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Effect of Maternal Intake of Organically or Conventionally Produced Feed on Oral Tolerance Development in Offspring Rats

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Supporting Information

ABSTRACT: The aim of this study was to investigate the effect of maternal consumption of organically or conventionally produced feed on immunological biomarkers and their offsprings' response to a novel dietary antigen. First-generation rats were fed plant-based diets from two different cultivation systems (organic or conventional) or a chow. Second-generation rats were exposed to ovalbumin (OVA) via their mother's milk and subsequently challenged with OVA after weaning onto the chow diet. In the chow diet group feeding the dams OVA resulted in suppression of the pups' anti-OVA antibody response to the OVA challenge (total OVA-specific IgG was 197 for the OVA-treated chow diet group and 823 for the control chow diet group (arbitrary ELISA units)). In contrast, OVA exposure of the dams from the plant-based dietary groups did not result in a similar suppression. Cultivation system had no effect on the immunological biomarkers, except for a higher spleen prostaglandin E_2 (PGE_2) concentration in pups originating from dams fed the conventional plant-based diet (223 ng/L) than from those fed the organic plant-based diet (189 ng/L).

KEYWORDS: *organic food, rats, tolerance, immunoglobulin, PGE_2*

INTRODUCTION

A shift in demand among consumers from conventionally to organically produced foodstuffs has progressed over the past decade. It appears to have arisen from a belief that organically grown foodstuffs have a superior nutrient profile and are healthier than conventionally produced foodstuffs.[1−3] The belief of "healthiness" of organic foods is a highly controversial topic in the scientific community. Some literature reviews conclude that organically and conventionally produced foodstuffs are broadly comparable in their nutrient profile,[4−6] whereas others conclude that organic produce has higher levels of nutritionally desirable compounds, as well as lower levels of undesirable compounds.[7−9] Variation in the nutritional content of foodstuffs have also been found to depend on factors such as harvest year, field location, and time of harvest, and these may be masking the differences between cultivation systems.[10−15] However, limited research has been performed to study the potential health effects of organic foodstuffs, and reliable physiological markers or biomarkers are needed to measure the influence on health status.

Most studies comparing the impact of diets comprising organically or conventionally grown crops on health have focused on growth, reproduction, and antioxidant capacity,[4,16,17] but recent studies have demonstrated effects of cultivation system on immune parameters. A multigeneration study by Huber et al.[18] evaluated health-related outcomes in a chicken model and concluded that both innate and acquired immunity were affected by cultivation system, as was the catch-up growth after an immunological challenge. In addition, Lauridsen et al.[19] compared diets from food products derived from either low fertilizer without pesticides, low fertilizer input with pesticides, or high fertilizer with pesticides and found that serum IgG concentrations were higher in rats from the two low-fertilizer groups than in rats from the high-fertilizer group. In contrast, Jensen et al.[10] found that IgG levels were higher in rats eating diets made from conventionally produced crops than from organically produced crops. Other animal studies have observed changes in lymphocyte populations[20] and in lymphocyte function[21] due to cultivation system. In addition, recent epidemiological studies have suggested the "farm effect" from organic farming as a protective result of anthroposophic lifestyle that has been associated with a lower incidence of atopic disorders in young children.[22−24] The aforementioned studies indicate that consumption of feed from different cultivation systems can lead to differences in immunological health parameters, and it has been suggested that potential effects of cultivation system are to be found in the immune system of young individuals.[10,18]

It is well established that changes in the maternal diet during different sensitive stages of pregnancy and lactation can have pronounced effects on the embryo, fetus, and neonate, with long-term consequences of nutritional programming of the embryo and fetus[25,26] and long-term programming effects of early nutrition on health in adulthood.[27,28] Immune factors (i.e., antibodies, growth factors, cytokines, etc.), lymphoid and nonlymphoid cells, and antigens are transferred from mother to fetus via the placenta during pregnancy and/or via colostrum and milk during breastfeeding.[29,30] Passively transferred antibody not only confers protection against infectious agents

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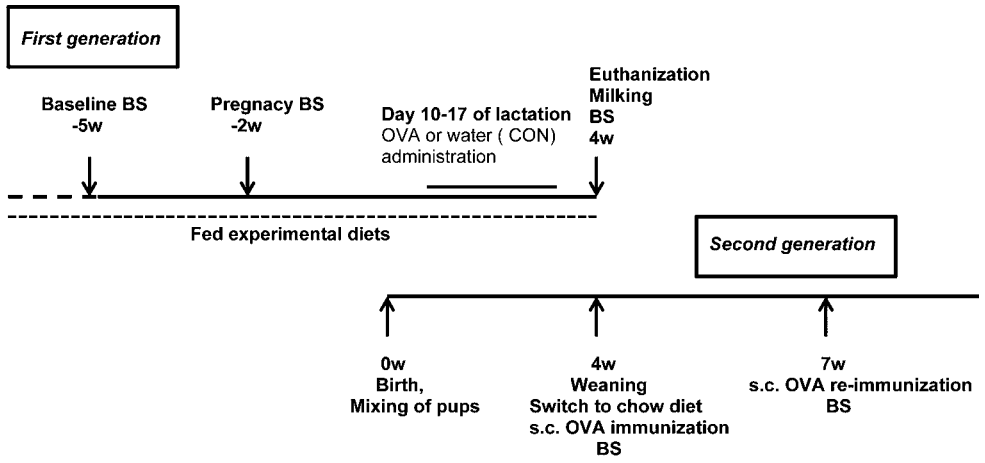

Figure 1. Time line (weeks) of the study for first- and second-generation rats showing the feed regimen, OVA administration, OVA immunization and reimmunization regimen, and blood sampling (BS).

but can also actively influence the immune system of the offspring.[31,32] In the current multigeneration rat study an oral tolerance test was applied as an "immunological challenge", to connect with the principles of organic agriculture of striving to maximize resilience, robustness, and adaptability in plants and animals to increase health.[10,33] Oral tolerance is a specific acquired mechanism, whereby prior feeding reduces an animal's ability to respond to subsequent presentation of that antigen. Failure to develop tolerance can result in damaging allergic reactions,[34] and the objective of the study was to investigate the effect of maternal consumption of organically or conventionally produced feed on modulation of immunological tolerance against an antigen (OVA) in the offspring. By including immunological analysis of the first generation, we were also able to compare effects on general immune parameters from the first exposure of the animals to the experimental feeds, that is, organic or conventionally grown feeds, as occurred in the first generation with effects in the indirectly exposed rats of the second generation.

MATERIALS AND METHODS

Chemicals. Albumin from chicken egg white (ovalbumin), grade V (≥98%), and Freund's complete and incomplete adjuvants were purchased from Sigma-Aldrich (St. Louis, MO, USA). The complete medium was obtained from Lonza (Basel, Switzerland). Antibodies and ELISA kits were purchased from Bethyl Laboratories (Montgomery, TX, USA), Southern Biotech (Birmingham, AL, USA), and Cayman Chemical (Ann Arbor, MI, USA).

Animals and Housing. The study comprised a blinded animal feeding experiment in which two generations of rats were fed diets of either organically or conventionally grown crops. The experimental protocol was approved by The Danish Animal Experiments Inspectorate, Ministry of Justice, Denmark. A two-generation design was chosen as it was reasoned that due to epigenetic mechanisms, the nutritional status of the mother may influence various physiological and immunological parameters of the rats in the next generation.[25,27]

In the current study, 54 female Wistar Hannover GALAS rats (Taconic Europe A/S, Laven, Denmark) (6 months of age) were recruited from a long-term feeding (6 rats per diet × 8 plant-based diets and 1 chow diet = 54 rats) study by Jensen et al.[10] comparing organically and conventionally grown feeds effect on health. The dams had been fed their assigned diets for approximately 6 months in the aforementioned study and were kept in their assigned groups (6 rats per diet) in the current study. The second generation was produced by introducing one male Wistar Hannover GALAS rat (Taconic Europe A/S) per three females within each dietary group for reproduction. The males were fed the rat chow until they were joined with the females, where they were fed the females assigned diets. The dams were placed in individual cages before birth. Twenty-nine dams produced viable offspring and were included in the study ($n_{mothers}$ = 29). If possible, cross-fostering within dietary groups took place within 48 h after birth with maximum of 6 pups per dam. The second generation consisted of 134 rats (n_{pups} = 134). Table S1 in the Supporting Information shows the number of rats in the first and second generation within each dietary group. The offspring were weaned at approximately 4 weeks and switched to the rat chow (Altromin, 1321; Altromin Spezialfutter GmbH & Co. KG, Lage, Germany). Rats from both generations were clinically evaluated each week and were weighed just before each blood sample. Figure 1 outlines the study duration of the first and second generation with the measurements relative to the age (weeks) of the rats.

Both generations were housed in clear polycarbonate cages (59 × 39 × 20 cm) with stainless steel wire lids (Scanbur A/S, Lellinge, Denmark), shelter, biting sticks, and nesting material (Tapvei, Kortteinen, Finland). Fresh water and feed were supplied ad libitum daily. Room temperature and humidity were kept at 24−25 °C and 50−60%, respectively, with alternating dark/light cycles of 12 h.

Crops and Diets. The current study was performed, using diets composed of crops from the long-term CropSys field study,[35] including 2 cultivation systems × 2 years × 2 locations = 8 diets. The diets originated from the Jensen et al.[10] study, and the two most extreme cultivation system practices were chosen for the current study: the organic cultivation system with green manure (legume-based cover crops) and without pesticides and the conventional with inorganic fertilizer (NPK) with pesticides. Crops used for the diets were potatoes (*Solanum tuberosum* L. cv. Sava), winter wheat (*Triticum aestivum* L. cv. Tommi), spring barley (*Hordeum vulgare* L. mixture of cvs. Simba, Smilla, and Power), and fava beans (*Vicia faba* L. cv. Columbo). The organic system was managed in full compliance with the Danish guidelines for organic farming outlined by the Danish Plant Directorate (http://www.pdir.fvm.dk/oekologi). The crops were grown in two successive years, 2007 and 2008 (years 1 and 2), at two different locations, Jyndevad and Foulum, in Denmark. For further descriptions of field study design and of field and soil characteristics, see Olesen et al.[35] and Laursen et al.[15] A control group was included and fed a rat chow diet (Supporting Information, Table S1) (Altromin 1321; Altromin Spezialfutter GmbH & Co. KG; http://www.altromin.de/altro_engl/kataloge/standard_ger.pdf).

The diet (Table 1) was composed to follow the nutrient requirement (NRC) recommendations for growing rats.[36] The plant-based diets were composed to contain the same percentage of each ingredient, to avoid elimination of any potential nutritional differences due to cultivation system, location, or year. However, a minimum vitamin/mineral/amino acid mix (18.4 g/kg diet) was added (kg^{-1} complete diets; 11.6 g of $CaCO_3$, 1.1 g of NaCl, 0.7 mg of retinol, 10.0

Table 1. Ingredient and Calculated Chemical Composition of the Plant-Based Diets

ingredient composition (kg^{-1} complete diets)			
fava beans	270 g		
barley	270 g		
wheat	150 g		
potatoes	162 g		
rapeseed oil	130 g		
vitamin/mineral/amino acid mixture[a]	18.4 g		
	conventional	organic	SEM
chemical composition (kg^{-1} complete diets)[b]			
dry matter	912 g	913 g	1.28
ash content	34.2 g	34.8 g	0.05
crude protein (N × 6.25)	142 g	136 g	2.92
starch	42.4 g	42.7 g	0.61
gross energy	19.0 MJ	18.9 MJ	0.03

[a] 11.6 g of $CaCO_3$, 1.1 g of NaCl, 0.7 mg of retinol, 10.0 mg of vitamin B_5, 6.0 mg of vitamin B_6, and 5.7 g of methionine. 150 mg of ammonium iron(III) citrate was added to the diet during reproduction to meet NRC requirements for reproduction of female rats.[36] [b] Cultivation system LSMEAN cultivation and SEM for the comparison of the diets within cultivation system.

mg of vitamin B_5, 6.0 mg of vitamin B_6, and 5.7 g of methionine) to avoid deficiency. The ingredients and chemical composition of the plant-based diets are given in Table 1, and the complete chemical composition of chow and the plant-based diets is described by Jensen et al.[10]

OVA Administration to the Dams and Immunization of the Second-Generation Rats. Figure 1 outlines the OVA administration procedure for the first generation and immunization time of the second generation. Blood samples were obtained from the tip of the tail of the first-generation rats before (baseline, −5 weeks) and during pregnancy (−2 weeks). To study the response to the dietary antigen OVA in the pups, the dams within each dietary group were given 1 mL of Milli-Q water either with 300 mg of OVA (OVA) or without (CON) by gastric intubation at the same time of the day on days 10−17 of lactation.[37] After weaning of the second-generation rats (week 4), the first-generation rats were weighed and then anesthetized with Hypnorm/Dormicum/water (1:1:2) (3 mL/kg BW), and an intraperitoneal injection of 0.2 mL of Oxytocin (10 IE/mL) was administered. The rats were milked manually, and the milk was collected in ice-cooled tubes and stored at −20 °C. The milk was collected at weaning, because we did not want to add stress to the first-generation rats during the suckling period. Milking was performed within 10 min, and immediately thereafter rats were euthanized by CO_2 asphyxiation; blood samples were collected by cardiac puncture (postpregnancy, 4 weeks).

At 4 weeks, the second-generation rats were weaned onto the rat chow. Blood samples were obtained from the tip of the tail, and the pups were immunized subcutaneously in the neck with OVA (100 μg OVA/100 μL complete Freund's adjuvant/isotonic NaCl (1:1)). Three weeks after the primary immunization (7 weeks), blood samples were drawn and the rats were boosted subcutaneously with OVA (100 μg OVA/100 μL incomplete Freund's adjuvant/isotonic NaCl (1:1)). At 10 weeks the rats were euthanized by CO_2 asphyxiation. Blood samples were drawn by cardiac puncture. The spleen and Peyer's patches (PP) were removed and stored on ice in complete medium (RPMI 1640 with L-glutamine, 25 mM Hepes, 100 U/mL penicillin, 50 μg/mL streptomycin, and 5% FCS).

All blood samples were centrifuged at 2000*g*, at 4 °C for 15 min, and plasma was obtained and stored at −20 °C except for blood samples for flow cytometry, which were analyzed immediately.

Immunoglobulin Quantification by ELISA. Total IgG, IgM, and IgA were measured in the plasma of the first-generation rats before (baseline, −5 weeks) and during pregnancy (−2 weeks) and after birth (postpregnancy, 4 weeks), in the milk (weaning, 4 weeks) and in the plasma of the second-generation rats at weeks 4 (weaning) and 10 (final). Commercially available rat ELISA kits were used.

OVA-Specific Quantification by ELISA. Plasma OVA-specific total IgG and OVA-specific subtypes IgG1 and IgG2a at 7 (reimmunization) and 10 weeks (final) of the second-generation rats were performed by indirect ELISA as follows: Microtiter plate wells were coated and incubated overnight at 4 °C with carbonate−bicarbonate coating buffer containing OVA (grade V, ≥ 98%, Sigma-Aldrich). After the wells had been washed with PBS−Tween (0.05% Tween) and addition of blocking buffer (PBS-Tween with 0.1% gelatin), dilutions of plasma were added to wells in triplicate. The plates were incubated for 1 h at 37 °C and were then washed with PBS−Tween (with 0.1% gelatin). Afterward, the plates were washed, and 100 μL/well detection antibody was then added. The OVA-specific levels were determined using horseradish peroxidase (HRP)-conjugated goat anti-rat IgG Fc (1/40000), HRP-conjugated mouse anti-rat IgG1 (1/10000), or HRP-conjugated mouse anti-rat IgG2a (1/16000) and were incubated for 1 h at 37 °C. After washing, 100 μL/well TMB substrate solution was added to each well. After a maximum 10 min of incubation in the dark at room temperature, the incubation was stopped (100 μL/well 1 M H_2SO_4) and the absorbance was measured at 450 nm. The OVA-specific IgG, IgG1, and IgG2a levels were calculated from a standard curve obtained with a pool of hyperimmune plasma and are expressed in arbitrary ELISA units (1000 EU).

Cell Isolation and Flow Cytometric Analysis. Spleen and PP cells were isolated by being squeezed through a metal strainer and a 70 μm cell strainer and washed in RPMI 1640. Red blood cells in the spleen were lysed using FACS Lysing Solution (BD, Franklin Lakes, NJ, USA) (8 mL lysis solution to 200 μL cell suspension). The cell number was adjusted to 1×10^7 cells/mL.

A four-color analysis was used to identify T cells (APC anti-CD3), B cells (FITC anti-CD45RA), and NK cells (PE anti-CD161a) within the CD45 (PE-Cy5 anti-CD45) positive leukocyte population. Four-color analysis was used to identify T cell subpopulations, using PE anti CD4 and FITC anti-CD8a, gated through CD3 positive T cells (APC anti-CD3) within the CD45 (PE-Cy5 anti-CD45) positive leukocyte population. (All antibodies were from BD Biosciences, San Jose, CA, USA.). Fifty microliters of whole blood or spleen or PP cell suspension was incubated with antibodies in the dark for 20 min at room temperature. Red cell lysis of the blood was achieved by addition of 1 mL of FACS Lysing Solution at this stage. Next, 1 mL of FACS buffer (PBS containing 0.2% sodium azid, 0.1% BSA, and 0.05% horse serum) was added to the cell/Ab solution, and after vortexing and centrifugation, the supernatant was removed. This step was repeated with 2 mL of FACS buffer, and finally the pellet was resuspended in 250 μL of FACS buffer. Samples were analyzed on the basis of 10000 acquired events using a CANTO (Becton-Dickinson, Franklin Lakes, NJ, USA). Analytic gates were chosen based on FSC and SSC to include small lymphocytes and exclude debris and dead cells.

ELISA for Cytokine, TNF-α, and PGE_2. Isolated spleen cells and blood from the pups (10 weeks) were stimulated with LPS (Sigma-Aldrich) (25 μg LPS/mL blood or 2 ng LPS/mL cell suspension, 5×10^6 cells/mL) for 4 h at 37 °C. The samples were centrifuged for 10 min at 2000*g* at 4 °C. The supernatant was stored at −80 °C. Spleen and blood TNF-α production was measured using a commercially available ELISA kit. Spleen PGE_2 concentrations were determined using a PGE_2 ELISA kit, following the manufacturer's protocol.

Statistical Analysis. The data analyzed for the general health and immunological parameters, Y_{ylsi}, were the individual observations for each rat for the measurements on the first-generation rats and the average across the measurements for the rats for the measurements on the second-generation rats. The responses were analyzed as a randomized block experiment with repeated measurements, where the four blocks were defined by the levels of year crossed with the levels of location. We fitted correspondingly the linear mixed model

$$Y_{ylsi} = \mu + \beta_{yl} + \gamma_s + \varepsilon_{yls} + \varepsilon_{ylsi} \quad \text{(I)}$$

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Table 2. TNF-α, PGE_2, and Plasma and Milk Antibody Concentrations in Rats[a]

	plant-based diets[b]			
	conventional	organic	p value	chow
plasma immunoglobulins (mg/L)				
first generation				
baseline IgG	6210 ± 1267	4468 ± 855	0.31	3072 ± 635
pregnancy IgG	4700 ± 1073	3731 ± 836	0.51	2358 ± 649
postpregnancy IgG	2233 ± 555	2041 ± 475	0.80	2212 ± 762
baseline IgA	27.8 ± 2.87	27.1 ± 2.44	0.87	32.1 ± 2.48
pregnancy IgA	30.9 ± 2.21	31.5 ± 2.15	0.87	50.0 ± 2.91
postpregnancy IgA	49.1 ± 6.27	50.2 ± 5.66	0.91	86.1 ± 33.7
baseline IgM	92.8 ± 7.46	82.8 ± 5.87	0.38	74.4 ± 9.32
pregnancy IgM	99.7 ± 8.69	81.4 ± 6.93	0.20	93.7 ± 13.9
postpregnancy IgM	47.7 ± 4.19	53.6 ± 4.13	0.39	56.1 ± 20.5
second generation				
weaning IgG	4694 ± 1525	2759 ± 886	0.30	1773 ± 88.2
final IgG	2736 ± 393	2382 ± 309	0.52	2165 ± 118
weaning IgA	6.43 ± 0.98	4.71 ± 0.71	0.24	12.0 ± 0.41
final IgA	35.8 ± 2.81	41.2 ± 2.72	0.27	48.2 ± 1.48
weaning IgM	27.9 ± 1.15x	23.2 ± 0.94y	0.05	28.0 ± 0.32
final IgM	42.5 ± 4.28	43.0 ± 4.18	0.93	49.6 ± 1.43
milk immunoglobulins (mg/L)				
IgG	413 ± 85.0	320 ± 61.7	0.42	403 ± 138
IgA	288 ± 87.7	270 ± 65.8	0.88	675 ± 284
IgM	3.60 ± 0.49	3.45 ± 0.43	0.87	4.67 ± 0.55
plasma TNF-α (ng/L)	4751 ± 682	4073 ± 522	0.49	5052 ± 704
spleen TNF-α (ng/L)	122 ± 19.4	87.5 ± 14.0	0.17	71.0 ± 9.02
spleen PGE_2 (ng/L)	223 ± 11.9x	189 ± 10.2y	0.05	209 ± 20

[a]ELISA values are antilog data, and data are presented as LSMEANS ± SEM. [b]Cultivation system values with different letters (x, y) within the same row are significantly different ($p < 0.05$).

where μ is the generalized intercept, β_{yl} (y = year 1, year 2; and l = Foulum, Jyndevad) the block effect, and γ_s (s = conventional, organic) is the effect of cultivation system. The errors (ε) are considered independently and normally distributed and represent corresponding variance components.

For the analysis of the oral tolerance data, we used a similar model, a split plot design, where the cultivation systems play the role of the whole plots and the first-generation rats the role of the split plots on which repeated observations via the second-generation rats are made. We fitted the corresponding model

$$Y_{ylsmp} = \mu + \beta_{yl} + \gamma_s + \alpha_h + (\gamma\alpha)_{sh} + \varepsilon_{yls} + \varepsilon_{ylsm} + \varepsilon_{ylsmp} \quad \text{(II)}$$

where α_h (h = OVA, CON) is the effect of OVA treatment and $(\gamma\alpha)_{sh}$ the interaction between cultivation system and OVA treatment. In this case we did not aggregate the measurements across the second-generation rats per first-generation rats, and therefore had a variance component ε_{ylsm} for the first-generation rats m and ε_{ylsmp} for the second-generation rats p.

For the oral tolerance test, observations were also available for the chow diet group besides those from the cultivation systems. Because the chow diet was not exposed to the same experimental setup (no year × location × cultivation system), we analyzed them separately with a simple one-way ANOVA taking into account the repeated measurements from the second-generation rats from each first-generation rat:

$$Y_{mp} = \mu + \alpha_h + \varepsilon_{ylsm} + \varepsilon_{ylsmp} \quad \text{(III)}$$

The pair-wise comparisons and their confidence intervals between the systems were adjusted to obtain a family-wise error rate of 5%. The model was fitted using the proc mixed procedure of SAS/STAT software (SAS (2008) *SAS/STAT 9.2 User's Guide*, SAS Institute Inc., Cary, NC, USA; URL http://support.sas.com/documentation/cdl/en/statugmixed/61807/PDF/default/statugmixed.pdf). All analysis on ELISA data is performed on log transformed data and is presented as LSMEANS and their standard error of mean for the antilog data. All other data are presented as LSMEANS and their standard error of mean.

RESULTS

Diets. Table 1 shows the ingredient composition and chemical composition of the plant-based diets. Crude protein content was just below NRC for reproduction and growth (139 vs 150 g/kg diet, respectively). The complete chemical composition of the diets used in the current study is described by Jensen et al.[10] Higher levels of dietary γ-tocopherol and rapeseed oil fatty acids C18:3, C20:2, and C22:0, and polyunsaturated fatty acids (PUFA) and a lower C18:1 were reported in the conventional plant-based diet than in the organic plant-based diet used in the current study. The essential amino acids, threonine, valine, leucine, lysine, and tryptophan, were just below NRC requirements for growing and reproducing rats in the plant-based diets. The chow diet was a commercial diet and had a completely different composition, and NRC for reproducing and growing rats were fulfilled.[10,36]

General Health Parameters. Although the rats seemed clinically healthy and performed well on both the plant-based diets and the chow diet throughout the study, six rats died in the first generation, five from the plant-based groups and one from the chow group; the autopsy revealed that two rats died due to pregnancy complications, whereas no cause was identified for the remaining four. In the second generation, two rats from the plant-based diet groups had clouded eye/eyes. Two rats were reported to have ring-tail at the time of

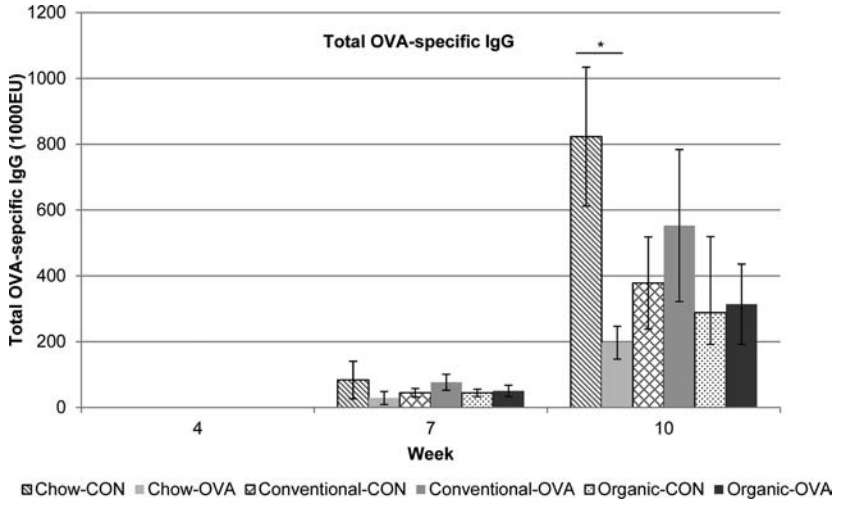

Figure 2. Total plasma OVA-specific IgG during oral tolerance development. Bars represent the LSMEAN ± SEM. ∗ indicates significant difference between OVA and CON treatment within the dietary treatments ($p < 0.05$). OVA, OVA-treated group; CON, control group.

Table 3. Plasma OVA-Specific IgG Subisotypes at Reimmunization (7 Weeks) and Euthanization (10 Weeks) in Second-Generation Rats after Oral Tolerance Induction[a]

	plant-based diets								
	conventional			organic			chow		
	OVA	CON	p value	OVA	CON	p value	OVA	CON	p value
OVA-specific IgG1									
7 weeks	542 ± 183x	231 ± 66y	0.04	192 ± 66	202 ± 50	0.89	128 ± 57	539 ± 225	0.12
10 weeks	2091 ± 766	1507 ± 495	0.34	1215 ± 427	1269 ± 373	0.91	957 ± 58x	3103 ± 159y	0.005
OVA-specific IgG2a									
7 weeks	63.0 ± 17x	24.3 ± 6.4y	0.02	32.8 ± 9.0	29.4 ± 6.8	0.75	21.6 ± 18	60.6 ± 49	0.36
10 weeks	557 ± 189	229 ± 64	0.05	310 ± 100	193 ± 48	0.24	208 ± 104	531 ± 254	0.25
OVA-specific IgG1/IgG2a ratio									
	8.40 ± 2.2	11.6 ± 2.2	0.33	6.81 ± 2.4	10.4 ± 2.0	0.25	6.74 ± 3.5	9.83 ± 3.3	0.58

[a]OVA, OVA treated group; CON, control group. ELISA values are antilog data and are expressed in arbitrary ELISA units (1000 EU). Data are presented as LSMEANS ± SEM. None of the groups expressed anti-OVA specific antibodies at weaning. Values with different letters (x, y) within the same dietary treatment (CON versus OVA) are significantly different ($p < 0.05$).

reimmunization (7 weeks), from the blood samples being drawn from the tail veins. There was no statistical difference in final body weight and relative spleen weight (g spleen kg^{-1} final weight) of the first- or second-generation rats between the cultivation systems (Supporting Information, Table S2).

Immunological Biomarkers in the First and Second Generations. No statistical difference was observed between cultivation systems in plasma or milk IgG, IgA, and IgM in the first generation. Plasma IgM at weaning (4 weeks) was higher in the conventional group than in the organic group in the second generation ($p = 0.05$), whereas no difference was seen for plasma IgG or IgA (Table 2).

Spleen PGE_2 production was, however, significantly higher in pups born to rats fed the conventional plant-based diet than in those born to rats fed the organic plant-based diet after LPS stimulation ($p = 0.05$) (Table 2), whereas the LPS-stimulated spleen or blood TNF-α production was not affected by cultivation system. The T cell subpopulation ratio and T cell/B cell/NK cell distribution in the blood, spleen, or PP in the second-generation rats were not affected by cultivation system (Supporting Information, Table S3).

Oral Tolerance Induction and OVA-Specific Antibodies in the Second Generation. Figure 2 (Supporting Information, Table S4) shows the total plasma OVA-specific IgG and Table 3 the OVA-specific IgG subisotypes of the second-generation rats at weeks 7 and 10. None of the groups (chow or plant-based) had anti-OVA antibodies in their blood at weaning (data not shown). In pups born to rats fed the chow, exposure to OVA via the milk from 10 to 17 days of age resulted in a reduced plasma antibody response to challenge by injection with OVA at weeks 4 and 7. These differences reached statistical significance for the IgG and IgG1 response at week 10 ($p < 0.05$ and $p < 0.005$, respectively). In contrast, there was no evidence for oral tolerance induction to OVA in the pups born to rats fed either of the plant-based diets (conventional or organic). Rather, a higher level of OVA-specific IgG1 was detected in the OVA-fed pups at week 7 ($p = 0.04$) as was a higher level of anti-OVA IgG2a in the OVA-fed pups at both weeks 7 ($p = 0.02$) and 10 ($p = 0.05$) in the CON-conventional plant-fed group than in the OVA-conventional plant-fed group. No statistical differences were found in plasma OVA-specific IgG1 and IgG2a between CON and OVA organic plant-fed group.

DISCUSSION

The present study showed that there were no major differences in the effects of maternal dietary intake of organically or conventionally produced crops during pregnancy and lactation

on selected immunological parameters in the offspring. There were, however, marked differences between the plant-fed groups and the group fed chow in their responses to a novel protein antigen. The study contributes to the knowledge of the health effects of organic food because of the well-controlled field study, including both cultivation systems, year, and location, in combination with a well-designed animal study including a large number of rats (n_p = 134 in the second generation) and the two-generational rat model.

Rats from the first generation in the current study originated from the Jensen et al.[10] long-term feeding study, in which the rats were fed their assigned plant-based diets or chow diet for 6 months, and data on growth in the current study were comparable to data from the Jensen et al.[10] study. The reduction from 54 to 29 rats included in the first generation and 134 rats in the second generation could be due several reasons, such as a relatively high age of the females at reproduction, a mating strategy with one male per three females, or cannibalism of the pups due to stress of the dams. A possible explanation for the observed occurrence of the shrouded eyes could be a deficiency in tryptophan, which is known to cause cataract formation and corneal vascularization.[36] However, the pups were switched to the chow diet at weaning, and this would alleviate any eventual deficiencies.

In the current study we investigated if the maternal diet during pregnancy and lactation could affect the health of the offspring, and response to a novel dietary antigen, OVA. Interestingly, in pups born to rats fed chow, feeding OVA resulted in the induction of oral tolerance, whereas feeding OVA to both groups fed plant-based diets resulted in significantly greater responses to subsequent OVA challenge. We have not attempted to address the mechanisms that may underlie these differences in response, but earlier studies in several species may shed some light. Following the introduction of a new dietary antigen, it has been shown that animals pass through a brief phase of hypersensitivity prior to the development of tolerance.[38,39] It is possible therefore that in the present study the development of tolerance is delayed in the plant-based diet groups. This could be due to compounds influencing the capability of inducing oral tolerance. Several factors have been shown to abrogate or delay the induction of mucosal tolerance including age, genetics, microbial flora, and protein malnutrition (for a review see ref 40). Earlier studies of oral tolerance induction in protein-malnourished mice showed an enhanced tolerance induction in animals fed a 4% protein diet compared to those on a standard 15% diet.[41] It is therefore unlikely that the slightly reduced protein level of the plant-based diets (13.9 versus 15.0%) in the present study would have delayed the induction of tolerance in these groups. The plant-based diets contained a generally high level of fat (~15%) compared to the rat chow (4%),[42] which might have influenced the protein permeation of the intestinal epithelium of the first-generation rats, where the initial uptake of the antigen occurred. In addition, it is known that the weaning process itself is a challenge for the animal, as is any change in diet.[43] In the current study, the second-generation rats originating from first-generation rats fed the plant-based diets, that is, organic and conventional diets, were weaned onto the chow diet, to ensure that the effect of cultivation system was a reflection of the diet of the mothers during pregnancy and lactation and not that of the diet of the second-generation rats themselves. This was also the same reason for not feeding the males used for breeding the experimental diets prior to the reproduction time. Second-generation rats from the chow diet group were kept on the feed of their mothers, whereas second-generation rats from the plant-based dietary groups had to adapt to a diet that their mothers had never digested. This change in diet at weaning could have affected the mucosal immune system in the gastrointestinal tract and influenced simultaneous reactions to the OVA immunizations, possibly leading to the observed difference in oral tolerance development between the plant-based diet groups and the chow diet group. Finally, tryptophan metabolism by DCs are associated with tolerance development,[44] and as mentioned previously tryptophan levels in the plant-based diets in the current study were below NRC. This could potentially have influenced the tryptophan status in the second-generation rats and thus have influenced the oral tolerance development in the second generation. An oral tolerance study in mice by Mito et al.[45] showed an increase in OVA-specific IgG1 and a trend decrease in OVA-specific IgG2 and hence a higher OVA-IgG1/IgG2a ratio in the oral tolerance induced mice compared to the control mice. The opposite was observed in the current study with a statistically higher plasma IgG1 concentration in the chow-CON group compared to the chow-OVA group at week 10. However, because IgG2a was not affected, it did ultimately not lead to a change in the OVA-IgG1/IgG2a ratio. The difference seen in OVA-specific IgG1 at week 7 in the conventional plant-fed group actually did follow the reported pattern by Mito et al.;[45] this was, however, not sustained in week 10.

Plasma IgM concentration at weaning was higher in the second-generation rats from the conventional plant-based group than from the organic plant-based group; however, this picture was not observed in the milk from their mothers or later in the final IgM. None of the other measured antibodies in the plasma or milk in both the first and second generations was affected by cultivation system. The level of antibodies in the milk is however affected by the lactation time, and the antibody levels could have been different at other times during the lactation period.[46] In the Jensen et al.[10] study, a higher baseline total plasma IgG was detected in rats fed the conventional plant-based diet than in rats fed the organic plant-based diet. It was not possible to replicate the results from the Jensen et al.[10] study, even though the rats came from the same long-term feeding study; hence, the individual variation appears to mask any possible effect of cultivation system. In addition, in another rat study the opposite result was observed with a lower plasma IgG in rats eating a high-fertilizer plus pesticide diet, compared to rats eating low fertilizer input plus/minus pesticide diets.[19] Change in total plasma immunoglobulin levels is a reflection of the combined effects of maturation of the immune system and the degree of antigenic exposure. Total immunoglobulin levels in healthy full-grown animals/individuals might not be a good health biomarker for investigating the influence of agricultural cultivation methods on health. Instead, it has been suggested that focus should be given to the immune system of young individuals, where the immune system is developing, and to include an immunological challenge.[10,18,33]

The B cell/T cell/NK cell ratio and T cell subpopulation ratio was not affected by cultivation system, which coincides with results from a study on rats fed either organically or conventionally grown carrots, where no difference was observed in spleen T cell subpopulation ratio or in spleen and PP NK cell activity.[47] However, in a recent study by Roselli et al.,[20] changes in lymphocyte populations were observed in mice eating carrots grown under different cultivations systems, suggesting a

positive effect of organic products. On the other hand, it should be noted that year effect and maybe location were stronger than the cultivation system in the study by Roselli et al.,[20] which is in agreement with previous studies.[10,12,47] TNF-α production was not affected by cultivation system in the current study, which is in accordance with results on pro- and anti-inflammatory cytokine production from the Roselli et al. study.[20]

Modifications in the fatty acid composition (n-6/n-3 ratio) of the diet are known to influence the immune cell function and modify cell membranes and signal transduction pathways or to alter the production of lipid mediators, such as PGE_2.[48] PGE_2 is a potent modulator of dendritic cell (DC) function, which is critical for the inclusion of primary immune responses and immunological tolerance.[37,49,50] Spleen PGE_2 production was higher in the second-generation rats from mothers eating the conventional plant-based diet than in those having eaten the organic plant-based diet, suggesting that there has been a change in the lipid mediator production due to cultivation system. The possible effect of cultivation system on PGE_2 and DC maturation should be further investigated.

In conclusion, our data indicate that oral tolerance development in the offspring is influenced by the mothers' dietary intake during the sensitive stages of pregnancy and lactation. However, no apparent effect was found on the general immune parameters, nor was an improvement of the absent oral tolerance induction from the intake of either conventionally or organically grown diets observed. However, epidemiological studies have suggested a relationship between living an anthroposophic lifestyle, including mothers eating organic food and especially drinking organic milk, and a lower incidence of atopic diseases in children.[22,23] We therefore suggest that future studies on the effect of cultivation system practices on health and immune system development and function should focus on the influence of the n-6/n-3 ratio and gut microbiota on the oral tolerance development in offspring, based on the mother's diet.

■ ASSOCIATED CONTENT

Supporting Information

Number of animals within each dietary group and LSMEANS and their standard error of mean for cultivation system of the growth and cell population percentage variables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

M.M.J. performed the animal study and the overall manuscript drafting. U.H. and M.M.J. performed the statistical analysis. C.L. was project coordinator and revised the manuscript draft. C.R.S. revised the manuscript draft and assisted in oral tolerance data interpretation.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CON, control treatment; DC, dendritic cells; FCS, fetal calf serum; HRP, horseradish peroxidase; IEL, intraepithelial lymphocytes; LPL, lamina propria lymphocytes; LSMEANS, least square of means; NRC, nutrient requirement recommendations; OVA, ovalbumin; PGE_2, prostaglandin E_2; PP, Peyer's patches; PUFA, polyunsaturated fatty acids; TNF-α, tumor necrosis factor-alpha

■ REFERENCES

(1) Velimirov, A.; Huber, M.; Lauridsen, C.; Rembialkowska, E.; Seidel, K.; Bügel, S. Feeding trials in organic food quality and health research. *J. Sci. Food Agric.* **2010**, *90*, 175−182.

(2) Yiridoe, E. K.; Bonti-Ankomah, S.; Martin, R. C. Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: a review and update of the literature. *Renew. Agric. Food Syst.* **2005**, *20*, 193−205.

(3) Nelson, L.; Giles, J.; MacIlwain, C.; Gewin, V. Organic FAQs. *Nature* **2004**, *428*, 796−798.

(4) Bourn, D.; Prescott, J. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 1−34.

(5) Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventinally grown foods − results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281−293.

(6) Dangour, A. D.; Dodhia, S. K.; Hayter, A.; Allen, E.; Lock, K.; Uauy, R. Nutritional quality of organic foods: a systematic review. *Am. J. Clin. Nutr.* **2009**, *90*, 680−685.

(7) Magkos, F.; Arvaniti, F.; Zampelas, A. Organic food: nutritious food or food for thought? A review of the evidence. *Int. J. Food Sci. Nutr.* **2003**, *54*, 357−371.

(8) Worthington, V. Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J. Altern. Complement. Med.* **2001**, *7*, 161−173.

(9) Hoogenboom, L. A. P.; Bokhorst, J. G.; Northolt, M. D.; de Vijver, L.; Broex, N. J. G.; Mevius, D. J.; Meijs, J. A. C.; Van der Roest, J. Contaminants and microorganisms in Dutch organic food products: a comparison with conventional products. *Food Addit. Contam. Part A−Chem.* **2008**, *25*, 1195−1207.

(10) Jensen, M. M.; Jørgensen, H.; Halekoh, U.; Olesen, J. E.; Lauridsen, C. Can agricultural cultivation methods influence the healthfulness of crops for foods? *J. Agric. Food Chem.* **2012**, 6383−6390.

(11) Eppendorfer, W. H.; Eggum, B. O. Effects of sulfur, nitrogen, phosphorus, potassium, and water-stress on dietary fiber fractions, starch, amino-acids and on the biological value of potato protein. *Plant Foods Hum. Nutr.* **1994**, *45*, 299−313.

(12) Jørgensen, H.; Brandt, K.; Lauridsen, C. Year rather than farming system influences protein utilization and energy value of vegetables when measured in a rat model. *Nutr. Res. (N.Y.)* **2008**, *28*, 866−878.

(13) Brunsgaard, G.; Kidmose, U.; Sorensen, L.; Kaack, K.; Eggum, B. O. The influence of variety and growth-conditions on the nutritive-value of carrots. *J. Sci. Food Agric.* **1994**, *65*, 163−170.

(14) O'Brien, L. Genotype and environment effects on feed grain quality. *Aust. J. Agric. Res* **1999**, *50*, 703−719.
(15) Laursen, K. H.; Schjoerring, J. K.; Olesen, J. E.; Askegaard, M.; Halekoh, U.; Husted, S. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *J. Agric. Food Chem.* **2011**, *59*, 4385−4396.
(16) Crinnion, W. J. Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Altern. Med. Rev.* **2010**, *15*, 4−12.
(17) Dangour, A. D.; Lock, K.; Hayter, A.; Aikenhead, A.; Allen, E.; Uauy, R. Nutrition-related health effects of organic foods: a systematic review. *Am. J. Clin. Nutr.* **2010**, *92*, 203−210.
(18) Huber, M.; de Vijver, L. P. L. V.; Parmentier, H.; Savelkoul, H.; Coulier, L.; Wopereis, S.; Verheij, E.; van der Greef, J.; Nierop, D.; Hoogenboom, R. A. P. Effects of organically and conventionally produced feed on biomarkers of health in a chicken model. *Br. J. Nutr.* **2010**, *103*, 663−676.
(19) Lauridsen, C.; Yong, C.; Halekoh, U.; Bügel, S. H.; Brandt, K.; Christensen, L. P.; Jørgensen, H. Rats show differences in some biomarkers of health when eating diets based on ingredients produced with three different cultivation strategies. *J. Sci. Food Agric.* **2008**, *88*, 720−732.
(20) Roselli, M.; Finamore, A.; Brasili, E.; Capuani, G.; Kristensen, H. L.; Micheloni, C.; Mengheri, E. Impact of organic and conventional carrots on intestinal and peripheral immunity. *J. Sci. Food Agric.* **2012**, *92*, 2913−2922.
(21) Finamore, A.; Britti, M. S.; Roselli, M.; Bellovino, D.; Gaetani, S.; Mengheri, E. Novel approach for food safety evaluation. Results of a pilot experiment to evaluate organic and conventional foods. *J. Agric. Food Chem.* **2004**, *52*, 7425−7431.
(22) Kummeling, I.; Thijs, C.; Huber, M.; van, d. V.; Snijders, B. E.; Penders, J.; Stelma, F.; van Ree, R.; van den Brandt, P. A.; Dagnelie, P. C. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. *Br. J. Nutr.* **2008**, *99*, 598−605.
(23) Alfven, T.; Braun-Fahrlander, C.; Brunekreef, B.; von, M. E.; Riedler, J.; Scheynius, A.; van, H. M.; Wickman, M.; Benz, M. R.; Budde, J.; Michels, K. B.; Schram, D.; Ublagger, E.; Waser, M.; Pershagen, G. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle − the PARSIFAL study. *Allergy* **2006**, *61*, 414−421.
(24) Floistrup, H.; Swartz, J.; Bergstrom, A.; Alm, J. S.; Scheynius, A.; van Hage, M.; Waser, M.; Braun-Fahrlander, C.; Schram-Bijkerk, D.; Huber, M.; Zutavern, A.; von Mutius, E.; Ublagger, E.; Riedler, J.; Michaels, K. B.; Pershagen, G. Parsifal Study Group. Allergic disease and sensitization in Steiner school children. *J. Allergy Clin. Immunol.* **2006**, *117*, 59−66.
(25) Symonds, M. E.; Stephenson, T.; Gardner, D. S.; Budge, H. Long-term effects of nutritional programming of the embryo and fetus: mechanisms and critical windows. *Reprod. Fert. Dev.* **2007**, *19*, 53−63.
(26) Rebel, J. M. J.; Van Hemert, S.; Hoekman, A. J. W.; Balk, F. R. M.; Stockhofe-Zurwieden, N.; Bakker, D.; Smits, M. A. Maternal diet influences gene expression in intestine of offspring in chicken (*Gallus gallus*). *Comp. Biochem. Physiol. A: Physiol.* **2006**, *145*, 502−508.
(27) Demmelmair, H.; von Rosen, J.; Koletzko, B. Long-term consequences of early nutrition. *Early Hum. Dev.* **2006**, *82*, 567−574.
(28) Lauridsen, C.; Stagsted, J.; Jensen, S. K. n-6 and n-3 fatty acids ratio and vitamin E in porcine maternal diet influence the antioxidant status and immune cell eicosanoid response in the progeny. *Prostaglandins Other Lipid Mediat.* **2007**, *84*, 66−78.
(29) Nguyen, T. V.; Yuan, L. J.; Azevedo, M. S. P.; Jeong, K. I.; Gonzalez, A. M.; Saif, L. J. Transfer of maternal cytokines to suckling piglets: In vivo and in vitro models with implications for immunomodulation of neonatal immunity. *Vet. Immunol. Immunopathol.* **2007**, *117*, 236−248.
(30) Verhasselt, V.; Milcent, V.; Cazareth, J.; Kanda, A.; Fleury, S.; Dombrowicz, D.; Glaichenhaus, N.; Julia, V. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat. Med.* **2008**, *14*, 170−175.
(31) Lundin, B. S.; Dahlman-Hoglund, A.; Pettersson, I.; Dahlgren, U. I. H.; Hanson, L. A.; Telemo, E. Antibodies given orally in the neonatal period can affect the immune response for two generations: evidence for active maternal influence on the newborn's immune system. *Scand. J. Immunol.* **1999**, *50*, 651−656.
(32) Haverson, K.; Corfield, G.; Jones, P. H.; Kenny, M.; Fowler, J.; Bailey, M.; Stokes, C. R.; Miller, B. G. Effect of oral antigen and antibody exposure at birth on subsequent immune status. *Int. Arch. Allergy Immunol.* **2009**, *150*, 192−204.
(33) Huber, M.; Bakker, M. H.; Dijk, W.; Prins, H. A.; Wiegant, F. A. The challenge of evaluating health effects of organic food; operationalisation of a dynamic concept of health. *J. Sci. Food Agric.* **2012**, *92*, 2766−2773.
(34) Strobel, S. Immunity induced after a feed of antigen during early life: oral tolerance v. sensitisation. *Proc. Nutr. Soc.* **2001**, *60*, 437−442.
(35) Olesen, J. E.; Askegaard, M.; Rasmussen, I. A. Design of an organic farming crop-rotation experiment. *Acta Agric. Scand., Sect. B* **2000**, *50*, 13−21.
(36) *Nutrient Requirements of the Laboratory Rat*; National Academy Press: Washington, DC, 1995; pp 11−79.
(37) Strobel, S.; Mowat, A. M. Immune responses to dietary antigens: oral tolerance. *Immunol. Today* **1998**, *19*, 173−181.
(38) Stokes, C. R.; Newby, T. J.; Bourne, F. J. The influence of oral immunization on local and systemic immune responses to heterologous antigens. *Clin. Exp. Immunol.* **1983**, *52*, 399−406.
(39) Bailey, M.; Haverson, K.; Miller, B.; Jones, P.; Sola, I.; Enjuanes, L.; Stokes, C. R. Effects of infection with transmissible gastroenteritis virus on concomitant immune responses to dietary and injected antigens. *Clin. Diagn. Lab. Immunol.* **2004**, *11*, 337−343.
(40) Newby, T. J.; Stokes, C. R. In *Local Immune Responses of the Gut*; Stokes, C. R., Ed.; CRC Press: Boca Raton, FL, 1984; p 249.
(41) Stokes, C. R.; Swarbrick, E. T. The immune effect of ingested antigens in mice. In *Protein Trasmission Through living Membranes*; Hemmings, W. A., Ed.; Elsevier/North-Holland Biomedical Press: Amsterdam, The Nethelands, 1979; p 309.
(42) Jensen, M. M.; Jorgensen, H.; Halekoh, U.; Olesen, J. E.; Lauridsen, C. Can agricultural cultivation methods influence the healthfulness of crops for foods? *J. Agric. Food Chem.* **2012**, *60*, 6383−6390.
(43) Mowat, A. M. Oral tolerance: an overview. In *Oral Tolerance: The Response of the Intestinal Mucosa to Dietary Antigens*, 1st ed.; Eurekah.com/Landes Biosciences and Kluwer Academic/Plenum Publishers: New York, 2004; pp 12−38.
(44) Mellor, A. L.; Munn, D. H. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol.* **2004**, *4*, 762−774.
(45) Mito, N.; Kaburagi, T.; Yoshino, H.; Imai, A.; Sato, K. Oral-tolerance induction in diet-induced obese mice. *Life Sci.* **2006**, *79*, 1056−1061.
(46) Hanson, L. A.; Korotkova, M.; Lundin, S.; Haversen, L.; Silfverdal, S. A.; Mattsby-Baltzer, I.; Strandvik, B.; Telemo, E. The transfer of immunity from mother to child. In *Immune Mechanisms and Disease*; New York Academy of Sciences: New York, 2003; Vol. 987, pp 199−206.
(47) Jensen, M. M.; Jørgensen, H.; Halekoh, U.; Watzl, B.; Thorup-Kristensen, K.; Lauridsen, C. Health biomarkers in a rat model after intake of organically grown carrots. *J. Sci. Food Agric.* **2012**, 2936−2943.
(48) Calder, P. C. The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2008**, *79*, 101−108.
(49) Steinbrink, K.; Paragnik, L.; Jonuleit, H.; Tuting, T.; Knop, J.; Enk, A. H. Induction of dendritic cell maturation and modulation of dendritic cell-induced immune responses by prostaglandins. *Arch. Dermatol. Res.* **2000**, *292*, 437−445.
(50) Haverson, K.; Singha, S.; Stokes, C. R.; Bailey, M. Professional and non-professional antigen-presenting cells in the porcine small intestine. *Immunology* **2000**, *101*, 492−500.