

IgE reactivity to $\alpha 1$ and $\alpha 2$ chains of bovine type I collagen in children with bovine gelatin allergy

Masahiro Sakaguchi, PhD,^a Hisae Hori, PhD,^c Shunji Hattori, PhD,^d Shinkichi Irie, PhD,^d Atsushi Imai, DVM,^f Makoto Yanagida, PhD,^f Hiroshi Miyazawa, PhD,^e Masako Toda, PhD,^a and Sakae Inouye, MD^b Tokyo and Gunma, Japan

Background: Anaphylactic reactions to measles, mumps, and rubella vaccines, including gelatin as a stabilizer, have been reported. It had been found that most of these reactions to live vaccines are caused by the bovine gelatin included in these vaccines. Gelatin mainly includes denatured type I collagen, which consists of $\alpha 1$ and $\alpha 2$ chains.

Objective: The current study was designed to investigate the IgE reactivity to $\alpha 1$ and $\alpha 2$ chains of bovine type I collagen in gelatin-sensitive children.

Methods: Serum samples were taken from 10 children who had anaphylaxis to the vaccines and high levels of specific IgE to bovine gelatin. Bovine type I collagen was isolated from bovine skin and then separated to $\alpha 1$ and $\alpha 2$ chains by column chromatography. IgE reactivity to denatured type I collagen and its $\alpha 1$ and $\alpha 2$ chains was analyzed by immunoblotting, ELISA, and histamine release from the mast cells passive sensitized with IgE antibodies in pooled serum of the children.

Results: All children had specific IgE to bovine type I collagen. Furthermore, IgE antibodies in their sera reacted with the $\alpha 2$ chain but not with the $\alpha 1$ chain. Similarly, the mast cells sensitized with pooled sera in the children showed $\alpha 2$ chain-specific histamine release but not $\alpha 1$ chain-specific histamine release.

Conclusion: In gelatin allergy denatured bovine type I collagen is a major allergen and IgE-binding sites exist in the $\alpha 2$ chain of type I collagen. (*J Allergy Clin Immunol* 1999;104:695-9.)

Key words: Allergen, anaphylaxis, cross-reactivity, IgE, gelatin, vaccine

Anaphylactic reactions to measles, mumps, and rubella vaccines and the combined measles-mumps-rubella (MMR) vaccines have been reported and have been suggested to be caused by allergy to egg proteins present in the vaccines.¹ However, the anaphylactic reactions also have been reported to occur after administration of the MMR vaccine in the children who tolerated eggs.²⁻⁶

Abbreviations used

CM: Carboxymethyl

MMR: Combined measles-mumps-rubella

SCF: Stem cell factor

Kelso et al⁷ reported that a child who had anaphylaxis to MMR vaccine had IgE antibody to gelatin. Also, in previous studies⁸⁻¹⁰ we found that most children who had the systemic immediate-type reactions, including anaphylaxis, to measles, mumps, and rubella vaccines had anti-gelatin IgE. It is suggested that most of the systemic immediate-type reactions after vaccination are caused by gelatin present in the live vaccines as a stabilizer. Furthermore, we found that the systemic reactions to varicella and Japanese encephalitis vaccines that contain gelatin are caused by the gelatin in the vaccines.^{11,12} It has been reported that gelatin causes food allergy.⁹ Recently, we found that anaphylaxis to erythropoietin products containing gelatin was caused by the gelatin.¹³

Gelatin has long been believed to be nonimmunogenic and nonallergic to humans, and therefore gelatin has been widely used as a stabilizer in vaccines.¹⁴ In Japan, most of the live vaccines contain bovine gelatin.¹⁴ Gelatin, obtained from bone and skin, consists mainly of denatured type I collagen, which is composed of 2 $\alpha 1$ and 1 $\alpha 2$ chains.^{15,16}

The current study was designed to investigate the IgE reactivity to $\alpha 1$ and $\alpha 2$ chains of bovine type I collagen and its $\alpha 1$ and $\alpha 2$ chains in gelatin-sensitive children.

MATERIAL AND METHODS

Children

Physicians and vaccine manufacturers submitted serum samples from 10 children with anaphylaxis to live vaccines to the Japan National Institute of Infectious Diseases (former National Institute of Health). Table 1 lists 10 children (mean age $\pm$ SD, 2 years 4 months $\pm$ 1 year) who had anaphylaxis on vaccination. Two children (No. 7 and 8) had food allergy to gelatin.

As a negative control, serum samples from 26 children (14 boys, 12 girls) (mean age $\pm$ SD, 2 years 4 months $\pm$ 1 year) who had no reaction to measles vaccine containing gelatin (1 mg/dose) were collected.

Gelatin and collagen

Gelatin for ELISA was prepared as described previously.⁸ Bovine type I collagen was prepared from skin dermis by 0.5 mol/L

From the Department of Immunology^a and the Infectious Disease Surveillance Center,^b National Institute of Infectious Diseases, the Division of Adult Diseases, Medical Research Institute, Tokyo Medical and Dental University,^c the Nippi Research Institute of Biomatrix,^d the Department of Medical Technology, Kyorin University,^e Tokyo, and the Pharmaceutical Development Laboratory, Kirin Brewery,^f Gunma, Japan.

Received for publication Apr 20, 1999; revised May 28, 1999; accepted for publication May 28, 1999.

Reprint requests: Masahiro Sakaguchi, PhD, Department of Immunology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/100514

TABLE I. Children with anaphylaxis to vaccine and antigelin IgE levels in serum

Children			Vaccines		Reaction to vaccine	Time of onset (min)	Antigelin IgE (Ua/mL)
No.	Age	Sex	Vaccine	Amount of gelatin (mg/shot)			
1	3 y 6 mo	M	Mumps	1.0	Urticaria, wheezing	30	34.0
2	1 y 11 mo	M	Measles	1.5	Urticaria, airway obstruction with wheezing	10	251
3	1 y 6 mo	M	Mumps	1.0	Urticaria, wheezing cough	5	15.0
4	1 y 8 mo	F	Measles	1.5	Urticaria, airway obstruction with wheezing	15	42.5
5	4 y 11 mo	F	Mumps	1.0	Urticaria, wheezing cough	15	30.7
6	2 y 1 mo	M	Measles	1.5	Urticaria, cough	15	11.0
7	3 y 11 mo	M	Measles	1.0	Urticaria, hypotension	10	11.8
8	2 y 9 mo	M	Rubella	1.0	Urticaria, wheezing cough	15	62.9
9	1 y 7 mo	F	Measles	1.5	Urticaria, airway obstruction with wheezing	20	24.4
10	1 y 8 mo	F	Measles	1.5	Urticaria, cough	15	28.0

Serum that had more than 100 Ua/mL as specific IgE was diluted and measured.

acetic acid extraction and purified by differential salt precipitation.¹⁷

Isolation of α 1 and α 2 chains from collagen type I

The α 1 and α 2 chains from heat-denatured type I collagen were isolated by carboxymethyl (CM) cellulose (CMC 32, Whatman, Maidstone, UK) chromatography at 42°C and 0.06 mol/L sodium acetate buffer, pH 4.8, with use of a linear salt gradient described by Piez et al.¹⁸ Eluted fractions were monitored at 230 nm. Identification and assessment of purity were routinely achieved by sodium SDS-PAGE.^{19,20}

SDS-PAGE and immunoblotting

SDS-PAGE was performed according to the procedure of Laemmli.²⁰ The sample was applied to SDS-PAGE (5% gel) under reducing conditions. The gel then was stained with coomassie blue or the proteins in the gel were transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories, Hercules, Calif). The membrane was rinsed in PBS and blocked with 1% BSA in PBS. After another rinse with PBS containing 0.02% Tween 20, it was cut into strips, which were incubated overnight with the pooled serum of patients diluted 1:2. After washing, the strip was incubated with antihuman IgE antibody-conjugated alkaline phosphatase (Quidel, San Diego, Calif) overnight and stained with 5-bromo-4-chloro-3-indolyl phosphate/N-benzoyl-L-tyrosyl.²¹

Measurement of specific IgE antibody to gelatin and denatured collagen

The Pharmacia CAP system (Pharmacia, Uppsala, Sweden) was used to determine the concentration (unit allergen [Ua] per milliliter) of IgE antibody to gelatin (Wako Pure Chemical Industries, Osaka, Japan).⁸

Specific IgE antibodies to gelatin or denatured collagen were determined by a fluorometric ELISA as described previously.^{9,22} Microplates were coated with gelatin or denatured collagen (5 μ g/mL). After incubation overnight at 4°C, the serum sample was added to the wells. Each child's serum was diluted (1:10-1:200) to approximately 1 Ua/mL as the level of specific IgE to gelatin. Negative sera were diluted 1:10. After incubation for 3 hours at room temperature, antihuman IgE antibody conjugated with β -D-galactosidase (Pharmacia) was added. The enzyme reaction substrate was 0.1 mmol/L 4-methylumbelliferyl- β -D-galactoside (Sigma, St Louis, Mo). The fluorescence unit was measured on a fluorometric microplate reader (Fluoroskan, Flow Laboratory, McLean, Va).

Human mast cell

Cultured human mast cells were prepared as described previously.²³ Briefly, mononuclear cells were obtained from umbilical cord blood and cultured in minimum essential medium containing 20% FCS in the presence of recombinant human stem cell factor (SCF) (100 ng/mL) and IL-6 (10 ng/mL) (Kirin Brewery, Gunma, Japan). When the mast cells reached 100% purity, they were used for experiments.

Allergen-specific histamine release from cultured mast cells passive-sensitized with children's sera

Allergen-specific histamine release from mast cells was conducted as described previously.²⁴ Briefly, the mast cells were washed 3 times in minimum essential medium containing SCF (100 ng/mL) and IL-6 (10 ng/mL) and then incubated overnight with the sera at 37°C. During sensitization SCF and IL-6 were added to the cultures at 100 and 10 ng/mL, respectively. After 3 washes they were suspended in Tyrode-HEPES buffer (pH 7.4) containing 0.1% BSA in the presence of SCF (10 ng/mL) and then were preincubated at 37°C for 10 minutes. Then the cells were challenged with gelatin or heat-denatured collagen (5 μ g/mL) or antihuman IgE monoclonal antibody (4 μ g/mL) (Chemicon, Temecula, Calif) at 37°C for 30 minutes. The histamine contents in the supernatants and in the cell pellets were determined by an automated fluorometric procedure. The spontaneous release was assessed by the addition of a buffer instead of gelatin or collagen or anti-IgE mAb. The percentage of histamine release was calculated according to the following equation:

$$\frac{\text{Released histamine contents}}{\text{Total histamine contents}} \times 100 = \text{Percentage of histamine release}$$

RESULTS

Specific IgE to gelatin and denatured type I collagen in children with anaphylaxis to vaccines

We measured specific IgE to gelatin and denatured type I collagen in 10 children who had anaphylaxis on vaccination. All the children had specific IgE to gelatin (Table I). Similarly, all children had specific IgE to type I collagen, which mainly is included in gelatin (Fig 1). As a negative control, all 26 children who showed no reaction to measles vaccines containing gelatin had no specific IgE to bovine gelatin and type I collagen.

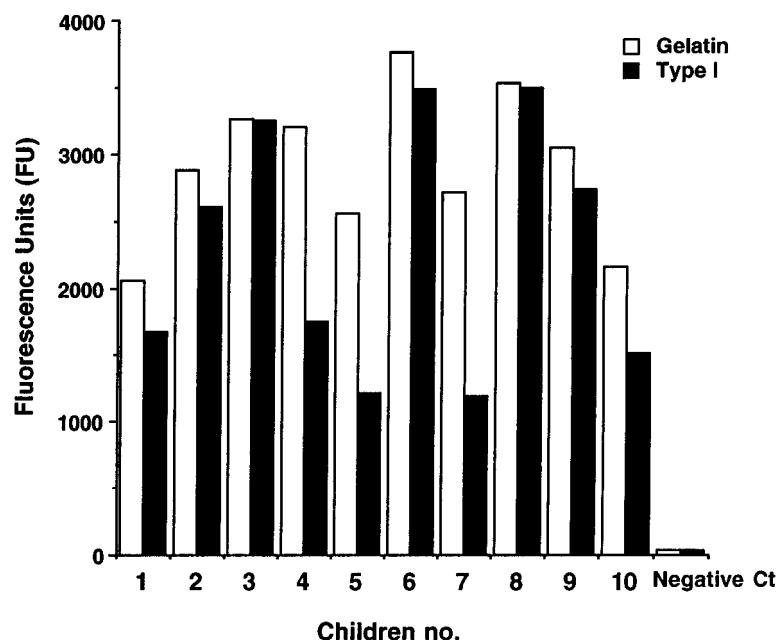


FIG 1. IgE reactivity to bovine gelatin and denatured type I collagen. Each child's serum was diluted to approximately 1 Ua/mL as level of specific IgE to gelatin.

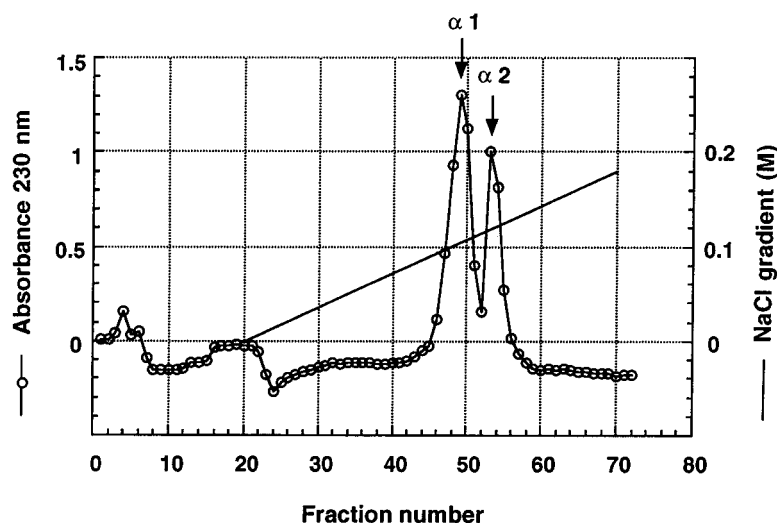


FIG 2. CM column chromatography of type I collagen. NaCl, Sodium chloride.

Specific IgE to $\alpha 1$ and $\alpha 2$ chains in type I collagen

For isolation of $\alpha 1$ and $\alpha 2$ chains, we used CM column chromatography with a linear gradient of sodium chloride (Fig 2). Type I collagen preparations showed 2 bands of $\alpha 1$ and $\alpha 2$ chains in SDS-PAGE (Fig 3, a). The first peak (fraction No. 49) showed a band of $\alpha 1$ chain and the second peak (fraction No. 53) showed a band of $\alpha 2$ chain on SDS-PAGE (Fig 3, a). We confirmed separation and purity of $\alpha 1$ and $\alpha 2$ chains from type I collagen by SDS-PAGE (Fig 3, a). IgE from pooled serum of

3 patients reacted only with $\alpha 2$ chain on immunoblotting (Fig 3, b). Furthermore, we measured specific IgE to $\alpha 1$ and $\alpha 2$ chains of bovine type I collagen in sera of the children by ELISA. Their IgE antibodies reacted with $\alpha 2$ chain but not with $\alpha 1$ chain (Fig 4). As the negative control, all 26 children had no specific IgE to $\alpha 1$ and $\alpha 2$ chains.

Denatured type I collagen-specific histamine release

Denatured type I collagen-specific histamine release assay was carried out with cultured mast cells passive sen-

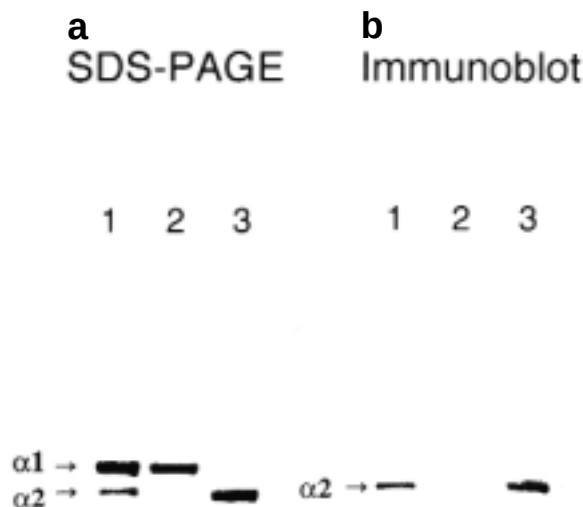


FIG 3. Analysis of SDS-PAGE (a) and immunoblotting (b). Lane 1, Denatured type I collagen; lane 2, $\alpha 1$ chain; lane 3, $\alpha 2$ chain.

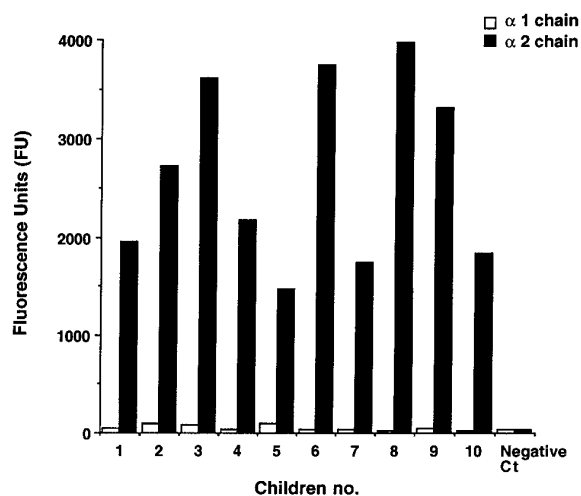


FIG 4. IgE reactivity to $\alpha 1$ and $\alpha 2$ chains. Each child's serum was diluted to approximately 1 Ua/mL as level of specific IgE to gelatin.

sitized with pooled serum (Fig 5). The mast cells sensitized with their pooled sera showed bovine gelatin-specific histamine release. Furthermore, the mast cells sensitized with pooled sera in the children showed $\alpha 2$ chain-specific histamine release but no $\alpha 1$ chain-specific histamine release. As a positive control, the mast cells challenged with gelatin and antihuman IgE showed histamine release.

DISCUSSION

Many children who showed anaphylaxis to bovine gelatin containing live virus vaccines had specific IgE to bovine gelatin.⁷⁻¹⁰ Gelatin mainly consists of denatured type I collagen. It was expected that the children with anaphylaxis to vaccines had specific IgE to gelatin also

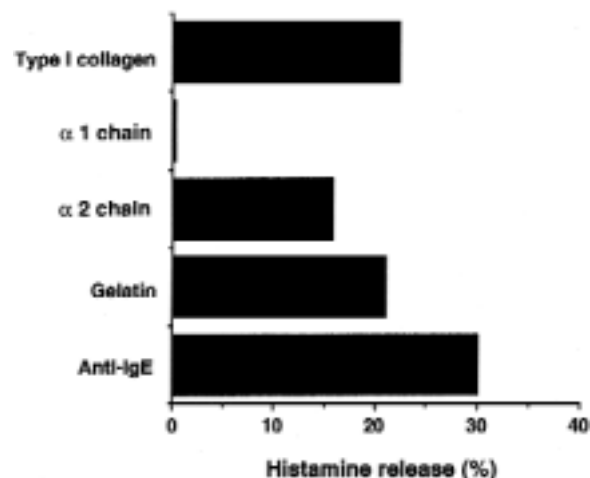


FIG 5. Histamine release from mast cells passive sensitized with sera of bovine-sensitive children.

demonstrated specific IgE to denatured type I collagen. As expected, in this study the children sensitized with gelatin strongly reacted with type I collagen, which mainly is contained in gelatin. It is suggested that type I collagen is a major allergen in gelatin allergy.

Bone, tendon, and skin is rich in type I collagen.¹⁶ The molecule is a heterotrimer composed of two $\alpha 1$ chains and one $\alpha 2$ chain.¹⁶ To examine whether $\alpha 1$ and $\alpha 2$ chains have allergenicity, we separately isolated $\alpha 1$ and $\alpha 2$ chains from type I collagen. IgE antibodies in children who had gelatin allergy strongly reacted with $\alpha 2$ chain but not with $\alpha 1$ chain. Furthermore, mast cells sensitized with pooled serum in the children showed only $\alpha 2$ chain-specific histamine release. We found that $\alpha 2$ chain in type I collagen has allergenicity.

In our previous study we reported the complementary DNA coding sequence for $\alpha 2$ chain of bovine type I collagen.²⁵ The encoded amino acid sequence shows 93% identity to the $\alpha 2$ chain of human type I collagen.²⁶ It was reported that the amino acid sequence of the $\alpha 1$ chain of bovine type I collagen has 98% identity to the $\alpha 1$ chain of human type I collagen.^{27,28} From these data we found that homology (98%) between $\alpha 1$ chains of human and bovine type I collagen is higher than that (93%) between the $\alpha 2$ chain of human and bovine type I collagen. These homology data might suggest that the $\alpha 2$ chain has higher allergenicity than the $\alpha 1$ chain.

Anaphylaxis to vaccines, drugs, and food containing gelatin have been reported, and it has been suggested that the anaphylaxis is caused by allergy to the gelatin present in them.⁷⁻¹³ Gelatin with no or low allergenicity needs to be developed. In this study we found that there are IgE-binding sites in the $\alpha 2$ chain of bovine type I collagen. In the future, to develop a gelatin that has no or low allergenicity to humans, we will analyze IgE-binding sites in the $\alpha 2$ chain of bovine type I collagen. Anaphylaxis caused by gelatin in vaccines might be reduced by digestion of IgE-binding sites in gelatin.

We thank the physicians and the staffs of the vaccine manufacturers for their help in the serum sampling.

REFERENCES

- Zimmerman B, Zimmerman RS. Adverse reactions to vaccines. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, eds. *Allergy: principles and practice*. St Louis: Mosby-Year Book; 1993. p 469-528.
- Aukrust L, Almeland TL, Refsum D, Aas K. Severe hypersensitivity or intolerance reactions to measles vaccines in six children. *Allergy* 1980;35:581-7.
- Pollok TM, Morris J. A 7-year survey of disorders attributed to vaccination in North West Thames region. *Lancet* 1983;1:753-7.
- Thurston A. Anaphylactic shock reaction to measles vaccine. *J R Coll Gen Pract* 1987;37:41.
- Jantunen-Backman K, Peltola H, Backman A, Saol OP. Safe immunization of allergic children against measles, mumps, and rubella. *Arch Pediatr Adolesc Med* 1987;141:1103-5.
- Businco L. Measles, mumps, rubella immunization in egg-allergic children: a long-lasting debate. *Ann Allergy* 1994;72:1-3.
- Kelso JM, Jones RT, Yunginger JW. Anaphylaxis to measles, mumps and rubella vaccine mediated by IgE to gelatin. *J Allergy Clin Immunol* 1993;91:867-72.
- Sakaguchi M, Ogura H, Inouye S. IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. *J Allergy Clin Immunol* 1995;96:563-5.
- Sakaguchi M, Nakayama T, Inouye S. Food allergy to gelatin in children with systemic immediate-type reactions including anaphylaxis to vaccines. *J Allergy Clin Immunol* 1996;98:1058-61.
- Sakaguchi M, Yoshida T, Asahi T, Aoki T, Miyatani Y, Inouye S. Development of IgE antibody to gelatin in children with systemic immediate-type reactions to vaccines. *J Allergy Clin Immunol* 1997;99:720-1.
- Sakaguchi M, Yoshida M, Kuroda W, Harayama O, Inouye S. Systemic immediate-type reactions to gelatin including in Japanese encephalitis vaccines. *Vaccine* 1997;15:121-2.
- Sakaguchi M, Yamanaka T, Ikeda K, Sano Y, Fujita H, Miura T, et al. IgE-mediated systemic reactions to gelatin included in the varicella vaccine. *J Allergy Clin Immunol* 1997;99:263-4.
- Sakaguchi M, Kaneda H, Inouye S. A case of anaphylaxis to gelatin included in erythropoietin products. *J Allergy Clin Immunol* 1999;103:349-50.
- Hashizume S. Vaccine components and side effects [in Japanese]. *Clin Virol* 1994;22:140-4.
- Budavari S. Gelatin. In: Budavari S, O'Neil MJ, Smith A, Heckelman PE, Kinneary JF, editors. *The Merck index*. Rahway (NJ): Merck; 1996. p 742.
- Birk D, Cohen R, Geiger B, Goodenough D, Gumbiner B, Hynes R, et al. In: Albertrts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD, editors. *Cell junctions, cell adhesion, and the extracellular matrix. Molecular biology of the cell*. New York: Garland Publishing; 1994. p 978-9.
- Miller EJ, Rhodes RK. Preparation and characterization of the different types of collagen. *Methods Enzymol* 1982;82:33-64.
- Piez KA, Eigner EA, Lewis MS. The chromatographic separation and amino acid composition of the subunits of several collagens. *Biochemistry* 1963;2:58-66.
- Hori H, Keene DR, Sakai, LY, Wirtz MK, Bachinger HP, Godfrey M, et al. Repeated helical epitopes of defined amino acid sequence in human type III collagen identified by monoclonal antibodies. *Mol Immunol* 1992;29:759-70.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Miyazawa H, Fukamachi H, Inagaki Y, Reese G, Daul CB, Lehrer SB, et al. Identification of the first major allergen of a squid (*Todardes pacificus*). *J Allergy Clin Immunol* 1996;98:948-53.
- Sakaguchi M, Hori H, Ebihara T, Irie S, Yanagida M, Inouye S. Response of IgE in bovine gelatin-sensitive children to gelatins from various animals. *Immunology* 1999;96:286-90.
- Yanagida M, Fukamachi H, Ohgami K, Kuwaki T, Ishii S, Uzumaki H, Tokiwa T, et al. Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells. *Blood* 1995;86:3705-14.
- Yanagida M, Fukamachi H, Takei M, Hagiwara T, Uzumaki H, Tokiwa T, et al. Interferon-g promotes the survival and FcεRI-mediated histamine release in cultured human mast cells. *Immunology* 1996;89:547-52.
- Shirai T, Hattori S, Sakaguchi M, Inouye S, Kimura A, Ebihara T, et al. The complete cDNA coding sequence for the bovine proα2(I) chain of type I procollagen. *Matrix Biol* 1998;17:85-8.
- Wet WD, Bernard M, Benson-Chanda V, Chu ML, Dickson L, Weil D, et al. Organization of the human pro-α2(I) collagen gene. *J Biol Chem* 1987;262:32-6.
- Bernard MP, Chu ML, Myers JC, Ramirez F, Eikenberry EF, Prockop DJ. Nucleotide sequences of complementary deoxyribonucleic acids for the proα1 chain of human type I procollagen: statistical evaluation of structures that are conserved during evolution. *Biochemistry* 1983;22:213-23.
- Bornstein P, Traub W. The chemistry and biology of collagen. In: Naurath H, Hill RL, editors. *The protein*. Vol 4. New York: Academic Press; 1979. p 411-632.

Correction

In the June issue, in the article by Bar-Sela et al (Bar-Sela S, Reshef T, Mekori YA. IgE antithyroid microsomal antibodies in a patient with chronic urticaria. 1999;103:1216-7) the last 3 references were omitted.

- Rumbyrt JS, Katz JL, Schocket AL. Resolution of chronic urticaria in patients with thyroid autoimmunity. *J Allergy Clin Immunol* 1995;96:901-5.
- Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997;99:461-5.
- Guo J, Rapoport B, McLachlan SM. Thyroid peroxidase autoantibodies of the IgE class in thyroid autoimmunity. *Clin Immunol Immunopathol* 1997;82:157-61.