

Research article

Transcriptional response of human mast cells stimulated via the FcεRI and identification of mast cells as a source of IL-11

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Abstract

Background: In asthma and other allergic disorders, the activation of mast cells by IgE and antigen induces the cells to release histamine and other mediators of inflammation, as well as to produce certain cytokines and chemokines. To search for new mast cell products, we used complementary DNA microarrays to analyze gene expression in human umbilical cord blood-derived mast cells stimulated via the high-affinity IgE receptor (FcεRI).

Results: One to two hours after FcεRI-dependent stimulation, more than 2,400 genes (about half of which are of unknown function) exhibited 2–200 fold changes in expression. The transcriptional program included changes in the expression of IL-11 and at least 30 other cytokines and chemokines. Human mast cells secreted 130–529 pg of IL-11/10⁶ cells by 6 h after stimulation with anti-IgE.

Conclusion: Our initial analysis of the transcriptional program induced in in vitro-derived human mast cells stimulated via the FcεRI has identified many products that heretofore have not been associated with this cell type, but which may significantly influence mast cell function in IgE-associated host responses. We also have demonstrated that mast cells stimulated via the FcεRI can secrete IL-11. Based on the previously reported biological effects of IL-11, our results suggest that production of IL-11 may represent one link between IgE-dependent mast cell activation in subjects with allergic asthma and the development of a spectrum of structural changes in the airways of these individuals; such changes, collectively termed "airway remodeling," can constitute an important long term consequence of asthma.

Background

In asthma and other allergic disorders, the activation of

mast cells by IgE and antigen induces the cells to release histamine and other mediators of inflammation [1,2], as

well as to produce certain cytokines and chemokines [1–4]. To search for new mast cell products, we used cDNA microarrays to analyze the transcriptional program in highly pure populations of human mast cells that had been stimulated via the FcεRI. While others have analyzed transcriptional profiles in certain rodent or human mast cell populations using microarray-based or alternative approaches [6–10], the transcriptional profile of human mast cells stimulated via the FcεRI has not yet been reported.

One of the many transcripts we found to be substantially induced in human mast cells stimulated via FcεRI was that for IL-11, a pleiotropic cytokine that has been implicated in airway inflammation and remodeling [11–13]; mast cells stimulated via the FcεRI also secreted IL-11 protein. These results indicate that analysis of the transcriptional profile of human mast cells stimulated by aggregation of FcεRI may be useful in suggesting new potential therapeutic targets in asthma and other IgE-associated allergic disorders.

Results and discussion

FcεRI-dependent activation of human mast cells results in substantially altered expression of over 2,400 genes

The two mast cell preparations shown in Fig. 1 released 56.6 ± 4.1 or $63.0 \pm 1.8\%$ of their total histamine content by 1 h after anti-IgE stimulation (vs. 1.7 ± 0.1 or $3.4 \pm 0.2\%$, respectively, in controls not stimulated with anti-IgE, $p < 0.001$ for either comparison), which is a strong secretory response in this mast cell type [15]. The transcriptional responses in these two populations were quite similar, with 2,478 genes exhibiting a 2–200 fold change in expression over baseline levels; about half of these 2,478 genes represent unnamed expressed sequence tags (ESTs) of genes of unknown functions (Fig. 1A). The reliability of the observed changes was tested by including replicate array elements representing the same gene for ~25% of the genes; these generally gave excellent agreement. A searchable version of Fig. 1A, raw array images and the actual quantitative array data from these experiments are available on the Stanford Microarray Database web site [http://genome-www.stanford.edu/mast/].

We observed changes in expression for 18 cytokines and one cytokine-related molecule (tumor necrosis factor-α converting enzyme, or TACE) (Fig. 1B), as well as 13 chemokines (Fig. 1C), including two CXC chemokines (IL-8 and Gro2), one C chemokine (lymphotactin), and ten CC chemokines (16) (Fig. 1C); several of these responses have not previously been reported (see symbols in Fig. 1B & 1C). Several genes involved in potential interactions with T cells, B cells or dendritic cells were upregulated, and one, the CD40 ligand, was substantially downregulated (Fig. 1D). Anti-IgE activated human mast

cells also altered the expression of several adhesion molecules (Fig. 1E).

The results presented in Fig. 1 from two mast cell populations, and those from another experiment (donor 3) in which the mast cells were derived using a different cell culture protocol and were challenged with anti-IgE at 1.0 μg/ml (see website for details), indicate that umbilical cord blood-derived mast cells from different donors may exhibit similar transcriptional profiles after IgE-dependent stimulation. However, additional studies will be required to characterize the extent of the similarities and differences of the transcriptional profiles in mast cell populations derived from different donors, different sources of progenitor cells or different conditions of culture.

Secretion of IL-11 by mast cells activated via the FcεRI

To assess whether increased expression of IL-11 mRNA (Fig. 1B; 3.0 fold and 4.7 fold increases in mRNA expression vs. baseline levels at 2 h after stimulation in donors 1 & 2, respectively) was associated with synthesis of the corresponding protein, we measured IL-11 secreted into the medium at 6 h after the FcεRI-dependent stimulation of mast cells (> 99% purity) derived from 3 different donors. The populations released 68.3 ± 4.7 , 63.9 ± 2.6 or $34.8 \pm 1.2\%$ of the total histamine within 1 h of stimulation (vs. 2.1 ± 0.2 , 1.7 ± 0.2 or $2.2 \pm 0.1\%$ for cells not stimulated with anti-IgE, $p < 0.001$ for each comparison) and secreted 130–529 pg of IL-11/10⁶ cells at 6 h after stimulation with anti-IgE, whereas little or no IL-11 was released by the control cells (Fig. 2A). In a separate set of experiments, we found that the maximal levels of IL-11 production were detected 12 h after stimulation with anti-IgE (Fig. 2B).

It has been reported that the expression of IL-11 can be greatly increased in both airway epithelial cells and resident inflammatory cells in subjects with severe asthma [11]. Moreover, IL-11 mRNA was co-localized with major basic protein (MBP) in inflammatory cells in the subepithelial layer of the airway [11]. Since the publication of that study, mast cells have been identified as a potential source of MBP [10]. Taken together with our results, this raises the possibility that both mast cells and eosinophils represent sources of IL-11 in asthma. Transgenic mice that overexpress IL-11 in the lung develop thickening of airway walls, subepithelial fibrosis, and other local structural changes [12]. Production of IL-11 thus may represent an important link between IgE-dependent mast cell activation in subjects with allergic asthma and the development of a spectrum of structural changes in the airways of these individuals; such changes, that are collectively termed "airway remodeling", constitute an increasingly recognized long term consequence of asthma [2,12,13].

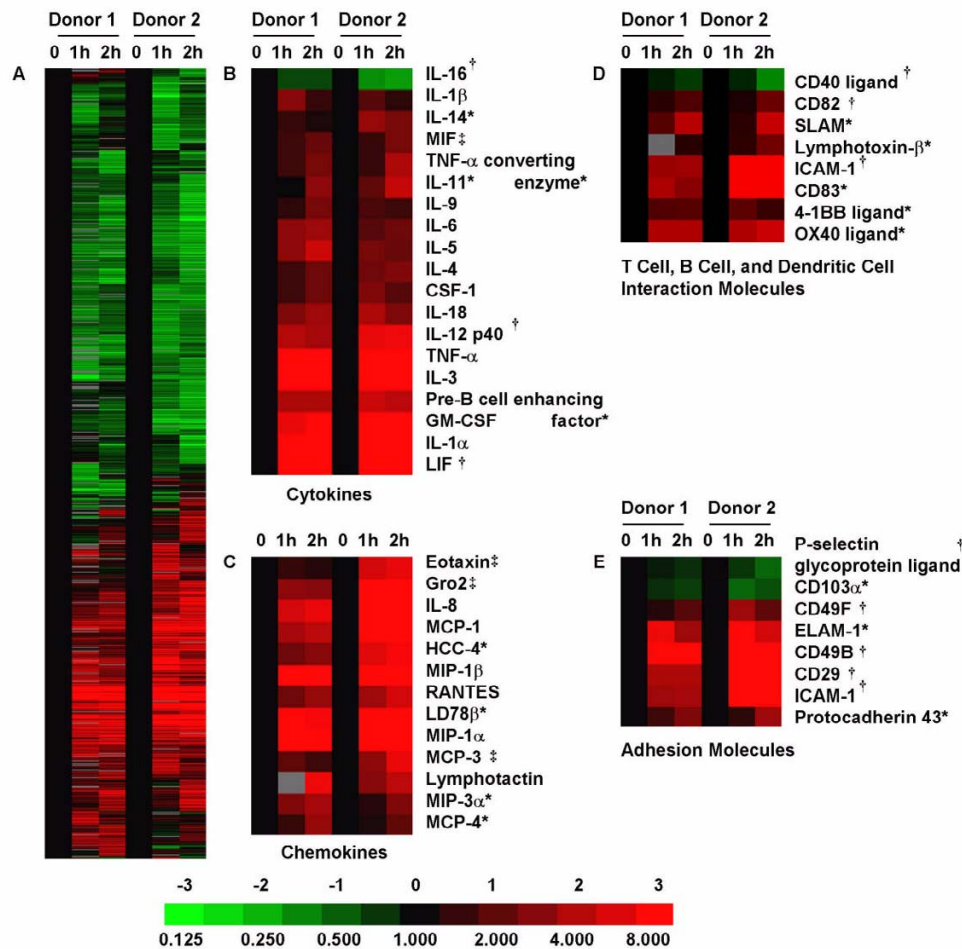


Figure 1

Changes in gene expression in human mast cells stimulated via the Fc ϵ RI (A) Clustering of 2,478 genes that exhibited a 2–200 fold change in expression over baseline "0" levels in two human umbilical cord blood-derived mast cell populations (> 99% purity) that were stimulated via the Fc ϵ RI (with anti-IgE at 10 μ g/ml) for 1 or 2 h. The microarrays were scanned on a GenePix 4000 microarray scanner (Axon Instruments, Foster City, CA) and analyzed using Scan Alyze (M. Eisen; [http://rana.stanford.edu/software/]) or GENEPIX PRO (Axon Instruments). Single spots or areas of the array with obvious blemishes were flagged and excluded from subsequent analyses. All non-flagged array elements for which the fluorescent intensity in each channel was greater than 1.4 times the local background were considered well measured. A set of clones that consistently behaved poorly across arrays (time course) was identified and excluded from all analyses. The ratio values (experimental vs. reference) were normalized according to the baseline (time 0) value of each donor (so that the level of baseline mRNA expression would appear black in our color scale) and log-transformed (base 2). Hierarchical clustering was applied using Cluster (M. Eisen; [http://rana.stanford.edu/software/]). Genes were selected for this analysis if their expression level deviated from that in the unstimulated (time 0) mast cells by at least a factor 2 in at least 1 time point. The values from two experiments from two different donors (Donor 1 and 2) were analyzed simultaneously. The results were displayed with Tree View (M. Eisen; [http://rana.stanford.edu/software/]). Changes in gene expression were depicted according to the color scale shown at the bottom. The results are displayed in a table format, in which each row represents a series of measurements of mRNA levels for a single gene, and each column represents the measured mRNA levels for all of the genes in a single sample of cells. Each cell is colored to reflect expression of the corresponding gene in a specific cell sample, relative to its expression level prior to treatment with anti-IgE. Green color represents decreased expression; red color represents increased expression. As indicated, the scale extends from ratios of 0.125 to 8 (-3 to 3 in log base 2 units). Gray cells in the table indicate missing or excluded data. Genes for (B) cytokines, (C) chemokines, (D) molecules that can regulate potential interactions with T cells, B cells, or dendritic cells, and (E) adhesion molecules whose expression changed significantly (same populations as in (A)). * = Expression (or \ddagger = change in expression after Fc ϵ RI dependent stimulation) not previously reported for either human or rodent mast cells; \ddagger = expression not previously reported for human mast cells.

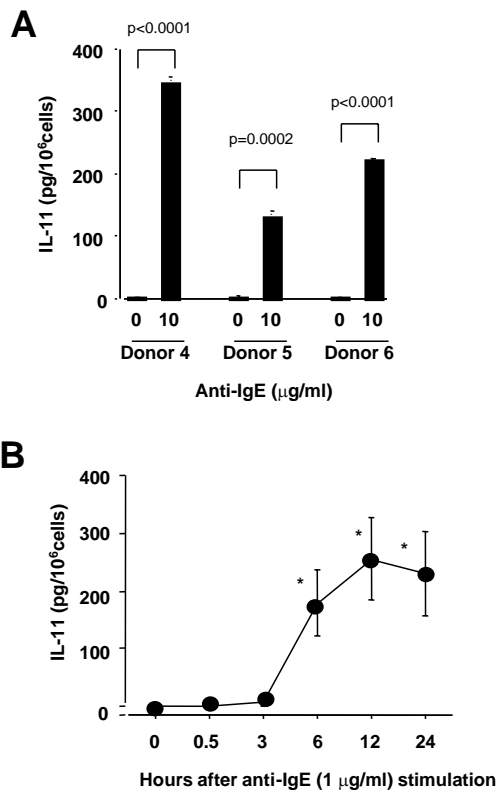


Figure 2
Human mast cells stimulated via the FcεRI secrete IL-11 (A) Production of IL-11 by 3 different populations (from Donors 4–6) of human umbilical cord blood-derived mast cells (> 99% purity) that were incubated for 6 h without anti-IgE ("0") or were maintained for 6 h after stimulation via the FcεRI with anti-IgE at 10 µg/ml. Data represent mean ± s.e.m. for 2–3 determinations for each population. (B) Kinetics of IL-11 production in human umbilical cord blood-derived mast cells after stimulation via the FcεRI with anti-IgE at 1 µg/ml. Data represent mean ± s.e.m. for 3 different populations of human mast cells (from Donors 7–9). Values for anti-IgE stimulated vs. control cells were analyzed for statistical significance by the Student's *t* test, 2-tailed. Note: In ~20 experiments of this type, little or no IL-11 secretion was detected in supernatants of mast cell preparations that were sensitized with IgE and then incubated without anti-IgE for 6 h.

Conclusions

Our initial analysis of the transcriptional program induced in *in vitro*-derived human mast cells stimulated via the FcεRI has identified many products that heretofore have not been associated with this cell type, but which may significantly influence mast cell function in IgE-associated host responses. We also have demonstrated that mast cells stimulated via the FcεRI can secrete IL-11, a cytokine that may contribute to some of the tissue altera-

tions that occur in the airways of patients with asthma. In future studies, it will be important to attempt to characterize the expression of IL-11 and the other potential products identified by our transcriptional analysis by specific mast cell populations *in vivo*. It of course will also be important to examine the potential roles of the individual mast cell-associated products identified in our cDNA microarray analyses, including IL-11, in the inflammation, immune modulation and tissue remodeling associated with allergic disorders and immune responses to parasites, as well as in the alterations of mast cell proliferation, survival and phenotype that can occur in these settings. Finally, it will be of great interest to define the programs of gene expression induced in mast cells by the many other immunological and non-immunological factors that can mediate activation of this cell type independently of IgE. Such studies may provide additional insights into the roles of this cell in health and disease.

Materials and methods

Umbilical Cord Blood-Derived Human Mast Cells

In vitro-derived human mast cells were obtained essentially as described in Kempuraj *et al.* [14]; details of the culture methods are available on the Stanford Microarray Database web site (<http://genome-www.stanford.edu/mast/>). Cells from 11–19 wk cultures were used; mast cells represented > 99% of the total cells according to light microscopic morphology in preparations stained with Kimura's stain and 98% (1 population) or > 99% (all the others) of the cells by immunohistochemistry for mast cell-associated tryptase [15]. In addition, these preparations consist of cells that have high levels of c-kit expression by flow cytometry (data not shown). Umbilical cord blood was obtained under the guidelines of the Stanford University Institutional Review Board.

Stimulation of Mast Cells via the FcεRI

Mast cells were incubated for 4 days with purified human myeloma IgE (5 µg/ml, Biodesign International, Kennebunk, ME) and rhIL-4 (10 ng/ml, PeproTech, Rocky Hill, NJ) to enhance surface expression of FcεRI [15]. Dose response studies indicated that substantial levels of histamine release, reflecting a high level of FcεRI-dependent mast cell activation, could be achieved in these preparations by stimulating the mast cells with 10 or 1 µg/ml of anti-human IgE antibody (Sigma); these concentrations were used for all the experiments reported herein. Cells were harvested immediately before or at 1 or 2 h after stimulation with anti-IgE for microarray analyses. Histamine release was assessed using the histamine enzyme immunoassay kit (Immunotech, Westbrook, ME) and IL-11 secreted into the medium was measured with a human IL-11 immunoassay kit (R&D Systems, Minneapolis, MN).

cDNA Microarray Procedures

The cDNA microarray used in these experiments represents a set of ~14,000 genes related to immune function [5]. All analyses were performed using poly(A) + mRNA (extracted using the Fast Track kit, Invitrogen, Carlsbad, CA) [17]. The experimental samples were our human mast cell preparations; the control "reference" samples represented a pool of mRNA isolated from a human mast cell line (HMC-1, ref. 18), a T cell line (Jurkat), and a B cell line (Raji). For each cell type, the mRNA used for the pool consisted of that derived from unstimulated cells (80% of the total) and stimulated cells (20% of the total); the control cell lines were stimulated with 1 μ M Ionomycin (Sigma) and 25 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma) for 2 h at 37°C. The use of common control cDNA probe allows the relative expression of each gene to be compared across all samples.

Authors' contributions

KS and KM participated in the design of the experiments and performed the cell culture of human mast cells, cDNA microarray analyses and ELISA immunoassays, with KS taking the lead role and also helping to write the first draft of the manuscript. MD produced the microarrays and participated in the design of the experiments and analysis of the microarray data. CL carried out some of the cDNA microarray analysis. SYT participated in the design of the study and the analysis of the microarray results. MT participated in the design and coordination of the study and the drafting of the manuscript. POB and DB participated in experimental design, interpretation of the microarray data, and revision of the manuscript. SJG conceived of the study, participated in its design and coordination, including interpretation of data, and with KS, drafted the manuscript.

All authors read and approved the final version of the manuscript.

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