Improvement of ELISA method for antigen-specific IgE in human sera

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Abstract

[Purpose] Radioallergosorbent test (RAST), ELISAs and fluorescence enzyme immunoassay (CAP-FEIA) are generally used to measure specific IgE. We have been monitoring IgE to novel food proteins in the sera of Japanese patient with food allergy. We present a convenient ELISA for antigen-specific IgE in human sera.

[Method] As coating-antigens, we used CP4-EPSPS, Cry9C, PAT (phosphinothricin-N-acetyltransferase) proteins that are present in genetically modified foods. The sera we used were purchased allergic patient’s sera from USA (Group A, 23 samples), Japanese allergic patient’s sera [Group B, 32 samples (1990-1992); Group C, 29 samples (2000-2002)] and normal sera (Group D, 9 samples). As for ELISA, antigen-specific IgE antibody in 20 times-diluted sera was detected by fluorescence or colorimetric method.

[Results] The optical density of ELISA for Cry 9C or CP4-EPSPS antigen-specific IgE in Group A and C sera was comparable to or less than 5 SD of the mean value of Group D. On the other hand, the optical density of ELISA for PAT antigen-specific IgE in 3 sera of Group C was apparently higher than Group D. To avoid the non-specific binding of antibody to antigen-coated plate, we added a washing process with 1N NaCl after incubation with patient serum. By adding this process, the optical density of ELISA for PAT-specific IgE in all of Group C sera was decreased. That in Group A and B sera was also less than 5 SD of the mean value of Group D. These values obtained were extremely low compared with the optical density with an immunized-mouse serum to PAT.

[Conclusions] An improved ELISA method to avoid non-specific binding of antibody to allergen-coated plate was developed. Using this method, we confirmed IgE antibody to the three proteins was not detected in the examined sera of Japanese food allergy-patients.