

## **Oral ingestion of cow's milk immunoglobulin G stimulates some cellular immune systems and suppresses humoral immune responses in mouse**

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### **Abstract**

Four-week-old male C3H/HeN mice were bred with diets consisting of ovalbumin alone (OVA, control diet) or mixtures of OVA and cow's milk immunoglobulin G (IgG-added diets) as a protein source for 4 or 5 weeks, and both the cellular and humoral immune properties of the mice were investigated. The number of interleukin (IL)-12<sup>+</sup>CD11b<sup>+</sup> cells in spleens and the formation of superoxide by peritoneal macrophages were higher in mice given the IgG-added diet than in those given the control diet. The number of natural killer cells in Peyer's patches or spleens and the cytotoxic activity of spleen cells toward an erythroleukemia cell line, K562, were also higher in mice given the IgG-added diet. In contrast, the numbers of interferon- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells in Peyer's patches or spleens and the levels of total or OVA-specific intestinal IgA and serum IgG were significantly lower in mice given the IgG-added diet than in those given the control diet. In addition, the number of cells expressing CD19 in spleens was significantly higher in mice given the IgG-added diet. These results indicate that oral ingestion of cow's milk IgG may stimulate some innate cellular immune systems, while suppressing humoral adaptive immune responses in the mouse.

*Keywords: Cow's milk IgG; Macrophages; Natural killer cells; CD4<sup>+</sup> cells; Antibody responses*

## **1. Introduction**

The concept of protecting a host with passively derived antibodies is not new. One of the valuable sources of passive antibodies may be cow's milk, a nutrient available to most of the human population. Milk immunoglobulin G (IgG) in cows accounts for most of the circulating antibodies in the suckled newborn, and may also contribute to local immunity in the gastrointestinal tract [1]. Hence, it seems only logical to attempt to replicate the IgG in order to supplement the diet of infants or others who are either deprived of this natural protection or who are exposed to infectious agents without the benefit of active immunity.

Oral administration of the IgG-rich fraction from cow's milk immunized with enteropathogenic and enterotoxigenic microorganisms has been demonstrated to provide effective protection against infections from microorganisms in humans [2]. A crucial event in pathogenesis appears to be the adherence of enteropathogenic microorganisms to the intestinal epithelial cells. The most important role of milk IgG has been thought to be either its ability to inhibit such adherence or its possible action of some kind, since IgG is not absorbed from the human intestinal tract [3].

Some of the intestinal epithelial cells of most mammals have IgG receptors on their surface that can bind IgG-antigen complexes, such as IgG-pathogen and IgG-food protein [4]. IgG-antigen complexes are actively adsorbed and transported to dendritic cells [5]. Ishida et al. [6] demonstrated that oral administration of milk IgG significantly enhanced the immunological functions of gastrointestinal associated lymphoid tissue (GALT) cells. Kobayashi et al. [7] found that the level of IgA in the supernatant of Peyer's Patch cell cultures was higher in mice given cow's milk containing relatively higher IgG than in mice that ingested cow's milk with relatively lower IgG. Moreover, Parreño et al. [8] observed that colostrum-acquired maternal antibodies modulated the systemic and mucosal antibody responses to rotavirus in calves experimentally challenged with bovine rotavirus. These facts indicate that the oral ingestion of cow's milk IgG may trigger the active immune responses in animals. However, there is little information on the active immunomodulatory effects of cow's milk IgG.

In cattle, on the other hand, serum IgG consists of two subclasses, IgG1 and IgG2, in approximately equal amounts. However, IgG1 accounts for more than 90% in milk, although milk IgG is transported from bloodstream [1]. This fact suggests that IgG1 plays a special role in milk. In a previous paper [9], the authors demonstrated that most of the antigen-binding and protein G-binding activities of cow's milk IgG1 might functionally act in intestinal tracts when mice orally ingested cow's milk IgG preparation.

Thus, in this work, the authors investigated the cellular and humoral immunological properties of the mice given diets containing ovalbumin (OVA) alone (control diet) or mixtures of OVA and cow's milk IgG (IgG-added diets) as a protein source for 4 or 5 weeks.

## **2. Materials and methods**

This experiment was conducted in accordance with the guidelines for the Regulation of Animal Experimentation at the Faculty of Agriculture, Shinshu University, and according to Law No. 105 and Notification No. 6 of the Japanese government.

### 2.1. Materials

A defined protein-free purified diet (PM15765) was obtained from Purina Mills (St. Louis, MO). Ovalbumin (OVA, grade II) and bovine serum albumin (BSA, fraction V) were purchased from Q.P. Corporation (Tokyo, Japan) and Sigma Chemical (St. Louis, MO), respectively. Horseradish peroxidase-labeled rabbit anti-bovine IgG (H+L) was from Rockland (Gilbertsville, PA). Horseradish peroxidase-labeled sheep anti-bovine IgG1 heavy chain, anti-bovine IgG2 heavy chain, goat anti-mouse IgG, and goat anti-mouse IgA were from Bethyl Laboratories (Montgomery, TX). BioLegend (San Diego, CA) was the supplier of phycoerythrin (PE)-labeled anti-mouse IL-4 monoclonal antibodies (mAb, clone 11B11), PE-labeled anti-mouse interleukin (IL)-12 p40/p70 mAb (clone C15.6), PE-labeled anti-mouse interferon (IFN)- $\gamma$  mAb (clone XMG1.2), PE-labeled anti-mouse CD49b mAb (clone DX5), biotin-labeled anti-mouse CD11b mAb (clone M1/70), biotin-labeled anti-mouse CD4 mAb (clone RM4-5), biotin-labeled anti-mouse CD19 mAb (clone MB19-1) and phycoerythrin/cyanine 5 (PE/Cy5)-labeled streptavidin. Brefeldin A (BFA), ionomycin, and phorbol 12-myristate 13-acetate (PMA) were from Wako Pure Chemical Industries (Osaka, Japan). Human erythroleukemia cell line K562 (TKG 0210) was from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. Defined fetal bovine serum (FBS) was from HyClone Laboratories (Road Logan, UT). RPMI-1640 and thioglycollate medium were from Nissui Pharmaceutical (Tokyo, Japan). Guava ViaCount Reagent was from Guava Technologies (Hayward, CA). 2-Methyl-6-p-methoxyphenylethynylimidazopyranizone (MPEC) was from ATTO (Tokyo, Japan).

### 2.2. IgG

IgG was prepared from 33% saturated ammonium sulfate precipitates of cow's defatted colostrum by anion-exchange chromatography [10]. The IgG was confirmed by immunoblotting analysis to consist of 95.9% IgG1 and 4.1% IgG2 using antibodies specific to bovine IgG and its subclasses after polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate [11].

### 2.3. Feeding procedure

Male C3H/HeN mice were obtained from Japan SLC (Shizuoka, Japan) at 3 weeks of age. The mice were assigned to test regimens and fed commercial mouse pellets (MF, Oriental Yeast Company, Tokyo, Japan) for 1 week. They were then fed for 4 or 5 weeks on PM15765 supplemented with 25% OVA (control diet), a mixture of 0.005% IgG and 24.995% OVA (0.005% IgG-added diet) or a mixture of 0.05% IgG and 24.95% OVA (0.05% IgG-added diet). The detailed composition of each diet is shown in Table 1. The diets were continuously available in columnar form from stainless-steel feeders. Water was provided *ad libitum* in their drinking bottles. The mice were maintained at  $22 \pm 2$  °C under 12 h-light/12 h-dark cycle.

### 2.4. Cell suspensions

Spleen and Peyer's patches were removed immediately after mouse death by an overdose of ether. A single spleen cell suspension was prepared by disrupting the organ in RPMI-1640 medium containing 5% FBS, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin as previously reported [12]. The cell suspension was washed three times in

**Table 1.**

this medium, suspended, and adjusted to  $1 \times 10^6$  viable cells/ml. Peyer's patches were disrupted in RPMI-1640 medium containing 5% FBS, 100 U/ml penicillin, and 100  $\mu\text{g/ml}$  streptomycin, and treated with dispase II (1.5 mg/ml, Boehringer Mannheim, Mannheim, Germany) at 37 °C for 30 min. Peyer's patch cells were collected, washed 3 times, resuspended in the medium, and adjusted to  $1 \times 10^6$  viable cells/ml.

Peritoneal macrophage suspensions were prepared from mice given the control diet or IgG-added diets for 4 weeks and further given the diets for 4 days after intraperitoneal injection with 2 ml of 4.05% thioglycollate medium as previously reported [13]. Peritoneal cells were collected by lavage and incubated at  $2 \times 10^5$  cells/cm<sup>2</sup> in plastic dishes. After a 1-h incubation, non-adherent cells were removed by washing and adherent cells were suspended in RPMI-1640 medium containing 5% FBS, 100 U/ml penicillin, and 100  $\mu\text{g/ml}$  streptomycin or HEPES-saline buffer (17 mmol/l 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid, 120 mmol/l sodium chloride, 5 mmol/l potassium chloride, 5 mmol glucose, 1 mmol magnesium sulfate, and 0.5 mmol/l calcium chloride, pH 7.4). Viable macrophages were confirmed to occupy 95% of the adherent cells by esterase and Giemsa staining, and adjusted to  $1 \times 10^6$  cells/ml.

### 2.5. Cell function analyses

Peyer's patch cells and cell surface marker antigens of spleen cells (which, in case of a reaction with anti-mouse CD19 mAb were previously incubated with mouse Fc block for 15 min at 4 °C) were reacted with biotin-labeled anti-mouse mAbs specific to CD4 (clone RM4-5), CD11b (clone M1/70) or CD19 (clone MB19-1), or PE-labeled anti-mouse mAb specific to CD49b (clone DX5) for 15 min at 4 °C, and visualized by incubation with PE/Cy5-labeled streptavidin for 15 min at 4 °C. The visualized cells were analyzed by means of a Guava Personal Cell Function Analyzer (Guava Technologies, Hayward, CA).

Assay of intracellular cytokines was performed by permeabilization of PE-labeled anti-mouse cytokine mAbs specific to IL-4, IL-12, and IFN- $\gamma$ . Briefly, the cells were incubated with 40  $\mu\text{g/ml}$  BFA, 4  $\mu\text{g/ml}$  ionomycin, and 40 ng/ml PMA for 4 h. The incubated cells were washed and fixed in IntraPrep reagent 1. After 15 min, the cells were washed again and permeabilized by incubation with IntraPrep reagent 2. The cells having cytokines were visualized with incubation of PE-labeled anti-mouse mAbs specific to IL-4, IL-12 p40/p70 or IFN- $\gamma$ , and were analyzed by means of a Guava Personal Cell Function Analyzer (Guava Technologies).

### 2.6. Determination of superoxide anion

A total of  $1 \times 10^6$  macrophages suspended in HEPES-saline was incubated in a humidified 5% CO<sub>2</sub> incubator for 30 min at 37 °C. After incubation, 300  $\mu\text{mol/l}$  MPEC was added to the macrophage suspensions. As a negative control, a total of  $1 \times 10^6$  macrophages in a suspension containing 30,000 U/ml superoxide dismutase (Biomedicals, Eschwege, Germany) was treated in parallel. An intensity of chemical luminescence was assayed by means of Luminescencer-PSN AB-2200 (ATTO).

### 2.7. Determination of phagocytosis

Phagocytosis as an indicator of latex bead ingestion was principally carried out according to the procedure described previously [13]. Fifty microliters of peritoneal

macrophages ( $1 \times 10^6$  cells/ml) suspended in HEPES-saline was placed on glass slides and incubated with latex beads (0.79  $\mu\text{m}$ , Polysciences, Warrington, PA) in a humidified 5%  $\text{CO}_2$  incubator at 37 °C for 2 h. Free latex beads were removed by rinsing slides three times with Hank's balanced salt solution. Slides were dried, and cells were fixed in ethanol containing 1% acetic acid for 5 min. Slides were dried again and cells were stained by Giemsa. Latex beads in the cell were counted under a microscope.

### 2.8. Cytotoxic activity

One ml of the spleen cell suspension ( $1 \times 10^6$  cells) was seeded per well in a 24-well microtiter plate. Erythroleukemia cell line K562 was added to the well to give spleen cells/ K562 ratios at 1, 20, 100, 200, and 1000. The plate was incubated at 37 °C in a humidified 5%  $\text{CO}_2$  incubator for 24 h. After incubation, 190  $\mu\text{l}$  of Guava ViaCount Regent was added to 10  $\mu\text{l}$  of cell suspension. Viability of K562 was determined by means of the Guava Cell Function Analyzer with ViaCount software. At each spleen cells/ K562 ratio, triplicate wells of spleen cells and K562, and triplicate wells of K562 alone were established in parallel. Eight replicates of the K562 alone as well as four wells of media alone were established for every experiment to determine the background.

### 2.9. Antibody assay

Serum samples and intestinal tracts (duodenum to rectum excluding Peyer's patches) consisting of tissue and the contents were carefully collected on the 35<sup>th</sup> day after the start of feeding with the control or IgG-added diets. The intestinal tract (1 g) was ground for 20 min on ice with sea sand (1.5 g) in 2.5 ml of phosphate-buffered saline (PBS). The grounded material was centrifuged at 3,000 rpm for 30 min at 4 °C, and the supernatant was collected as the intestinal extract.

An enzyme-linked immunosorbent assay (ELISA) was principally carried out according to the procedure described previously [12]. Wells of microtiter plates (Nunc, Roskilde, Denmark) were coated with OVA, goat anti-mouse IgG or goat anti-mouse IgA. A serum sample or intestinal extract of optimal dilution was added to each well. Horseradish peroxidase-labeled anti-mouse IgG, or IgA was used as a second antibody. Ortho-penylenediamine was used to detect the peroxidase reaction, which was stopped by the addition of  $\text{H}_2\text{SO}_4$ . The plates were read at 490 nm on a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Hercules). The antibody level was represented as an antibody index calculated by the following formula:

$$\text{Antibody index} = \frac{\text{ELISA value (A490 nm) for sample from IgG-added diet group}}{\text{ELISA value (A490 nm) for sample from control diet group}}$$

### 2.10. Statistical analysis

All data were expressed as means  $\pm$  standard deviation. The significance of the difference between the control group and IgG-added diet group was tested by Student's *t*-test.

## 3. Results

### 3.1. $\text{CD11b}^+$ and $\text{IL-12}^+\text{CD11b}^+$ cells in Peyer's patches and spleens of mice given IgG-added diets

Four-week-old male C3H/HeN mice were bred with control or IgG-added diets for 4

or 5 weeks. No significant difference was observed in body weights gained between mice given the control and IgG-added diets (data not shown). Figure 1 shows numbers of CD11b<sup>+</sup> and IL-12<sup>+</sup>CD11b<sup>+</sup> cells in Peyer's patches and spleens of mice given control (non-IgG) and 0.005% or 0.05% IgG-added diet for 4 weeks. The both numbers of CD11b<sup>+</sup> and IL-12<sup>+</sup>CD11b<sup>+</sup> cells in spleens were significantly higher in mice given the IgG-added diets than in those given the control diet. In particular, the number of CD11b<sup>+</sup> cells in spleens was more than 7 times higher in mice given the 0.05% IgG-added diet than in those given the control diet. In contrast, the number of CD11b<sup>+</sup> cells in Peyer's patches was significantly lower in mice given the IgG-added diets than in those given the control diet, while the number of IL-12<sup>+</sup>CD11b<sup>+</sup> cells in Peyer's patches was also lower in mice given the 0.05% IgG-added diet.

### 3.2. Activities of superoxide formation and latex bead ingestion by peritoneal macrophages of mice given IgG-added diets

Figure 2 shows activities of superoxide formation and latex bead ingestion by peritoneal macrophages of mice given the control and IgG-added diets for 4 weeks. The formation of superoxide by the macrophages was significantly higher in mice given the IgG-added diets than in those given the control diet. In contrast, the number of latex beads ingested in the macrophages was lower in mice given the IgG-added diets.

**Fig. 2.**

### 3.3. CD49b<sup>+</sup> (DX5) cells in Peyer's patches and spleens of mice given IgG-added diets

Figure 3 shows the number CD49b<sup>+</sup>(DX5) cells in Peyer's patches and spleens of mice given the control and IgG-added diets for 4 weeks. The number of CD49b<sup>+</sup>(DX5) cells in both the Peyer's patches and spleens was significantly higher in mice given the IgG-added diets than in those given the control diet.

**Fig. 3.**

### 3.4. Viability of K562, an erythroleukemia cell line, in the presence of spleen cells of mice given IgG-added diets

Figure 4 shows the viabilities of K562 in the presence of spleen cells of mice given the control and IgG-added diets for 4 weeks. The viability of K562 in cultures with the spleen cells was significantly lower in mice given the IgG-added diets than in those given the control diet. The viability decreased with an increase in the spleen cells/K562 ratio.

**Fig. 4.**

### 3.5. Numbers of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells of Peyer's patches and spleens of mice given IgG-added diets

Figure 5 shows numbers of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells in Peyer's patches and spleens of mice given the control and IgG-added diets for 4 weeks. Numbers of both IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells in Peyer's patches and spleens were significantly lower in mice given the IgG-added diets than in those given the control diet.

**Fig. 5.**

### 3.6. Total or OVA-specific intestinal IgA and serum IgG levels of mice given IgG-added diets

Figure 6 shows total or OVA-specific intestinal IgA and serum IgG levels of mice given the control and 0.05% IgG-added diets for 5 weeks. The levels of both total or OVA-specific intestinal IgA and serum IgG were significantly lower in mice given the IgG-added diets than in those given the control diet.

**Fig. 6.**

### 3.7. CD19<sup>+</sup> cells in spleens of mice given IgG-added diets

Figure 7 shows numbers of cells expressing low density CD 19 (CD19<sup>low</sup> cells) and high-density CD19 (CD19<sup>high</sup> cells) in spleens of mice given the control and IgG-added diets for 4 weeks. The total number of CD19<sup>high</sup> and CD19<sup>low</sup> cells was highest in mice given the 0.05% IgG-added diet, while that was lowest in mice given the control diet.

**Fig. 7.**

## 4 . Discussion

The amount of diet ingested by mice per day and the average body weight of mice in this study were about 2 g and 19.5 to 28.4 g, respectively. Hence, the oral average ingestion amount of IgG per mouse was 0.1 mg per day in the 0.005% IgG-added diet group. This IgG amount corresponds to 0.21 g in humans with 60 kg of body weight and to the IgG amount present in approximately 300 ml of mature cow's milk. Many of us can easily drink 300 ml of cow's milk a day.

The number of spleen CD11b<sup>+</sup> and IL-12<sup>+</sup>CD11b<sup>+</sup> cells was significantly higher in mice given the IgG-added diets than in those given the control diet, whereas that of Peyer's patches was lower in mice given the IgG-added diets (Fig. 1). The superoxide formation of peritoneal macrophages was significantly higher in mice given the IgG-added diets than in those given the control diet (Fig. 2). CD11b is a typical cell surface antigen of monocytes/macrophages and dendritic cells [14]. IL-12 is one of major cytokines produced from monocytes/macrophages and dendritic cells [15]. Macrophages and dendritic cells have IgG receptors for the Fc region such as Fcγ receptor (FcγR) I, FcγRII, and FcγRIII. Of these Fcγ receptors, FcγRI displays high affinity for free IgG as well as IgG-antigen complexes [16]. The binding of free IgG or IgG-antigen complex to FcγRI on macrophages and dendritic cells may activate the cells, and induce the production of IL-12 from the cells [17]. The activated cells may migrate from Peyer's patches to secondary lymphocyte tissues [18]. In addition, the binding of free IgG or IgG-antigen complex to macrophages via FcγRI stimulates superoxide production [19]. Hence, the dietary cow's milk IgG may bind to FcγRI on macrophages and dendritic cells in Peyer's patches, activate the cells, and accelerate their migration from Peyer's patches to secondary lymphoid tissues.

The number of CD49b<sup>+</sup>(DX5) cells in both the Peyer's patches and spleens was significantly higher in mice given the IgG-added diets than in those given the control diet (Fig. 3). Monoclonal antibody specific to CD49b (DX5) is a typical anti-pan natural killer (NK) cells [20]. NK cells are known to display cytotoxic effects on tumor cells. This work showed that the spleen cells had a significantly higher cytotoxic activity toward an erythroleukemia cell line, K562, in mice given the IgG-added diets than in those given the control diet (Fig. 4). The proliferation and activation of resting NK cells are induced by IL-12 [21]. As already mentioned, IL-12<sup>+</sup>CD11b<sup>+</sup> cells in spleens are higher in mice given the IgG-added diet than in those given the control diet. Hence, the increments of CD49b<sup>+</sup>(DX5) cell numbers and cytotoxic activity may be attributable to the increment of IL-12<sup>+</sup>CD11b<sup>+</sup> cell numbers in spleens. Thus, the oral ingestion of cow's milk IgG is concluded to enhance the innate cellular immunity via the numbers and functions of macrophages, dendritic cells, and NK cells.

On the other hand, the numbers of IFN-γ<sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells in Peyer's patches and spleens decreased significantly in mice given the IgG-added diets than in those given



the control diet (Fig. 5). The levels of total or OVA-specific serum IgG and intestinal IgA were significantly lower in mice given the IgG-added diets than in those given the control diet (Fig. 6). In addition, the total number of CD19<sup>high</sup> and CD19<sup>low</sup> cells in spleens were higher in mice given the IgG-added diet than in those given the control diet (Fig.7). IFN- $\gamma$ <sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cells are type 1 helper T (Th1) and type 2 helper T (Th2) cells, respectively, and Th2 cells stimulate antibody responses [22]. The differentiations of T cells to Th1 or Th2 cells and the functions of Th1 and Th2 cells are suppressed by regulatory T cells [23]. The regulatory T cell induces and maintains the oral tolerance to antigens, and the oral tolerance in turn suppresses the production of antigen-specific immunoglobulins [23-26]. Moreover, CD19 is a surface marker of both B cells and plasma cell precursors, but not plasma cells [27]. It is reported that the density of CD19 is higher in conventional B cells (CD19<sup>high</sup>) than in plasma cell precursors (CD19<sup>low</sup>) [27]. The regulatory T cell may suppress the response of B cells as well as the differentiation of Th1 and Th2 cells [28]. On the other hand, the induction of regulatory T cells in the mucosal tolerance requires Fc $\gamma$ RIIb signaling on dendritic cells [29]. Fc $\gamma$ RIIb displays high affinity for IgG-antigen complex, but little affinity for free IgG [16]. Some ingested cow's milk IgGs may form IgG-antigen complexes with intestinal bacteria. Thus, the decrease of IFN- $\gamma$ <sup>+</sup> and IL-4<sup>+</sup>CD4<sup>+</sup> cell numbers in Peyer's patches or spleens, and the lower immunoglobulin level in mice given the IgG-added diets may be attributable to the binding of cow's milk IgG-antigen complexes to Fc $\gamma$ RIIb on dendritic cells. At present, however, the authors have little data to explain this mechanism, and further study is currently in progress to clarify this mechanism.

In conclusion, we propose that the oral ingestion of cow's milk IgG stimulates some natural cellular immune systems, while suppressing humoral adaptive immune responses in mice.

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## Figure legends

Fig. 1. Numbers of CD11b<sup>+</sup> cells (upper) and IL-12<sup>+</sup>CD11b<sup>+</sup> cells (lower) in Peyer's patches and spleens of mice given IgG-free (control), 0.005% IgG-added, or 0.05% IgG-added diets for 4 weeks. Values significantly differ from those of control diet at \* $P < 0.05$  or \*\*\* $P < 0.001$ .

Fig. 2. Formation of superoxides and ingestion of latex beads by peritoneal macrophages of mice the given control, 0.005% IgG-added, or 0.05% IgG-added diet for 4 weeks. Values significantly differ from those of control diet at \*\*\* $P < 0.001$ .

Fig. 3. Numbers of CD49<sup>+</sup>(DX5) cells in Peyer's patches and spleens of mice given the control, 0.005% IgG-added, or 0.05% IgG-added diet for 4 weeks. Values significantly differ from those of control diet at \* $P < 0.05$ .

Fig. 4. Viability of erythroleukemia cell line, K562, in the presence of spleen cells of mice given control ( ), 0.005% IgG-added ( ), or 0.05% IgG-added diet ( ) for 4 weeks. Values significantly differ from those of control diet at \* $P < 0.05$ , \*\* $P < 0.01$  or \*\*\* $P < 0.001$ .

Fig. 5. Numbers of IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> (upper) and IL-4<sup>+</sup>CD4<sup>+</sup> cells (lower) in Peyer's patches or spleens of mice given the control, 0.005% IgG-added, or 0.05% IgG-added diet for 4 weeks. Values significantly differ from those of control diet at \* $P < 0.05$ , \*\* $P < 0.01$  or \*\*\* $P < 0.001$ .

Fig. 6. Total or OVA-specific intestinal IgA and serum IgG in mice given the control, or 0.05% IgG-added diet for 5 weeks. Values significantly differ from those of control diet at \*\* $P < 0.01$  or \*\*\* $P < 0.001$ .

Fig. 7. Cells expressing low density CD 19 (CD19<sup>low</sup> cells) and high density CD19 (CD19<sup>high</sup> cells) in spleens of mice given the control, 0.005% IgG-added, or 0.05% IgG-added diet for 4 weeks.

Table 1. Composition of diets

	Control diet	IgG1 added diet	
		0.005%	0.05%
		%	
IgG1	0.000	0.005	0.050
Ovalbumin	25.000	24.995	24.950
Dextrin	32.738	32.738	32.738
Sucrose	27.112	27.112	27.112
RP mineral mix #10 <sup>†</sup>	3.750	3.750	3.750
Corn oil	3.750	3.750	3.750
Lard	3.750	3.750	3.750
Powdered cellulose	2.250	2.250	2.250
RP vitamin mix <sup>‡</sup>	1.500	1.500	1.500
Choline chloride	0.150	0.150	0.150
Total	100.000	100.000	100.000

<sup>†</sup>RP mineral mix #10: calcium, 0.60%; phosphorus, 0.40%; potassium, 0.40%; magnesium, 0.07%; sodium, 0.21%; chlorine, 0.24%; fluorine, 5.0 ppm; iron, 60 ppm; zinc, 21 ppm; manganese, 65 ppm; copper, 15.0 ppm; cobalt, 3.2 ppm; iodine, 0.57 ppm; chromium, 3.0 ppm; molybdenum, 0.82 ppm; selenium, 0.23 ppm. <sup>‡</sup>RP vitamin mix: vitamin K, 10.4 ppm; thiamin hydrochloride, 20.6 ppm; riboflavin, 20.0 ppm; niacin, 90 ppm; pantothenic acid, 55 ppm; choline chloride, 1400 ppm; folic acid, 4.0 ppm; pyridoxine, 16.5 ppm; biotin, 0.4 ppm; vitamin B12, 20 mcg/kg; vitamin A, 22.1 IU/g; vitamin D3, 2.2 IU/g; vitamin E, 50 IU/kg; ascorbic acid, 0.0 ppm.

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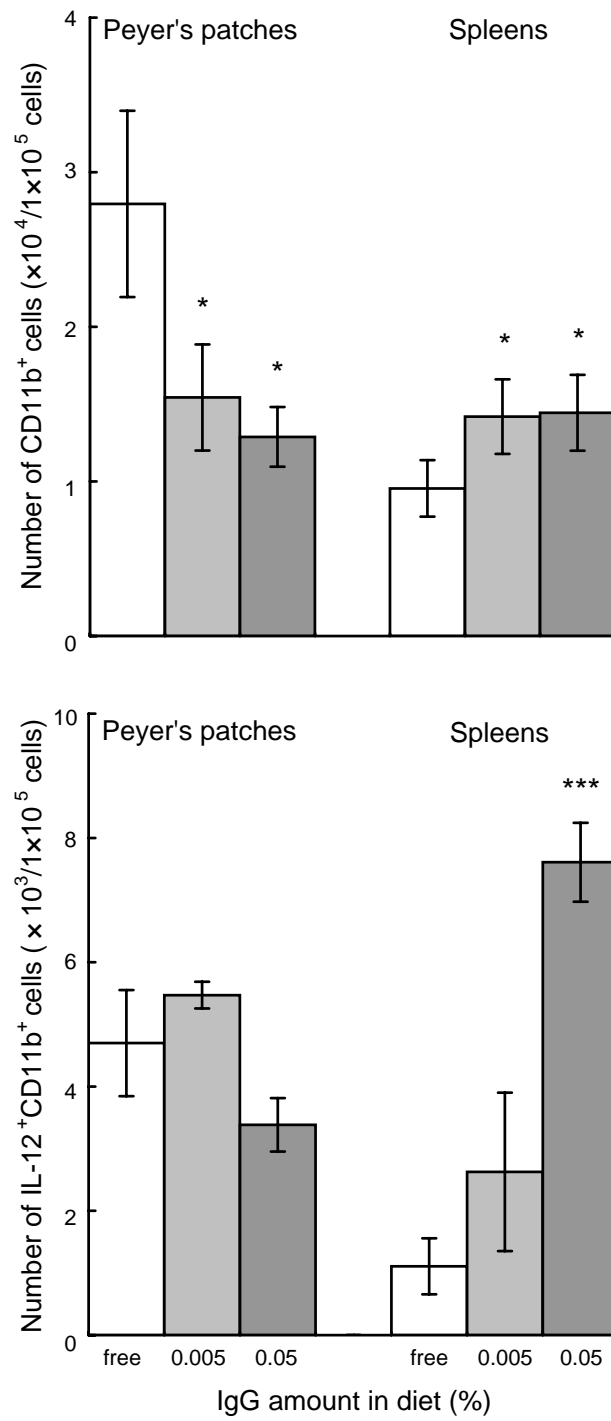


Fig. 1

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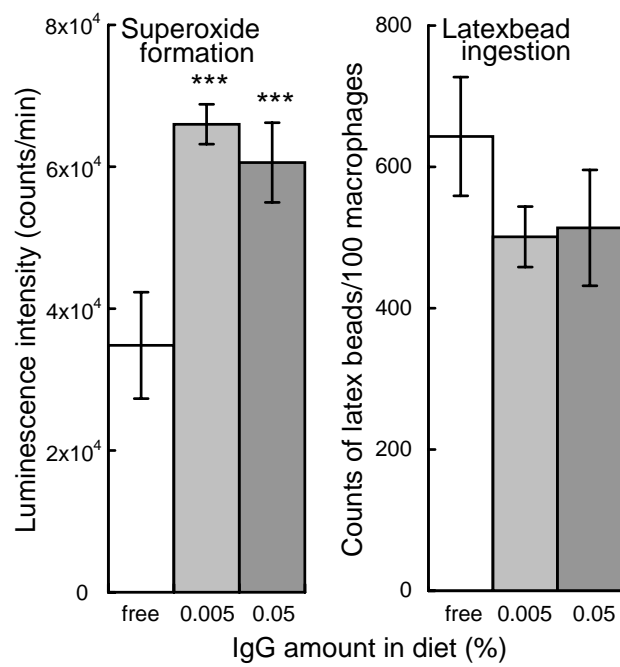


Fig. 2

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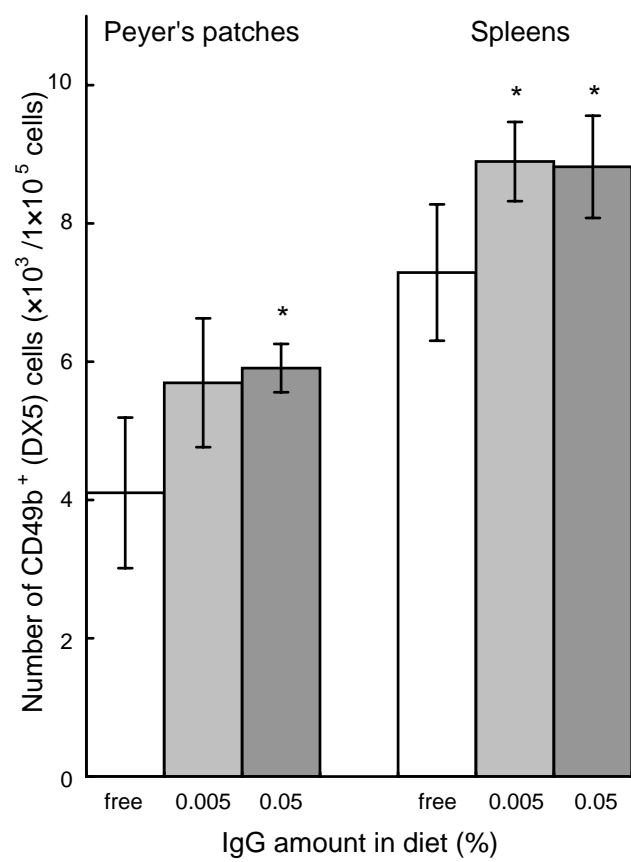


Fig. 3

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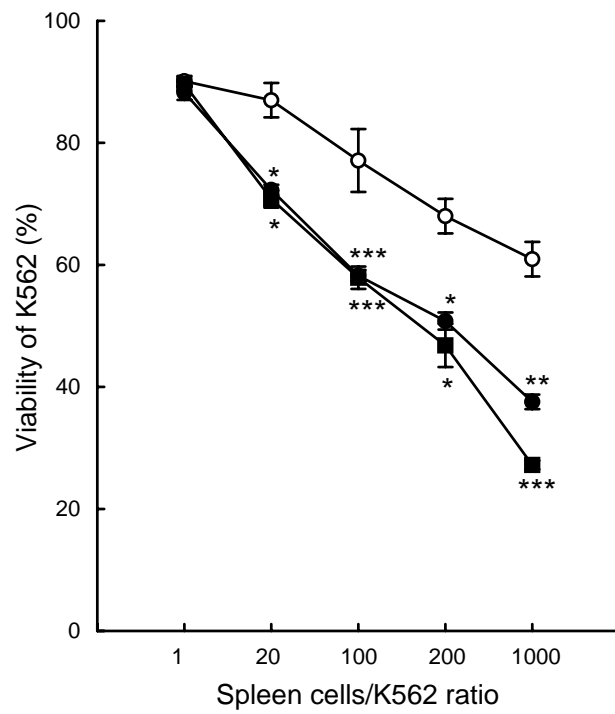


Fig. 4

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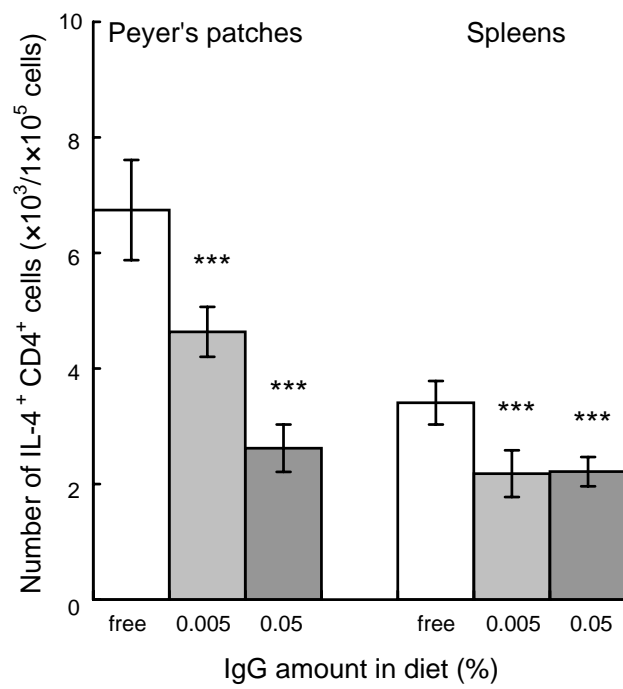
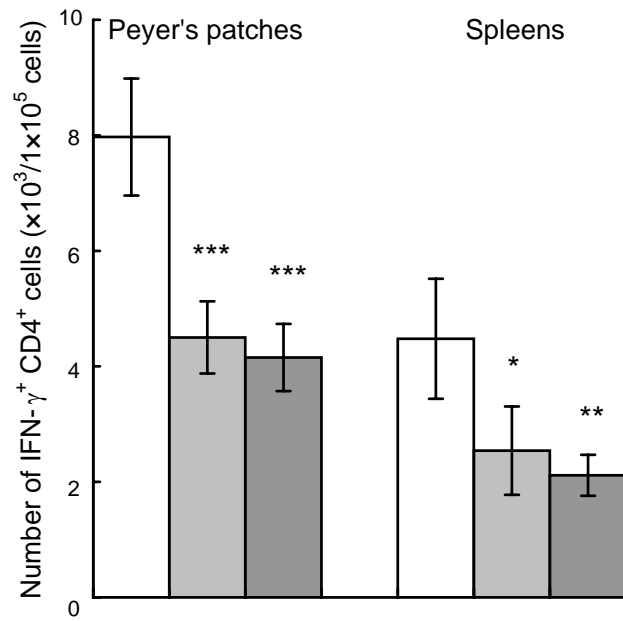


Fig. 5

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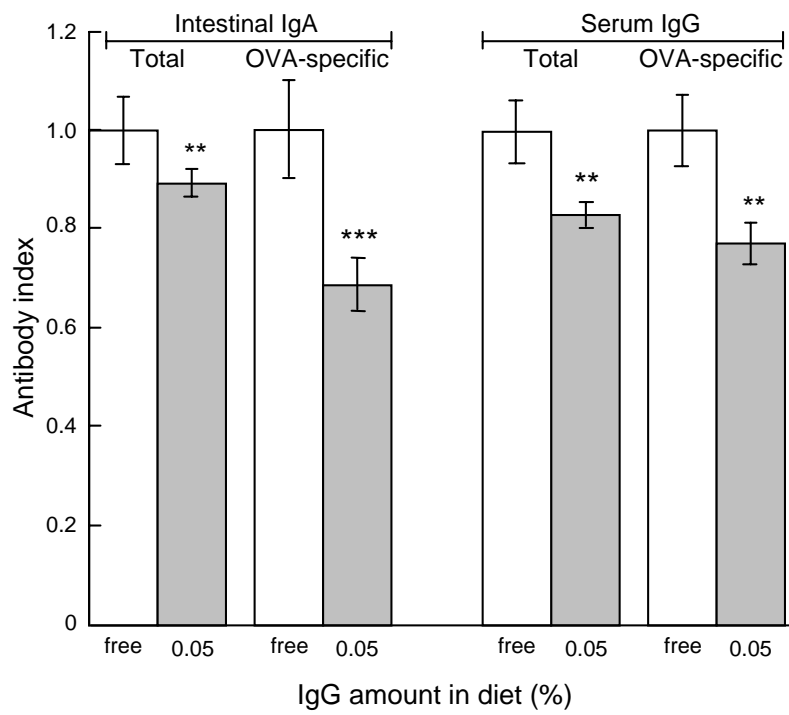


Fig. 6

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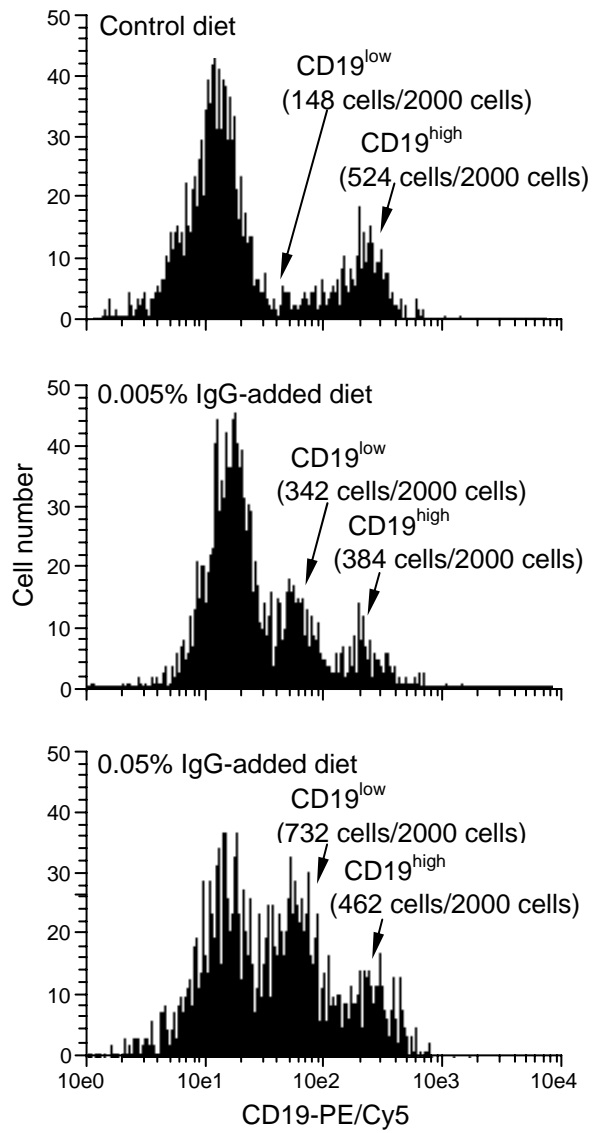


Fig. 7

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