following exercise. In addition, processed cheese but not milk induced anaphylaxis following ingestion of aspirin. Moreover, we identified a new IgE-binding antigenic protein present in cheese extracts stable even under the presence of pepsin.

A 40-year-old Japanese man was referred to us with a 20-year history of occasional anaphylactic episodes, characterized by urticaria and occasional loss of consciousness. The episode occurred after ingestion of pizza, cakes, pastas, sandwiches or cheese. He was otherwise healthy. The radio-immunosorbent test for total IgE antibodies was 408 IU ml^{-1} (the normal value is <250). Radioallergosorbent testing (RAST) for specific IgE reactions revealed no reactivity to egg white, egg yolk, and shrimp, borderline reactivity to alpha-lactalbumin and beta-lactoglobulin, and positive reactivity to cow's milk, cheese, casein and wheat.

To further address its pathology, prick tests were performed. Reactions were positive for milk and milk proteins such as casein, lactoferrin, alpha-lactalbumin, and beta-lactoglobulin, but negative for wheat, egg white, buckwheat, and peanut (Fig. 1a, left and middle). The patient also exhibited strong positive reactions to dairy products, including cow's milk, cheese and yogurt (Fig. 1a, right).

The patient was admitted to our hospital. Provocation tests were performed with (A) food ingestion followed by the step-ladder exercise and (B) food ingestion preceded by aspirin intake. Ingestion of milk (500 ml) or wheat (100 g) alone, or preceded by 333 mg aspirin or followed by exercise provoked no allergic symptoms. Ingestion of processed cheese (100 g) followed by the step-ladder exercise for 30 min did not induce any symptom. However, when the patient consumed aspirin before ingestion of food, he exhibited generalized urticaria, nasal obstruction, hypotension, and dyspnea (Fig. 1b).

We then sought to identify the IgE-binding antigenic proteins present in cheese extracts by immunoblot analysis. On immunoblot analysis, serum from the patient had IgE antibodies that reacted with a protein possessing a molecular weight of about 75-kDa substantially in milk and cheese extracts, and in minute concentrations in lactoferrin and casein (Fig. 2a). The sera from the healthy controls did not have these antibodies. In addition, the 75-kDa protein was apparently observed in milk and cheese (Fig. 2b).

Oddly, despite the fact that the 75-kDa protein was found in milk and cheese, only the cheese and aspirin combination, but not the milk and aspirin combination, induced anaphylaxis. In order to understand the reason for this, we compared the efficacy of peptic digestion of milk and cheese. Milk and cheese were hydrolyzed with 0.1N HCl, and a 1 mg/ml milk solution and a 0.1 g/ml cheese solution were digested with pepsin (50 μg/ml) for 0, 15, 30 and 45 min at

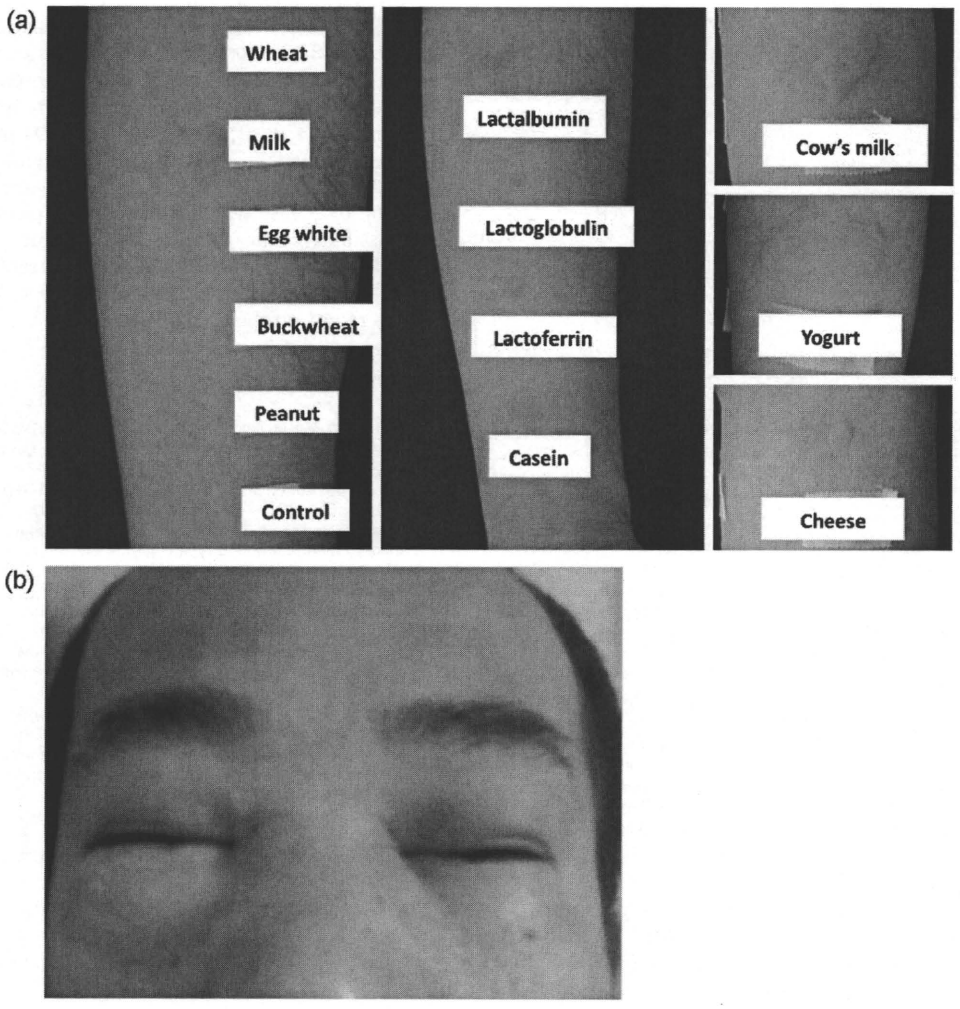

Fig. 1. Prick tests and provocation tests. Reactions were positive for milk (a, left panel), and milk proteins such as casein, lactoferrin, lactalbumin, and lactoglobulin (a, middle panel), but negative for wheat, egg white, buckwheat, and peanut (a, left panel). In addition, reactions were positive for dairy products, such as cow's milk, cheese and yogurt (a, right panel). The patient exhibited generalized urticaria, nasal obstruction, hypotension, and dyspnea induced by the combination of oral administration of aspirin followed by ingestion of processed cheese (b).

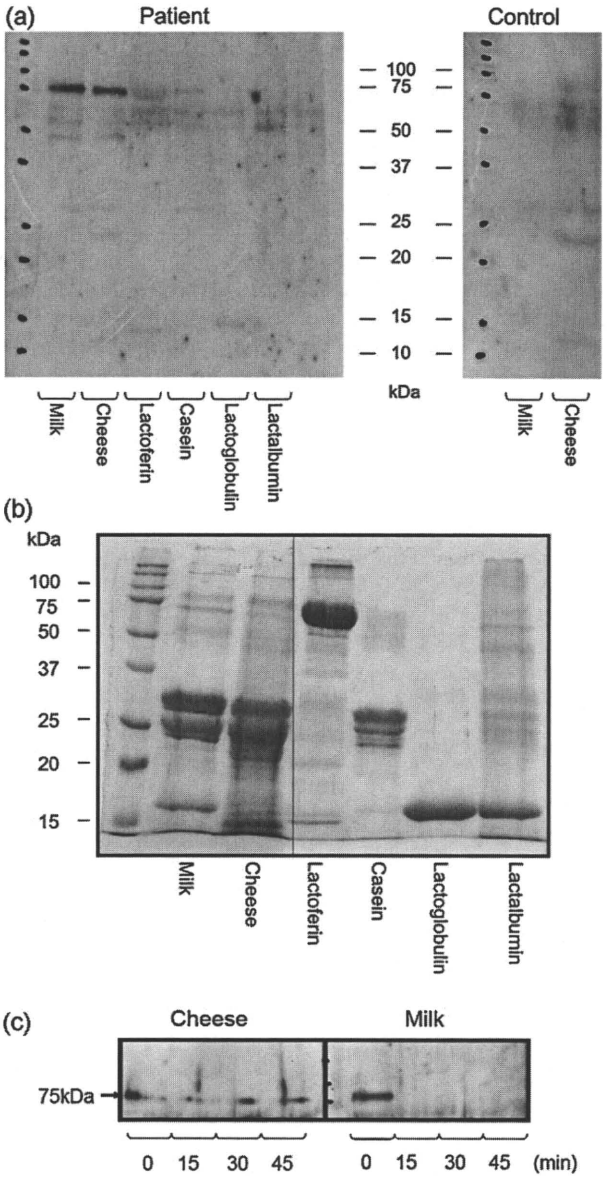

Fig. 2. IgE immunoblot analysis, CBB-stained proteins, and peptic digestion. (a) IgE immunoblot analysis. The proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels were transferred onto a nitrocellulose membrane under semidry conditions. Bound IgE antibodies were detected using horseradish peroxidase-conjugated goat anti-human IgE antibodies and an ECL-Western blotting kit. A protein with molecular weight of about 75-kDa in milk and cheese extracts reacts strongly with IgE antibodies from the patient's sera, but not with sera from healthy control. (b) The test proteins were separated by SDS-PAGE. This CBB stain of the total proteins of milk, cheese, lactoferrin, casein, lactoglobulin, and lactalbumin shows that the 75 kDa protein is detected in milk and cheese, but not in casein, lactoferrin, lactalbumin, and lactoglobulin. (c) The effects of peptic digestion on the 75-kDa protein are compared between milk and cheese. The 75-kDa protein in milk is digested by pepsin within 15 min, but the protein in cheese remains incompletely digested even after 45 min of incubation with pepsin.

37 °C. The 75-kDa protein in milk was digested by pepsin within 15 min, but the same protein in cheese remained incompletely digested even after 45-min incubation with pepsin (Fig. 2c).

In this case report, anaphylaxis was induced in the subject by the combination of orally-administered aspirin and processed cheese but not by the combination of aspirin and milk. Food-dependent salicylate-induced anaphylaxis (FDSIA) describes a condition wherein anaphylactic symptoms are caused by the combination of aspirin and certain foods. Accordingly, aspirin and NSAIDs have been described as precipitating factors in FDEIA [3,7]. It has been speculated that aspirin triggers allergic reactions in patients with certain food allergies [4]. However, the precise mechanism underlying FDSIA remains unclear.

The present patient showed positive reactions on RAST and prick tests to milk and cheese, suggesting that dairy products were responsible for his symptoms. However, the provocation tests yielded the puzzling result that the patient only developed anaphylaxis from the cheese and aspirin combination, not the milk and aspirin combination. By immunoblot analysis, we found that the patient had circulating IgE antibodies specifically immunoreactive to a 75-kDa protein. This protein was different from casein, lactoferrin, lactalbumin, and lactoglobulin, and it was present in significant concentrations in cheese and milk. The detection of weak concentrations of this protein in the lactoferrin and casein preparations used for the immunoblot analysis seems to indicate that this protein is present in small amounts in these substances. Thus, it may be possible that this allergen is a minor but allergically important protein in dairy products.

This is the first report on a unique 75-kDa protein in dairy products that is a possible cause of food-dependent anaphylaxis. In the present case, ingestion of cheese, but not milk, preceded by aspirin induced anaphylaxis. This discrepancy may be explained by the different rates at which each of these foods is digested. Therefore, acidic microenvironment may be responsible for digestion of this protein and may expose a dichotomy regarding the different allergic responses to ingested milk and cheese. Because of the ability of aspirin to promote intestinal absorbance [8], it appears that aspirin allows a 75-kDa protein to be rapidly absorbed through the intestinal mucous membranes. Interestingly, our patient had previous episodes of anaphylaxis following ingestion of cheese without preceding aspirin intake. This raises the possibility that certain dairy products contain salicylate derivatives as preservatives. We are currently trying to purify this 75-kDa protein and identify its amino acid sequences and we hope to report on this in the future.

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Letter to the Editor

CD109 expression levels in malignant melanoma

To the Editor

Malignant melanoma is one of the most aggressive cancers. Recently, an increasing number of biomarkers for malignant melanoma like S100, MART-1, and gp100/HMB45 have been identified [1]. We also provided the possibility that Zinc finger protein 28 could be a biomarker for malignant melanoma [2]. These biomarkers are useful for a more detailed diagnostic categorization for malignant melanoma.

The CD109 gene encodes a glycosyl-phosphatidylinositol-anchored glycoprotein that is a member of the alpha (2)-macroglobulin/C3, C4, C5 family of thioester-containing proteins [3]. CD109 interacts directly with the type I transforming growth factor-beta (TGF-ß) signaling receptor and negatively modulates TGF-ß signaling [4]. CD109 transcript is highly expressed in squamous cell carcinoma and glioblastoma cell lines, but their levels are undetectably low in neuroblastoma and small-cell lung carcinoma cell lines [5]. However, there is very limited information about CD109 in malignant melanoma. In this study, we demonstrated that CD109 is expressed in malignant melanoma in humans as well as in mice and is a potential biomarker of malignant melanoma.

We previously established RFP–RET-transgenic mice of line 304/B6 (RET-mice) in which RET is expressed under the control of *metallothionein-I* promoter and enhancer [6]. In RET-mice, systemic hyperpigmented skin, benign melanocytic tumors and malignant melanoma developed stepwise accompanying metastasis [7,8]. A previous study revealed increased levels of CD109 transcript in oncogenic RET (RET-MEN2B)-transfected NIH3T3 cells *in vitro* [5]. In this study, we examined whether CD109 transcript expression levels are also increased in tumors from RET-mice *in vivo* (Fig. 1a). As shown in Fig. 1a, the CD109 transcript expression levels increased with increase in tumor size. Interestingly, the CD109 transcript expression levels in malignant melanoma were 14–700-fold higher than those in benign melanocytic tumors in the RET-mice. The mean value of CD109 transcript expression levels in malignant melanoma was more than 80-fold higher than that in benign melanocytic tumors (Fig. 1b). The difference between CD109 expression levels in benign tumors and malignant melanoma in the RET-mice was statistically ($P < 0.01$) significant (Fig. 1b). These results suggest that CD109 is associated with oncogenic RET-mediated melanomagenesis. We then prepared a murine melanocyte cell line (melan-a; lane 1 in Fig. 1c) and a Mel-ret cell line derived from a tumor in a RET-mouse (lane 2 in Fig. 1c) [7] and compared the CD109 expression levels. CD109 transcript expression levels in Mel-ret cells were more than 300-fold higher than those in

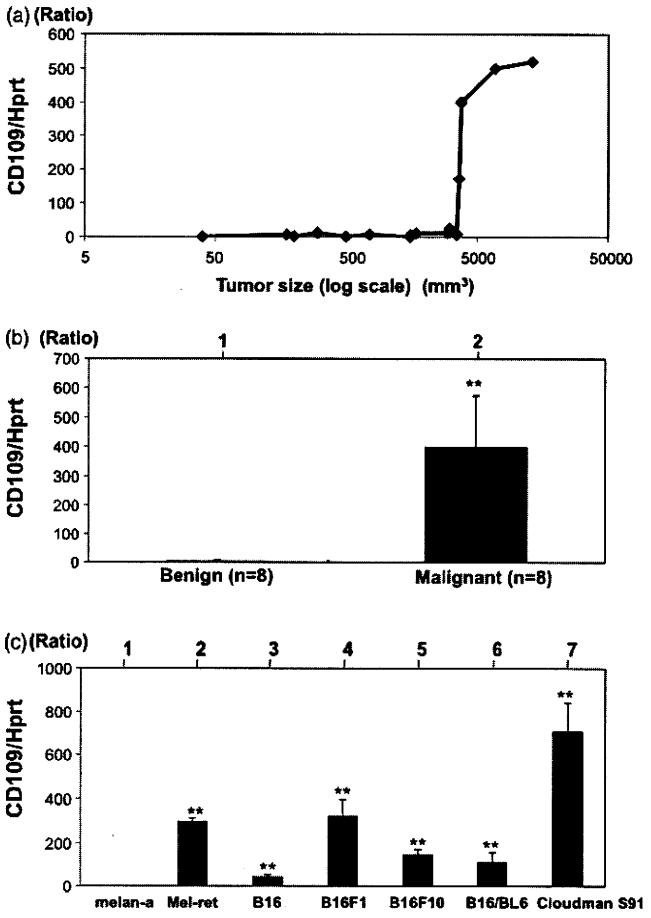

Fig. 1. CD109 transcript expression levels in murine cell lines and tissues. (a–c) CD109 transcript expression levels in murine cells and tissues were measured by quantitative polymerase chain reaction (Q-PCR) using the primers TGATAACGGGAAACTCAACCTT and TGCCTGTCTCCTTGGTTCAT for CD109 and the primers CTTTGCTGACCTGCTGGATT and TATGTCCCCCGTTGACTGAT for Hprt. (a) CD109 transcript expression levels in tumors of various sizes (40–17500 mm^3) from RFP–RET-transgenic mice of line 304/B6 (RET-mice). The result derived from one set of experiment is shown. (b) CD109 transcript expression levels (mean ± SD) in histopathologically defined benign melanocytic tumors ($n = 8$) and malignant melanomas ($n = 8$) from RET-mice. ** Significantly different ($P < 0.01$) from the control by the Mann-Whitney *U*-test. (c) CD109 expression levels (mean ± SD) in murine melanocytes (malan-a) and 6 melanoma cell lines (Mel-ret, B16, B16F1, B16F10, B16/BL6, Cloudman S91). Q-PCR was performed following the previous method using Hprt as an internal control [9]. ** Significantly different ($P < 0.01$) from the control by the Kruskal–Wallis test. Representative results of three independent experiments with consistent results are shown.