

Original article

Innate immunomodulatory effects of cereal grains through induction of IL-10

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Background: Interactions between the gastrointestinal immune system and the luminal environment play critical roles in maintaining immune homeostasis and in diseases such as inflammatory bowel disease. Although immunomodulation by microbial factors has been studied extensively, little attention has been given to the potential immunomodulatory effects of ingested foods.

Objective: We characterized the effects of cereal grains on the immune response in human subjects and investigated the mechanism.

Methods: PBMCs from healthy individuals were incubated with cereal grain extracts, and cytokine levels in cell-free supernatants were measured. The cellular source of IL-10 and the role of monocytes were investigated by means of flow cytometry and cell-depletion/reconstitution experiments.

Results: Extracts of cereal grains, including rice and wheat, induced marked IL-10 production from PBMCs. Intracellular cytokine staining and cell-depletion experiments showed that CD14⁺ monocytes produced IL-10. Importantly, when PBMCs were stimulated with concanavalin A, cereal grains concentration-dependently inhibited their production of IL-5, IL-13, and IFN- γ ; neutralizing IL-10 or removing the monocytes abrogated this inhibitory effect. This cereal grain-induced IL-10 response was polymyxin B sensitive, heat resistant, and inhibited by blocking the Toll-like receptor 4.

Conclusion: Cereal grains have strong innate immunomodulatory effects by inducing marked production of IL-10 from CD14⁺ monocytes *in vitro*. LPS or LPS-mimicking activity in cereal grains might be responsible. The potential immunomodulatory effects of cereal grains need further study *in vivo*.

Clinical implications: Exposure to grain-derived LPS-like activity might be important in disease processes and in immune homeostasis. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Rice, wheat, cereal grains, food, IL-10, immunomodulation, lipopolysaccharide

Abbreviations used

Con A: Concanavalin A
DSS: Dextran sulfate sodium
FITC: Fluorescein isothiocyanate
HDM: House dust mite
PE: Phycoerythrin
TLR: Toll-like receptor

The interactions between the mucosal immune system and the luminal environment play a key role in the pathogenesis of epithelial inflammation in the intestine.^{1,2} To characterize the intestinal immune system, recent research has focused on the interactions with commensal and pathogenic bacteria, with little attention given to the immunologic effects of ingested foods. As staples, cereal grains, especially rice and wheat, are consumed extensively worldwide³; their estimated world production in 2003 reached 584 and 560 million tons, respectively.⁴ The ingestion of grains has increased in the United States.⁵ Cereal grains can be beneficial, with their nutrients and high fiber contents,⁵ although they can induce hypersensitivity reactions, including food allergy and celiac disease.⁶ Overall, there is little information on the immune effects of grains.

During our investigation of allergen-induced cytokine production from human PBMCs,^{7,8} we discovered that allergen extracts of cereal grains, but not other foods, induce marked IL-10 production from PBMCs. IL-10 is a prototypical regulatory cytokine, exerting several immunomodulatory effects by suppressing both T_H1 and T_H2 cytokines.^{9,10} This altered production of IL-10 might be important in disease pathogenesis, such as inflammatory bowel disease,^{11,12} allergies,¹³ and autoimmune disorders.¹⁴ IL-10-deficient mice spontaneously have chronic colitis,¹⁵ but IL-10 administration inhibits this colitis.^{11,12} In dextran sulfate sodium (DSS)-induced colitis, IL-10 neutralization exacerbates and IL-10 administration ameliorates the colitis.¹⁶ Because PBMCs incubated with cereal grain extracts produce IL-10, we hypothesized that cereal grain extracts possess immunomodulatory actions. Herein, we used human PBMCs to characterize the *in vitro* immunomodulatory properties of cereal grains and investigated the mechanisms.

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K.Y. was supported by National Institutes of Health (NIH) grant T32 AI07047.

J.A.M. was supported by NIH grants R01 DK57982 and R01 DK071003.

H.K. was supported by NIH grants R01 AI49235 and P01 AI50494.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication February 5, 2007; revised August 8, 2007; accepted for publication August 8, 2007.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.08.031

METHODS

Reagents

All lyophilized allergen extracts, including egg, soy, milk, rice, wheat, rye, buckwheat, oat, corn, *Aspergillus fumigatus*, house dust mite (HDM), and short ragweed pollen, were from Greer Laboratories (Lenoir, NC). After the manufacturer delipidated the raw materials with ether or acetone, allergens were extracted with ammonium bicarbonate, dialyzed, filter sterilized, and lyophilized. Allergen stock solutions (1 mg/mL) were prepared in RPMI 1640 medium (Celox, St Paul, Minn), filter sterilized (0.45 μ m; MILLEX-HV; Millipore, Billerica, Mass), stored at -30°C , and used within 2 weeks. Except as noted, all experiments used lyophilized allergen extracts. Glycerinated extracts (liquids) were from 4 sources: ALK-Abelló, Inc (Round Rock, Tex); Hollister-Stier (Spokane, Wash); Allergy Laboratories, Inc (Oklahoma City, Okla); and Antigen Laboratories, Inc (Liberty, Mo). These extracts were prepared similarly to the lyophilized extracts (ie, with delipidation and aqueous extraction) and dissolved in 50% glycerin. Because extracts were produced by means of delipidation, followed by aqueous extraction, they contained only water-soluble components; they did not contain water-insoluble components, such as gluten. We also prepared extracts of cereal grain foods purchased from a local grocery store: short-grain rice, long-grain rice, wild rice, rice flour, wheat flour, white bread, whole wheat bread, wheat cereal, soy flour, and soybeans. After homogenization with a mortar and pestle, the homogenates or flours (1 g) were suspended in 10 mL of PBS and rotation extracted for 20 hours at 4°C . After centrifugation (1300g), the supernatants were collected, filtered (0.45 μ m), and stored at -30°C . As a control, 10 mL of PBS was processed as above.

Details for other reagents are provided in the [Online Repository at www.jacionline.org](http://www.jacionline.org).

Allergen extract–induced cytokine production by cultured PBMCs

PBMCs were isolated from heparinized peripheral blood of healthy volunteers by using a density gradient (Histopaque-1077; Sigma, St Louis, Mo); these donors did not have allergen-specific IgE against the foods and allergens studied. The Institutional Review Board of the Mayo Foundation approved the study protocol, and written informed consent was obtained. PBMCs (2×10^6 cells/mL) were incubated with 50 μ g/mL allergen extracts for 96 hours at 37°C and 5% CO_2 (details are provided in the [Online Repository at www.jacionline.org](http://www.jacionline.org)). Allergens tested include the following: 9 lyophilized food extracts, including 6 cereals (wheat, rice, rye, buckwheat, oat, and corn) and 3 noncereals (egg, milk, and soy), and 3 aeroallergens (HDM, short ragweed pollen, and *A fumigatus*). In certain experiments PBMCs were incubated with glycerinated extracts of rice and wheat or glycerin control. For polyclonal stimulation of cytokine production, a mitogen, concanavalin A (Con A; 5 μ g/mL), was used as a positive control.

Effects of cereal grain extracts on Con A–induced cytokine production from PBMCs

PBMCs (2×10^6 cells/mL) were incubated with Con A (5 μ g/mL) and lyophilized wheat or rice extracts (0–50 μ g/mL) for 96 hours. To neutralize IL-10 and TGF- β , we used a goat anti-human IL-10 (R&D Systems, Minneapolis, Minn) and a rabbit anti-human pan-specific TGF- β (R&D Systems), respectively, and studied the cereal grain–induced changes in IL-5, IL-10, IL-13, and IFN- γ production.

Cytokine measurement

Details on cytokine measurements are provided in the [Online Repository at www.jacionline.org](http://www.jacionline.org).

Intracellular IL-10 staining and flow cytometric analysis of PBMCs

PBMCs (2×10^6 cells/mL) were cultured with or without rice extract (10 μ g/mL) or Con A (5 μ g/mL). After 12 hours, 2 μ L of Brefeldin A (BD GolgiPlug; BD Biosciences, San Diego, Calif) was added (0.1 μ g/mL), and PBMCs were incubated for 8 more hours. Cells were harvested, and surface antigens were stained with fluorescein isothiocyanate (FITC)–conjugated anti-CD4, anti-CD8, anti-CD14, anti-CD19, and anti-CD56. After washing, cells were fixed and permeabilized with Cytotfix/Cytoperm solution and stained with phycoerythrin (PE)–conjugated anti-IL-10 or control IgG. Cells were analyzed with a flow cytometer (FACScan; Becton-Dickinson, San Jose, Calif).

Monocyte depletion and reconstitution

Monocytes were depleted with a CD36/glycophorin A–based kit (RosetteSep human monocyte depletion cocktail; StemCell Technologies, Inc, Vancouver, British Columbia, Canada). Briefly, whole blood was incubated with the monocyte depletion cocktail for 20 minutes, diluted in PBS with 2% FBS, and layered above the Histopaque-1077 (Sigma). After centrifugation at 1200g for 20 minutes at room temperature, the cells from the Histopaque-plasma interface were collected. PBMCs or monocyte-depleted PBMCs (2×10^6 cells/mL) were incubated with rice or wheat extracts (10 μ g/mL) for 24 hours, and cytokine levels in the supernatants were determined.

To reconstitute monocytes, we isolated CD4 $^{+}$ T cells and CD14 $^{+}$ monocytes from PBMCs by means of negative selection with the RosetteSep kit with CD4 $^{+}$ T-cell enrichment cocktail and the CD14 $^{+}$ monocyte enrichment cocktail (StemCell Technologies), respectively. CD4 $^{+}$ T-cell and CD14 $^{+}$ monocyte purities were greater than 98% and greater than 95%, respectively. CD4 $^{+}$ T cells (2×10^6 cells/mL) were incubated with different numbers of CD14 $^{+}$ monocytes (0.2×10^6 to 2×10^6 cells/mL) and stimulated with Con A (5 μ g/mL) with or without wheat extract (10 μ g/mL) for 96 hours, as above.

Characterization of immunomodulatory activity in cereal grains

Because LPS induces IL-10 production from PBMCs,^{17,18} we assessed roles for LPS or LPS-like activity in cereal grain extracts using biochemical and immunologic approaches. For sensitivity to polymyxin B, PBMCs were incubated with rice extract (1 μ g/mL) or LPS (1 ng/mL) with or without polymyxin B (10 μ g/mL) for 24 hours, and IL-10 levels in the supernatants were measured. For heat sensitivity, the rice extract was boiled for 10 minutes, and its effects on IL-10 production from PBMCs were examined. We assessed the effects of blocking a coreceptor for LPS, Toll-like receptor (TLR) 4, and a receptor for lipoteichoic acid/proteoglycan, TLR2, on IL-10 production.^{19,20} PBMCs were preincubated with or without anti-TLR4 (10 μ g/mL), anti-TLR2 (10 μ g/mL), or control IgG for 15 minutes at room temperature and then stimulated with rice (1 μ g/mL), wheat (1 μ g/mL), or LPS (1 ng/mL). After 24 hours, the cell-free supernatants were collected, and IL-10 levels were determined.

Measurement of LPS activity in allergen extracts

Details for LPS measurement are provided in the [Online Repository at www.jacionline.org](http://www.jacionline.org).

Statistical analyses

Data were expressed as the mean and SE. Statistical differences were examined by using the Mann-Whitney *U* test, the paired *t* test, or the Wilcoxon signed-rank test. A *P* value of less than .05 was considered significant. A statistical software package (StatView 4.0.2; Abacus Concepts, Berkeley, Calif) was used.

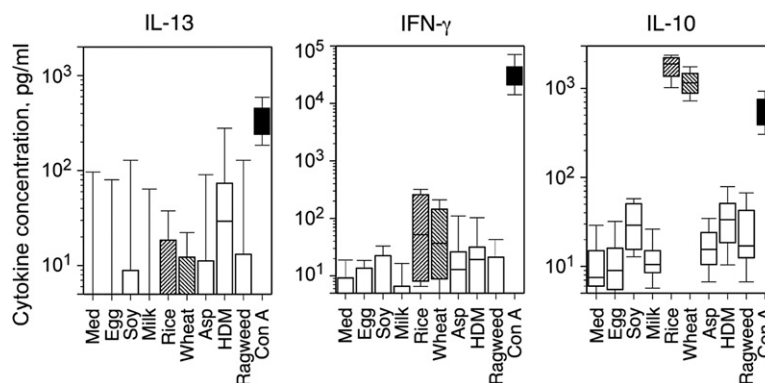


FIG 1. Allergen extract- and Con A-induced cytokine production from PBMCs. PBMCs were incubated with allergen extracts or Con A. The box plots summarize 20 healthy individuals, the line represents the median (50th percentile), and the lower whisker, lower hinge, upper hinge, and upper whisker represent the 10th, 25th, 75th, and 90th percentiles, respectively. Asp, *Aspergillus fumigatus*.

RESULTS

Rice and wheat extracts induce marked IL-10 production from PBMCs

PBMCs from healthy individuals were incubated with lyophilized extracts of 5 food allergens (egg, soy, milk, rice, and wheat), 3 aeroallergens (*A. fumigatus*, short ragweed pollen, and HDM), or Con A. These extracts induced no or minimal IL-13 (median, <30 pg/mL) and IFN- γ (median, <50 pg/mL) production from these PBMCs ($n = 20$, Fig 1). Rice and wheat extracts induced marked IL-10 production (median, >1000 pg/mL), but the other foods and aeroallergens induced minimal IL-10 production (median, <30 pg/mL). A polyclonal stimulus, Con A, also induced production of IL-13, IFN- γ , and IL-10. Thus cereal grains, but not other foodstuffs, might induce IL-10 production from PBMCs.

We also investigated whether glycerinated, fluid-form cereal grain extracts from different commercial sources induce IL-10 production from PBMCs. These extracts are in extensive clinical use for allergen skin tests (eg, skin prick tests) and immunotherapy. All glycerinated extracts from 4 (rice) and 3 (wheat) sources induced marked IL-10 production from PBMCs at levels comparable with those induced by lyophilized rice or wheat extracts (Fig 2, A). A glycerin solution (control) did not induce IL-10 (<10 pg/mL). Lyophilized extracts of 4 other cereal grains (ie, rye, buckwheat, oat, and corn), but not HDM or milk, induced marked IL-10 production (Figure 2, B).

Cellular source of cereal grain-induced IL-10 in PBMCs

To identify the cellular source or sources of grain-induced IL-10, we used intracellular cytokine staining and cell-surface labeling. After stimulation with rice extract or Con A, PBMCs were stained with FITC-conjugated anti-CD4, anti-CD8, anti-CD56, anti-CD19, and anti-CD14 and then stained intracellularly with PE-conjugated anti-IL-10. With rice extract, only the CD14⁺ population shows intracellular IL-10 staining (Fig 3, A), suggesting

that monocytes are involved. No intracellular IL-10 staining was detected in the CD4⁺, CD8⁺, CD19⁺, or CD56⁺ cell populations (data not shown). In contrast, with Con A, only the CD4⁺ (and not the CD14⁺) population shows IL-10 staining.

To verify the monocyte origin of IL-10, we depleted monocytes and stimulated the remaining PBMCs with rice or wheat extracts. Using CD36/glycophorin A-based monocyte depletion, we eliminated 99.5% $\pm$ 0.1% of the CD14⁺ monocytes from PBMCs (mean $\pm$ SD, $n = 3$). Monocyte depletion completely abrogated rice- and wheat-induced IL-10 production (Fig 3, B). Unexpectedly, when stimulated with rice or wheat, these monocyte-depleted PBMCs produced increased levels of IFN- γ (Fig 3, B). Thus cereal grain extracts activate CD14⁺ monocytes to produce IL-10; when PBMCs are stimulated with cereal grains, this monocyte-produced IL-10 might inhibit IFN- γ production from the nonmonocytic cells.

Effects of rice and wheat extracts on Con A-induced production of IL-5, IL-13, and IFN- γ

As a prototypical regulatory cytokine, IL-10 can suppress both T_H1 and T_H2 immune responses.^{9,10} We examined the effects of rice and wheat extracts on Con A-stimulated cytokine production from PBMCs isolated from 4 healthy (without wheat or rice hypersensitivity) donors. Wheat extract concentration-dependently inhibited Con A-induced production of IL-5, IL-13, and IFN- γ (Fig 4); in some individuals, it (10 μ g/mL) completely abolished IL-5, IL-13, or IFN- γ production. In contrast, wheat extract concentration-dependently enhanced IL-10 production at 10 μ g/mL or higher. Rice extract showed similar results (data not shown).

We examined the roles of IL-10 and monocytes in the immunomodulatory effects on T_H1 and T_H2 cytokine production. First, we examined whether neutralizing IL-10 by means of antibody treatment influences the ability of cereal grains to inhibit cytokine production. As expected, wheat extract almost abolished Con A-induced IL-5, IL-13, and IFN- γ production (Fig 5). Adding neutralizing

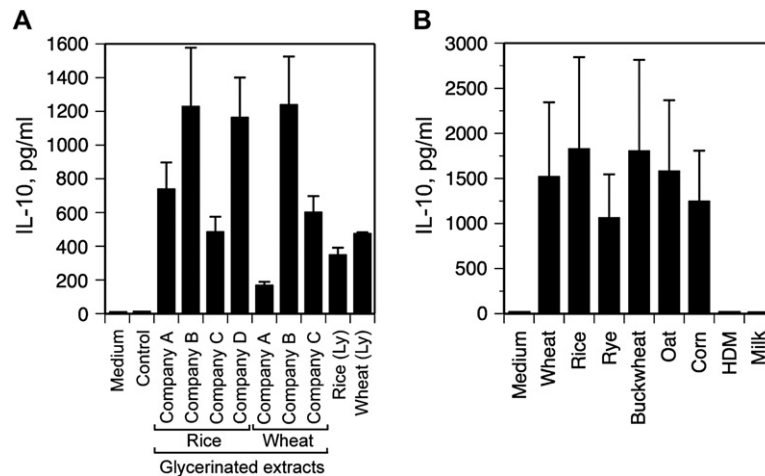


FIG 2. Effects of glycerinated cereal grain extracts (**A**) and lyophilized cereal grain and nongrain extracts (**B**) on IL-10 production from PBMCs. In Fig 2, A, PBMCs were incubated with glycerinated or reconstituted lyophilized (Ly) wheat or rice extracts. Glycerinated extracts were from 4 sources (A-D). In Fig 2, B, PBMCs were incubated with 6 grains (wheat, rice, rye, buckwheat, oat, and corn), HDM, and milk extracts. Values are presented as means and SEs ($n = 3$ different individuals).

anti-IL-10, but not control IgG, concentration-dependently reversed the wheat-induced inhibition of IL-5, IL-13, and IFN- γ production; 25 $\mu\text{g/mL}$ anti-IL-10 rescued IL-5 and IL-13 production by about 60% and 80%, respectively. Anti-IL-10 at 5 or 25 $\mu\text{g/mL}$ enhanced IFN- γ production greater than 100%, which is similar to monocyte depletion (Fig 3, B, right panel). Anti-TGF- β showed no effects on cereal grain-mediated inhibition of IL-5, IL-13, or IFN- γ production (data not shown).

Second, we examined whether monocytes are necessary for the inhibitory effects of cereal grains. We isolated CD4 $^{+}$ T cells and CD14 $^{+}$ monocytes from PBMCs. CD4 $^{+}$ cells were stimulated with 5 $\mu\text{g/mL}$ Con A alone or Con A plus wheat extract (10 $\mu\text{g/mL}$) plus increasing numbers of monocytes. Stimulation of CD4 $^{+}$ cells (2×10^6 cells/mL) with Con A alone induced increased levels of IL-5, IL-13 and IFN- γ (Fig 6). However, unlike the results with PBMCs (Figs 3 and 4), when wheat extract was added to CD4 $^{+}$ cells, it did not inhibit IL-5 or IL-13 production; wheat markedly enhanced the IFN- γ production by CD4 $^{+}$ cells (Fig 6). Importantly, adding more CD14 $^{+}$ cells ($0.2\text{--}2 \times 10^6$ cells/mL) to CD4 $^{+}$ cells incubated with Con A and wheat inhibited the production of IL-5, IL-13, and IFN- γ concentration dependently. These inhibitory effects were observed with as low a ratio as 0.1 CD14 $^{+}$ cells to CD4 $^{+}$ cells. When CD14 $^{+}$ cells were added to CD4 $^{+}$ cells, which were incubated with Con A plus wheat extract, production of IL-10 increased. Thus the IL-10 produced by CD14 $^{+}$ cells might play a major role in the immunomodulatory effects of cereal grains.

Partial characterization of immunomodulatory activity in cereal grains

We started to characterize the component or components of cereal grains that are responsible for IL-10

production. Because bacterial colonization is common in cereal grains^{21,22} and plants themselves can produce LPS-like molecules,²³⁻²⁶ we suspected that LPS or an LPS-mimicking molecule or molecules associated with cereal grains might induce IL-10. TLR4 plays critical roles in the monocyte response to LPS.^{19,20} Anti-TLR4 inhibited the IL-10 produced by PBMCs in response to rice and wheat extracts by 70% and 93%, respectively ($P < .05$); anti-TLR4 also inhibited the IL-10 produced in response to LPS by 60% (Fig 7). Anti-TLR2 antibody did not affect cereal grain- or LPS-induced IL-10 production (data not shown). When polymyxin B (10 $\mu\text{g/mL}$) was coincubated with rice or LPS, the IL-10 produced by PBMCs was nearly abolished (ie, >95%; $n = 3$; $P < .05$). Boiling the rice extract and LPS for 10 minutes did not affect their abilities to induce IL-10 from PBMCs (data not shown). A limulus test detected LPS activity in lyophilized rice and wheat extracts (120 and 575 EU/mg dry weight, respectively). Collectively, LPS or LPS-like components associated with cereal grains likely play a major role in IL-10 production from PBMCs.

Extracts of common food products induce IL-10 production from PBMCs

We examined whether extracts prepared in our laboratory from cereal grain food products, such as flour, and processed food, such as bread, could induce IL-10 production. PBS extracts of rice- and wheat-containing foods (20 $\mu\text{g/mL}$) induced IL-10 production (100 to 1500 pg/mL; see Fig E1 in the Online Repository at www.jacionline.org). Both food ingredient (eg, flour) and processed product (eg, bread) extracts showed strong activity. In contrast, the extracts of noncereal food products (ie, soy flour and soybean) induced minimal IL-10 production.

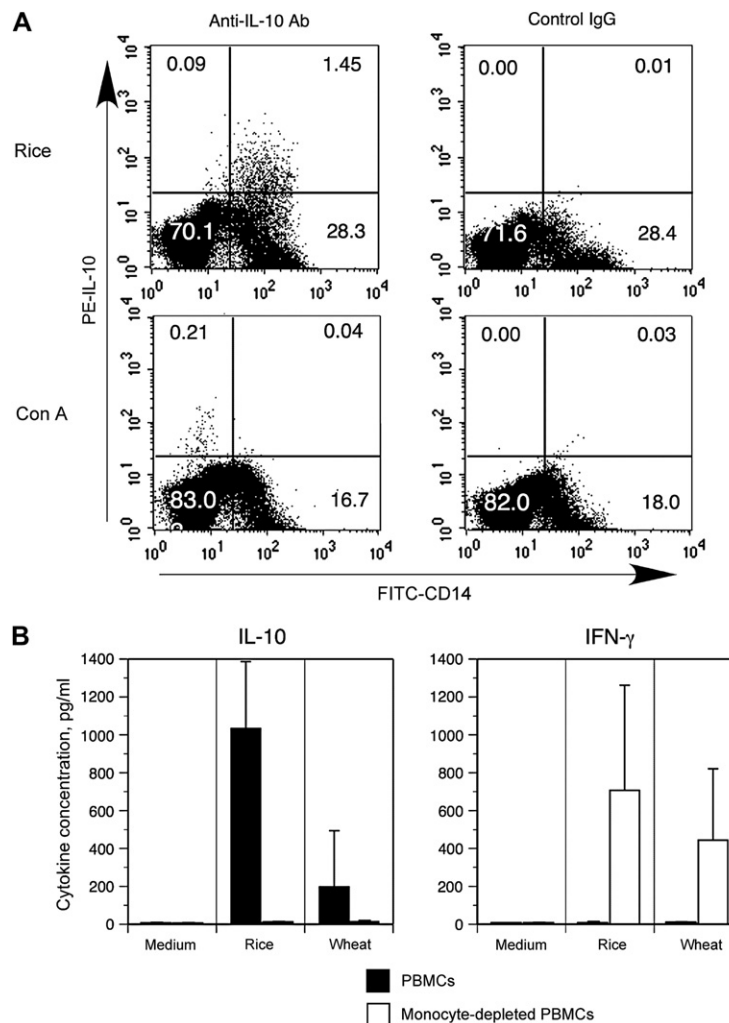


FIG 3. Monocytes in PBMCs stimulated with grain extracts produce IL-10 and might inhibit IFN- γ . **A**, PBMCs were stimulated with rice extract or Con A and stained intracellularly with PE-conjugated anti-IL-10 and extracellularly with FITC-conjugated anti-CD14. Representative dot plots show 1 donor from 3 different individuals. **B**, PBMCs and monocyte-depleted PBMCs were incubated with rice or wheat extract. Values are presented as means and SEs ($n = 3$ different individuals). Ab, Antibody.

DISCUSSION

Two cereal grains, rice and wheat, which are predominant sources of worldwide nutrition, exert potent immunomodulatory effects *in vitro*. Extracts of these grains, as well as rye, buckwheat, oat, and corn, induced marked IL-10 production from human PBMCs. This IL-10 production by monocytes strongly inhibited T-cell production of both T_H1 (IFN- γ) and T_H2 (IL-5 and IL-13) cytokines, suggesting fine tuning of acquired immune response by innate immunity. In the absence of monocytes or when IL-10 was blocked, these cereal extracts induced IFN- γ from other immune cells (Figs 3, B; 5; and 6). Furthermore, commercial foods containing rice and wheat potently induced IL-10 production. Thus the immunomodulatory effects of cereals might permit us to tolerate readily foods that would otherwise be potent

immunogens. Alternately, derangements in these immunomodulatory effects or their mechanisms could explain how cereals might induce a deleterious proinflammatory response.

Perhaps LPS or an LPS-mimicking molecule, acting on monocytes, induces IL-10 production and the subsequent suppression of T_H1 and T_H2 cytokines from T cells. The reasons are as follows: (1) LPS induces IL-10 from monocytes^{17,18}; (2) bacterial colonization or infection is ubiquitous in plants, including grains^{21,22}; (3) the IL-10-inducing activity in grains is polymyxin B sensitive, heat insensitive, and mediated largely through TLR4; and (4) authentic plant lectins, such as PHA or wheat germ agglutinin, do not inhibit production of IL-5 or IL-13 (see Fig E2 in the Online Repository at www.jacionline.org). The source of LPS or LPS-mimicking molecules could be bacteria, cereal grain plants, or both. Expressed

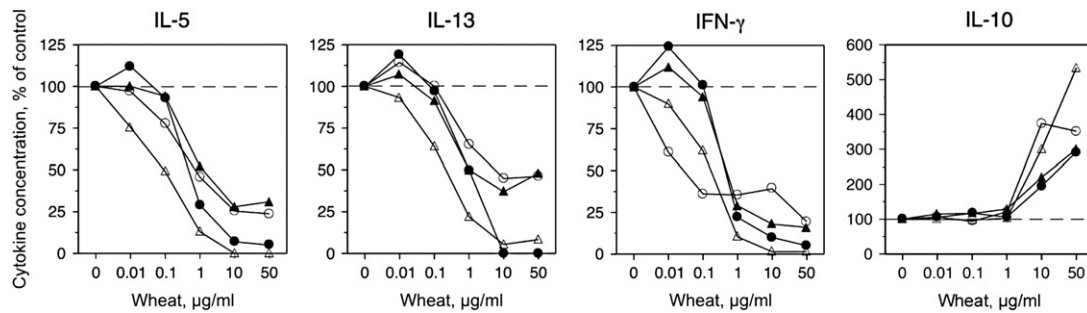


FIG 4. Wheat extract inhibits Con A–induced production of IL-5, IL-13, and IFN- γ and enhances IL-10 production from PBMCs. PBMCs were incubated with Con A and various concentrations of wheat extract. Each line represents one of 4 different healthy donors; results are normalized to no wheat as 100%.

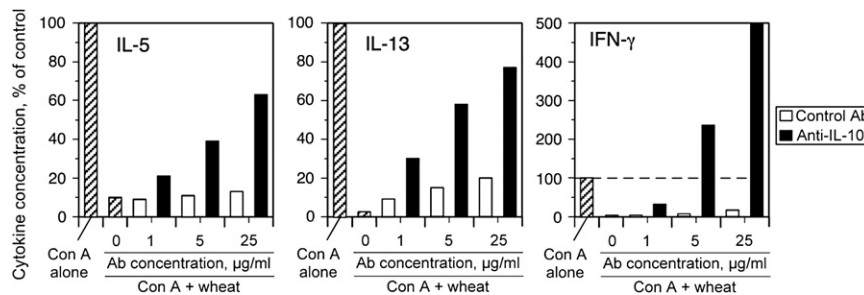


FIG 5. Anti-IL-10 reverses the wheat-induced inhibition of Con A–stimulated cytokine production. PBMCs from healthy donors were incubated with Con A with or without wheat extract and anti-human IL-10 or control IgG. Data are normalized to the levels induced by Con A as 100%. Results show 1 representative experiment of 3 experiments. Ab, Antibody.

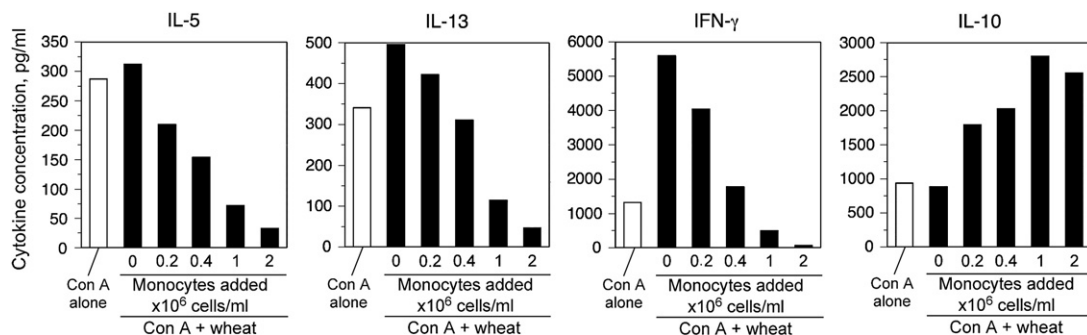


FIG 6. Effects of monocyte reconstitution on Con A–induced production of cytokines by CD4⁺ T cells. Isolated CD4⁺ T cells were incubated with Con A or Con A plus wheat extract. Different numbers of CD14⁺ monocytes were reconstituted into the CD4⁺ T cells. Results show 1 representative experiment of 3 experiments.

sequence tags databases indicate that *Arabidopsis thaliana* and many other plants, including rice, contain homologues of *Escherichia coli* genes, encoding the biosynthesis of lipid A, an active LPS component.²³ Furthermore, a non-bacterial, wheat flour–specific LPS has been reported.^{24–26} On the other hand, the ability of various food products to induce IL-10 production did not always correlate with the concentrations of LPS in their extracts (data not shown). Therefore LPS or LPS-mimicking molecules likely work in concert with other immunostimulatory or immunoregulatory

molecules in the cereals, such as carbohydrate polymers or lectins, to induce robust IL-10 production.

The physiologic relevance of our results for immunologic diseases in the gastrointestinal tract (eg, inflammatory bowel disease) and food-related diseases (eg, celiac disease and food allergies) awaits further study. First, the immunomodulatory effects might explain why most individuals who are at genetic risk for celiac disease (ie, 30% of white subjects) do not have celiac disease or inflammatory bowel disease.

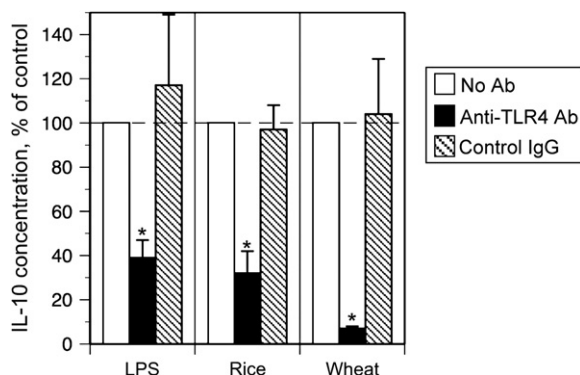


FIG 7. Anti-TLR4 inhibits cereal grain-induced IL-10 production from PBMCs. PBMCs were incubated with rice, wheat, or LPS with or without anti-TLR4 antibody or control IgG. Results are normalized to no antibody as 100%. Values are presented as means and SEs ($n = 3$ different individuals). * $P < .05$ compared with control IgG. Ab, Antibody.

Second, LPS activity in cereal grain foods might be quantitatively negligible compared with the immense bacterial load in the intestinal microflora. However, because bacterial LPS is localized in the cell membrane, direct interaction between membrane LPS and intestinal epithelia is likely blocked by the thick glycocalyx surrounding the bacteria; thus bacterial disintegration is required to liberate bacterial LPS.²⁷ In contrast, plant LPS or LPS-like molecules in grain-related foods might be soluble LPS that could interact with the intestinal immune system. Indeed, PBS extracted the IL-10-producing activity from foods (see Fig E1 in the Online Repository at www.jacionline.org).

Third, macrophages in the intestinal lamina propria might differ phenotypically and functionally from blood monocytes.^{28,29} Intestinal macrophages do not express CD14, an LPS coreceptor, and do not produce IL-10 in response to LPS, whereas blood monocytes express CD14 and produce IL-10 in response to LPS.²⁸ Interestingly, about one third of the mononuclear phagocytes in the inflamed intestinal mucosa are likely newly recruited monocytes from the blood.^{28,30-32} These new monocytes might be exposed to cereal grain-associated LPS in inflamed tissue and might modulate diseases.

Finally, LPS signaling played a protective role in colitis³³⁻³⁵ and food allergy models.³⁶ In mice without intestinal microflora (after oral antibiotics), oral LPS ameliorated their DSS-induced colitis,³³ and TLR4-deficient mice had a more severe DSS-induced colitis.³³⁻³⁵ Furthermore, bacterial LPS might increase the suppressive effects of CD4⁺CD25⁺ regulatory cells through TLR4 on the T cells.³⁷ Thus grain-derived, LPS-like activity might be important in immune homeostasis and disease progression by stimulating monocytes and regulatory T cells.

In conclusion, cereal grain extracts (eg, rice and wheat) induce marked immunomodulation through robust production of IL-10 from CD14⁺ cells. Investigation of *in vivo* immunomodulation by cereal grain ingestion is warranted and might provide novel insights into strategies

to manipulate IL-10 levels in the gastrointestinal tract and other mucosal organs.

We thank Cheryl R. Adolphson for editorial assistance and LuRaye S. Eischens for secretarial help.

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METHODS

Other reagents

Con A, L-glutamine, HEPES, 2-mercaptoethanol, BSA, LPS (*Escherichia coli* O127:B8), and polymyxin B were from Sigma. FBS was from HyClone (Logan, Utah). Reagents for intracellular cytokine staining, as well as cell-surface labeling (including FITC-conjugated antibodies for CD4, CD8, CD56, CD19, and CD14; PE-conjugated anti-IL-10; control mouse IgG; and the BD Cytotfix/Cytoperm kit), were from BD Biosciences. Anti-TLR4, anti-TLR2, and control IgG were from eBioscience (San Diego, Calif).

Allergen extract-induced cytokine production by cultured PBMCs

PBMCs were washed and incubated in RPMI 1640 medium supplemented with 25 mmol/L HEPES, 10% FBS, 2 mmol/L L-glutamine, 50 μ mol/L 2-mercaptoethanol, 100 U/mL penicillin G, and 100 μ g/mL streptomycin sulfate (pH 7.3). The cell viability was greater than 98%, as determined by means of trypan blue dye

exclusion. The cell-free supernatants were collected and stored at -30°C until cytokine measurement.

Cytokine measurements

The levels of IL-5, IL-10, IL-13, and IFN- γ in culture supernatants were determined by means of ELISA with matched pair antibodies (Pierce, Rockford, Ill), according to the manufacturer's directions, except for the IL-13 ELISA, in which biotinylated second antibody was used at a dilution of 1:50,000. Poly-HRP streptavidin (Pierce) and high-binding microtiter plates (Immulon-4 HBX; Thermo Labsystem, Franklin, Mass) were used to increase the assays' sensitivities. The ELISA detection thresholds were as follows: 0.5 pg/mL for IL-5, IL-10, and IFN- γ and 4 pg/mL for IL-13.

LPS measurements

LPS activities in lyophilized allergen extracts were determined by using the Limulus ES-II test (Wako Pure Chemical Industries, Ltd, Osaka, Japan) with the Toxinometer ET-2000 (kinetic turbidimetric LAL assay; Wako). This assay specifically measures endotoxin activity and is not affected by β -glucan.

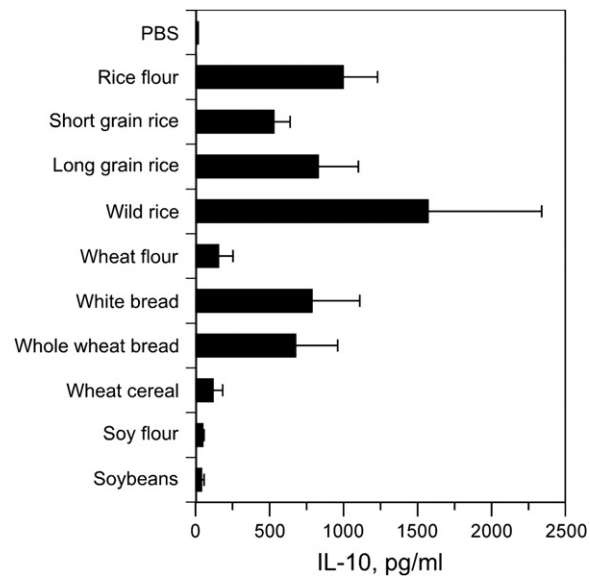


FIG E1. IL-10 production from PBMCs in response to commercial foods. Food extracts (cereal grains, flours, bread, cereals, and soy products) were incubated with PBMCs (2×10^6 cells/mL) for 24 hours. Results show means with SEs of 3 experiments using PBMCs from 3 different individuals. Mortar wash solution (PBS), treated similarly, was used as a control.

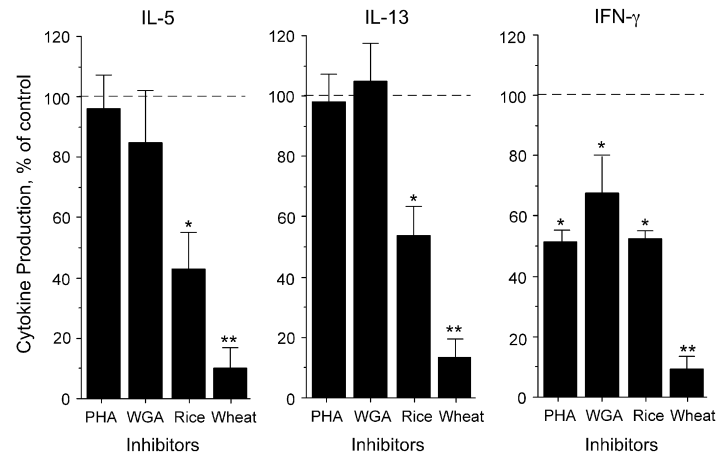


FIG E2. Effects of lectins, rice extract, and wheat extract on Con A–induced cytokine production from PBMCs. PBMCs (2×10^6 cells/mL) were incubated with Con A ($5 \mu\text{g/mL}$) with or without $10 \mu\text{g/mL}$ PHA, wheat germ agglutinin (*WGA*), rice extract, or wheat extract for 96 hours at 37°C . Each column represents means $\pm$ SEM from 3 experiments using PBMCs from healthy donors; results are normalized to Con A alone (no inhibitors) as 100%. * $P < .05$ and ** $P < .01$ compared with Con A alone.