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Significance of a Highly Specific and Sensitive Enzyme Linked Immunosorbent Assay on Evaluation of Environmental Toxicant-Mediated Allergic Responses

Hyoung Ah Kim[1] and Yong Heo[2,*]

[1]*Department Preventive Medicine, College of Medicine, The Catholic University of Korea*
[2]*Laboratory of Environmental and Occupational Immunology, Institute for Occupational and Environmental Health, 527-44 Inchang-dong, Kuri City, Kyonggi-do Province, Korea 417-010*

ABSTRACT *: Enhancement of antigen-specific IgE is a hallmark of allergic hyperresponsiveness, therefore it is necessary to adopt or develop a highly sensitive and specific assay for determination of allergen-specific IgE levels in vivo. In this presentation, we introduce an ELISA (enzyme linked immunosorbent assay) system developed to measure the levels of chicken egg ovalbumin (OVA)-specific IgE in serum. The ELISA method uses a commercially available purified rat anti-mouse IgE as a capture Ab and biotinylated OVA as a detection reagent. Avidin-peroxidase with its substrate is used for color development resulting in optical density measurement at 405 nm. The ELISA system produces a highly sensitive dose-response relationship between optical density levels and the dilution titer of the OVA-IgE standard serum but no cross-reaction with unrelated IgE or IgG. It is believed that the system is an efficient tool to delineate an adjuvant effect of environmental pollutants on development of asthmatic and atopic responses.*

Key Words *: ELISA, OVA-specific IgE, Allergy*

I. INTRODUCTION

Occurrence of allergic diseases including asthma and skin dermatitis has been continuously increased in past 30 years (Ishizaki *et al.*, 1987; Corbo *et al.*, 1993). Environmental pollution is considered as a significant contributing factor for the development of asthmatic and atopic responses (Peden DB, 2000). In order to define a role of environmental pollutant on progression of the allergic diseases, it may be critical to precisely evaluate level of allergen- specific IgE *in vivo* or *in vitro*. Elevation of allergen-specific IgE is one of the hallmarks of allergic airway or dermal disease (Blanco *et al.*, 1998; Hessel *et al.*, 1998). Concentration of IgE is extremely low compared with that of IgG in sera of human and rodent, in that nanogram level for IgE whereas milligram level for IgG (Janeway and Travers, 1994; Heo *et al.*, 1996). Passive cutaneous anaphylaxis method has been widely used to quantitate allergen-specific IgE in sera (Maejima *et al.*, 1997), but this assay is a bioassay demanding technical expertise. Therein, in this paper, we introduce an enzyme linked immunosorbent assay, which is simple for performance and valid for measurement of allergen-specific IgE. Chicken egg ovalbumin was used as a sample allergen.

*To whom correspondence should be addressed
List of abbreviations : ELISA, enzyme linked immunosorbent assay; OVA, chicken egg ovalbumin

II. MATERIALS AND METHODS

1. ELISA Procedure

Immulon 2® microplates (Dynatech, Chantilly, VA) are coated with purified rat anti-mouse IgE capture Ab (100 μl/ well; Biosource, Camarillo, CA) diluted to 2 μg/ml in a binding buffer (0.1 M Na_2HPO_4, pH 9.0), then incubated overnight at 4°C. The plates are washed three times with PBS containing 0.05% Tween-20 (Sigma, St. Louis, MO) followed by incubation with PBS containing 0.05% Tween-20 and 1% bovine serum albumin (200 μl/well) for 2 hours at room temperature to block non-specific Ab binding. After the blocking, the plates are washed three times, then diluted serum samples or standard sera are added to each well (100 μl/well) and incubated overnight at 4°C. The serum samples are appropriately diluted with the blocking

buffer. Biotinylated OVA (100 μl/well), which is diluted to 1.5 μg/ml with the blocking buffer, is added to each well following six times washing, and incubated at room temperature for 2 hours. OVA (Sigma) can be biotinylated using Biotin-X-NHS kit (Calbiochem, San Diego, CA). The plates are washed six times and further incubated with avidin-peroxidase (100 μl/well; 1.25 μg/ml; Sigma) for 1 hour before detection using the peroxidase substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma). The absorbance is determined at 405 nm using an ELISA reader.

2. Acquisition of the standard serum

To obtain the standard serum, mice (e.g. BDF1 mice, F1 progeny from the cross between C57BL/6 and DBA/2 mice; The Jackson Laboratory, Bar Harbor, ME) are intraperitoneally immunized with OVA (50 μg/ 200 μl/injection) for three times with a week interval. OVA is resuspended in 200 μl physiological saline containing 2 mg alum adjuvant (Pierce, Rockford, IL). Blood is collected by cardiac puncture at Day 56 after the first immunization and serum is prepared by centrifugation. The standard sera or serum samples are stored at 80°C freezer before use. This immunization method was reported to produce a significantly elevated level of OVA-specific IgE in BDF1 mice (Heo and Hankinson, 2000).

III. RESULTS AND DISCUSSION

The sandwich ELISA method developed can be recognized as a valid *in vitro* tool to determine serum levels of OVA-specific IgE, which should be extremely low in comparison that of OVA-specific IgG after immunization of mice with OVA (Hessel *et al.*, 1995), after confirming its efficiency in various ways. First, sensitivity of the ELISA system can be evaluated through analyzing relationship between levels of optical density and the dilution titer of the OVA-IgE positive standard serum (Fig. 1 left). When the standard serum diluted to 1/200 was defined to contain 100 units of OVA-specific IgE per ml, a significantly strong correlation (R^2=0.997) was obtained in our investigation. Furthermore, the optical density level reaching over 0.6 for the 1/200 diluted serum was quite impressive in comparison with that (approximately 0.15)

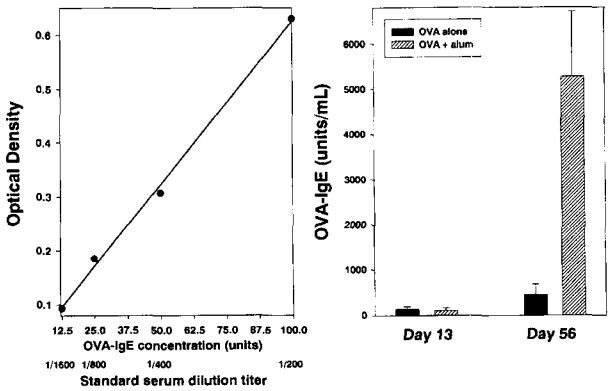

Fig. 1. Development of a sensitive sandwich ELISA assay to detect OVA-specific IgE in murine serum. The OVA-IgE positive standard serum was serially diluted from 1/200 to 1/1600, and assayed for OVA-specific IgE antibody levels (left). Mice were intraperitoneally immunized with 50 μg OVA with or without 2 mg alum three times with a week interval. Sera were collected at Day 13 and 56 after the first immunization. OVA-IgE levels were determined by the ELISA described in ***Materials and Methods*** (right). The result is expressed as the means±SD of two separate experiments for 6 mice per treatment.

reported from A/J mice immunized intraperitoneally with 10 μg OVA and challenged intratracheally with 1.5% OVA (Willis-Karp *et al.*, 1998). An adjuvant effect of alum, a well recognized adjuvant (Maejima *et al.*, 1997), on OVA-IgE production was also dramatically demonstrated with our immunization and ELISA detection system (Fig. 1 right).

Next, specificity of the ELISA system should be tested using IgE antibody generated against unrelated antigen or IgG, the most predominant immunoglobulin isotype in serum. When mouse IgE specific for trinitrophenol-keyhole limpet hemocyanin antigen was applied instead of the OVA-IgE positive standard serum (Fig. 2 left), levels of optical density were negligible under the ELISA system using biotinylated OVA instead of biotin-conjugated rat anti-mouse IgE mAb as a detection reagent. No cross-reactivity with IgG was found when mouse IgG serially diluted from 1.6 mg/ml to 6.25 ng/ml was applied to the system (Fig. 2 right). Cross-reaction with irrelevant IgE in serum from unimmunized BDF1 mice (data not shown) was also negligible.

Overall, the ELISA system introduced in this paper could meet the major requirements for an allergen-specific IgE detection ELISA method, in that our ELISA system provides significantly high sensitivity and specificity, and the amount of serum samples for the assay

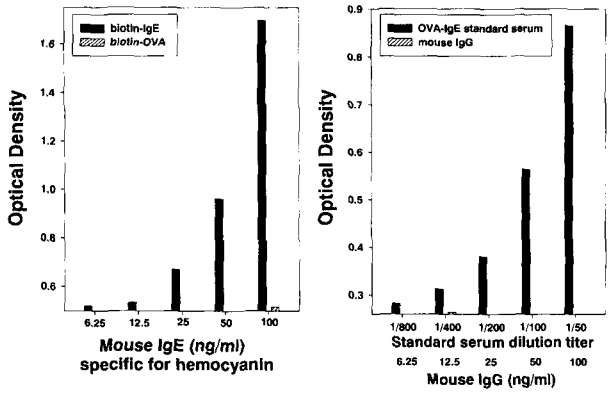

Fig. 2. No non-specific/ cross-reactive reaction with mouse IgG or IgE against hemocyanin under the sandwich ELISA system. Mouse IgE specific for keyhole limpet hemocyanin was added to an ELISA plate coated with IgE capture Ab, followed by detection with biotin-IgE or OVA (left). The OVA-IgE positive standard serum serially diluted from 1/50 to 1/800 or IgG serially diluted from 100 to 6.25 ng/ml was applied to the system followed by detection with biotin-OVA (right). One representative result is shown for each figure.

is quite small resulting in lower than 10 μl needed. In conclusion, the ELISA system with minor modification could be a valuable tool to investigate effects of environmental pollutants such as diesel exhaust particles (Takano *et al.*, 1998; Heo and Hankinson, 2000) on allergen-induced airway or dermal hypersensitivity.

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REFERENCES

Blanco, C., Carrillo, T., Ortega, N., Alvarez, M., Dominguez, C. and Castillo, R. (1998): Comparison of skin-prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin. Exp. Allergy*, **28**, 971-976.

Corbo, G.M., Forastiere, F., DellOrco, V., Pistelli, R., Agabati, N., De Stefanis, B., Ciappi, G. and Perucci, C. A. (1993): Effects of environment on atopic status and respiratory disorders in children. *J. Allergy Clin. Immunol.*, **92**, 616-623.

Heo, Y. and Hankinson, O. (2000): Effect of diesel exhaust particles and their components on the allergen-specific IgE and IgG1 response in mice. *Toxicological Sciences* (in submission).

Heo, Y., Parsons, P.J. and Lawrence, D.A. (1996): Lead differentially modifies cytokine production *in vitro* and *in vivo*. *Toxicol. Appl. Pharmacol.*, **138**, 149-157.

Hessel, E.M., Van Oosterhout, A.J.M., Hofstra, C.L., De Bie, J.J., Garssen, J., Van Loveren, H., Verheyen, A.K.C.P., Savelkoul, H.F.J. and Nijkamp, F.P. (1995): Bronchoconstriction and airway hyperresponsiveness after ovalbumin inhalation in sensitized mice. *Eur. J. Phamacol.*, **293**, 401-412.

Hessel, E.M., Cruikshank, W.W., Van Ark, I., De Bie, J.J., Van Esch, B., Hofman, G., Nijkamp, F.P., Center, D.M. and Van Oosterhout, A.J.M. (1998): Involvement of IL-16 in the induction of airway hyper-respionsiveness and up-regulation of IgE in a murine model of allergic asthma. *J. Immunol.*, **160**, 2998-3005.

Ishizaki, T., Koizumi, K., Ikemori, K., Ishiyama, Y. and Kushibiki, E. (1987): Studies of prevalence of Japanese cedar pollinosis among the residents in densely cultivated area. *Ann. Allergy*, **58**, 265-270.

Janeway, C.A. and Travers, P. (1994): Immunobiology, Current Biology Ltd., London, p. 3:28.

Maejima, K., Tamura, K., Taniguchi, Y., Nagase, S. and Tanaka, H. (1997): Comparison of the effects of various fine particles on IgE antibody production in mice inhaling Japanese cedar pollen allergens. *J. Toxicol. Env. Health*, **52**, 231-248.

Peden D.B. (2000): Development of atopy and asthma: Candidate environmental influences and important periods of exposure. *Env. Health Perspect.*, **108(suppl 3)**, 475-482.

Takano, H., Ichinose, T., Miyabara, Y., Shibuya, T., Lim, H.-B., Yoshikawa, T. and Sagai, M. (1998): Inhalation of diesel exhaust enhances allergen-related eosinophil recruitment and airway hyperrespionsiveness in mice. *Toxicol. Appl. Pharmacol.*, **150**, 328-337.

Willis-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L. and Donaldson, D.D. (1998): Interleukin-13: Central mediator of allergic asthma. *Science*, **282**, 2258-2261.