Artificial Human Sera (ARTHUS) as a tool for validation and standardization of in-vitro diagnostic systems for the detection of specific IgE

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Introduction

Human pool sera are the first choice controls for most diagnostic applications, but they are strongly limited regarding availability, specificity, varying quality and high costs. The aim of this study was to circumvent these limitations. Artificial human sera (ARTHUS) of scalable IgE reactivity could represent an option for validation and standardization of in-vitro diagnostic systems for the detection of specific immunoglobulin E (sIgE). For quantitative analyses, sIgE levels should correspond to total IgE levels.

Methods

The extracellular domains (ECD) of the human high affinity IgG receptor FcγRI (CD64) were fused to human immunoglobulin Fc regions of the epsilon isotype (IgE). Recombinant adapter molecules comprising the FcγRI ECD and human Ig Fc domains (CD64-Ig Fc) were expressed in human HEK-293 cells.

Allergen-specific IgG antibodies were produced in rabbits by immunization with purified recombinant and native allergens as well as whole allergen extracts. Pre-incubation of polyclonal IgG with CD64-IgE Fc produced artificial allergen-specific reagents which were used in different types of commercially available allergen-specific immunoassays.

Results

A set of artificial human sera was generated and tested in established assay methods: ALLERG-O-LIQ, ALFA and ImmunoCAP for the detection of specific IgE and a total IgE ELISA for the detection of total IgE. Different concentrations of allergen specific rabbit IgGs were tested with a defined concentration of the CD64-IgE Fc molecule (6.25 IU/mL total IgE). Api m 1 and Ves v 5 specific ARTHUS were used to demonstrate that the ratio of the two components in ARTHUS is a useful parameter for defining the total IgE and specific IgE concentrations. The obtained results indicate a need for standardization of purified recombinant and native allergens as well as allergen extracts in assays for detection of sIgE. The data also confirmed that the total IgE concentration can be adjusted to match with the sIgE concentration, in particular when ARTHUS were produced by immunization with a purified allergen in contrast to an allergen extract.

Conclusion

ARTHUS, Allergen-specific rabbit IgG antibodies complexed with the IgG-specific CD64-Ig Fc adapter molecule, represent a useful tool for validation and standardization of in-vitro diagnostic systems for the detection of sIgE. The ratio of the two integral components in ARTHUS provides the opportunity to quantitatively adjust total IgE and specific IgE concentrations.