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Desensitization of mast cells' secretory response to an immuno-receptor stimulus

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Abstract

Knowledge of the desensitization process of responses to the type I receptor for IgE (FcεRI) is rather limited. We investigated whether mast cells' secretory response to this receptor's stimulus can be subjected to desensitization under protocols usually employed for hormonal or neural receptors, i.e. by excessive, prolonged or repetitive exposure to the stimulus. To study this we have employed the rat mucosal-type mast cells of the RBL-2H3 line, which enables a rigorous examination of the response to the FcεRI stimulus. These cells exhibited a marked decrease of both, secretion of granule-stored and de novo synthesized mediators to an optimal stimulation, when first exposed to prolonged FcεRI-IgE clustering by specific antigen (DNP_{11}-BSA) or by the IgE specific mAb 95.3 at concentrations that are below the threshold of inducing secretion. The extent of desensitization depended on the employed concentrations of IgE and on the clustering agents, as well as on the length of the desensitization period. The levels of cell surface FcεRI expression and of cell-bound IgE were determined following the desensitization period and no significant correlation has been observed between the extent of endocytosis and the observed desensitization. Thus, a different process, which interferes with FcεRI stimulus–response coupling network, is responsible for the observed desensitization.

Keywords: Physiological conditions; Monomeric IgE; Sub-threshold desensitization

1. Introduction

The activation via the type 1 receptor for IgE (FcεRI) by IgE and multivalent antigen clustering leads to three types of secretory responses: (i) degranulation, i.e. secretion of granule-stored mediators including histamine, serotonin, β-hexosaminidase and proteases [1]; de novo synthesis and secretion of (ii) arachidonic acid products (leukotrienes and prostaglandins) [2], and (iii) cytokines and chemokines, including IL-4 and TNF-α [3–5].

While current understanding of the FcεRI stimulus–response coupling network is relatively advanced, that of its control mechanisms is only emerging [6–11]. Desensitization is a widely studied regulatory process modulating cellular responses induced by diverse membrane receptors (e.g. for growth factors, hormones or for neurotransmitters) [12–18]. Nevertheless, knowledge of this process in the case of response to the multi-chain immuno-recognition receptors (MIRR), notably the FcεRI, is rather limited. Of particular interest is the question whether FcεRI-mediated secretory responses can be subjected to desensitization under conditions effective for other receptors, i.e. by excessive, prolonged or repetitive exposure to the stimulus. To date, only few studies have explored the validity of this approach in

Abbreviations: ATCC, american type culture colection; BSA, bovine serum albumine; DCCM, defined cell culture medium; DMEM, dulbecco's mod eagle medium; DMSO, dimethyl sulphoxide; DNP, dinitro phenyl; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; HPLC, high performance liquid chromatography; IL-4, interleukin 4; ITIM, immunoreceptor tyrosine-based inhibitory motif; mAb, monoclonal; NHS, *N*-hydroxy succinimide; PBS, phosphate buffer saline; RBL, rat basophilic leukaemia; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TNF-α, tumor necrosis factor α

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human basophils [19–22]. Moreover, the desensitization of the FcεRI-mediated response under physiological conditions has been rarely investigated [23], as most of the other experimental protocols in mast cells or basophils have employed essentially non-physiological conditions: exposing the cells to FcεRI clustering in the absence of extracellular calcium for varying time periods and monitoring their secretory response upon re-introduction of the latter ions [24–27]. Further, in these studies desensitization of degranulation has mainly been investigated, rather than that of the late phase response [28,29].

Here, we present results of experiments where we identified and characterized conditions leading to desensitization of FcεRI-mediated secretion of both granule-stored and de novo synthesized mediators under physiological conditions, in a manner analogous to that determined for most cell-membrane receptors. Cells of the RBL-2H3 line that allow a detailed quantitative analysis of the stimulus–response relation were employed. They were subjected to prolonged subthreshold treatment (threshold concentrations representing the lowest stimulus that yields a significantly measurable secretory response), followed by stimulation under optimal conditions. A brief preliminary report indicating feasibility of this approach has been presented earlier [30].

2. Materials and methods

2.1. Cells

RBL-2H3 cells, originally obtained from Dr. R. Siraganian (NIH, Bethesda, MD), were grown in DMEM (Gibco-BRL, NY, USA), supplemented with 10% heat inactivated fetal calf serum (Gibco-BRL, NY, USA), 2% L-glutamine (Biological Ind., Beit Haemek, Israel) and 0.2% combined antibiotics (Bio Lab, Jerusalem, Israel) at 37 °C, 7% CO_2 in humidified atmosphere. These cells were used in the majority of the experiments. In addition, an RBL-2H3 sub-line (generously provided by Dr. Z. Honda, University of Tokyo, Japan) was grown under the same conditions and used primarily in the experiments where desensitization of IL-4 secretion has been studied.

2.2. Reagents

(1) Antigen: BSA derivatized with an average of 11 residues of 2,4 dinitro phenyl per molecule (DNP_{11}-BSA) has been prepared in our lab by reacting fraction V of BSA (Sigma, MO, USA) with 2,4-dinitro-benzenesulphonic acid.
(2) Tyrode's buffer: 130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 20 mM HEPES buffer, pH = 7.3–7.4 and 0.1 mg/ml BSA. The buffer was freshly prepared before each experiment.
(3) DuoSet ELISA Development Kits for measurements of the secreted TNF-α and IL-4 were purchased from R&D Systems, MN, USA and all the antibodies and buffers were used according to the manufacturer's instructions.
(4) 3,3′,5,5′-Tetramethylbenzidine used as substrate in ELISA TNF-α and IL-4 assays was purchased as powder from Sigma. Stock solutions of 1 mg/ml were prepared in DMSO and stored frozen at −20 °C. The working solution has always been freshly prepared, by diluting 1 ml of the stock in the appropriate buffer (4.5 ml deionized water, 2.5 ml 0.1 M citric acid, 1.9 ml 0.2 M Na_2HPO_4, 0.9 μl H_2O_2).

2.3. Antibodies

(1) The hybridoma cells secreting the DNP-specific mAb A2IgE were purchased from ATCC and grown either as ascites in mice (cd1 nu strain) or in a synthetic serum-free medium (DCCM-1) obtained from Biological Ind., supplemented with 0.2% combined antibiotics (Bio Lab) and 1% sodium pyruvate (Sigma). The ascitic fluid or supernatants of confluent cultures respectively, were regularly harvested. The IgE was purified by chromatography on an affinity column of DNP-Lysine covalently conjugated to HiTrap™ NHS-activated HP column (Amersham Biosciences, NJ, USA). The oligomeric and monomeric fractions were separated by HPLC. The status of the monomeric fraction was verified by two different protocols:
   (i) Analytical: an aliquot of 5 μl (∼25 nM) was separated on 5% SDS-PAGE and silver stained according to the manufacturer's instructions (Amersham Biosciences) and shown to contain less than 1% oligomeric IgE by densitometric measurements using National Institute of Health Image (NIH Image 1.62) software.
   (ii) Functional: RBL-2H3 cells were incubated with a range of IgE concentrations (0.1–50 nM) of this monomeric fraction and assayed for their capacity to induce β-hexosaminidase secretion. None of the samples used in our experiments induced secretion even at the highest IgE concentration employed.
(2) The rat hybridoma cell line secreting the mouse IgE specific monoclonal antibody mAb 95.3 (IgG2 isotype) was grown in DCCM-1 containing 0.2% combined antibiotics (Bio Lab) and 1% sodium pyruvate (Sigma). The supernatant was harvested once a week and the protein was affinity chromatography purified on HiTrap™ Protein G HP column (Amersham Biosciences), according to the manufacturer instructions.
(3) Rat gamma globulin used to block the cells' FcγRIIb and FITC-conjugated F(ab′)2 fragments specific for mouse IgG light chains, also able to bind to the IgE light chains, were all purchased from Jackson ImmunoResearch, PA, USA. FITC was purchased from Molecular Probes OR, USA and conjugated with purified IgE in our lab, according to the manufacturer instructions.