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# 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)-$12^+CD11b^+$ 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-$\gamma^+CD4^+$ and IL-$4^+CD4^+$ 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; $CD4^+$ 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

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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)-γ 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 chromatograpy [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±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