PROF. CHANG KIM (Orcid ID : 0000-0003-4618-9139)

Article type : Letter to the Editor

Differential food protein-induced inflammatory responses in swine lines selected for reactivity to soy antigens

To the editor

Food protein-induced enterocolitis, commonly triggered by milk and soy protein, is on the rise, but immunological mechanisms of the disease are poorly understood (1). Most animal models of food allergy utilize mice which have significant limitations in obtaining translatable information (2). Here, we report a novel porcine model of soy-induced enteritis mimicking Food Protein-Induced Enterocolitis Syndrome (FPIES) that mainly affects neonates and young children (3-6). An advantage of using a swine model is their relative longer growing period during which induction and assessment of food allergy responses can be studied (7). Moreover, higher similarities in anatomy, immunology, and diet are also useful characteristics. Our model utilizes two related pig lines (L1 and L2), created by selective breeding for 8 generations based on their low (L1) and high (L2) responses to soy proteins injected in the hypodermis (8). L2 animals develop eosinophilic enteritis similar to human FPIES upon sensitization and subsequent oral challenges with soy proteins, while L1 animals develop moderate neutrophilia in the small intestine but do not develop clinically overt inflammatory responses. Enhanced responses of soy-reactive IL-4-producing $CD4^+$ T and non-T cells were detected in the intestine of L2, whereas low levels of Th2 but normal levels of Th1 cells were detected in L1 animals.

To induce food allergy responses, L1 and L2 animals were sensitized 3 times with a soy extract and cholera toxin (i.p.), and then orally challenged with soy-containing diet (Figure 1A). L1 and L2 had different levels of inflammation in the jejunum. While both L1 and L2 developed enteritis based on leukocyte infiltration in the jejunum, L2 developed a significantly higher inflammatory response, indicated by low villus heights and high mucosal layer destruction (Figure S1A, B; 1B, C), which is reminiscent of the small intestinal lesions of certain FPIES patients (4-6). Histological examination of the inflamed jejunum tissues revealed eosinophilic infiltration (some marked by black arrows), particularly in the lamina propria area of L2 animals (Figure 1B). In contrast, mononuclear phagocytes and neutrophils (green arrows) with small numbers of eosinophils infiltrated the jejunum of soy-challenged L1 animals.

To more quantitatively examine leukocytes, we determined the frequency of the infiltrating eosinophils and neutrophils in the soy-challenged animals by flow cytometry. $SWC1^{+}SIRP1\alpha^{+}$ cells represent neutrophils, whereas $SWC1^{-}SIRP1\alpha^{+}$ cells represent eosinophils in pigs (9). The frequency of eosinophils was greatly increased in the blood and jejunum of soy-challenged L2 animals (Figure S2A; 2A). In contrast, the frequency of neutrophils was increased in the jejunum of L1 animals upon soy challenge (Figure S2B).

GATA3 is a major transcription factor expressed by Th2 and innate type 2 lymphoid cells (ILC2). CCL11 is a chemoattractant for eosinophils. IL18 is also called interferon-gamma inducing factor and associated with Th1 responses. In line with the eosinophil response, *GATA3* and *CCL11* were highly up-regulated in the jejunum of L2, but *IL18* expression was up-regulated in the jejunum of L1 following soy-challenge (Figure S3A). In addition, L2 had lower expression of *IL17A* compared to L1 (Figure S3B).

Next, we examined the levels of Th1 and Th2 effector cells. L1 has higher steady-state levels of Th1 cells in the blood. Soy challenge decreased them in the blood but slightly increased them in the jejunum (Figure S3C, D). Th2 numbers were decreased in the blood of both lines following soy challenge but were considerably increased in the MLN and the jejunum of L2 animals only (Figure S3C, Figure 2B). Overall, the Th2/Th1 ratio was high in the blood of unchallenged and in the gut tissues of challenged L2 animals (Figure S3E). Soy challenge appears to shift effector T cells, particularly Th2 cells, from the blood to gut tissues.

We also detected soy-responsive $CD4^+$ T cells and non-$CD4^+$ cells in the blood of L2 animals challenged with soy diet (Figure S4A). Only L2, but not L1, $CD4^+$ and $CD4^-$ cells underwent proliferation ex vivo in the presence of soy antigens (Figure S4A; 2C). These cells expressed IL-4, but not IFN-γ, at increased levels (Figure S4B). These results confirm that L2 animals have increased numbers of soy protein-reactive Th2 cells. Non-T cells, such as innate lymphoid cells (ILCs), can also produce the Th1/2 cytokines. IL-4-, but not-IFN-γ–, expressing $CD3^-$ non-T cells were also increased in the jejunum of L2 (Figure S5A, B). L2 had higher frequencies of $FoxP3^+$ T cells than L1 animals upon soy challenges (Figure S5C, D). Thus, Tregs were not quantitatively suppressed in the L2 animals.

Importantly, soy-fed L2 animals displayed retarded growth during the 20-day feeding period (Figure 2D). Flow cytometry examination of intestinal tissues revealed increased frequencies of Th2, Th1, and $FoxP3^+$ T cells in the jejunum of soy-fed L2 pigs (not shown). These results indicate that natural soy exposure through the oral route can cause adverse immune responses in the intestine of L2 animals, leading to decreased growth performance.

We have established a swine model of food allergy. This model will be particularly useful in studying food protein-induced allergy responses in the intestine. This model is unique in that it employs two swine lines with a ~12% genetic relatedness among individual animals. Therefore, this model better mimics the genetically heterogeneous human populations. The two lines were different in immune responses to soy proteins in terms of Th2 cells, eosinophils and non-T cell IL-4 producers, which could be ILC2. Thus, the two lines represent individuals with high and low susceptibility to food protein-induced inflammatory responses. Especially, the L2 animals have heavy infiltration with eosinophils and Th2 cells in the small intestine, thus similar to the eosinophil type FPIES (4-6). We demonstrated that the increased sensitivity to soy antigens can deteriorate animal health evidenced by retarded growth. This model will be highly useful for developing pharmaceuticals for prevention or treatments of food allergy responses. It can also serve as a testing model for developing hypo-allergenic foods including baby formulas and animal feeds effective for growth. Future work includes generation of stable lines for in-depth immunological and genetic studies to understand underlying mechanisms.

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Seika Hashimoto-Hill, [1,*] Myunghoo Kim, [1,*] Leon Friesen,[1,2,3] Kolapo M. Ajuwon,[4] Eliot Herman, [5] Allan Schinckel,[4] and Chang H. Kim[2,3,]**

[1]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907; [2]Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI 48109; [3]Mary H. Weiser Food Allergy Center, University of Michigan School of Medicine, Ann Arbor, MI 48109; [4]Department of Animal Science, Purdue University, West Lafayette, IN 47907, U.S.A; [5]School of Plant Sciences, University of Arizona, Tucson, AZ

*Equal contribution

**Correspondence to Chang Kim, Department of Pathology and Mary H. Weiser Food Allergy Center, University of Michigan School of Medicine, Ann Arbor, MI 48109, USA; Phone: 734-647-8153; Email address: chhkim@umich.edu

Current address: MK (Department of Animal Science, Pusan National University 50463 Republic of Korea).

Author contributions: CHK, AS, and EH conceived the immunological study and obtained funding. Immunological characterizations of the animals were performed by SH, MK, LF and CHK. Animal derivation, maintenance, immunizations, oral challenge, and/or tissue preparation were carried out by AS, KMA, and SH. SH, MK and LF prepared the data figures. CHK drafted the manuscript, and all were involved in completing the manuscript for submission.

Funding information: This study was supported, in part, from grants from the Purdue College of Veterinary Medicine, USDA NIFA (Grant no. 2015-67017-23140), and NIH (1R01AI121302, 1R01DK076616, and R01AI080769). CK is the Kenneth and Judy Betz Endowed Professor at the Mary H. Weiser Food Allergy Center at University of Michigan.

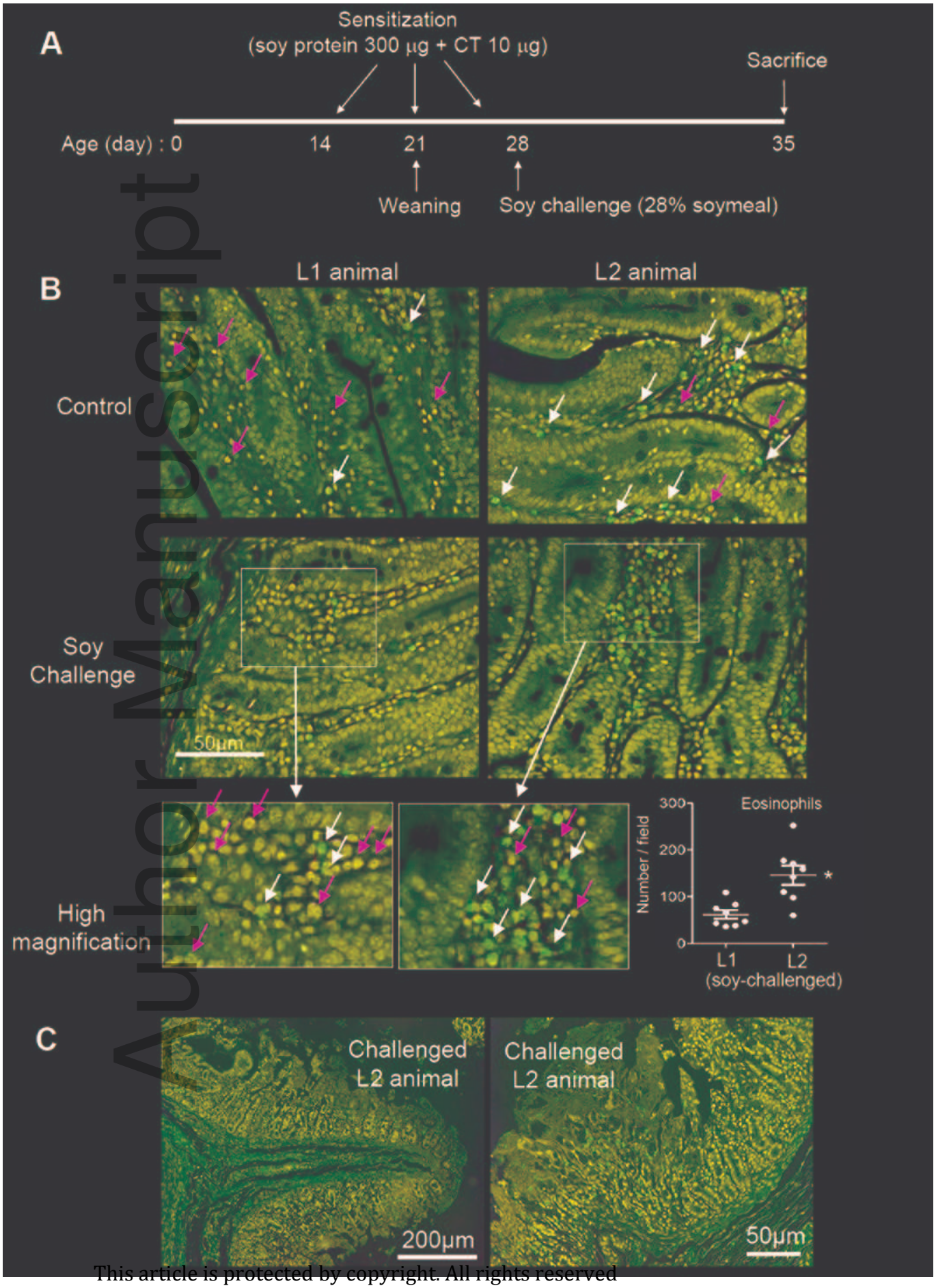
Conflict of interest: The authors declare no financial or commercial conflicts of interest.

Acknowledgments: We thank the Purdue animal science graduate students for their helpful assistance with animal experiments and tissue preparation.

Figure legends

Figure 1. Differential soy-induced inflammatory responses in two pig lines. (A) The soy challenge group was sensitized with immunization i.p. with soy protein extract (300 μg) and cholera toxin (CT, 20 μg) and then challenged with 28% soy meal. Control groups received CT only without soy proteins and were not challenged with soy. (B) Representative histological images of jejunum of L1 and L2 pigs with eosinophil counts in challenged animals. Representative eosinophils (black) and mononuclear cells/neutrophils (green) are highlighted with arrows. (C) Severe cases of intestinal inflammation in L2 animals. *Significant differences ($p<0.05$; n=8 per group).

Figure 2. Elevated levels of eosinophils and Th2 cells and soy-diet-induced growth retardation. Frequencies of eosinophils (A) and Th2 cells (B) in L2 animals. (C) Ex vivo proliferation of peripheral blood $CD4^+$ T cells in response to soy proteins. (D) Growth rates of L2 animals on soy diet. For panel A-C, the data from animals challenged again on day 41 and euthanized on day 42 were similar to those challenged once in Fig. 1A, and therefore the data were combined. For panel D, weaned L2 pigs were placed on soy-free diet for 7 days and then on soy-free or 18% soy diet for the next 21 days. *Significant differences ($p<0.05$; n=4-9 per group).



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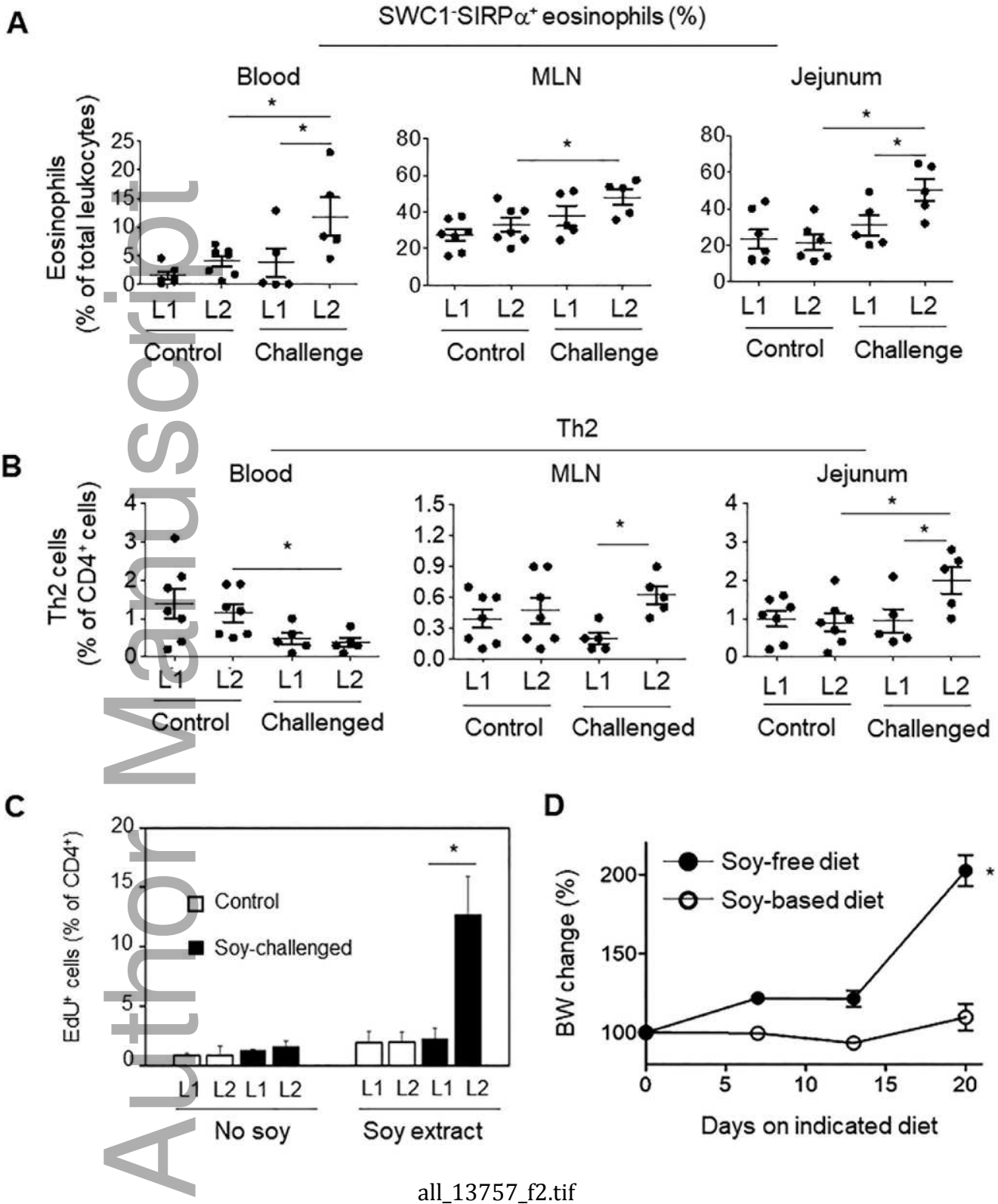

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