

# Cholesterol selectively enhances in vitro latex-specific IgE production in atopic dermatitis patients with latex allergy

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## Abstract

Effect of cholesterol on in vitro latex-specific IgE production by mononuclear cells from atopic dermatitis patients with latex allergy. Cholesterol enhanced latex-specific IgE production in a dose-dependent fashion, and maximal enhancement was achieved at 1 µg/ml. In contrast, cholesterol had no effect on latex -specific IgA or IgG4 production. Study for cytokine production revealed that cholesterol decreased latex-induced production of IFN-γ and IL-12, while it increased latex-induced production of IL-4, IL-10 and IL-13. These results indicate that cholesterol skews cytokine pattern toward Th2 type. Collectively, cholesterol may increase allergen-specific IgE production, which may in turn aggravate allergic symptoms.

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**Keywords:** Cholesterol; Latex-specific IgE production; Atopic dermatitis; Latex allergy

## Introduction

Patients with atopic dermatitis (AD) had frequently hypercholesterolemia (Kimata, 2001a), and hypercholesterolemia enhances allergen-induced skin wheal responses (Kimata, 2001b). In addition, cholesterol enhanced cytokine production in ionomycin-stimulated mouse mast cells (Baumruker et al., 2003). On the other hand, hypercholesterolemia leads Th2 responses and increase antigen-specific IgE production in mice (Robertson et al., 2004). Dietary restriction decreased IgE production in mice (Fan et al.,

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2001; Yoshino et al., 2003). These results suggest that cholesterol may be involved in allergic responses. However, direct effect of cholesterol on allergen-specific IgE production in human was not reported.

Peripheral blood mononuclear cells (MNC) from AD patients with latex allergy produced latex-specific IgE upon stimulation with latex allergen, while MNC from AD patients without latex allergy failed to do so (Kimata, 2001c, 2003). Using this in vitro system, the effect of cholesterol on latex-specific IgE production was studied.

## Materials and methods

### Subjects

After obtaining informed consents, peripheral blood was sampled from 15 AD patients with latex allergy. All of the patients had moderate AD according to SCORAD index (Kunz et al., 1997), and were

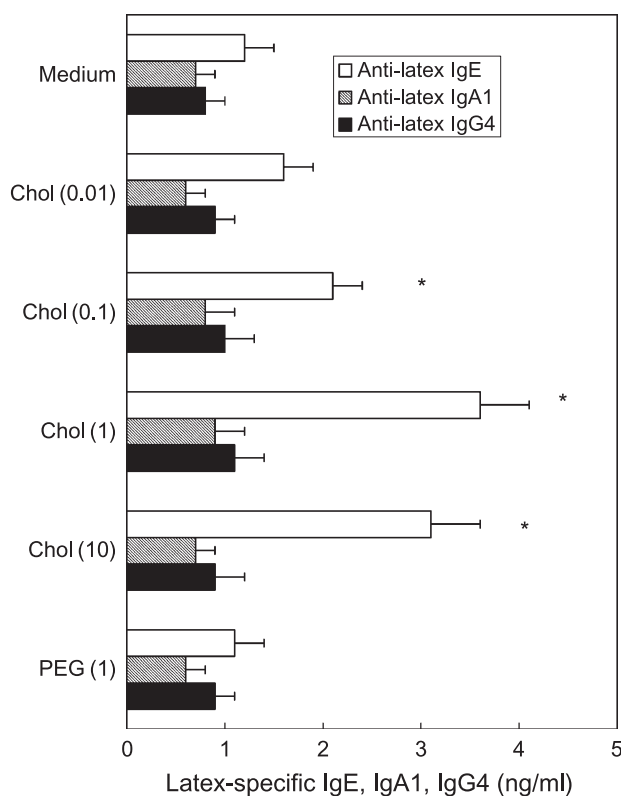


Fig. 1. Effect of cholesterol on latex-specific IgE, IgA1 and IgG4 production by mononuclear cells from AD patients with latex allergy. Mononuclear cells from AD patients with latex allergy were stimulated with latex (0.1 ng/ml) with or without indicated factors, and latex-specific IgE, IgA1 and IgG4 production was measured. Cholesterol (Chol) was used from 0.01  $\mu$ g/ml, 0.1  $\mu$ g/ml, 1  $\mu$ g/ml or 10  $\mu$ g/ml, as indicated as Chol (0.01), Chol (0.1), Chol (1), or Chol (10). Polyethylene glycol (PEG) was used at 1  $\mu$ g/ml as PEG (1) as control. Values are the means  $\pm$  SD from 3 experiments. \* Significant enhancement ( $P < 0.05$ ) compared to cultures with medium.

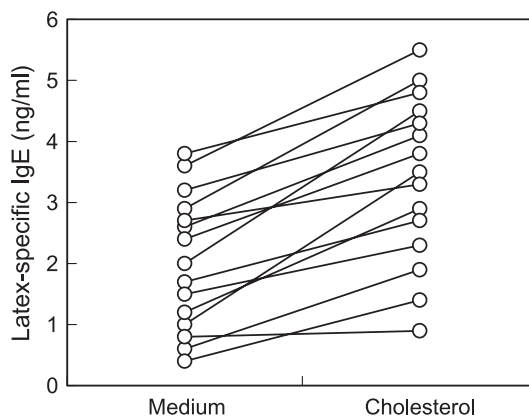


Fig. 2. Enhancement of latex-specific IgE production in 15 AD patients with latex allergy by cholesterol. Mononuclear cells from 15 AD patients with latex allergy were stimulated with latex (0.1 ng/ml) with or without cholesterol (1  $\mu$ g/ml), and latex-specific IgE production was measured. Each value is mean of triplicate cultures in each patient. \* Significant enhancement ( $P < 0.01$ ) compared to cultures with medium.

allergic to latex as determined by clinical symptoms to latex, positive skin prick test to latex, and positive serum latex-specific IgE (Kimata, 2003). In contrast, none of them had allergy to Japanese cedar pollen (JCP). Mean serum latex-specific IgE was 36.7 IU/ml, while serum JCP-specific IgE was not detected ( $< 0.34$  IU/ml). All of AD patients were treated with application of mixture of pinetar (Yoshida Pharmaceutical Co., Ltd. Tokyo, Japan) and zinc oxide ointment, but without oral medication (Kimata, 2004).

### Culture systems

Mononuclear cells (MNC) from blood were separated, and they were cultured ( $1 \times 10^5/0.2\text{ml/well}$ ) with 0.1 ng/ml of latex allergen (C-serum, kindly provided by Dr. Akira Akasawa, Department of

Table 1  
Effect of cholesterol on latex-induced cytokine production

| Cytokine      | Cytokine production (pg/ml) |             |           |             |           |             |
|---------------|-----------------------------|-------------|-----------|-------------|-----------|-------------|
|               | Patient 1                   |             | Patient 2 |             | Patient 3 |             |
|               | Medium                      | Cholesterol | Medium    | Cholesterol | Medium    | Cholesterol |
| IFN- $\gamma$ | 257 (52)                    | 178 (31)*   | 152 (25)  | 101 (17)*   | 348 (63)  | 211 (50)*   |
| IL-12         | 135 (24)                    | 92 (17)     | 197 (32)  | 121 (20)*   | 314 (72)  | 178 (45)*   |
| IL-4          | 489 (73)                    | 597 (98)**  | 536 (98)  | 781 (115)** | 468 (89)  | 634 (123)** |
| IL-10         | 244 (32)                    | 316 (54)**  | 317 (45)  | 418 (69)**  | 168 (43)  | 273 (58)**  |
| IL-13         | 326 (51)                    | 428 (63)**  | 257 (41)  | 345 (65)**  | 459 (94)  | 673 (117)** |

MNC from AD patients with latex allergy were cultured with latex (0.1 ng/ml) with medium or cholesterol (1  $\mu$ g/ml), and cytokine production was measured.

Values are the means (SD) of quadruplicate cultures in 3 patients. Although not shown, similar results were obtained in other 12 patients.

\* Significant decrease ( $p < 0.01$ ) compared to cultures with medium.

\*\* Significant increase ( $p < 0.01$ ) compared to cultures with medium.

Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo, Japan) (Kimata, 2001c, 2003). Cultures were stimulated with different concentrations of cholesterol coupled to polyethylene glycol (PEG) or PEG as control (Sigma, St. Louis, MO). After 14 days of culture, production of latex-specific IgE, IgA1 and IgG4 was determined by ELISA (Kimata, 2003; Kimata et al., 1996), while after 5 days of culture, production of IL-4, IL-10, IL-12, IL-13 and IFN- $\gamma$  was determined by ELISA (R and D Systems, Inc. Minneapolis, MN). In some experiment, MNC were also cultured with 0.1 ng/ml JCP (TORII PHAR, Co., Ltd., Tokyo, Japan) for 14 days, and JCP-specific IgE, IgA and IgG4 production was measured (Kimata, 2003; Kimata et al., 1996). The culture medium was serum-free medium, DME, supplemented with Ham's Nutrient (DME/F-12; Sigma Chemical Co. (ST. Louis. MO), 0.5% BSA, and 50  $\mu$ g/ml transferrin (Kimata et al., 1996). Statistical analysis was performed with Student's *t*-test. These studies were conducted with the approval of the Ethical Committee at the Ujitakeda Hospital.

## Results

As shown in Fig. 1, cholesterol coupled to PEG (for solubility reason) enhanced latex-specific IgE production in a dose-dependent fashion, and maximal enhancement was achieved at 1  $\mu$ g/ml of cholesterol. The carrier alone (PEG) had no effect in such assay. In contrast, cholesterol failed to enhance production of latex-specific IgA1 or IgG4 (Fig. 1). Although not shown, MNC stimulated with 0.1 ng/ml of latex failed to produce latex-specific IgG1, IgG2, IgG3, IgM or IgA2, and addition of cholesterol at concentrations from 0.01 to 10  $\mu$ g/ml failed to induce them.

In 15 patients, cholesterol significantly ( $P < 0.01$ ) enhanced latex-specific IgE production (Fig. 2). The levels of latex-specific IgE production (mean  $\pm$  SEM ng/ml) in cultures were  $2.0 \pm 0.3$  ng/ml (with medium) and  $3.4 \pm 0.4$  ng/ml (with cholesterol).

To confirm that this enhancement was allergen specific to which patients were allergic, MNC were cultured with JCP (0.1 ng/ml) to which patients were not allergic. In 15 patients, MNC did not produce JCP-specific IgE ( $< 0.3$  ng/ml), and addition of cholesterol at concentrations from 0.01 to 10  $\mu$ g/ml failed to induce JCP-specific IgE ( $< 0.3$  ng/ml).

Latex-specific IgE production was increased by Th2 cytokines, IL-4, IL-10 and IL-13, while it was decreased by Th1 cytokines, IFN- $\gamma$  and IL-12 as previously reported (Kimata, 2003). Therefore, effect of cholesterol on latex-induced cytokine production was studied. As shown in Table 1, cholesterol decreased Th1 cytokines, IFN- $\gamma$  and IL-12, while cholesterol increased Th2 cytokines, IL-4, IL-10 and IL-13 in 3 representative patients. Identical results were observed in other 12 patients (data not shown). These results indicate that cholesterol skewed cytokine pattern toward Th2 type, which in turn enhanced latex-specific IgE production.

## Discussion

These results demonstrated that cholesterol enhanced latex-specific IgE production without affecting latex-specific IgA1 or IgG4 production in AD patients with latex allergy. Moreover, enhancement was specific to allergen to which patients were allergic, since cholesterol failed to induce JCP-specific IgE production by JCP-stimulated MNC from 15 AD patients who were not allergic to JCP. The selective

enhancement of latex-specific IgE production is due to the cholesterol-induced skewing cytokine pattern toward Th2 type, since cholesterol increased IL-4, IL-10 and IL-13 production while it decreased IFN- $\gamma$  and IL-12 production in latex-stimulated cultures. This is not surprising. We and others have previously reported that skewing cytokine toward Th2 type differentially regulate IgE, IgG subclass or IgA subclass production (Akdis et al., 1998; Carballido et al., 1994; Kimata et al., 1991; Ohmori et al., 1990). Detailed mechanisms of selective enhancement of latex-specific IgE production are currently under investigation.

We have also studied the effect of cholesterol on other allergen-specific IgE production. In preliminary study, 15 AD patients with house dust mite (HDM) and JCP allergy, and 15 allergic rhinitis (AR) patients with JCP allergy but without HDM allergy, were studied. MNC from these AD patients or AR patients were cultured with HDM (0.1 ng/ml) or JCP (0.1 ng/ml) with or without cholesterol (1  $\mu$ g/ml). Cholesterol enhanced HDM-specific IgE production and JCP-specific IgE production from  $4.5 \pm 0.4$  ng/ml to  $7.9 \pm 0.6$  ng/ml and from  $3.4 \pm 0.3$  ng/ml to  $5.2 \pm 0.4$  ng/ml, respectively, in AD patients. On the other hand, cholesterol enhanced JCP-specific IgE production from  $2.2 \pm 0.2$  ng/ml to  $3.6 \pm 0.3$  ng/ml, while cholesterol failed to induce HDM-specific IgE production ( $< 0.3$  ng/ml) in AR patients. Collectively, these results indicate that cholesterol may enhance allergen-specific IgE production in allergic patients.

It was reported that cholesterol increased activation of mast cells, which may aggravate allergic symptoms (Baumruker et al., 2003; Sheets et al., 1999). Our results indicate that cholesterol also enhances allergen-specific IgE production, which may also increase mast cell activation. In fact, we have previously reported that hypercholesterolemia increased allergic skin wheal responses (Kimata, 2001b). Therefore, it is tempting to speculate that decrease in cholesterol may alleviate allergic symptoms. This is important since decrease in cholesterol may be useful in the treatment of allergic diseases. In fact, decrease in cholesterol by fasting improved skin symptoms in AD patients (Kimata, 2002). Moreover, decrease in cholesterol by vegetarian diet or short-term fasting improved skin symptoms of AD (Nakamura et al., 2003; Tanaka et al., 2001).

We are now studying the effect of decrease in cholesterol on allergen-specific IgE production in vivo and in vitro and clinical symptoms in large scale of AD patients.

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