

Allergen-Specific Transforming Growth Factor- β -Producing CD19(+)CD5(+) Regulatory B-Cell (Br3) Responses in Human Late Eczematous Allergic Reactions to Cow's Milk

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CD19(+)CD5(+) regulatory B cells produce transforming growth factor β (TGF- β) in both mouse and human B-cell leukemias. In this study, TGF- β was uniquely produced by normal human regulatory B cells. TGF- β -producing regulatory B-cell (Br3) responses were characterized through allergic responses to cow's milk. In total, 10 subjects allergic to milk and 13 milk-tolerant subjects were selected following double-blinded, placebo-controlled food challenges. Their peripheral blood mononuclear cells were stimulated *in vitro* with casein. Following allergen stimulation, the percentage of Br3s among CD5(+) B cells decreased from $11.5\% \pm 13.7\%$ to $8.0\% \pm 9.6\%$ ($P = 0.042$, $n = 5$) in the milk-allergy group and increased from $14.7\% \pm 15.6\%$ to $18.9\% \pm 20.1\%$ ($P = 0.006$, $n = 7$) in the milk-tolerant group. However, the numbers of Br3s increased only in the milk-tolerant group, from $1,954 \pm 1,058$ to $4,548 \pm 1,846$ per well ($P = 0.026$), whereas the numbers of Br3s in the milk-allergy group were unchanged [$2,596 \pm 823$ to $2,777 \pm 802$ per well ($P = 0.734$)]. The numbers of apoptotic events were similar to the numbers of total Br3 responses. The percentage of non-TGF- β -producing CD5(+) B cells with apoptotic changes increased from $13.4\% \pm 17.1\%$ to $16.4\% \pm 20.3\%$ ($P = 0.047$, $n = 5$) in the milk-allergy group and remained unchanged [from $9.9\% \pm 11.9\%$ to $9.3\% \pm 11.4\%$ ($P = 0.099$, $n = 7$)] in the milk-tolerant group. Using carboxyfluorescein succinimidyl ester labeling, we observed that the percentage of proliferating Br3s among CD5(+) B cells was unchanged [from $6.1\% \pm 2.8\%$ to $6.4\% \pm 2.9\%$ ($P = 0.145$)] in the milk-allergy group and increased from $6.8\% \pm 3.9\%$ to $10.2\% \pm 5.3\%$ ($P = 0.024$) in the milk-tolerant group. In conclusion, Br3s proliferated in response to allergen stimulation in the milk-tolerant group and not in the milk-allergy group. TGF- β -producing regulatory B cells (Br3) may be involved in allergy tolerance by negatively regulating the immune system with TGF- β , and this negative regulation may be controlled by apoptosis.

Introduction

FOOD ALLERGY PLAYS an important role in atopic dermatitis (AD) (Sampson 1983). In addition to food allergies, an imbalance in the Th1/Th2 ratio is known to be important in the development of AD (Grewe and others 1998). Food allergies are classified as immunoglobulin E (IgE)-mediated, non-IgE-mediated, or mixed-type allergies (Ramesh 2008). Because of the lack of available laboratory diagnostic tests, non-IgE-mediated food allergy has been underdiagnosed and undertreated (Sabra and others 2003). Recently, late eczematous reactions of the skin have been found to be typical diagnostic symptoms of non-IgE-mediated food allergy (Kemp 2007).

CD5 plays a key role in the generation and maintenance of tolerance (Raman 2002). A subset of CD5(+) B cells known as regulatory B cells negatively regulate immune responses through the production of interleukin-10 (IL-10) (Mizoguchi and Bhan 2006). A recent report described a role for IL-10-producing regulatory B cells (Br1) in the late eczematous reaction in human food allergy (Lee and others 2010). In addition to Br1, another regulatory B-cell subset that produces transforming growth factor β 1 (TGF- β 1) after *in vitro* stimulation with LPS has been also identified (Tian and others 2001). TGF- β is produced by both CD5(+) and CD5(-) B cells from autoimmune-prone NZB mice (Douglas and others 1997), and TGF- β is also expressed by human chronic lymphocytic leukemia cells and normal human B cells (Lotz and others 1994).

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TABLE 1. CHARACTERISTICS OF THE MILK-ALLERGY AND THE MILK-TOLERANT GROUPS

	Milk allergy group (positive)	Milk tolerant group (negative)	Unit
<i>n</i>	10	13	
M:F	4:6	6:7	
Mean age	12.5 ± 11.7	14.2 ± 14.8	year
Total IgE	1323 ± 1211	1195 ± 1318	IU/L
Blood eosinophil	8.6 ± 5.3	8.0 ± 5.1	%
Blood lymphocyte	39.1 ± 16.1	35.5 ± 13.8	%
Milk-specific IgE	Undetectable	Undetectable	kUA/L
Casein-specific IgE	Undetectable	Undetectable	kUA/L
Skin-prick test for milk	All negative	All negative	Grade

IgE, immunoglobulin E.

TGF- β plays an important role in the function of CD4(+)CD25(+) regulatory T cells and the induction of immune tolerance (Zhang and others 2006). TGF- β -producing regulatory B-cell (Br3) responses have not been previously reported to be associated with allergic disease. In this study, Br3 responses were characterized in human late eczematous allergic reactions to cow's milk.

Materials and Methods

Subjects and inclusion criteria

Subjects with a repeated history of late eczematous reactions or exacerbations of AD who visited the Department of Allergy and Clinical Immunology at the Seoul Allergy Clinic between March 2010 and April 2010 were screened for this study. They fulfilled the Hanifin and Rajka's AD criteria (Hanifin and Rajka 1980). In total, 10 subjects allergic to milk and 13 milk-tolerant subjects were selected and underwent double-blinded, placebo-controlled food challenges (DBPCFC) (Table 1). All subjects received blood and skin-prick tests as described below. The patients (or the parents of minor patients) were asked to sign a consent form. This study was approved by the Institutional Review Board of Chungnam National University Hospital, Daejeon, Korea.

DBPCFC tests were conducted for milk. The diagnostic criteria for late eczematous reactions to food allergens have been well described in previous reports (Lee and others 2010). These criteria are (1) a late eczematous reaction, including exacerbation of AD following DBPCFC (Fig. 1); (2) an onset of exacerbation at 4 h after the food challenge; and (3) a negative skin-prick test reaction and a negative food-specific IgE level for milk (Kvenshagen and others 2008).

Blood tests included a complete blood count with differential counts for the eosinophil fraction, the total serum IgE level, and the serum milk-specific IgE level, which was measured using a UniCAP method (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The threshold for detecting food-specific IgE levels in the serum was 0.35 kU A/L. Skin-prick tests were conducted on the patient's left forearm using commercial allergen extracts (Bencard, Brentford, England). Histamine hydrochloride (1 mg/mL; Bencard) was used as a positive control, and physiological saline was used as a negative control. Reactions were read after 15 min and classified as negative (0: no reaction; +: a reaction greater than the control reaction but less than half the level of the reaction caused by histamine) or positive (++: half the level; +++: equal to; and ++++: twice the level caused by histamine).

Diagnosis of late eczematous reactions to food allergens

For the diagnosis of milk allergies, a DBPCFC test was conducted according to the diagnostic methods described in our previous report (Noh and others 2007). Briefly, we instituted an elimination diet for all possible foods that may potentially induce the allergic AD.

A DBPCFC test was conducted just after the elimination diet. The DBPCFC test was performed using a vehicle, which was a mixed cereal flour consisting of brown rice flour, glutinous rice flour, and barley flour. Two challenges, separated by 5 days, were performed: one was the vehicle only, and the other contained the suspected food antigen in the vehicle. The randomization and preparation of the challenges were performed by dieticians in the clinical research unit. All reactions were scored regarding the type, time of onset, severity, and duration.

The signs and symptoms of the non-IgE food allergy appeared as either newly developed symptoms or an exacerbation of AD. Because of these signs and symptoms, the clinical severity was evaluated using the SCORing Atopic Dermatitis (SCORAD) index, a system used worldwide to assess the severity of atopic eczema (Kunz and others 1997). A diagnosis of food allergy was made if the clinical severity

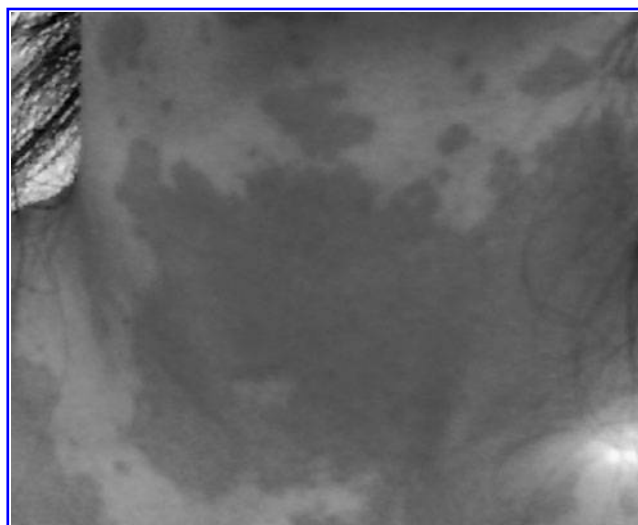


FIG. 1. Representative skin lesions of late eczematous reactions to cow's milk. Erythematous papular eruptions developed following food challenge.

score increased by >20% in subjects demonstrating a basal score of >50 points or if the score increased by over 10 points for those with a basal score of ≤ 50 points. Apparent new skin lesions and/or itching were regarded as positive reactions, which were required for the diagnosis of a food allergy by food challenge.

Casein stimulation of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood using density-gradient separation and Ficoll-Hypaque (Biomedicals, Aurora, OH). PBMCs were resuspended at 1×10^6 cells/mL in α -minimum essential medium (α -MEM; Irvine Scientific, Santa Ana, CA) and cultured with or without a 50 μ g/mL mixture of purified α -, β -, and κ -casein (Sigma, St. Louis, MO) in α -MEM in a 24-well plate. The selected casein concentration was that used in a previous study (Shreffler and others 2009). As unstimulated controls, cells were cultured without casein.

Cell-surface staining, intracellular staining, carboxyfluorescein succinimidyl ester labeling, and flow-cytometry analysis

After 24 h of culture, PBMCs with or without exposure to casein were stained with specific monoclonal antibodies. Cells were prepared at a concentration of 1×10^6 cells in

100 μ L of FACS staining buffer (eBioscience, San Diego, CA) in an Eppendorf tube. Allophycocyanin (APC)-labeled anti-CD19 (eBioscience), phycoerythrin-Cy7 (PE-Cy7)-labeled anti-CD5 (eBioscience), and FITC-conjugated Annexin V from an apoptosis-detection kit (eBioscience) were used for analysis of the B-cell subsets. Monoclonal antibodies were added at a concentration of 1 μ g/mL according to the manufacturer's instructions. The cells were incubated for 1 h in the dark and washed 3 times with FACS staining buffer. After cell-surface staining using the PE-Cy7-labeled anti-CD5, APC-labeled anti-CD19, and FITC-conjugated Annexin V, the cells were fixed and permeabilized using a fixation/permeabilization kit (eBioscience). The cells were stained with a PE-labeled anti-TGF- β rat monoclonal antibody (R&D Systems, Minneapolis, MN). The stained cells were then analyzed using a FACSCalibur (BD Biosciences, Milpitas, CA), and the data were analyzed using CellQuest software (BD Biosciences).

To observe the proliferation of Br3s, carboxyfluorescein succinimidyl ester (CFSE) labeling of PBMCs was performed as described elsewhere, with some modifications (Turcanu and others 2003). Briefly, immediately after isolation, PBMCs were incubated with CFSE (5 μ M) for 10 min at 37°C. Labeling was stopped with α -MEM and excess dye was washed away. After 24 h of culture, the CFSE-stained PBMCs were stained using PE-Cy7-labeled anti-CD5, APC-labeled anti-CD19, and PE-labeled anti-TGF- β .

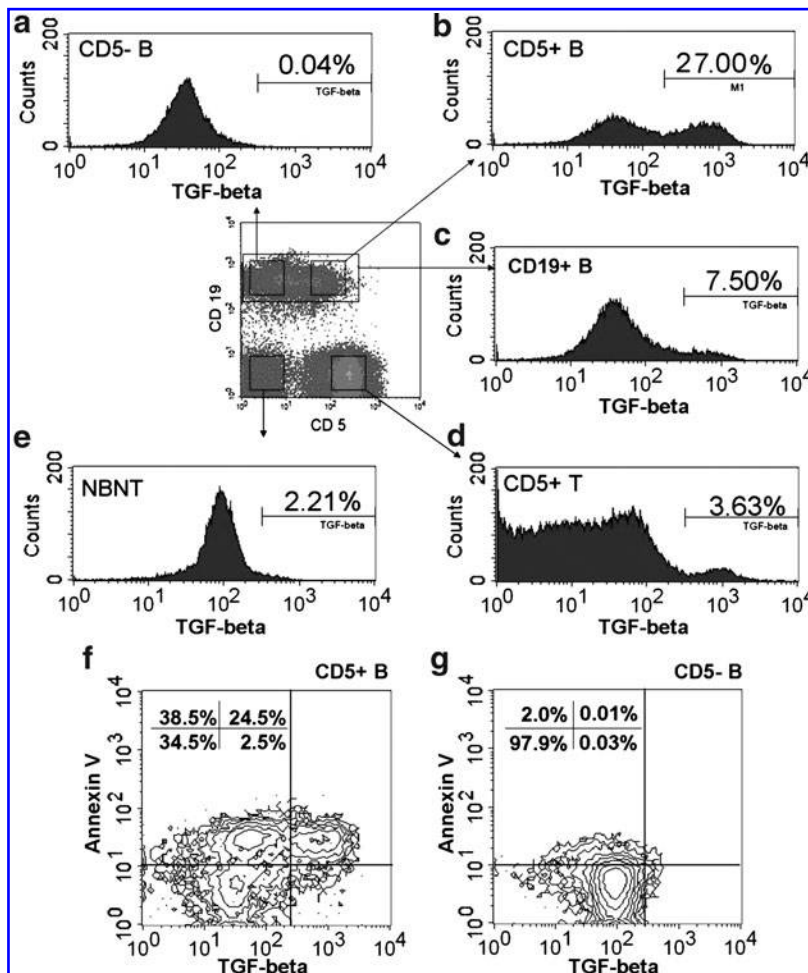


FIG. 2. TGF- β production and apoptosis of CD19(+)CD5(+) regulatory B cells. Representative histograms of TGF- β -producing cells (a-e) and double-stained plots of the CD19(+)CD5(+) (f) and CD19(+)CD5(-) B-cell populations (g) of another patient. (a) CD19(+)CD5(-) B cells; (b) CD19(+)CD5(+) B cells; (c) CD19(+) B cells; (d) CD19(-)CD5(+) T cells; (e) CD19(-)CD5(-) non-B, non-T cells; (f) CD19(+)CD5(+) B cells; (g) CD19(+)CD5(-) B cells. CD5- B, CD19(+)CD5(-) B cells; CD5+ B, CD19(+)CD5(+) B cells; CD5+ T, CD19(-)CD5(+) T cells; NBNT, CD19(-)CD5(-) cells. TGF- β , transforming growth factor- β .

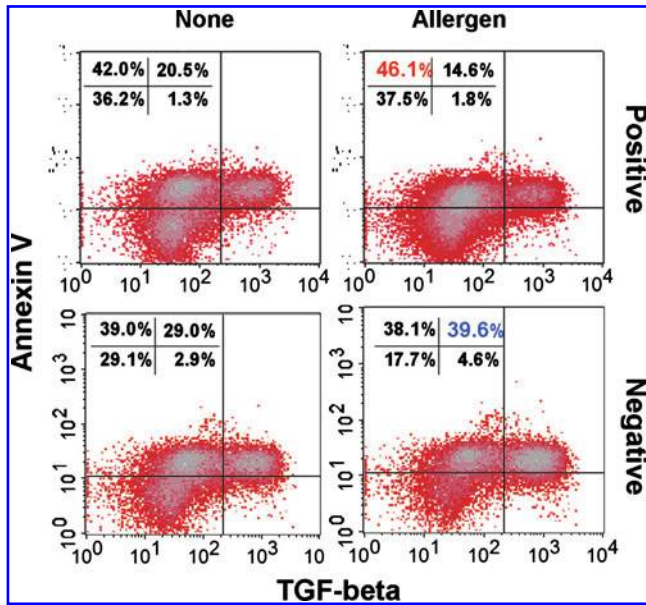


FIG. 3. Representative compositional changes in CD5(+) B-cell subtypes in response to allergen stimulation in the milk-allergy group and the milk-tolerant group. The percentage of each population is described in the upper-left quadrant. X-axis, TGF- β expression; Y-axis, Annexin V; None, no stimulation of PBMCs; Allergen, casein-stimulated PBMCs; Positive, milk allergy group; Negative, milk-tolerant group. PBMCs, peripheral blood mononuclear cells.

Statistical analysis

Statistical analyses were performed using the Wilcoxon signed rank test and the Mann-Whitney U test with Bonferroni corrections for individual groups using SigmaPlot software (Systat Software, San Jose, CA). P values of <0.05 were considered significant.

Results

TGF- β -producing CD19(+)CD5(+) regulatory B cells

TGF- β was produced by the B cells among the PBMCs, particularly the CD19(+)CD5(+) B cells (Fig. 2b). TGF- β -producing cells accounted for 7.5% of the CD19(+) B cells, and TGF- β -producing CD5(+) T cells accounted for 3.63%. Interestingly, TGF- β was produced by CD19(+)CD5(+) regulatory B cells (27.00%) and not by CD5(-) conventional B cells (0.04%).

TGF- β production and apoptosis were investigated in CD5(+) B cells. The CD19(+)CD5(+) regulatory B cells were gated and analyzed for TGF- β production and apoptosis. TGF- β was predominantly expressed in CD19(+)CD5(+) regulatory B cells (Fig. 2f), whereas CD19(+)CD5(-) conventional B cells did not show significant production of TGF- β (Fig. 2g). Moreover, based on the Annexin V staining, most of the Br3s studied exhibited apoptotic changes.

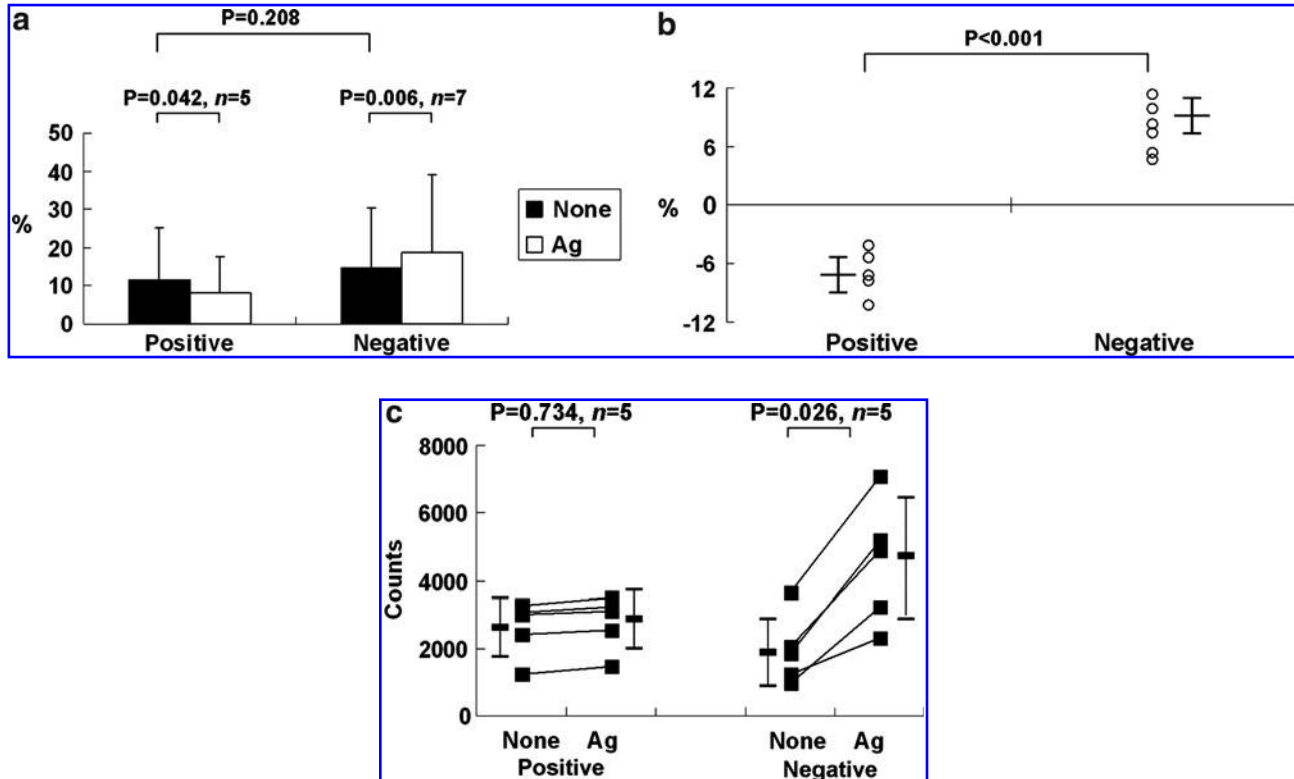


FIG. 4. Br3 responses in the milk-allergy and milk-tolerant groups following allergen stimulation. (a) Mean Br3 proportion in CD5(+) B cells before and after allergen stimulation. (b) Individual changes in Br3 proportions by allergen stimulation. (c) Total Br3 changes following allergen stimulation, as calculated by multiplying the total number of culture cells by the percentage of Br3 cells. Positive, milk-allergy group; Negative, milk-tolerant group; None, no stimulation of PBMCs; Ag, casein-stimulated PBMCs.

Br3 responses following allergen stimulation

Br3 responses were analyzed in the milk-allergy group and the milk-tolerant group, and the representative responses are shown in Fig. 3. Br3s with apoptotic changes (upper-right quadrant) were observed to increase in the milk-tolerant group. The Br3s included cells with apoptotic and nonapoptotic changes.

The proportion of Br3s decreased from $11.5\% \pm 13.7\%$ to $8.0\% \pm 9.6\%$ ($P=0.042$, $n=5$) in the milk-allergy group and increased from $14.7\% \pm 15.6\%$ to $18.9\% \pm 20.1\%$ ($P=0.006$, $n=7$) in the milk-tolerant group (Fig. 4a) after allergen stimulation. Mean changes in the Br3 proportions following allergen stimulation were $-7.0\% \pm 2.3\%$ in the milk-allergy group and $8.4\% \pm 2.9\%$ in the milk-tolerant group ($P<0.001$; Fig. 4b).

Through allergen stimulation, the cultured cell counts increased from $124,000 \pm 24,000$ to $213,000 \pm 43,000$ per well ($P<0.001$) in the milk-allergy group and $115,000 \pm 22,000$ to $265,000 \pm 49,000$ per well ($P<0.001$) in the milk-tolerant group. Br3 numbers were calculated by multiplying the percentage of Br3s among the cultured cells by the total number of cultured cells. Consequently, the Br3 numbers increased only in the milk-tolerant group [from $1,954 \pm 1,058$ to $4,548 \pm 1,846$ per well ($P=0.026$)], whereas the numbers in the milk-allergy group were going insignificantly from $2,596 \pm 823$ to $2,777 \pm 802$ per well ($P=0.734$; Fig. 4c).

Br3 responses include apoptotic changes following allergen stimulation

In response to allergen stimulation, the percentages of Br3s with apoptotic changes decreased from $10.3\% \pm 12.2\%$ to $6.9\% \pm 8.2\%$ ($P=0.038$, $n=5$) in the milk-allergy group and increased from $13.2\% \pm 14.1\%$ to $16.7\% \pm 17.8\%$ ($P=0.006$, $n=7$) in the milk-tolerant group (Fig. 5a). Mean changes of the cell proportions by allergen stimulation were $-6.9\% \pm 1.8\%$ in the milk-allergy group and $6.9\% \pm 2.5\%$ in the milk-tolerant group ($P<0.001$; Fig. 5b).

Responses of non-TGF- β -producing CD5(+) B cells with apoptotic changes following allergen stimulation

The percentages of non-TGF- β -producing CD5(+) B cells with apoptotic changes increased following allergen stimulation in the milk-allergy group (from $13.4\% \pm 17.1\%$ to $16.4\% \pm 20.3\%$, $P=0.047$, $n=5$) and were unchanged in the milk-tolerant group (from $9.9\% \pm 11.9\%$ to $9.3\% \pm 11.4\%$, $P=0.099$, $n=7$) (Fig. 5c). The mean changes in these cell proportions induced by allergen stimulation were $6.0\% \pm 2.6\%$ in the milk-allergy group and $-2.7\% \pm 3.5\%$ in the milk-tolerant group ($P<0.001$; Fig. 5d).

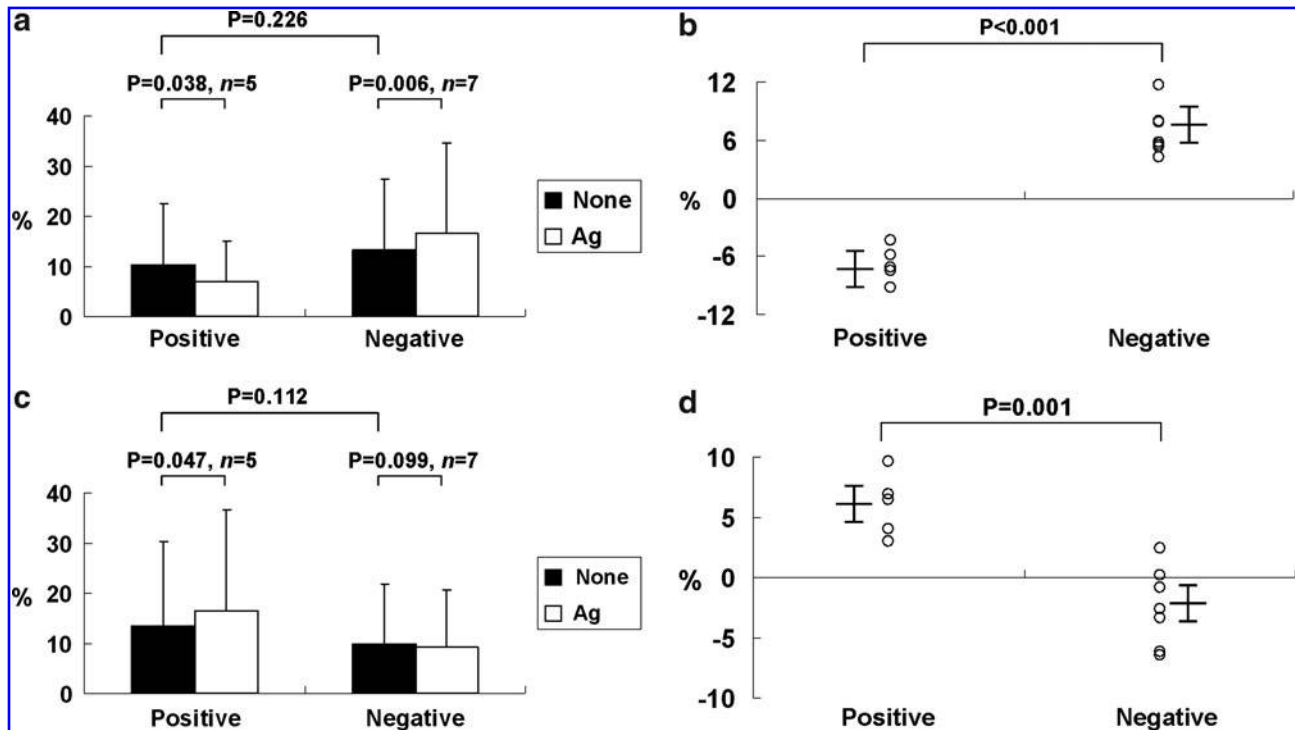


FIG. 5. Fractional changes in Br3s with apoptotic changes in CD5(+) B cells and non-TGF- β -producing CD5(+) B cells with apoptotic changes in CD5(+) B cells after allergen stimulation in the milk-allergy and milk-tolerant groups. (a) The mean Br3 proportions with apoptotic changes in CD5(+) B cells before and after allergen stimulation. (b) Individual proportional changes in Br3 with apoptotic changes in CD5(+) B cells after allergen stimulation. (c) Mean non-TGF- β -producing CD5(+) B-cell proportions with apoptotic changes in CD5(+) B cells before and after allergen stimulation. (d) Individual proportional changes in non-TGF- β -producing CD5(+) B cells with apoptotic changes in CD5(+) B cells by allergen stimulation. Positive, milk-allergy group; Negative, milk-tolerant group; None, no stimulation of PBMCs; Ag, casein-stimulated PBMCs.

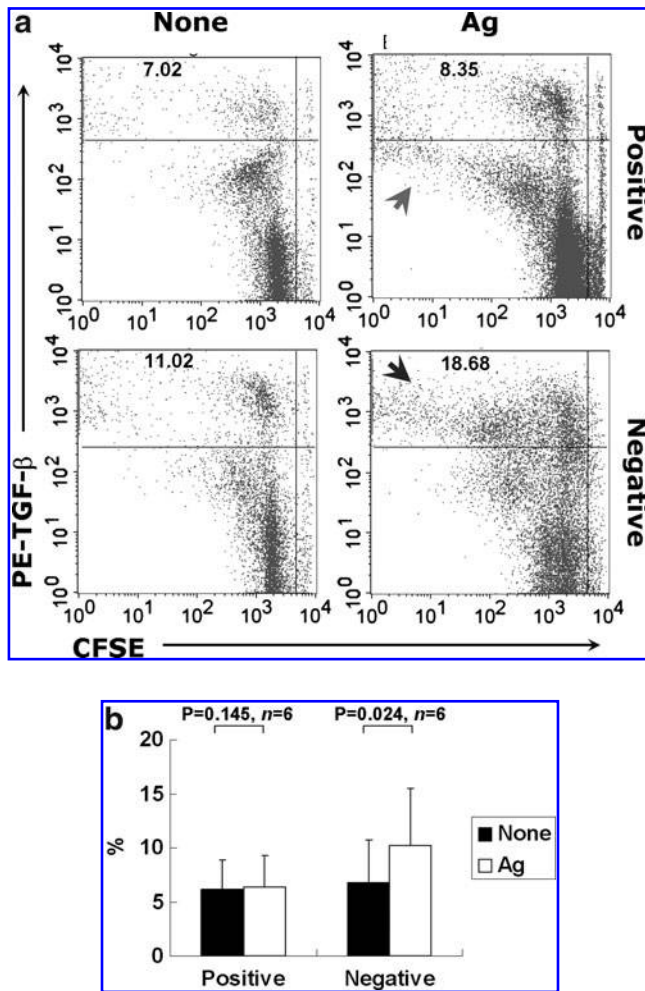


FIG. 6. Proliferation study using CFSE labeling. **(a)** Representative plots of the proliferation of Br3s in the milk-allergy and milk-tolerant groups by allergen stimulation. **(b)** Mean proliferating Br3 percentages in the milk-allergy and milk-tolerant groups by allergen stimulation. Positive, milk-allergy group; Negative, milk-tolerant group; None, no stimulation of PBMCs; Ag, casein-stimulated PBMCs; blue arrow, proliferation of TGF- β -producing Br3; red arrow, proliferation of non-TGF- β -producing CD5(+) B cells. CFSE, carboxyfluorescein succinimidyl ester.

Proliferation study by CFSE labeling

The proliferation of Br3s in the milk-tolerant group was confirmed by CFSE staining (Fig. 6a). After allergen stimulation, the percentage of proliferating Br3s remained unchanged [from $6.1\% \pm 2.8\%$ to $6.4\% \pm 2.9\%$ ($P = 0.145$)] in the milk-allergy group and increased from $6.8\% \pm 3.9\%$ to $10.2\% \pm 5.3\%$ ($P = 0.024$) in the milk-tolerant group (Fig. 6b), whereas non-TGF- β -producing CD5(+) B cells showed no significant changes, going from $49.4\% \pm 4.0\%$ to $52.1\% \pm 4.6\%$ ($P = 0.328$) in the milk-allergy group and from $52.1\% \pm 10.6\%$ to $48.0\% \pm 7.8\%$ ($P = 0.314$) in the milk-tolerant group.

Discussion

In addition to producing IL-10, according to a previous report (Noh and others 2010), normal human B cells also

produce TGF- β , predominantly in CD19(+)CD5(+) B cells (Fig. 2b). TGF- β has been previously reported to be produced in Th3 cells (Weiner 2001). CD5(+) B cells are a subset of B cells known as B1 cells, which have been shown to be closely related to autoimmune disease (Lemoine and others 2009). Recently, subsets of CD5(+) B cells have been characterized as regulatory B cells because of their participation in immune regulation (Bouaziz and others 2008). TGF- β was reported to be produced by both CD5(+) B cells and CD5(-) B cells in autoimmune-prone NZB mice (Douglas and others 1997). TGF- β expression has been reported in human chronic lymphocytic leukemia cells as well as in normal human B cells (Lotz and others 1994). The proportion of TGF- β -producing cells was highest among CD19(+)CD5(+) B cells compared with their proportions among T cells and other CD19(-)CD5(-) cells (Fig. 2b, d); CD5(-) conventional B cells appeared to produce no significant levels of TGF- β (Fig. 2a). In addition to the production of TGF- β in CD19(+)CD5(+) regulatory B cells, the most remarkable finding in this study was that most Br3s were undergoing apoptosis (Fig. 2f).

Regulatory B-cell responses induced by allergen stimulation were further investigated in milk allergy (Fig. 3). In response to allergen stimulation, the proportion of Br3s increased in the milk-tolerant group (Fig. 4a, b). Considering the total number of cultured cells and Br3 fractions among the total cultured cells, the Br3 counts were unchanged in the milk-allergy group and increased in the milk-tolerant group after allergen stimulation (Fig. 4c). Moreover, the percentage of proliferating Br3s increased in the milk-tolerant group and was unchanged in the milk-allergy group after allergen stimulation (Fig. 6). Overall, the data indicate that Br3s proliferated in response to allergen stimulation in the milk-tolerant group but not in the milk-allergy group, and these results suggest that Br3s may play a role in immune tolerance in food allergy (Fig. 7).

IL-10-producing regulatory B cells, Br1, have been reported to play an important role in delayed-type hypersensitivity by mediating the required early T-cell recruitment (Szczepanik and others 2003). They have been also shown to mediate airway hyperreactivity in nonatopic asthma (Kawikova and others 2004). Br1 have been shown to negatively regulate immune responses through the production of IL-10 (Mizoguchi and Bhan 2006). This is the first report of the involvement of Br3s in human allergic disease, and the results of the present study also support the previously reported results demonstrating that Br1 play a role in human late eczematous reactions in food allergy (Lee and others 2010).

TGF- β is an immunosuppressive cytokine secreted in an antigen-specific fashion by regulatory T cells, although it functions in a nonantigen-specific fashion (bystander suppression). TGF- β is a potent negative regulator of human lymphocytes (Kehrl and others 1991) and has been closely linked with CD4(+)CD25(+) regulatory T cells, which play an important role in the maintenance of immune tolerance and homeostasis (Zhang and others 2006). Br3 responses were antigen-specific in our study. The allergen-specific proliferation of Br3s in the milk-tolerant individuals suggests an antigen-specific immunosuppressive role in allergic effector cells, which could result in allergen-specific tolerance, and a nonspecific immunosuppressive role in addition to inducing proliferation and the maintenance of regulatory T cells.

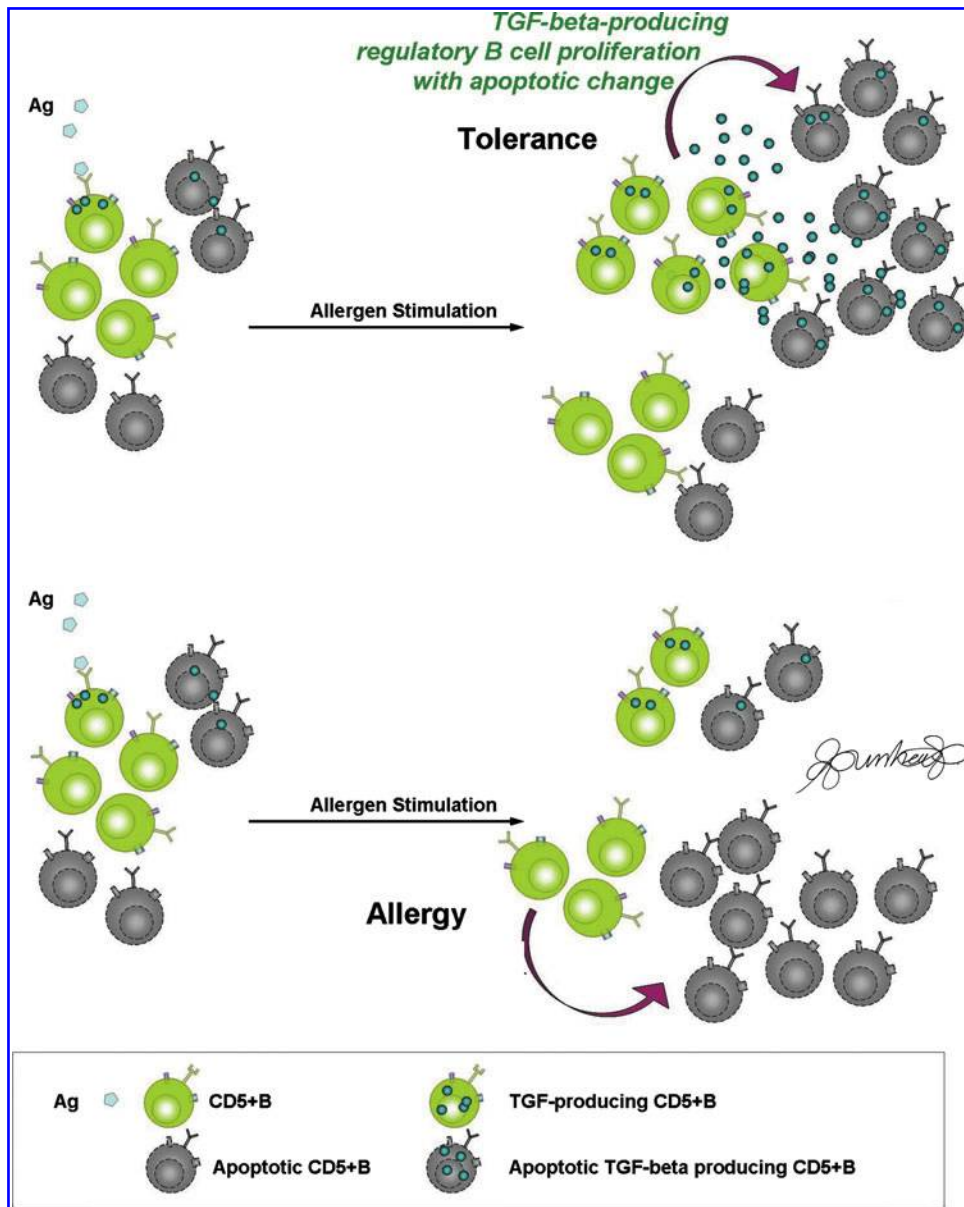


FIG. 7. A schematic diagram of the responses of Br3s in allergy and immune tolerance toward food allergens. TGF- β -producing regulatory B cells (Br3) proliferate in response to allergen in milk-tolerant subjects, and most of these cells simultaneously undergo apoptosis. CD5+B, CD5(+) B cells; Ag, allergen.

Br3 proliferation was accompanied simultaneously by apoptosis in this study (Figs. 3, 5). Although it is required for the development of plasma cells, TGF- β stimulates the apoptosis of B cells (Lebman and Edmiston 1999). Proliferating B cells are killed by CD4(+)CD25(+) T cells that are affected by TGF- β , and this mechanism is important in immune tolerance (Janssens and others 2003). Our results suggested the possibility that endogenous TGF- β might stimulate Br3s in an autocrine manner. Although the proliferation and apoptotic changes may appear contradictory, apoptosis may be an effective way to control and negatively regulate the proliferating immune cells. We also interpreted the simultaneous proliferative and apoptotic changes of Br3s observed in this study as the action of a negative regulatory mechanism by which Bregs, through the production of TGF- β , are controlled by the accompanying apoptosis (Fig. 7). B-cell chronic lymphocytic leukemia comprises mature CD5(+) B lymphocytes (Dighiero and others 1991). This possibility was

consistent with previous studies, which demonstrated that TGF- β negatively regulates chronic lymphocytic leukemia (Lagneaus and others 1997). Further, autocrine TGF- β secretion exhibits an antiproliferative effect on B-cell chronic lymphocytic leukemia cells (Schuler and others 1999). In mice, TGF- β can induce apoptosis in CD5(+) B cells (Douglas and others 1997). Activation-induced apoptosis is also an important negative regulatory mechanism (Stohl and Elliott 1995). It is questionable whether the negative regulation of Br3s by apoptosis was affected by autocrine TGF- β effects or antigen-dependent activation.

Apoptotic T cells also release TGF- β , which contributes to an immunosuppressive milieu (Chen and others 2001). The immunologic mechanism of TGF- β -producing T cells for the negative regulation of the immune system seems to be similar to that of the Br3s in this study. CD4(+) Foxp3(+)CD25(+) regulatory T cells have been thought to play a dominant role in immune tolerance (Lohr and others

2006). Regulatory T cells modulate immune reactions by secreting immunomodulatory cytokines, particularly TGF- β (Askenasy and others 2008). Similar to TGF- β -producing Th3, TGF- β -producing Br3 seems to be involved in immune tolerance, including allergy tolerance. With respect to immune regulation, the B-cell subset structure, with TGF- β -producing Br3 as well as IL-10-producing Br1, may resemble the T-cell subset structure, which consists of IL-10-producing Tr1 (Battaglia and others 2006) cells and TGF- β -producing Th3 (Weiner 2001) or other regulatory T-cell subsets.

In conclusion, TGF- β is produced in normal human CD19(+)CD5(+) regulatory B cells. Moreover, Br3 seems to react to allergens in food-allergy tolerance, whereas non-TGF- β -producing CD5(+) B cells react to allergens in milk allergy. A negative regulation for the control of immune suppression by Br3s in allergy tolerance may be controlled by apoptosis, which ensures protection from the excessive negative regulation of Br3 by producing TGF- β . Further studies examining the mechanisms underlying the role of Br3 in immune tolerance toward food antigens are required.

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Author Disclosure Statement

No competing financial interests exist.

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