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Short Communication

# Increased expression of α4β7 integrin on food allergen-stimulated CD4+ T cells in active food allergic enterocolitis

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## Abstract

We used flow cytometry to investigate the expression of α4β7 integrin on peripheral blood CD4+ T cells stimulated with αs-casein, one of the major allergens in milk allergy, in patients with milk-induced enterocolitis. In the active state of the disease, the levels of α4β7 integrin on αs-casein-stimulated CD4+ T cells, as well as the numbers of positive cells, were higher than in the age-matched control. Upon outgrowing milk allergy, α4β7 integrin levels decreased to the same levels as in the healthy control. The proliferative responses of peripheral blood mononuclear cells to αs-casein in the active state did not differ from those in the outgrown state, suggesting that the expression of α4β7 integrin on milk allergen-activated T cells is a marker of the activation state leading to the pathogenesis of milk-allergic enterocolitis.

**Key words:** α4β7 integrin, αs-casein, cow's milk allergy, enterocolitis, flow cytometry, T cell.

## Introduction

Recent studies investigating the mechanisms that determine the tissue specificities of particular subsets of lymphocytes have demonstrated that the tissue-selective homing of lymphocytes is mainly regulated at the level of the interaction between homing receptors on lymphocytes and their ligands on post-capillary venular endothelial cells. The lymphocyte/endothelial cell adhesion molecule pairs thought to participate in tissue-selective lymphocyte homing include skin-selective homing receptor, cutaneous lymphocyte antigen, the peripheral lymph node homing receptor L-selectin, the gut-homing receptor α4β7 and αEβ7 integrin.[1–6]

Milk-induced enterocolitis is frequently observed in infants with milk allergy. Although it has been suggested that cellular immunity is involved,[7–12] the mechanisms for the development of milk allergic enterocolitis remain to be determined. Several investigators have reported α4β7 integrin to act as a putative homing receptor to the gut,[4–6] suggesting that this integrin may be involved in the development of food-induced enterocolitis. However, its role in such pathological conditions so far has not been demonstrated. In the present study we investigated the expression of α4β7 integrin on T cells from patients with milk-induced enterocolitis by stimulating them with αs-casein, a major allergen in milk proteins.

## Methods

Profiles of subjects studied are presented in Table 1. Informed consent was obtained from all subjects after the purpose of the study had been explained. Milk allergy was diagnosed by cessation of diarrhea after milk elimination and the occurrence of diarrhea after a milk challenge test.

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Upon milk challenge test, patient (P)1 and P3 also presented mild skin rash. Patient 2 presented diarrhea but not skin rash only after the intake of large amounts of milk. Two hundred thousand peripheral blood mononuclear cells (PBMC) were cultured for 7 days with or without 200 μg/mL αs-casein, a major allergen in milk, in 96-well U-bottom plates at 37°C under 7% $CO_2$ in RPMI 1640 supplemented with 2 mmol/L L-glutamine, antibiotics, $2 \times 10^{-5}$ mol/L mercaptoethanol and 10% human AB serum. Cells were collected and washed with RPMI 1640 and incubated either with anti-human α4β7 integrin (Act-I)[13] or with control mouse $IgG_1$ antibody (Becton Dickinson, Mountain View, CA, USA) for 30 min on ice. After washing with cold phosphate-buffered saline, cells were incubated for 30 min with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (KPL, Gaithersburg, MD, USA), then incubated with phycoerythrin-labeled monoclonal anti-CD4 antibody (Becton Dickinson). After washing, cells were analyzed by flow cytometry on a FACStar using CELLQUEST software (Becton Dickinson). The cellular proliferation of PBMC after 7 days culture was measured using a [$^3$H]-TdR assay as described previously.[14,15]

## Results and Discussion

When PBMC from P1 were stimulated with αs-casein, the $CD4^+$ T cells expressed high levels of α4β7 integrin compared with unstimulated T cells (Fig. 1Aa). Patient 2, who suffered diarrhea after the intake of large amounts of milk, displayed a small but identifiable fraction of $CD4^+$ T cells high in α4β7 integrin (Fig. 1Ab). Patient 1 became tolerant to milk ingestion (i.e. outgrew milk allergy) several months later and, at this time, the levels of α4β7 integrin expression on αs-casein-stimulated $CD4^+$ T cells did not increase but rather decreased compared with unstimulated T cells (Fig. 1Ac). The pattern of α4β7 integrin expression on $CD4^+$ T cells when P1 became tolerant to milk was similar to the pattern seen on healthy controls (H)1 and H2 (Fig. 1Ad,e). Peripheral $CD4^+$ T cells from P3 contained α4β7 integrin-high populations following stimulation with αs-casein (Fig. 1Ba). After the clinical outgrow of milk allergy, this high α4β7 integrin population disappeared (Fig. 1Bb). The number of $CD4^+$ T cells expressing L-selectin at the time of active

Table 1. Subjects in the present study

| Subjects | Age (months) | Sex | IgE RAST score to milk |
|---|---|---|---|
| P1 | 6 | M | 2 |
| P2 | 24 | F | 0 |
| P3 | 8 | F | 2 |
| H1 | 8 | M | 0 |
| H2 | 12 | F | 0 |

P, patient; H, healthy control.

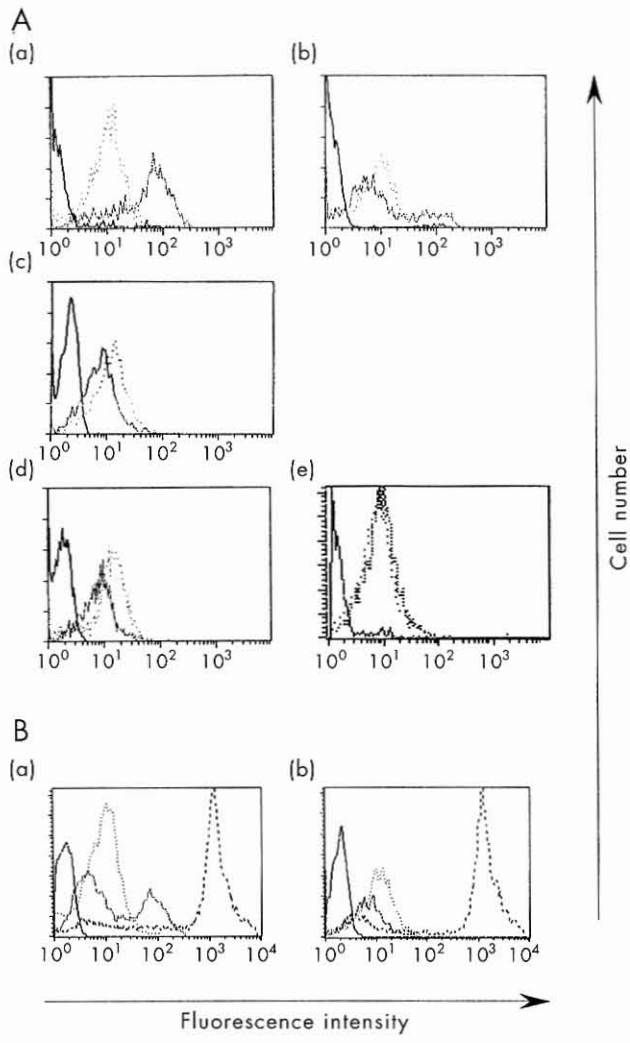


**Fig. 1** Expression of α4β7 integrin on $CD4^+$ T cells 7 days after *in vitro* treatment of peripheral blood mononuclear cells with or without 200 μg/mL αs-casein. (-------), $CD4^+$ T cells cultured with αs-casein and stained with anti-α4β7 integrin (Act-I); (- - - -), $CD4^+$ T cells cultured without αs-casein and stained with Act-I; (——), $CD4^+$ T cells cultured with αs-casein and stained with control IgG1; (– – –), $CD4^+$ T cells cultured with $\alpha_s$-casein and stained with anti-L-selectin. (Aa) P1 during periods of active milk allergy; (Ab) P2; (Ac) P1 after becoming tolerant to milk ingestion; (Ad) H1; (Ae) H2. (Ba) P3 during periods of active milk allergy; (Bb) P3 after becoming tolerant to milk ingestion.

milk allergy and that when tolerance to milk was established did not differ. These results suggest that the milk allergen-induced expression of α4β7 integrin on T cells may be involved in the pathogenesis of milk-induced enterocolitis.

While the levels of α4β7 integrin on αs-casein-stimulated CD4$^+$ T cells are high in milk allergic enteritis patients, similar levels of proliferative responses of PBMC to αs-casein were observed both in active milk allergy and outgrown milk allergy (Fig. 2). The magnitude of the proliferative response of the healthy control to αs-casein was higher than that of patients. However, there was no statistically significant difference among proliferative responses when the stimulation index ([$^3$H]-TdR incorporation with αs-casein-stimulation divided by [$^3$H]-TdR incorporation without αs-casein) was compared (data not shown). These results indicate that αs-casein-reactive T cells are neither anergized nor deleted in healthy subjects or patients who outgrow milk allergy. The results further suggest that the presence of circulating αs-casein-reactive T cells does not simply lead to the development of milk-induced enterocolitis, but rather that certain activation states, such as high expression levels of gut-homing receptor on milk allergen-specific T cells, may be involved in the pathogenesis of milk-induced enterocolitis. There may be at least two populations in αs-casein-reactive T cells in patients with active milk allergic enterocolitis; one with high α4β7 integrin expression and another with low α4β7 integrin expression on antigenic stimulation. The former population may be involved in the pathogenesis in enterocolitis and tolerance of this population may lead to the outgrow of the allergy. A recent report by Hesterberg *et al.*,[16] showing that injection of antibody to α4β7 integrin rapidly resolved spontaneous colitis of tamarins, suggests that this integrin is important in the pathogenesis of inflammatory diseases of the gut.

It has been reported that PBMC from patients with allergic enterocolitis produce high levels of tumor necrosis factor (TNF)-α compared with healthy controls[12] and that TNF-α may directly injure the gut epithelium, leading to enterocolitis. Thus, it is interesting to study whether T cells that express high levels of α4β7 integrin from patients with active milk-induced enterocolitis produce TNF-α. Further investigations are needed to determine whether TNF-α induces or enhances the expression of α4β7 integrin on T cells or a counter-receptor for α4β7 integrin, MAdCAM-1, on post-capillary venular endothelial cells in the gut.

In summary, we have shown here that the abnormal upregulation of α4β7 integrin on food allergen-reactive T cells may be involved in the development of allergic enterocolitis. More detailed studies of the mechanism and regulation of gut-homing receptor expression on T cells will contribute to a better understanding of the pathogenesis and treatment of food-induced enterocolitis.

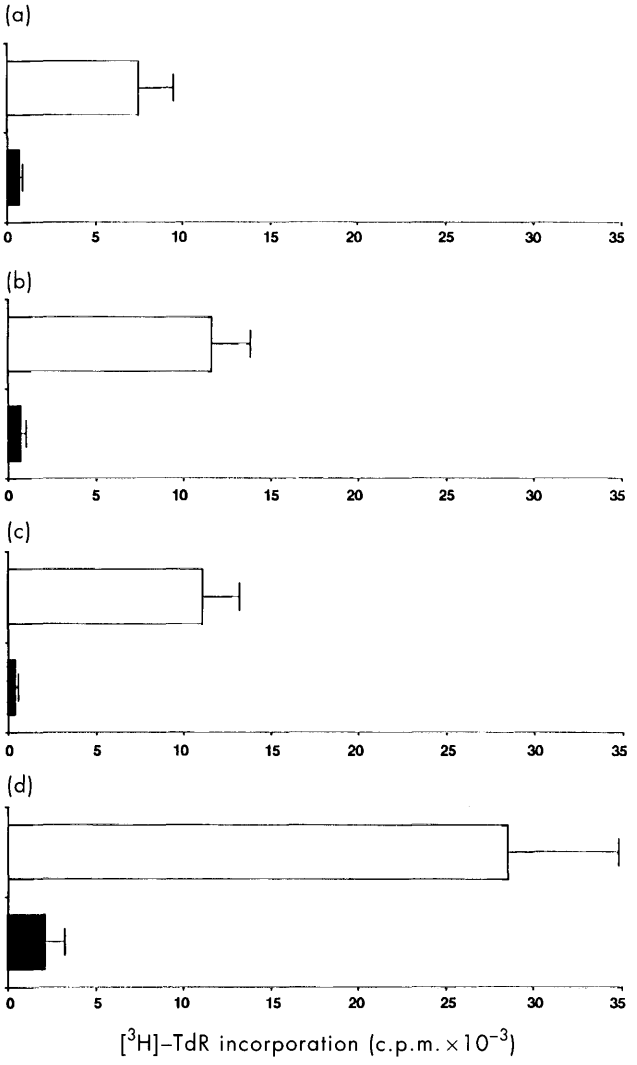


**Fig. 2** *In vitro* proliferative responses of peripheral blood mononuclear cells from subjects in Fig. 1A to αs-casein. Peripheral blood mononuclear cells were cultured with (□) or without (■) 200 μg/mL αs-casein for 7 days and [$^3$H]-Tdr incorporation was measured by liquid scintillation counting. Data are the mean ± SD of triplicate cultures. A proliferation assay was performed at the same time as the flow cytometry analysis in Fig. 1A. (a) P1 during a period of active milk allergy; (b) P2; (c) P1 after becoming tolerant to milk ingestion; (d) H1.

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