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ORIGINAL ARTICLE

Applying Surface Plasmon Resonance to Monitor the IgE-Mediated Activation of Human Basophils

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ABSTRACT

Background: The histamine releasing test which detects histamine released from basophils *in vitro* is safe, sensitive and widely used for clinical examination in the field of allergy. However, basophils of certain individuals do not release histamine, because of dysfunctions in their intracellular signal transduction (non-responder). To overcome potential shortcomings of the histamine releasing test, we applied surface plasmon resonance (SPR) to detect the activation of basophils.

Methods: Basophils of patients with allergy, and those of non-allergic volunteers were isolated from peripheral blood. A batch of basophils obtained from a healthy volunteer was treated with lactic acid and IgE of a patient with atopic dermatitis in order to replace their endogenous IgE. They were fixed on the sensor chip of the SPR apparatus, pretreated with or without various inhibitors for intracellular signal transduction, and exposed to the antigens or anti-IgE antibody.

Results: When basophils were sensitized with antigen specific IgE, they immediately caused the increase of resonance angle (AR) in response to either anti-IgE antibody or corresponding antigens, even when they did not release histamine. Moreover, the dose dependent reactions of basophils were reflected by the increase of AR as well as the release of histamine. The increase of AR in response to anti-IgE antibody was reduced by pre-treatment of basophils with inhibitors for intracellular signal transduction, but not more than the level for histamine release.

Conclusions: SPR biosensors may be superior to the histamine release test for studying functions of human basophils including those not releasing histamine.

KEY WORDS

basophil, biosensor, histamine release, IgE, surface plasmon resonance

INTRODUCTION

The identification of antigen that provokes mast cell activation is crucial to avoid anaphylactic shock and the aggravation of atopic diseases, such as atopic dermatitis, allergic rhinitis and asthma. The detection of antigen specific IgE in serum implies hypersensitivity against the antigen. Thus, a variety of immunological methods, such as CAP-RAST™, Ala-STAT™ and ADVIA Centaur™, to detect antigen specific IgE have been developed and utilized in clinical practice.[1] However, there are often substantial discrepancies between these serological tests and clinical symptoms.[1] *In vivo* tests, such as skin tests and antigen challenge tests, are more reliable in reflecting the clinical scenario. However, these tests may be painful, and could potentially evoke anaphylactic shock when a patient is extremely sensitive to a particular antigen.[2] Moreover, the intradermal injection of an antigen may sensitize subjects who are not sensitive to the antigen. Both mast cells and basophils in subjects sensitized with the same repertoire of IgE release histamine in response to the antigen. Therefore, the *in vitro* histamine release test with basophils of peripheral blood is sensitive, safe and gives reliable information regarding antigen that causes type I hypersensitivity.

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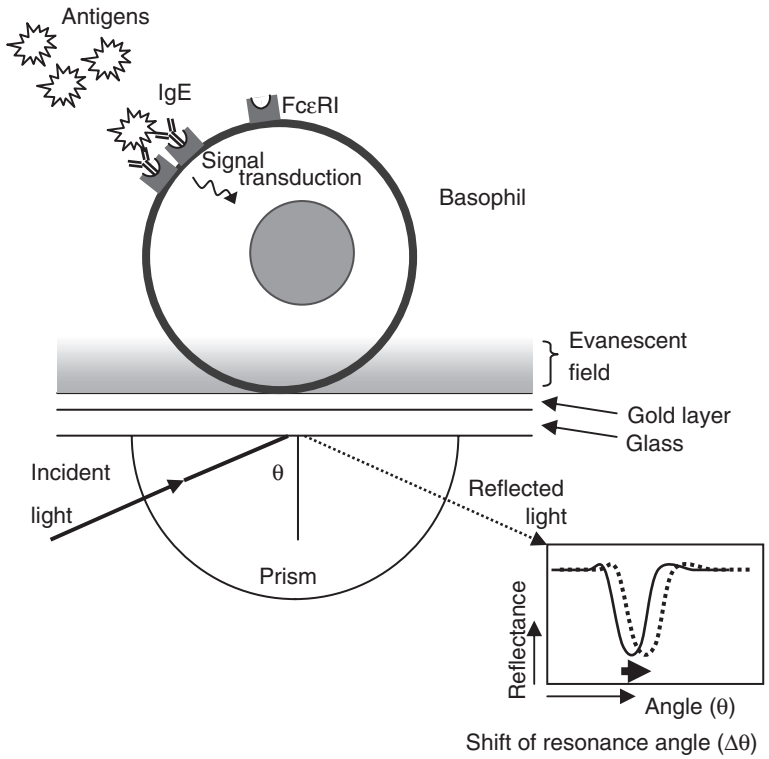

Fig. 1 Principle of the activation of basophils via the high affinity IgE receptor and analysis of SPR signals. Basophils were fixed to the surface of gold layer coated on a glass plate. The laser beam is directed toward the cell via a prism and reflected from there to the monitor which detects the shift of the resonance angle. The angle of resonance is proportional to the reflex index in the evanescent field, a few hundred nanometers from the surface. The reflex index reflects the amount (density) of the molecules in the evanescent field.

Griese *et al*.[3] reported that the histamine release test showed higher sensitivity and specificity than the skin test or RAST analysis based on the comparison with bronchoprovocation of extrinsic asthmatic children. However, basophils of a certain population of individuals, who showed type I hypersensitivity *in vivo*, do not release histamine upon the activation of the IgE receptors *in vitro*.[4] Except for the histamine release from basophils, there is no apparent clinical difference between such patients and ordinary basophil-reactive subjects.

The surface plasmon resonance (SPR) biosensor detects a change of the reflex index on the surface of a sensor chip in a real time fashion with high sensitivity. Therefore, it has been widely utilized for the analysis of binding and dissociation of a ligand to its receptor fixed on a sensor chip.[5] We recently found that degranulation of mast cells on the sensor chip caused unexpectedly large changes of SPR signal.[6,7] In this study we developed a method to detect basophil activation by a SPR biosensor (Fig. 1).

METHODS

REAGENTS

Chemicals were from the following sources: human serum albumin (HSA), 4,4'-dithio dibutyric acid (DDA), N-hydroxysuccinimide (NHS), and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) from Sigma-Aldrich Japan (Tokyo, Japan); genistein, piceatannol, PP1 and wortmannin from Calbiochem (San Diego, CA, USA); fetal calf serum (FCS) from Biowest (Paris, France); RPMI-1640 medium, DMEM and penicillin streptomycin liquid from Invitrogen (Carlsbad, CA, USA); Goat anti-human IgE antibody from Seikagaku Co. (Tokyo, Japan). BA312 antibody[8] was kindly provided by Shionogi Pharmaceutical, Co., Ltd. (Osaka, Japan) and was used for capturing basophils on sensor chips. A monoclonal antibody (25H3) against human IgE was provided as a kind gift from Wakunaga Pharmaceutical Co., Ltd. (Osaka, Japan) and was used for purification of human IgE from a serum of a patient with atopic dermatitis. Mite antigen (300 AU/ml) in 0.04% phenol and 0.05% glycerol