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Gene expression profiling of dexamethasone-treated RBL-2H3 cells: induction of anti-inflammatory molecules

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Abstract

Glucocorticoids are well known for their anti-inflammatory effect through the regulation of gene expression in many types of immune cells, including mast cells. However, the genes that are involved in suppression of mast cell-mediated inflammation by glucocorticoids have not been fully identified. Therefore, we examined the dexamethasone (Dex)-responsive genes in RBL-2H3 mast cells using a high-density oligonucleotide microarray technique. Gene expression profiling revealed that the antigen-induced up-regulation of pro-inflammatory factors, including monocyte chemoattractant protein-1, was markedly inhibited by 100 nM Dex. On the other hand, Dex treatment itself caused the substantial up-regulation of many genes, including phenylethanolamine-*N*-methyl transferase (PNMT) and cytokine-inducible SH2-containing protein (CISH), in the mast cells. The expression of these two genes significantly increased 6 h after Dex exposure and lasted for more than 24 h. Considering that PNMT is the rate-determining enzyme in epinephrine synthesis and that CISH is a suppressor of cytokine signaling, these Dex-responsive genes may be potential anti-inflammatory factors. Thus, gene expression profiling suggested that Dex might exert its anti-inflammatory effect through two pathways in mast cells: the suppression and induction of potentially pro- and anti-inflammatory factors, respectively.

Keywords: Mast cells; Dexamethasone; DNA microarray

1. Introduction

Glucocorticoids are the major anti-inflammatory agents presently in clinical use. These drugs act not only on lymphocytes, but also on various types of inflammatory cells, including mast cells [1,2]. Glucocorticoids exert their effects through intracellular receptors, which act as potent transcriptional activators of genes that possess glucocorticoid responsive elements (GREs) [1]. By regulating gene expression, glucocorticoids suppress the production of pro-inflammatory proteins, such as cytokines, chemokines and some enzymatic mediators [3]. A number of studies have focused on the cellular and molecular mechanisms of the anti-inflammatory effects of glucocorticoids. However, the genes that are involved in the anti-inflammatory effects on mast cells remain unidentified.

Using a DNA microarray technique, we have identified several genes, including those for cytokines and signal transducers, that were up-regulated in mast cells after stimulation with antigen or an antioxidant (DTBHQ) [4]. In a previous study, we demonstrated that the mRNA expression of monocyte chemoattractant protein (MCP)-1, which is a CC chemokine that activates monocytes to infiltrate inflammatory tissues, was markedly enhanced when RBL-2H3 mast cells were stimulated with IgE and the specific antigen,

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suggesting the importance of this chemokine in mast cell-mediated inflammation. Another previous study by our group showed that antigen-induced MCP-1 up-regulation was completely suppressed by overnight treatment with 100 nM of dexamethasone (Dex) [5]. Although Dex inhibits antigen-induced mast cell activation, its inhibitory mechanism(s) has not been fully examined. In the present study, we profiled a comprehensive expression of Dex-responsive genes in RBL-2H3 mast cells using a DNA microarray technique and identified several genes that might be involved in the inhibitory mechanism(s) of Dex.

2. Materials and methods

2.1. Reagents and cells

Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Co. (Tokyo, Japan), and fetal bovine serum was from Sigma (St. Louis, MO). Murine anti-dinitrophenyl (DNP) monoclonal IgE antibody (IgE-53-569) and dinitrophenylated BSA (DNP_7-BSA) were prepared as described previously [6]. TRIzol reagent and the SuperScript Choice system were purchased from Invitrogen Corp (Carlsbad, CA). TaqMan Gold RT-PCR kit and TaqMan Universal PCR Master Mix were purchased from Applied BioSystems (Foster City, CA). The RBL-2H3 cells were a kind gift of Dr. R.P. Siraganian (National Institute of Dental Research, National Institutes of Health, Bethesda, MD); the cells were grown in DMEM supplemented with 10% heat-inactivated fetal calf serum at 37 °C under 5% CO_2 in a humidified atmosphere, as described previously [6]. Rabbit polyclonal IgG for bovine PNMT (CA-401 bMTrab) was purchased from Protos Biotech (New York, NY), and goat polyclonal IgG for human CIS (N19) (sc-1529) was from Santa Cruz Biotechnology (Santa Cruz, CA). These antibodies were described by the manufacture as being cross-reactive to rat PNMT and rat CISH, respectively.

2.2. Mast cell activation and assay for Ca^{2+} response, degranulation and MCP-1 release

RBL-2H3 cells were pretreated with the indicated concentrations of Dex for 15 h and simultaneously sensitized with 1:800 diluted anti-DNP IgE; after washing twice with DMEM, they were stimulated with 10 μg/ml of antigen (DNP_7-BSA) at 37 °C for 30 min (for degranulation) or 3 h (for MCP-1 assay) in the presence or absence of Dex.

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using Fura-2 AM and a fluorophotometer (RF-5300PC, Shimadzu), as described previously [7]. Degranulation from the RBL-2H3 cells was measured as the activity of β-hexosaminidase, as described previously [7]. MCP-1 release was measured using a rat MCP-1 immunoassay kit (BioSource International, California), following the manufacturer's protocol.

2.3. GeneChip analysis

Total RNA from the control or antigen-stimulated RBL-2H3 cells (1×10^7) cultured in the presence or absence of 100 nM of Dex was prepared using a TRIzol reagent, according to the manufacturer's instructions ($n = 3$, respectively). The RNA was processed as described previously [4] and analyzed using GeneChip Rat Genome U34A arrays, a fluidics station, and a scanner (Affymetrix). The 12 RNA samples from the four different conditions were separately hybridized to the arrays. The expression signals (Signal) were automatically calculated using Microarray Suite (MAS, Ver. 5.0; Affymetrix) with scaling to 2500 as a median, and the data were imported into GeneSpring analysis software (Ver. 6.2; Silicon Genetics, Redwood City, CA) for further analysis. All expression signals were normalized across all the genes and all the arrays using the GeneSpring standard normalization algorithm.

Among the genes that were significantly up-regulated by antigen stimulation in the absence of Dex, those that were further up- or down-regulated by antigen stimulation in the presence of Dex were selected by MAS and GeneSpring as follows: (1) genes whose Detection was "P (Present)" in at least three out of six experiments (three controls and three antigen-stimulated); (2) genes whose maximum average Signal under the three conditions (control, antigen, or antigen + Dex) was more than 1000; (3) genes those passed statistical analysis (Welch's *t*-test, $P < 0.05$); (4) genes whose average fold change in treated (antigen or antigen + Dex) to non-treated samples was more than three (for up-regulated genes) or less than one-third (for down-regulated genes). Genes that were significantly up- or down-regulated by Dex treatment only were selected using the same criteria.

2.4. Quantitative PCR

RBL-2H3 (1×10^7) cells were treated with 100 nM of Dex for 0, 1, 3, 6, 12 and 24 h to determine the effects of the Dex treatment time. In some cases, RBL-2H3 cells were treated with the indicated concentrations of Dex and stimulated with or without antigen. The cells were then harvested and the total RNA was prepared using TRIzol reagent. The amounts of PNMT, CISH, and GAPDH mRNA were measured by TaqMan PCR using an ABI PRISM™ 7700 sequence detector (PE Applied BioSystems, Foster City, CA), according to the manufacturer's instructions. For each experiment, 100 ng of total RNA, 0.3 μM of forward primer, 0.3 μM of reverse primer, and 0.3 μM of fluorescent probe were used. The PCR conditions were as follows: 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 s, and 60 °C for 1 min.

Primers and probes used for TaqMan analysis were as follows: PNMT forward primer, 5′-GCT TCC GGC AGG CTT TG-3′; PNMT reverse primer, 5′-GGC CCC GAT GAG AAG GA-3′; PNMT TaqMan probe, 5′-VIC-TCA TAT CAC GAC GCT GCT GAG GCC-Tamra-3′; CISH forward primer, 5′-TGA GAA TGA ACC GAA GGT GCT A-3′;