spleen cells by Yukihikari, whereas it increased by Kirara397. In accordance with *in vitro* results, Yukihikari-fed mice exhibited more GC B cells in PPs and MLNs than that of Kirara397-fed mice, indicating that humoral immunity was suppressed by Yukihikari. Regulatory T cells—master regulators of the *FoxP3* gene, which were upregulated *in vitro* by Yukihikari slightly increased *in vivo* by Yukihikari in comparison with Kirara397, although it is an insignificant difference. Compared to the Kirara397-fed mice, serum anti-DNA antibodies, which might be involved in autoimmune diseases were more reduced in Yukihikari-fed $IgA^{-/-}$ mice than in. These effects of Yukihikari on the immune system may justify previous clinical findings, which stated that changing dietary rice from Yuhikikari to Kirara397 relieved atopic dermatitis.

Compared with Yukihikari-fed mice, Kirara397-fed mice increased more in body weight. Since Yukihikari possesses anti-inflammatory functions, inflammation may be reduced in Yukihikari-fed mice. This might

be a reason for reduced weight because inflammation induces the anti-inflammatory hormone, cortisol, which is responsible for fat storage in visceral fat tissues [24]. Further studies will be required to clarify this mechanism.

Yukihikari modulates the gut microbiota and augments the gut barrier function [7]. However, in this study, we showed that Yukihikari negatively regulated spleen cells *in vitro*, it acts on the immune system directly. Since dendritic cells can capture luminal molecules through the intestinal epithelium and migrate to PPs and lymph nodes, possibly Yukihikari directly regulates the immune system *in vivo* in a gut microbiota independent manner.

In conclusion, Yukihikari possesses immune-suppressive and anti-inflammatory functions, which may prevent allergies and various inflammation-oriented diseases, such as lifestyle diseases and major neurocognitive disorders. Further studies are required to justify these functions and clarify their mechanisms.

Declarations

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Author contributions

T.A. and K.K. designed the research and wrote the manuscript; K.K. (Figures 1–6), K.T. (Table 1) and T.A. performed the experiments, analyzed the data and prepared the figures; S.M. and K.K. prepared rice cultivars. All authors reviewed the manuscript.

Competing interests

The authors declare no financial or commercial conflict of interest.

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Tables

Table 1. Gene ontology of DNA microarray analysis of “Yukihikari” and “Kirara397”-treated spleen cells. Top 50 are shown.

term	p-value	GeneNumber(Up)	GeneNumber(Down)
response to virus	4.60E-09	0	14
defense response to virus	6.38E-08	3	21
cellular response to interferon-beta	2.72E-07	0	11
response to bacterium	1.97E-06	2	19
organic substance metabolic process	1.04E-05	3	1
immune response	1.89E-05	4	20
cellular response to lipopolysaccharide	2.15E-05	3	16
regulation of ribonuclease activity	5.19E-05	1	4
negative regulation of translation	6.58E-05	7	5
negative regulation of viral genome replication	0.000167951	1	7
cellular response to interferon-gamma	0.000182935	3	9
endothelial cell apoptotic process	0.000218687	0	3
postsynaptic neurotransmitter receptor internalization	0.000571946	2	2
blood vessel development	0.00063116	6	4
positive regulation of transcription by RNA polymerase II	0.00063473	32	35
innate immune response	0.000647949	5	23
regulation of protein binding	0.000697694	4	2
acute inflammatory response	0.000831953	1	3
cellular response to interferon-alpha	0.000831953	1	3
neutrophil chemotaxis	0.000898239	1	8
negative regulation of B cell apoptotic process	0.00103181	0	3
negative regulation of plasminogen activation	0.00103181	1	2
mitochondrion organization	0.001046295	4	6
chemokine-mediated signaling pathway	0.001150069	0	7
positive regulation of cytokinesis	0.001206942	1	5
immune system process	0.001224521	9	23

positive regulation of lamellipodium assembly	0.001254603	2	3
activation of GTPase activity	0.00126514	7	3
killing of cells of other organism	0.001427898	0	6
regulation of myosin II filament organization	0.00147053	1	1
alpha-tubulin acetylation	0.00147053	1	1
negative regulation of protein localization to cilium	0.00147053	1	1
protein localization to nuclear inner membrane	0.00147053	2	0
negative regulation of fibrinolysis	0.001754199	1	2
positive regulation of axon extension involved in axon guidance	0.001754199	2	1
positive regulation of transforming growth factor beta production	0.001754199	0	3
regulation of establishment of endothelial barrier	0.001754199	1	2
positive regulation of interleukin-6 production	0.001985611	2	6
nucleosome disassembly	0.002092637	1	3
positive regulation of GTPase activity	0.002187725	8	6
protein transport	0.002285396	18	20
substrate adhesion-dependent cell spreading	0.002513666	3	4
endoplasmic reticulum organization	0.002631888	2	4
histone H2A acetylation	0.002706195	2	2
movement of cell or subcellular component	0.002706195	2	2
myeloid progenitor cell differentiation	0.002726874	2	1
inflammatory response	0.002840959	7	17
positive regulation of interleukin-8 production	0.003282421	1	4
coronary vasculature development	0.003461795	3	3
hemopoiesis	0.003808756	4	5

Table 2. Gene ontology of RNA sequencing analysis of “Yukihikari” and “Kirara397”-treated spleen cells. Top 50 are shown.

term	over_represented_pvalue	GeneNumber(Up)	GeneNumber(Down)
inflammatory response	7.62E-17	25	40
innate immune response	2.59E-16	26	27
defense response to virus	6.3E-14	35	10
immune response	8.72E-14	27	20
defense response to Gram-positive bacterium	2.13E-11	10	14
defense response to protozoan	5.65E-11	13	3
cellular response to lipopolysaccharide	3.81E-10	17	14
humoral immune response mediated by circulating immunoglobulin	6.69E-10	1	10
positive regulation of cytokine secretion	9.64E-10	8	10
oxidation-reduction process	1.06E-09	54	90
cellular response to hypoxia	1.51E-09	22	4
response to lipopolysaccharide	2.23E-09	22	24
neutrophil chemotaxis	2.36E-09	7	12
negative regulation of cysteine-type endopeptidase activity involved in apoptotic process	4.39E-09	20	11
positive regulation of ERK1 and ERK2 cascade	4.58E-09	18	16
negative regulation of apoptotic process	1.32E-08	60	42
response to mechanical stimulus	3.17E-08	14	9
positive regulation of peptidyl-tyrosine phosphorylation	3.58E-08	13	13
defense response to Gram-negative bacterium	4.24E-08	3	12
cellular response to interferon-beta	1.39E-07	14	0

negative regulation of viral genome replication	2.13E-07	10	4
activation of cysteine-type endopeptidase activity involved in apoptotic process	4.2E-07	19	9
carbohydrate phosphorylation	6.74E-07	6	4
negative regulation of growth of symbiont in host	1.01E-06	6	5
myeloid dendritic cell differentiation	1.18E-06	6	5
positive regulation of glycolytic process	1.39E-06	8	1
wound healing	1.54E-06	13	13
response to hypoxia	2.63E-06	28	21
negative regulation of interleukin-6 production	2.9E-06	7	5
transmembrane transport	5.33E-06	25	31
B cell homeostasis	5.76E-06	4	8
defense response to bacterium	6.7E-06	9	13
response to estradiol	7.34E-06	20	9
cell adhesion	7.45E-06	21	43
phagocytosis, recognition	7.5E-06	0	7
response to organonitrogen compound	8.14E-06	7	6
L-amino acid transport	8.92E-06	4	4
prostaglandin biosynthetic process	9.35E-06	6	4
positive regulation of peptidyl-serine phosphorylation	9.58E-06	13	8
negative regulation of cellular protein metabolic process	9.86E-06	5	1
response to estrogen	1.19E-05	12	11

positive regulation of tumor necrosis factor production	1.21E-05	6	8
mucosal immune response	1.48E-05	0	5
positive regulation of angiogenesis	1.49E-05	13	14
aging	1.75E-05	17	17
positive regulation of chemokine (C-X-C motif) ligand 2 production	1.96E-05	5	1
positive regulation of interferon-gamma biosynthetic process	2.09E-05	3	5
response to glucocorticoid	2.31E-05	19	12
negative regulation of gene expression	2.34E-05	10	10

Figures

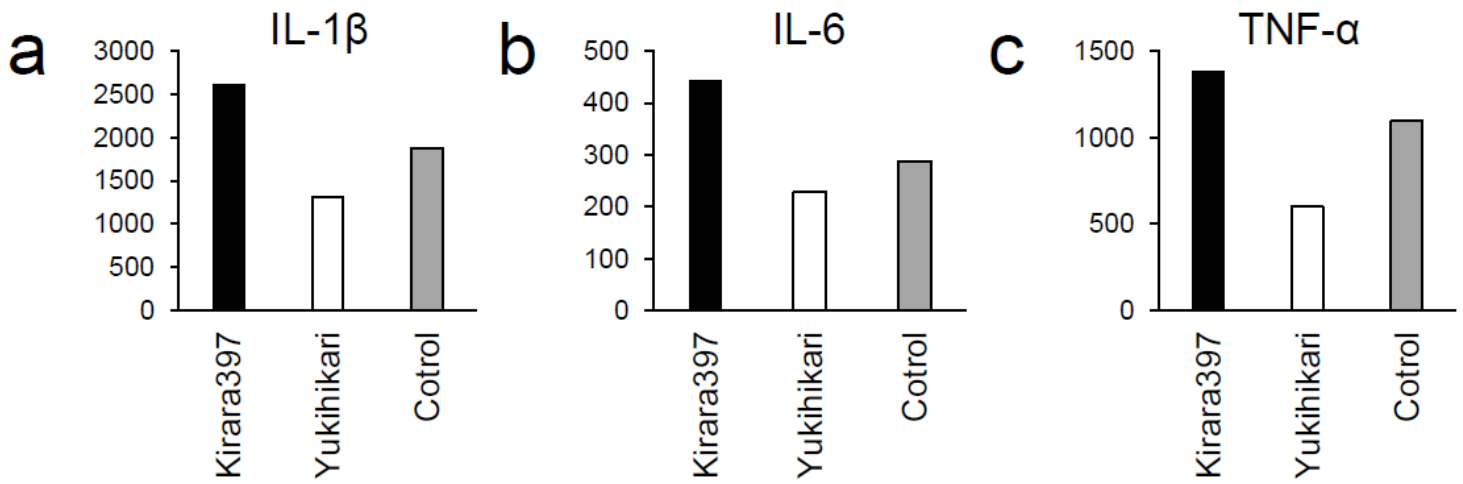

Figure 1

Representative results of gene expression levels of spleen cells treated with Yukihikari and Kirara397using DNA microarray analysis. (a) IL-1β, (b) IL-6, and (c) TNF-α. Cells were prepared as shown in Fig.1 and total RNA was prepared and subjected to DNA microarray analysis. (a) Heat maps (Kirara397 versus control, Yukihikari versus control, and Yukihikari versus Kirara397) and (b) plot of expression levels between Yukihikari versus Kirara397.

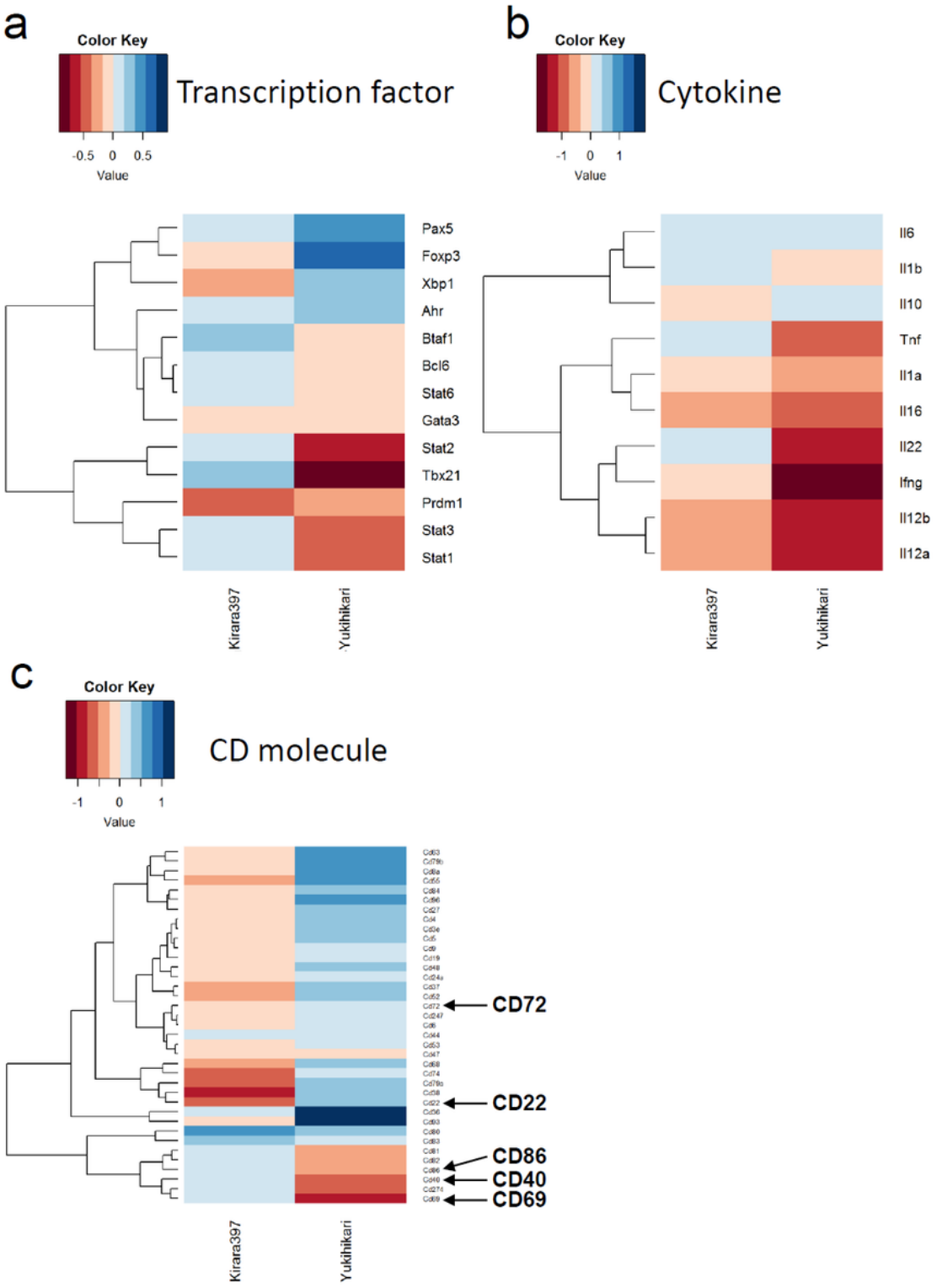

Figure 2

Comparison of gene expression levels in spleen cells treated with Kirara397 and Yukihikari. Heat maps of representative gene expression of (a) transcription factors (b) cytokines, and (c) CD molecules.

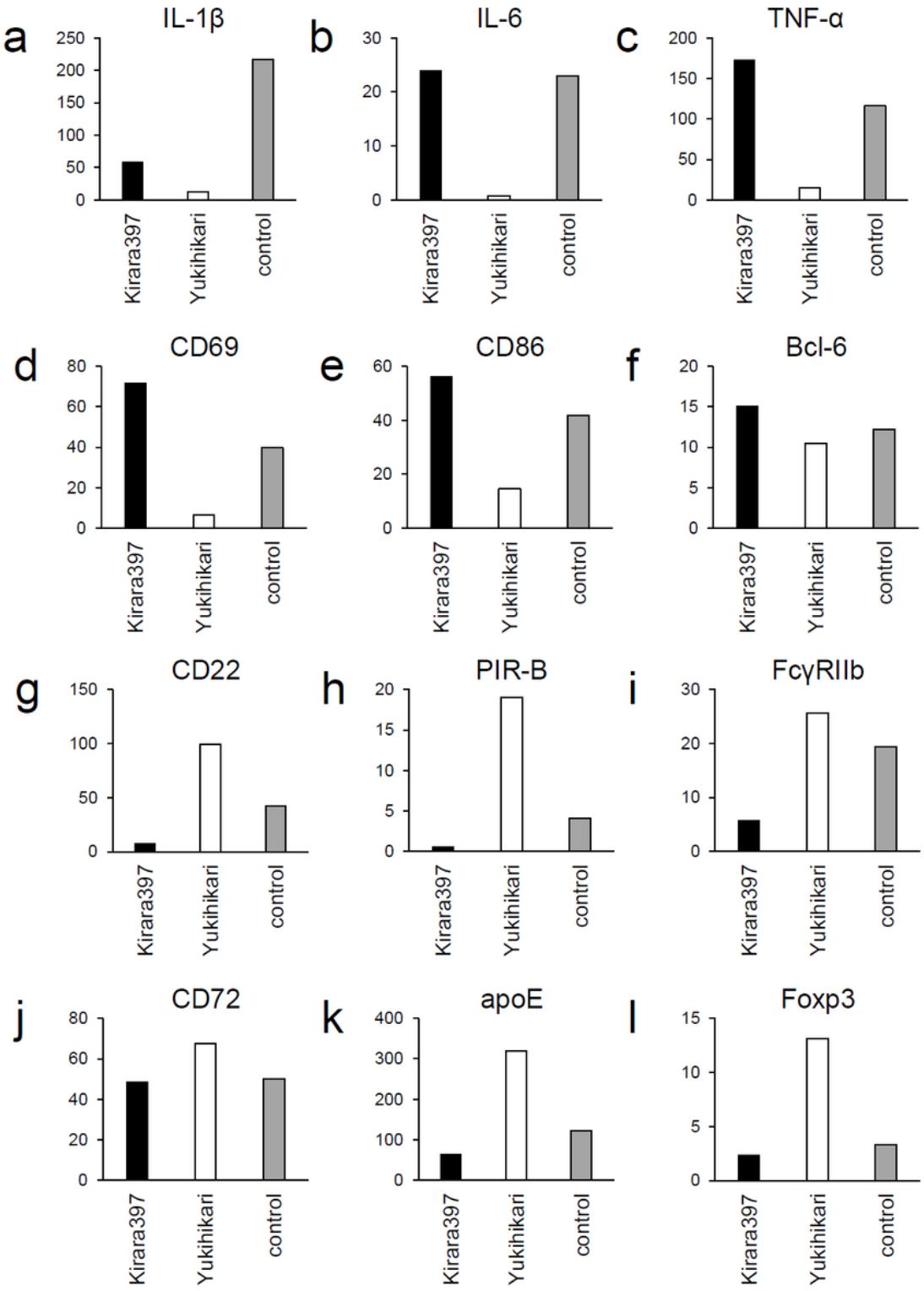

Figure 3

Representative results of gene expression levels of spleen cells treated with Kirara397 and Yukihikari by RNA sequencing analysis. Gene expression levels of (a) IL-1β, (b) IL-6, (c) TNF-α, (d) CD69, (e) CD86, (f) Bcl-6, (g) CD22, (h) PIR-B, (i) FcγIIB, (j) CD72, (k) ApoE, and (l) FoxP3. Differences in gene expression levels of Bcl-6 and CD72 are insignificant. Cells were prepared as shown in Fig.1 and total RNA was

prepared and subjected to RNA sequencing analysis. The vertical axis indicates FPKM (Fragments per kilobases per million reads) based on read counts from HT-seq (v.0.6.1) 25.

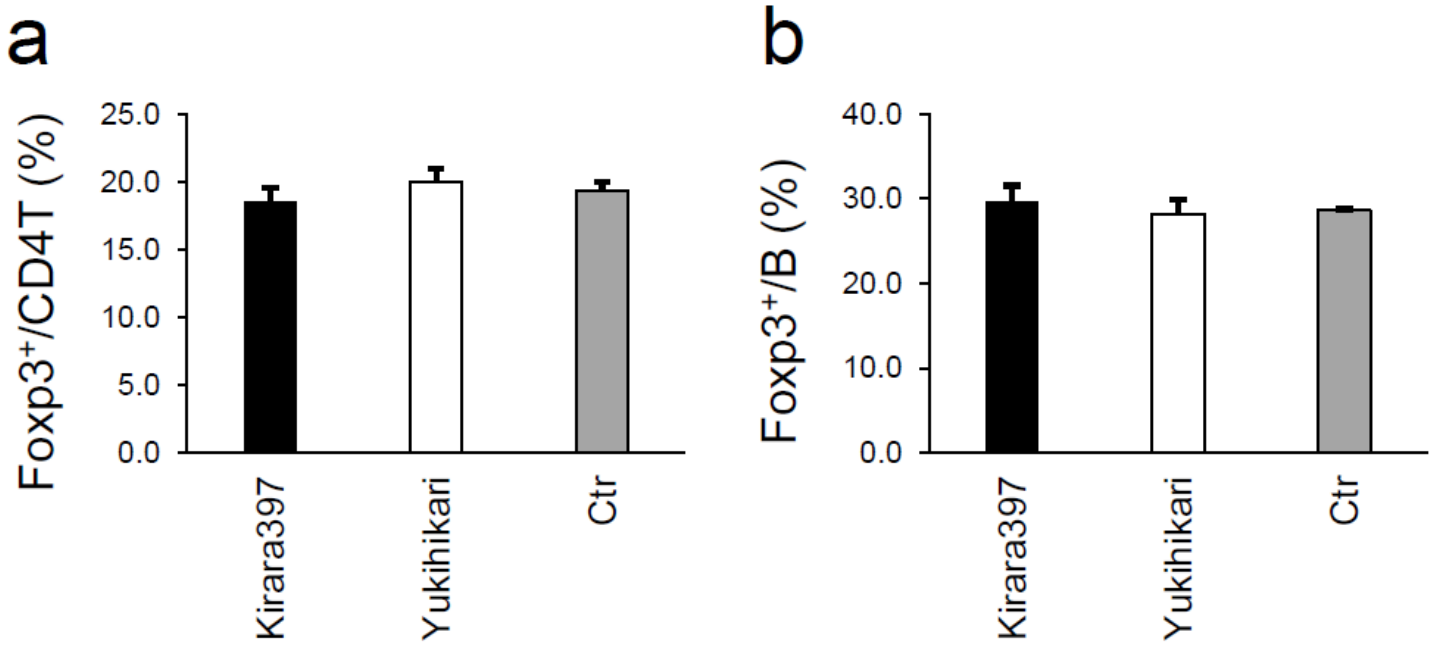

Figure 4

Effect of Yukihikari and Kirara397 on regulatory CD4 T cells in vitro. Spleen cells of C57BL/6 mice were cultured for 2 d with or without 20-μg rice powder (Yukihikari or Kirara397). (a) The ratio of FoxP3+ CD4 T cells and (b) FoxP3+ B cells. Bars indicate mean ± standard deviation (S.D).

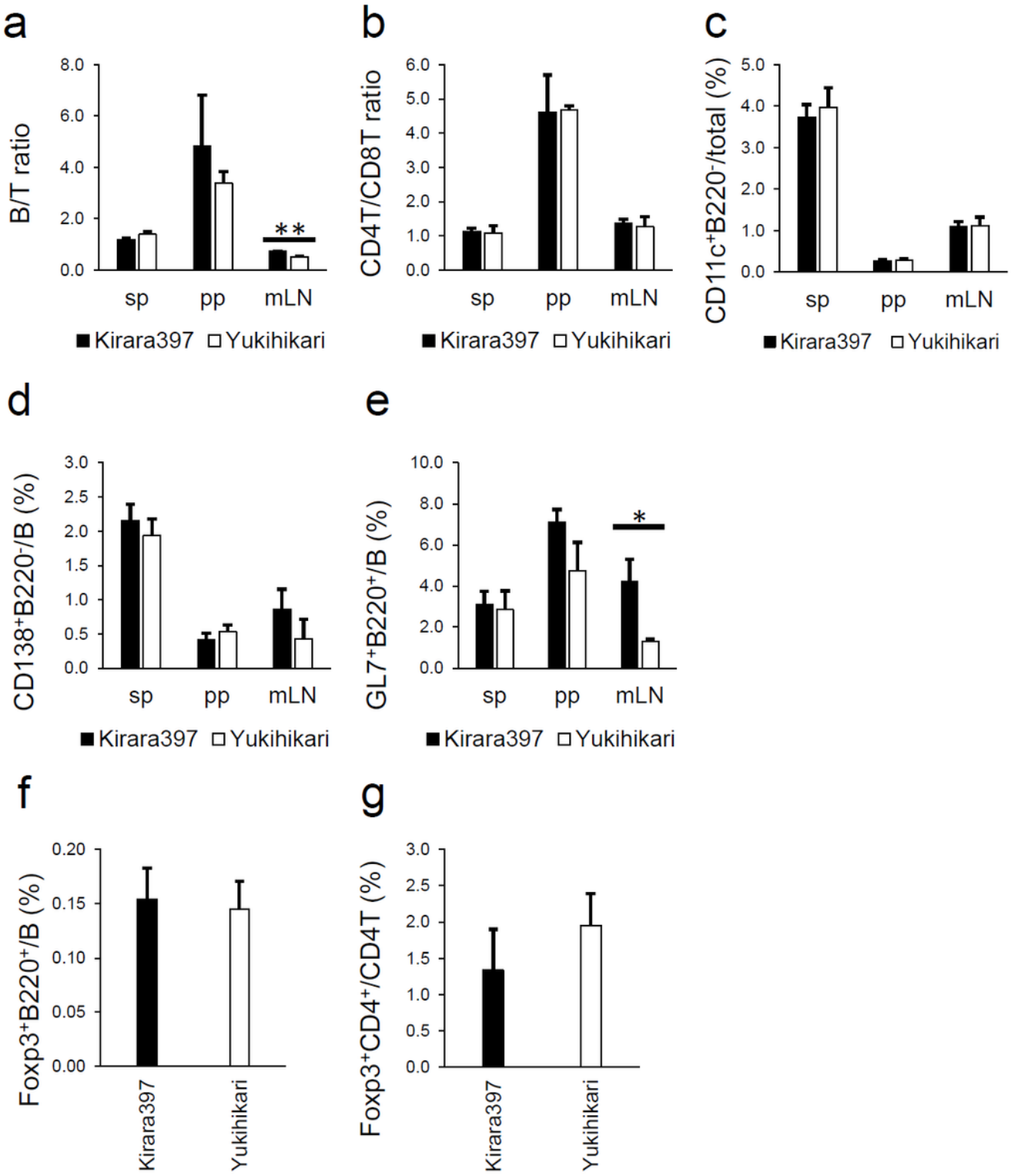

Figure 5

Effect of Yukihikari and Kirara397 on immune cells of IgA deficient (IgA−/−) mice in vivo. (a) The ratio of B cells and T cells (B/T), (b) the ratio of CD4 T and CD8 T cells (CD4T/CD8T), (c) the ratio of DC (CD11c+B220−) in total cells, (d) CD138+ cells, (e) GL-7+ cells, (f) in B cells, FoxP3+ B cells, and (g) FoxP3+ CD4 T cells. A diet containing 30% rice powder (Kirara397 or Yukihikari) was fed to C57BL/6 mice for 6 weeks (n = 3 each). Bars indicate mean ± standard deviation (S.D). *p < 0.05 using t-test.

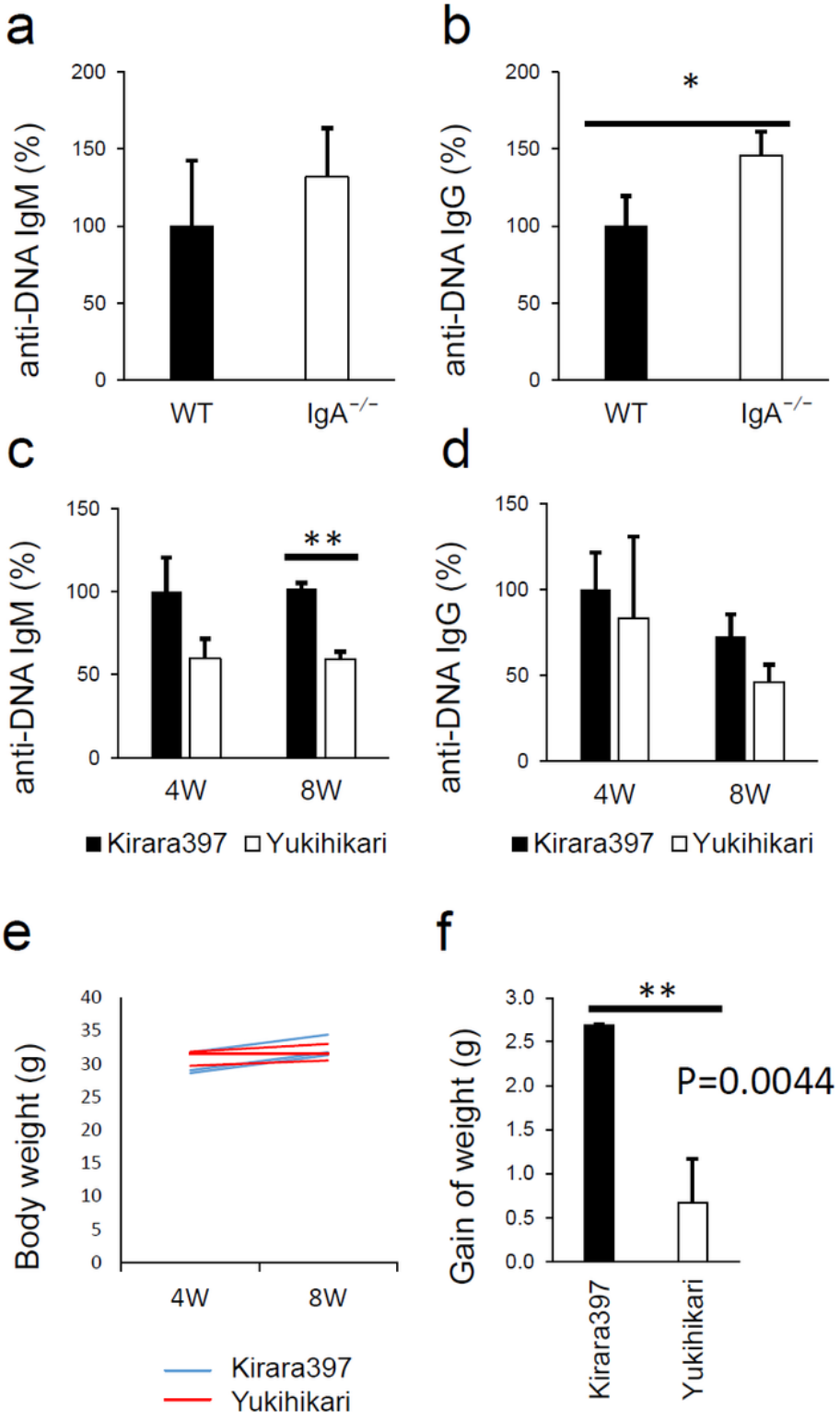

Figure 6

Serum anti-DNA antibodies and body weight of Yukihikari- or Kirara397-fed IgA deficient (IgA−/−) mice. (a) Serum anti-DNA IgM antibodies and (b) IgG in wild-type and IgA−/− mice fed with conventional diet (n = 3 each). (c and d) A diet containing 30% rice powder (Kirara397 or Yukihikari) was fed to IgA−/− mice for 8 weeks (n = 3 each). (c) Serum anti-DNA IgM and (d) IgG levels were measured by ELISA (n = 3). (e)

Body weight of mice fed rice-containing diet at 4 and 8 weeks and (f) body weight gain for 4 weeks. Bars indicate mean ± standard deviation (S.D). *p < 0.05 using t-test.

Supplementary Files

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