

Histamine Release from the Basophils of Control and Asthmatic Subjects and a Comparison of Gene Expression between “Releaser” and “Nonreleaser” Basophils¹

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Most human blood basophils respond to FcεRI cross-linking by releasing histamine and other inflammatory mediators. Basophils that do not degranulate after anti-IgE challenge, known as “nonreleaser” basophils, characteristically have no or barely detectable levels of the Syk tyrosine kinase. The true incidence of the nonreleaser phenotype, its relationship (if any) to allergic asthma, and its molecular mechanism are not well understood. In this study, we report statistical analyses of degranulation assays performed in 68 control and 61 asthmatic subjects that establish higher basal and anti-IgE-stimulated basophil degranulation among the asthmatics. Remarkably, 28% of the control group and 13% of the asthmatic group were nonreleasers for all or part of our 4-year long study and cycling between the releaser and nonreleaser phenotypes occurred at least once in blood basophils from 8 (of 8) asthmatic and 16 (of 23) control donors. Microarray analysis showed that basal gene expression was generally lower in nonreleaser than releaser basophils. In releaser cells, FcεRI cross-linking up-regulated >200 genes, including genes encoding receptors (the FcεRI α and β subunits, the histamine 4 receptor, the chemokine (C-C motif) receptor 1), signaling proteins (Lyn), chemokines (IL-8, RANTES, MIP-1α, and MIP-1β) and transcription factors (early growth response-1, early growth response-3, and AP-1). FcεRI cross-linking induced fewer, and quite distinct, transcriptional responses in nonreleaser cells. We conclude that “nonreleaser” and “cycler” basophils represent a distinct and reversible natural phenotype. Although histamine is more readily released from basophils isolated from asthmatics than controls, the presence of nonreleaser basophils does not rule out the diagnosis of asthma. *The Journal of Immunology*, 2007, 178: 4584–4594.

Basophils, which represent <1% of human peripheral blood leukocytes, are effector cells in IgE-mediated inflammatory and allergic reactions. Basophil granules contain potent inflammatory mediators, including histamine, neutral proteases, proteoglycans, and lysosomal enzymes. Cross-linking IgE-bound FcεRI on the basophil surface with anti-IgE Ab or polyvalent allergen elicits calcium-dependent degranulation, as well as cytoskeletal reorganization, membrane ruffling, the up-regulation of β₁ integrin adhesive activity, and the de novo synthesis of leukotrienes and cytokines (1). Basophils from a minority of subjects fail to secrete histamine, increase integrin-mediated adhesion, or produce IL-4 in response to FcεRI cross-linking (2–8). The unresponsiveness of “nonreleaser” basophils has been linked to the increased proteasomal degradation of Syk, an essential tyrosine kinase in the FcεRI-signaling cascade (5, 9). Partial responses are recovered

in nonreleaser basophils after prolonged IL-3 treatment, suggesting that changes in transcriptional regulation may underlie the differences between the releaser and nonreleaser phenotypes (6, 9).

The role of basophils in asthma has never been resolved clearly. Lung mast cells are generally thought to be the main source of histamine and other mediators in acute asthmatic responses (10). However, a 10-fold increase in basophil numbers was observed in lung tissue sections of patients who died from asthma in comparison with other causes of death (11), and infiltrating basophils have been implicated as a potential additional source of mediators in the asthmatic lung, especially in late phase responses (12–16).

In this study, basal and FcεRI-mediated histamine release were measured repeatedly over a 4-year period in peripheral blood basophils from two pools of donors, 68 healthy nonatopic nonasthmatics and 61 atopic asthmatics, to compare basophil releasability between these two populations and to determine whether the nonreleaser phenotype is limited to nonasthmatics. We report that basophils from asthmatics show higher rates of spontaneous and stimulated degranulation than controls and, unexpectedly, that nonreleaser basophils occur among asthmatic subjects, although less often than among controls. “Cycling” between releaser and nonreleaser status was observed in both groups. Microarray analysis was used to compare gene expression between resting and activated releaser and nonreleaser basophils purified from the peripheral blood of several of these donors.

Materials and Methods

Reagents

Allergy skin test kits were obtained from Greer Labs. Percoll was purchased from Pharmacia, and affinity-purified goat anti-human IgE (anti-IgE) was from

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BioSource International. MidiMacs separation columns and MidiMacs magnetic columns were from Miltenyi Biotec. ELISA kits for IL-8, MIP-1 α , MIP-1 β , and RANTES were from BioSource International. The IL-3 ELISA kit was from R&D Systems, and the human histamine kits were from Beckman Coulter. Gene expression profiling was performed using GeneChip Human Genome U133A oligonucleotide microarrays (Affymetrix), comprised of 22,283 probe sets corresponding to human genes or expressed sequence tags. For microarray analyses, RNA was prepared with the RNeasy minikit (Qiagen), RNA concentrations were determined using the RiboGreen RNA Quantitation Reagent (Molecular Probes), and RNA quality was assessed using the RNA 6000 Nano or Pico LabChip assay with the Agilent 2100 Bioanalyzer. Diethyl pyrocarbonate-treated water, RNA 6000 ladder, RNase inhibitor, ammonium acetate, MEGA script T₇-Kits, linear polyacrylamide, and RNase ZAP were obtained from Ambion. SuperScript II, dNTPs mix, 5 $\times$ second strand buffer, DNA ligase, DNA polymerase I, RNase H, T4 DNA polymerase, and random primers were from Invitrogen Life Technologies. T7-oligo(dT) Promotor Primer (Enzo Diagnostics) BioArray High Yield RNA Transcript labeling kits and Gene Chip Sample Cleanup modules were from Affymetrix. β -Mercaptoethanol was purchased from Sigma-Aldrich. For PCR, RNA was isolated using RNA-Bee (IsoTex Diagnostics). Semiquantitative PCR was performed using the Qiagen OneStep RT-PCR Kit and an Eppendorf Mastercycler gradient Thermocycler. Real-time PCR was performed using the Qiagen QuantiTect Probe RT-PCR Kit and an Applied Biosystems 7000 Sequence Detection System. TaqMan Primer Express 2.0 Software was used for probe and primer design. Primers, purchased from DNA Research Services of the University of New Mexico Health Sciences Center, were as follows: Fc ϵ RI β (5'-cag ctc ggt taa tga aaa aat gg-3'; 5'-gga tga ggc cga ctt caa tag tc-3'); FosB (5'-gca caa act cca gac gtt cct t-3'; 5'-ccg gga acg aaa taa act agc a-3'); and GADPH (5'-ctc tgc ccc ctc tgc tga T-3'; 5'-cac gat acc aaa gtt gtc atg gat-3'). Probes, purchased from Integrated DNA Technologies, were as follows: Fam-GADPH-black hole probe (5'-/56-FAM/ATG CCT CCT GCA CCA CCA ACT GCT TAG/3BHQ_1/-3'); Fam-FOS-b black hole probe (5'-/56-FAM/TCC AGG CGG AGA CAG ATC AGT TGG A/3BHQ_1/-3'); and Fam-Fc ϵ RI β -black hole probe (5'/56-FAM/AGG AGC CTT CCA GTG TGC CTG CAT T/3BHQ_1/-3').

Subjects

A cohort of young, healthy, nonsmoking volunteers was recruited with approval by the Human Research Review Committee at the University of New Mexico. The subjects' current medications (self-reported) did not include systemic glucocorticosteroids, but did include inhaled, nasal and skin steroids, inhaled β_2 agonists, oral contraceptives and antihistamines. Nonsmoking status was confirmed with a serum cotinine level of <14 ng/ml. The subjects were classified as either atopic asthmatic or nonatopic nonasthmatics based on results of allergy skin tests using aeroallergens common in the Albuquerque area (Fremont cottonwood, juniper-mountain cedar, Bermuda grass, bluegrass, fescue, Russian thistle, Kochia, western ragweed, cat dander, and house dust mite) plus methacholine challenges performed under physician supervision. A subject was considered atopic if he or she had two or more positive skin prick tests (≥ 3 mm in wheal diameter and no wheal in response to normal saline). Asthma was defined as either a $\geq 15\%$ increase of forced expiratory volume in one second (FEV₁)³ in response to 200 μ g of albuterol or increased sensitivity to inhaled methacholine. A positive methacholine test was defined as a decrease of the FEV₁ of at least 20% after inhalation of ≤ 16 mg/ml methacholine (17). Control subjects had no history of asthma or upper respiratory tract atopy. They had negative immediate type skin tests and normal spirometry. Most (45 of 68) had methacholine challenges, which were all (45 of 45) negative.

Cell isolation and activation for histamine release assays

Histamine release was measured as previously described (6, 8) with slight modifications. Briefly, basophil-enriched cell populations (1–55% basophils) were prepared by Percoll gradient centrifugation of anticoagulated blood (usually 110 ml). Portions of the Percoll-enriched cells were incubated in duplicate at 37°C with no addition (to measure spontaneous degranulation) or with four concentrations of anti-human IgE Ab (10 ng/ml, 100 ng/ml, 1 μ g/ml, and 10 μ g/ml; to capture maximum Fc ϵ RI-mediated degranulation) and with 1 μ g/ml of the calcium ionophore A23187 (a Syk-independent secretagogue causing >40% release from both releaser and nonreleaser basophils; used as an internal control). After 30 min, reactions were terminated by dilution in ice-cold HBSS and centrifugation, and his-

tamine in cell pellets and supernatants was measured by ELISA. Total histamine was measured in supernatants generated by lysis of identical cell aliquots using three freeze-thaw cycles. The percentage of Fc ϵ RI-mediated histamine release was defined as the histamine release induced by treatment with the optimal concentration of anti-IgE (1 μ g/ml in most cases) minus the spontaneous release divided by the total histamine content of the sample. Each donor contributed blood at least four times over the 4 years of this study. Each result was treated as an independent measure.

Statistical analysis of histamine release data

The histamine release data were not skewed, so that logarithmic transformation was not required. A *t* test was used to compare the continuous variables, percent histamine release and age, between the two groups of patients. The proportion of females in each group was compared by Fisher's exact test. The binary variable, releaser status, was analyzed by multivariate logistic regression with group, gender, age, and medications as predictor variables. The "best" logistic model was determined by stepwise methods. The continuous variable percent histamine release was analyzed by multiple regression methods, including stepwise regression to include the covariates gender, age, and medications. The suspected bimodality of the maximum percent release data set was examined both graphically and using the maximum likelihood method programmed in SAS (18). In addition to the maximum likelihood method, we used a receiver operator curve to select a cut-score for maximal release percent for a definition of non-releaser status for each sample. The relationship between spontaneous release and total histamine content was compared between groups by analysis of covariance.

Cell isolation and activation for gene expression profiling

Following Percoll gradient centrifugation, cells for gene expression studies were further enriched to >98% basophils by negative selection using MidiMacs separation columns as described previously (8). Subsequent flow sorting based on the forward light scatter/side light scatter characteristics increased the purity to >99.5% basophils for microarray analyses. Yields ranged from 6.4×10^5 to 2.1×10^6 cells per donor, with an average of 1.63×10^6 .

Cells for microarray analyses (10⁶ basophils/ml; $0.4\text{--}1 \times 10^6$ basophils per condition) were activated by incubation in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin for indicated times at 37°C in a 5% CO₂ incubator with or without 1 μ g/ml anti-IgE. For most experiments, media (with or without anti-IgE) were replaced after 30 min to limit continued exposure to basophil-secreted products. Cell viability was assessed by trypan blue exclusion and exceeded 99% for all incubation times and conditions. Total cellular RNA was prepared with the Qiagen RNeasy minikit.

Microarray analysis

RNA targets were amplified from 100 ng of total RNA. Two rounds of RT-PCR were used to prepare cDNA according to the small sample target labeling protocol from Affymetrix. After purification, double-stranded cDNA underwent in vitro transcription in the presence of biotinylated nucleoside triphosphates using the Enzo high-yield T7 RNA Polymerase kit. The biotin-labeled cRNA was purified on Qiagen RNeasy minikit columns and quantified using RiboGreen. The average cRNA yield after two cycles of cDNA synthesis and in vitro transcription reactions was 74.49 ± 12 μ g. cRNA (20 μ g) was fragmented to an average size of 300 nt following the Affymetrix protocol and hybridized with a probe array for 20 h at 45°C. After hybridization, the chips were washed, stained using the Euk-GE-WS2 fluidics protocol, and scanned at 488 nm with an HP Gene Array Scanner.

Data filtering and analysis

Affymetrix GeneChips were scaled in the Affymetrix MAS 5.0 software to a target fluorescence of 500. The per-chip values were imported into GeneSpring 6.0 software for analysis. Pairwise replicate comparisons were made between unstimulated samples and samples that were cross-linked for 2 or 4 h with anti-IgE. All samples were normalized per gene to the median of the unstimulated releaser samples.

Two different data mining strategies were used. The first used a fluorescence threshold filter to identify a 2-fold up or down change in the expression when compared with the control sample. For the induced genes, the threshold value was set to exclude all genes that had a raw fluorescence <1000 relative fluorescence units in at least half of the samples. For the repressed genes, the fluorescence threshold was set at 1200 relative fluorescence units for at least half the samples. This strategy is referred to as the basic filter set. The second strategy combined the conventional filter with a statistical filter. For this four-step analysis, the first step is to use the

³ Abbreviations used in this paper: FEV₁, forced expiratory volume in one second; Egr, early growth response; HRF, histamine-releasing factor; TCTP, translationally controlled tumor protein; VEGF, vascular endothelial growth factor.

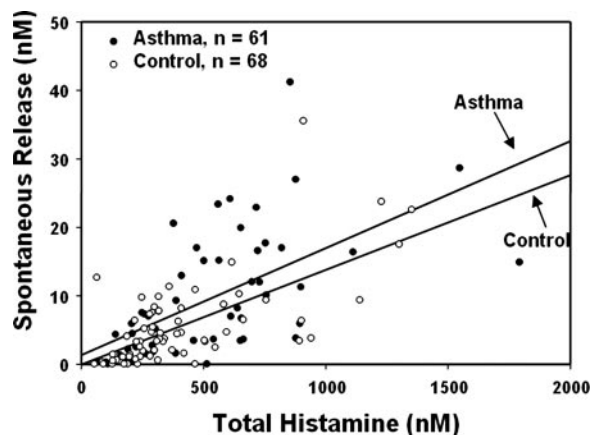


FIGURE 1. Relationship between total histamine in basophils and spontaneous histamine release. The total amount of histamine was the same between the two groups (t test, $p = 0.053$), but spontaneous histamine release was higher in the asthmatic group ($p = 0.008$). Data represent the first assay performed for each donor.

GeneSpring "Flag" filter that is based on the Affymetrix algorithm for a present, marginal, or absent categorization for each gene's expression. This parameter was set so that half of the samples under consideration had a present or marginal call. The second step was an expression threshold cutoff of 500 for half the samples. The next step used the one-way Anova filter in GeneSpring to determine whether a statistically significant difference was present between the genes in the cross-linked and unstimulated samples. The p value maximum was set at 0.05. Finally, a 2-fold change filter was used to select only genes that changed >2 -fold, either up or down. The identities, fold change, accession number, and description details of individual genes were obtained from Affymetrix (www.affymetrix.com/index.affx).

Chemokine ELISA measurements

Percoll-enriched, negatively selected ($>98\%$ pure) releaser and non-releaser basophils (2×10^5 cells/ml) were incubated at 37°C in a $5\% \text{CO}_2$ incubator in RPMI 1640 medium supplemented with $10\% \text{FBS}$, 2 mM L-glutamine, 100 U/ml penicillin, and $100 \mu\text{g/ml}$ streptomycin with or without the addition of various concentrations of anti-IgE or with 5 ng/ml of the Ca^{2+} ionophore A23187. Culture supernatants were harvested and analyzed by ELISA for IL-8, MIP-1 α , MIP-1 β , and RANTES according to the manufacturer's instructions.

Polymerase chain reaction

RNA was purified from flow-sorted ($>99.5\%$ pure) basophils using RNA-Bee. Primers and probes were designed with Primer Express 2.0 software using the TaqMan Probe and Primer Design Option with default parameters, except that amplicon size was set to a minimum of 100 bp and a maximum of 200 bp. The selected primers bridged splice sites or were located on either side of a splice site. Semiquantitative PCR used the Qiagen OneStep RT-PCR kit according to the manufacturer's instructions, except for the use of $20\text{-}\mu\text{l}$ final reaction volumes. All reagents were proportionally adjusted. Total RNA ($0.1 \mu\text{g}$) was used as a template. Amplifications were performed using an Eppendorf Mastercycler gradient thermocycler. Real-time PCR was performed using Qiagen's QuantiTect Probe RT-PCR kit according to the manufacturer's instructions, except for the use of $25\text{-}\mu\text{l}$ final reaction volumes. Total RNA ($0.1 \mu\text{g}$) was used as a template. Amplifications were performed using an Applied Biosystems 7000 Sequence Detection System. Relative levels of transcripts were determined based on the number of cycles needed to detect the specified product.

Measuring serum IL-3

Four-milliliter serum aliquots were concentrated to $800 \mu\text{l}$ using Ultrafree MC Centrifugal Filter Units, with a molecular mass cutoff of 10 kDa , in a microfuge, then analyzed using an ELISA kit sensitive to 7.4 pg/ml IL-3.

Results

The study population

We recruited 61 atopic asthmatics (aged 27.3 ± 1.2 years, consisting of 25 males and 36 females) and 68 nonatopic controls

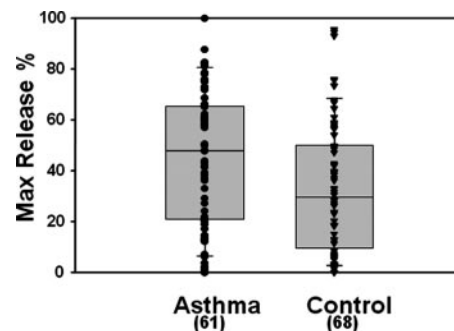


FIGURE 2. Higher anti-IgE-stimulated histamine release from the basophils of asthmatic donors. The central box represents the values from the lower to the upper quartile (25th and 75th percentile), the middle line the median, and the whiskers the 5th and 95th percentile. Value of $p = 0.02$, asthma group compared with the control group (t test). Data represent the first assay performed for each donor.

(aged 26.4 ± 1.4 years, consisting of 21 males and 47 females) for our studies. There was no difference between the groups in age (t test) or gender distribution (Fisher's exact test). Most of the asthmatic subjects had mild disease as demonstrated by normal spirometry. Forty-five of the 61 asthmatics were using inhaled β_2 agonist, but only 3 had a diminished FEV_1 that was increased after inhalation of a β_2 agonist. The remaining 58 asthmatics had baseline normal FEV_1 but increased reactivity to inhaled methacholine. The controls had negative allergy skin tests, normal spirometry, and negative methacholine challenges. Basophils were isolated for histamine release assays from each donor at least four times over the course of the study.

Histamine release data

There was no difference in the total amount of histamine between the two groups (t test, $p = 0.053$). Histamine release was compared between the groups based on the first measure of spontaneous and stimulated degranulation for each donor. Asthmatics showed an increase in the spontaneous release of histamine in comparison to nonasthmatics ($p = 0.008$; Fig. 1). Anti-IgE-stimulated percent histamine release from peripheral blood basophils was also higher when the donors were asthmatic ($p = 0.02$; Fig. 2). The differences were modest in both cases, and there was considerable overlap between the groups.

Statistical analysis of the entire data set (original plus repeated measurements; over 500 separate histamine release assays)

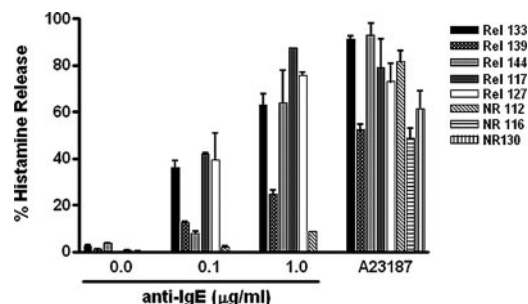


FIGURE 3. Histamine release from the five releasers and three non-releasers basophils on the same day as RNA purification. Percoll-enriched releaser and nonreleaser basophils were incubated for 30 min in various concentrations of anti-IgE or A23187 (5 ng/ml), and histamine levels were measured in the incubation supernatants using ELISA. Results, corrected for spontaneous release, show the range of duplicate assays. Anti-IgE induced no histamine release from NR116 and NR130.

Table I. Detecting typical basophil genes^a

Function	Accession	Description
IgE receptor Subunits	BC005912	FcεRI α polypeptide
	D10583	FcεRI β polypeptide
	NM_004106	FcεRI γ polypeptide
Granule contents	NM_002112	Histidine decarboxylase
	NM_002727	Proteoglycan 1, secretory granule
	NM_000521	β-Hexosaminidase (β polypeptide)
	NM_001828	Charcot-Leyden crystal
Kinases	A1356412	Tyrosine kinase Lyn
Receptors	NM_002183	IL-3R α (low affinity)
	AF056979	IFN-γR 1
	L41944	IFN (α, β, and ω) receptor 2
	AF348491	Chemokine (C-X-C motif), receptor 4 (fusin)
Cytokines and chemokines	NM_000584	IL-8
	NM_000589	IL-4
	M15330	Human IL-1β mRNA
	NM_002983	Small inducible cytokine A3 (homologous to mouse Mip-1α)
	M21121	RANTES
	AF022375	VEGF
Other	A1721229	TCTP (p23) (HRF, IgE-dependent)

^a RNA was prepared from the basophils (>99% pure) of three different releaser donors. Two Affymetrix Genechip arrays were used for each sample.

showed there was no effect of medication on the percent maximal release, with one exception: use of β₂ agonists was associated with increased maximal percent release. This association was absent when the asthmatics using β₂ agonists were compared with the asthmatics not using β₂ agonists. Therefore, the apparent association in the entire data occurred because only asthmatics used β₂ agonists, and asthmatics exhibited higher percent maximal release.

Basophils that released little or no histamine were identified in both the asthma and nonasthma groups. This led to concern over the definition of a nonreleaser. Previously, we and others (3–9) have used different arbitrary cut points (usually 5–10%) to distin-

guish releasers from nonreleasers. We applied statistical methods to the entire data set to make an objective determination of the best cut point. We found that the data distribution of percent maximal release was compatible with a mixture of two distributions. There is an exponential distribution for the small values and a broad normal distribution for the higher values. The transition point of 12.7% between these two distributions was verified by maximum likelihood methods. Applying 12.7% as the cut point between releasers and nonreleasers, we found that 13% of the asthmatic subjects were nonreleasers compared with 28% of the control subjects ($p = 0.02$, χ^2).

FIGURE 4. Heat map and Venn diagram representations of gene expression in resting and activated releaser and nonreleaser basophils. The four heat maps represent genes that were up or down-regulated by 2-fold or greater in four separate experiments. Differential expression was detected using either the basic filtering method or the Anova incorporating filter method. Each row represents one gene and each column one stimulus. A color scale represents the expression ratios. *A* represents 253 genes that were differentially expressed in releaser basophils from three different donors in response to FcεRI cross-linking. *B* represents 205 genes that were differentially expressed in releaser basophils from two different donors in response to FcεRI cross-linking with medium exchange after 30 min of activation. *C* displays 96 genes that were differentially expressed between unstimulated releaser (five donors) and nonreleaser (two donors) basophils. *D* represents the gene expression profile of nonreleaser basophils from two different donors in response to FcεRI cross-linking. The Venn representations of the filtering results indicate numbers of genes identified as 2-fold or greater up or down-regulated only when using the basic filter (red), only when using the Anova incorporating filter (green) and when using both filters (overlap, yellow).

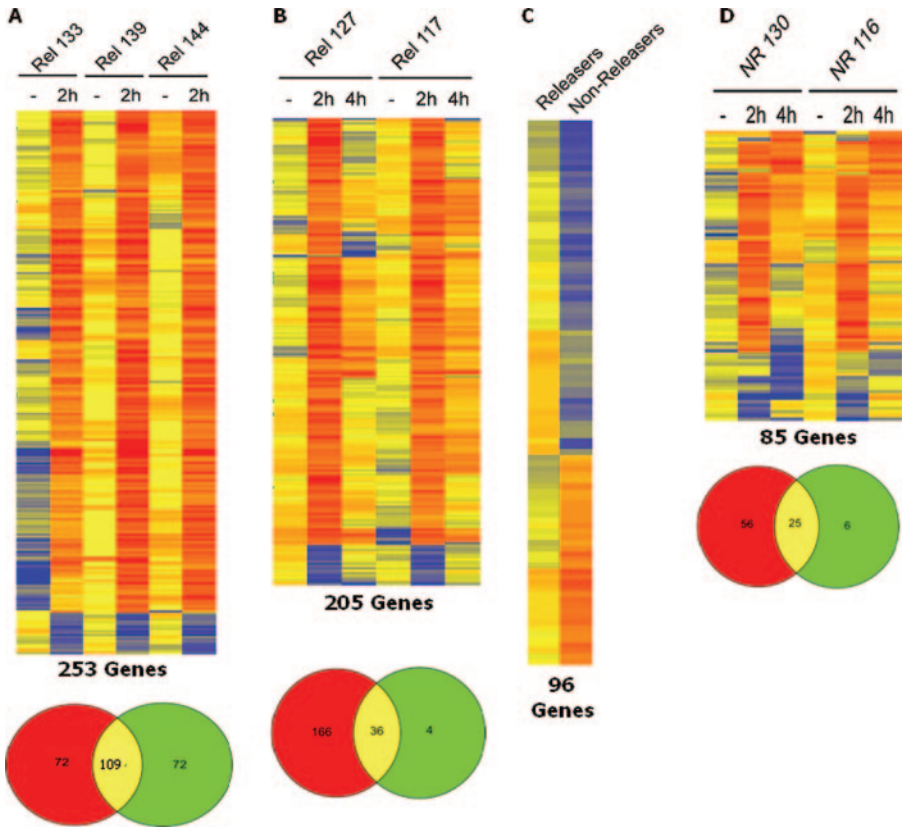


Table II. *Human basophil genes that are differentially expressed in response to FcεRI cross-linking^a*

Group	Fold Change	Accession	Description
Cytokines and chemokines	2.85	*AF022375	VEGF
	3.09	NM_002983	Homologous to mouse Mip-1α
	11.01	*M21121	RANTES
Receptors	7.38	NM_003807	TNF (ligand) superfamily, member 14
	0.22	NM_001837	Chemokine (C-C motif) receptor 3
	2.55	*NM_001736	Complement component 5 receptor 1 (C5a ligand)
	0.36	NM_014879	G protein-coupled receptor 105
	2.39	AK021780	Phosphatidylserine receptor
	2.21	X74039	Plasminogen activator, urokinase receptor
	2.23	NM_006018	Putative chemokine receptor; GTP-binding protein
GTPases and associated proteins	3	*BC001188	Transferrin receptor (p90, CD71)
	3.88	*NM_014504	Putative Rab5 GDP/GTP exchange factor homolog
	3.16	*D42063	RAN-binding protein 2
	2.79	*AL137335	RAN-binding protein 7
	2.07	AF054183	RAN member RAS oncogene family
	3.18	*J03198	Guanine nucleotide-binding protein G(k), α subunit
	3.32	NM_002068	Guanine nucleotide-binding protein (G protein), α 15
Kinases	2.5	*NM_006496	Guanine nucleotide-binding protein (G protein), α-inhibiting activity polypeptide 3
	4.18	NM_004073	Cytokine-inducible kinase
	0.44	U86453	Phosphoinositide-3-kinase, catalytic, δ polypeptide
	0.41	NM_002647	Phosphoinositide-3-kinase, class 3
	2.24	NM_003600	Serine/threonine kinase 15
	2.41	NM_003158	Serine/threonine kinase 6
	2.51	*NM_005433	Tyrosine kinase Lyn
Phosphatases	5.18	*NM_001827	CDC28 protein kinase 2
	4.15	*NM_004417	Dual specificity phosphatase 1
	10.54	*NM_004418	Dual specificity phosphatase 2
Pro-/Antiapoptosis	4.13	U16996	Dual specificity phosphatase 5
	4.75	NM_004049	BCL2-related protein A1
	0.26	NM_004346	Caspase-3, apoptosis-related cysteine protease
	3.61	U60521	Caspase-9, apoptosis-related cysteine protease
	3.05	*NM_015675	Growth arrest and DNA damage inducible, β
	5.23	*NM_021960	Myeloid cell leukemia sequence 1 (BCL2-related)
	0.39	NM_006265	RAD21 (<i>S. pombe</i>) homolog
Other signaling	2.15	L07555	CD69 Ag (p60, early T cell activation Ag)
	5.51	NM_004233	CD83 Ag
	0.38	NM_004688	N-myc (and STAT) interactor
	2.09	*NM_004556	NF-κβ inhibitor, ε
	2.44	*NM_002852	Pentaxin-related gene, rapidly induced by IL-1β
	0.19	BC005352	TNF-induced protein
	2.48	NM_001675	Activating transcription factor 4
Transcription	2.14	NM_005760	CCAAT-box-binding transcription factor
	5.88	AB017493	Core promoter element-binding protein
	5.85	*NM_001964	Egr-1
	9.14	NM_004430	Egr-3
	15.21	NM_006732	FOS-B
	3.71	NM_002135	Nuclear receptor subfamily 4, group A, member 1
	4	L03411	RD RNA-binding protein
	2.46	NM_012421	Rearranged L-myc fusion sequence
	6.54	*NM_005655	TGF-β-inducible early growth response
	3.21	NM_017544	Transcription factor NRF
Translation	2.7	*NM_012323	Transcription factor MAFF
	2.98	NM_003452	Zinc finger protein 189
	2.07	NM_018555	Zinc finger protein 331; zinc finger protein 463
	2.94	*NM_014670	Basic leucine-zipper protein BZAP45
	2.32	NM_001412	Eukaryotic translation initiation factor 1A
	2.21	*BC002719	Eukaryotic translation initiation factor 3, subunit 1 (α, 35 kDa)
	2.8	BC006210	Eukaryotic translation initiation factor 4A, isoform 1
	3.08	*NM_004730	Eukaryotic translation termination factor 1
	3.29	*BC000451	Splicing factor, arginine/serine-rich 10
	7.76	*AF107405	Splicing factor, arginine/serine-rich 3
Ubiquitination	3.26	NM_005744	UbcH7-binding protein
	4.66	NM_014501	Ubiquitin carrier protein
	2.86	*AF064801	Ring finger protein 139
Cytoskeleton	2.06	NM_005721	ARP3 homolog
	2.76	*BC000665	T-complex 1
	8.53	NM_001069	Tubulin, β polypeptide
Heat shock	2.05	NM_003370	Vasodilator-stimulated phosphoprotein
	4.28	*NM_003379	Villin 2 (ezrin)
	3.74	*NM_001539	DnaJ (Hsp40) homolog, subfamily A, member 1
	14.05	*NM_006145	DnaJ (Hsp40) homolog, subfamily B, member 1
	2.53	NM_006736	DnaJ (Hsp40) homolog, subfamily B, member 2
	4.34	*NM_012328	DnaJ (Hsp40) homolog, subfamily B, member 9
	12.28	*NM_006644	Heat shock 105 kDa
	10.43	*NM_002157	Heat shock 10-kDa protein 1 (chaperonin 10)
	5.68	*NM_002156	Heat shock 60-kDa protein 1 (chaperonin)
	23.53	NM_005346	Heat shock 70-kDa protein 1B
	14.56	NM_002155	Heat shock 70-kDa protein 6 (HSP70B')
	5.19	*AF352832	Heat shock 70-kDa protein 8
	2.85	*BC000478	Heat shock 70-kDa protein 9B (mortalin-2)
	13.99	*NM_005348	Heat shock 90-kDa protein 1, α
	4.92	*AF275719	Heat shock 90-kDa protein 1, β
	2.6	*BC002446	Similar to <i>MRJ</i> gene for a member of the DNAJ protein family
	2.19	NM_006948	Stress 70 protein chaperone, 60 kDa

^a Basophils from three different releaser donors were cultured for 2 h in medium with or without 1 μg/ml anti-IgE, then RNA was isolated for microarray analysis. In these basophils, 1 μg/ml anti-IgE caused the release of 63% (rel 133), 25% (rel 139), and 63.9% (rel 144) of total histamine (corrected for spontaneous) in 30 min. Two Affymetrix Genechip arrays were used for each sample. This list reports genes that could be assigned to relevant functional categories among the 253 that were differentially expressed in 2-h activated cells using either of two filtering approaches. Asterisks identify genes that were significantly induced or down-regulated using both filtering approaches.

Table III. Human basophil genes that are differentially expressed in response to FcεRI cross-linking, with medium exchange^a

Group	Fold Change	Accession	Description
Cytokines and chemokines	3.51	NM_000584	IL-8
	3.02	NM_002983	MIP-1α
Receptors	2.69	U62027	Complement component 3a receptor 1
	2.52	NM_001295	Chemokine (C-C motif) receptor 1
	2.74	NM_001736	Complement component 5 receptor 1 (C5a ligand)
	3.38	AF312230	Histamine H4 receptor
	2.25	BC005912	FcεRIα polypeptide
	2.46	NM_000527	Low-density lipoprotein receptor
	3.38	NM_002355	Mannose-6-phosphate receptor
	2.15	D10583	FcεRIβ polypeptide
	2.07	X83858	PGE receptor 3 (subtype EP3)
	8.75	NM_006018	Putative chemokine receptor; GTP-binding protein
GTPases and associated proteins	2.82	*D42063	RAN-binding protein 2
	2.53	NM_006834	RAB32, member RAS oncogene family
	2.67	NM_000161	GTP cyclohydrolase 1 (dopa-responsive dystonia)
	4.9	NM_014504	Putative Rab5 GDP/GTP exchange factor homolog
	2.53	NM_004310	Ras homolog gene family, member H
	2.62	AF030107	Regulator of G-protein signaling 13
Kinases	0.3	NM_005308	G protein-coupled receptor kinase 5
	3.03	*M24779	Pim-1 oncogene
	3.26	*M79321	Tyrosine kinase Lyn
Phosphatases	4.86	*BC003143	Dual specificity phosphatase 6
	2.13	AF014403	Phosphatidic acid phosphatase type 2A
Pro- or antiapoptosis	3.3	U37546	Baculoviral IAP repeat-containing 3
	6.29	BC002461	BCL2/adenovirus E1B 19-kDa interacting protein 2
	2.16	AF005775	CASP8 and FADD-like apoptosis regulator
	2.57	U69546	CUG triplet repeat, RNA-binding protein 2
	6.82	NM_021960	Myeloid cell leukemia sequence 1 (BCL2-related)
Other signaling	8.2	NM_004226	Serine/threonine kinase 17b (apoptosis-inducing)
	3.27	NM_003853	IL-18R accessory protein
	2.44	NM_002182	IL-1R accessory protein
	2.74	NM_020651	Pellino (<i>Drosophila</i>) homolog 1
	2.28	NM_003473	Signal transducing adaptor molecule 1
	2.31	NM_005625	Syndecan-binding protein (syntenin)
	4.19	U59863	TNFR-associated factor family member-associated NFKB activator
	8.68	*NM_005655	TGFB-inducible early growth response
	3.98	NM_002852	Pentaxin-related gene, rapidly induced by IL-1β
	6.48	*NM_000700	Annexin A1
Transcription	3.23	*NM_001784	CD97 Ag
	2.58	NM_001675	Activating transcription factor 4
	0.29	NM_005197	Checkpoint suppressor 1
	8.83	M82882	E74-like factor 1
	7.38	NM_001964	Egr-1
	6.84	NM_004430	Egr-3
	17.03	NM_006732	FOS-B
	4.42	*BC001124	H3 histone, family 3B (H3.3B)
	2.39	NM_005354	Jun D proto-oncogene
	3.3	*NM_006792	Mortality factor 4
Translation	3.12	*NM_005384	NF, IL-3 regulated
	2.36	AF118094	TAF11 RNA polymerase II
	0.23	NM_015833	Adenosine deaminase, RNA specific, B1
	2.1	NM_003670	Basic helix-loop-helix domain containing, class B, 2
	2.1	NM_014670	Basic leucine-zipper protein BZAP45
	2.91	NM_001356	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3
	2.69	NM_012141	Deleted in cancer 1; RNA helicase HDB/DICE1
	2.16	NM_003750	Eukaryotic translation initiation factor 3, subunit 10
	2.44	BC006210	Eukaryotic translation initiation factor 4A, isoform 1
	3.76	*M75715	Eukaryotic translation termination factor 1
Ubiquitination	3.14	*AF083441	Putative translation initiation factor
	2.12	NM_005875	Translation factor sui1 homolog
	5.38	NM_006758	U2(RNU2) small nuclear RNA auxiliary factor 1
	3.62	AF157323	F-box and leucine-rich repeat protein 5
	2.83	NM_002803	Proteasome 26S subunit, ATPase, 2
	2.91	NM_002793	Proteasome subunit, β type, 1
	2.42	AL136733	Ubiquitin associated protein
Cytoskeleton/Motility/Adhesion	3.01	*AB009010	Ubiquitin C
	4.73	*NM_006825	Cytoskeleton-associated protein 4
	2.54	NM_012325	Microtubule-associated protein
	3.02	NM_006915	Retinitis pigmentosa 2
	4.1	*NM_000655	Selectin L (lymphocyte adhesion molecule 1)
	2.14	NM_021103	Thymosin, β 10
	2.66	BC005946	Tubulin α 6
	4.21	*AF141347	Tubulin, α 3
	2.54	BC006481	Tubulin, α, ubiquitous
	3.93	*BC004188	Tubulin, β, 2
Heat shock	3.78	NM_006086	Tubulin, β, 4
	2.3	AL080081	DnaJ (Hsp40) homolog
	0.28	NM_005346	Heat shock 70-kDa protein 1B
	2.92	NM_006948	Stress 70 protein chaperone

^a The experiment was as described in Table II, except that the medium was exchanged after 30 min to remove basophil-secreted products. Data are from duplicate analyses of RNA from two donors. A total of 1 μg/ml anti-IgE induced the release of 75.7% (rel 127) and 87.2% (rel 117) of total histamine (corrected for spontaneous) from these basophils. This list reports genes that could be assigned to relevant functional categories among the 205 that were differentially expressed in 2-h activated cells using either of two filtering approaches. Asterisks identify genes that were significantly induced or down-regulated using both filtering approaches.

Table IV. Comparison of gene expression profiles of unstimulated releaser and nonreleaser basophils^a

Group	Fold Change	Accession	Description
Receptors	0.457	NM_006018	Putative chemokine receptor
	0.39	BC001188	Transferrin receptor (p90, CD71)
	0.302	NM_001736	Complement component 5 receptor 1 (C5a ligand)
	2.084	NM_021624	Histamine H4 receptor
Kinases	0.397	U08839	Plasminogen activator, urokinase receptor
	2.987	AF092132	Serine threonine kinase
	0.449	NM_006888	Calmodulin 1 (phosphorylase kinase, δ)
	0.325	NM_005204	MEK kinase 8
	0.397	NM_002350	Tyrosine kinase Lyn
Other Signaling	0.383	NM_006435	IFN-induced transmembrane protein 2 (1-8D)
	2.396	NM_007115	TNF, α -induced protein 6
	0.349	NM_000574	Decay accelerating factor for complement
	0.452	AB005043	JAK-binding protein
	0.414	NM_001784	CD97 Ag
Transcription	0.43	AJ240085	TCR-interacting molecule
	2.176	NM_021813	Transcription regulator protein BACH 2
	0.489	NM_005324	H3 histone, family 3B (H3.3B)
	2.266	NM_004993	Spinocerebellar ataxia 3
	2.126	NM_002432	Myeloid cell nuclear differentiation Ag
	0.374	NM_006164	NF (erythroid-derived 2)-like 2
	2.5	D87684	UBX domain-containing 2
Translation	2.261	AF055376	Transcription factor MAF
	3.095	U30884	Splicing factor, arginine/serine-rich 5
	0.438	NM_000985	Ribosomal protein L17
	0.484	NM_000990	Ribosomal protein L27a
	0.489	L05095	Ribosomal protein L30
Ubiquitination	0.467	NM_001997	Ubiquitin like-S30 fusion protein
	0.34	AF064801	Ring finger 139
Cytoskeleton/Motility/Adhesion	2.385	Z22780	Cylicin, basic protein of sperm head cytoskeleton 1
	0.485	NM_021103	Thymosin, β 10
	0.385	AF141347	Tubulin, α 3
	0.486	BC006379	Tubulin, α , ubiquitous
Heat shock	0.46	AF028832	Heat shock 90-kDa protein 1, α

^a Basophil mRNA was isolated from five releaser and two nonreleaser donors. Data were filtered using the Anova incorporating method alone. Known genes that could be assigned to relevant functional categories are listed. Fold change refers to transcript level in nonreleasers relative to releasers.

Remarkably, 8 (of 8) asthmatic nonreleasers and 16 (of 23) nonasthmatic nonreleasers cycled at least once to releaser status over 4 years. Cycling was not related to the classification of the subject (both asthmatics and control subjects cycled), nor to medications or season of the year. In the case of two asthmatics, increased reactivity to inhaled methacholine was measured when the basophils were in both releaser and nonreleaser status, suggesting that the transition to nonreleaser basophils does not

alter hallmarks of established asthma. In all cases, nonreleaser status was associated with the loss of detectable Syk by Western blotting (data not shown; illustrated previously (5, 6, 8)).

Changes in serum IL-3 levels have been proposed as a possible reason for cycling because in vitro culture with IL-3 can convert nonreleaser to releaser basophils (6, 9). Estimates of IL-3 concentrations in serum are sparse, but values in the range of 5 pg/ml for normal subjects have been reported (19). Our direct measurements

Table V. Genes that are differentially expressed between resting and stimulated nonreleaser basophils^a

Group	Fold Change	Accession	Description
Receptors	2.65	NM_003853	IL-18 receptor accessory protein
	2.48	*NM_006018	Putative chemokine receptor; GTP-binding protein
	2.76	AJ240085	TCR-interacting molecule
	2.15	BC001188	Transferrin receptor (p90, CD71)
GTPases and associated proteins	2.53	NM_013285	Nucleolar GTPase
	2.84	*NM_014504	Putative Rab5 GDP/GTP exchange factor homolog
Kinases	2.49	M27319	Calmodulin 1 (phosphorylase kinase, δ)
	0.47	NM_019884	Glycogen synthase kinase 3 α
	2.97	*NM_002350	Tyrosine kinase Lyn
Phosphatases	12.37	BC005047	Dual specificity phosphatase 6
	3.88	NM_002714	Protein phosphatase 1, regulatory subunit 10
Cytoskeleton/Motility/Adhesion	3.62	NM_006825	Cytoskeleton-associated protein 4
	0.67	L35594	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)
	3.09	AF141347	Tubulin, α 3
	2.98	*NM_000655	Selectin L (lymphocyte adhesion molecule 1)
Heat shock proteins	2.15	AL080081	DnaJ (Hsp40) homolog, subfamily B, member 9
	2.86	*NM_006948	Stress 70 protein chaperone, microsome-associated, 60 kDa

^a The table focuses on genes that could be assigned to relevant functional categories, among the 85 that were differentially expressed in 2- or 4-h activated nonreleaser cells, using either of two filtering approaches. Asterisks mark genes that were identified using both filtering approaches.

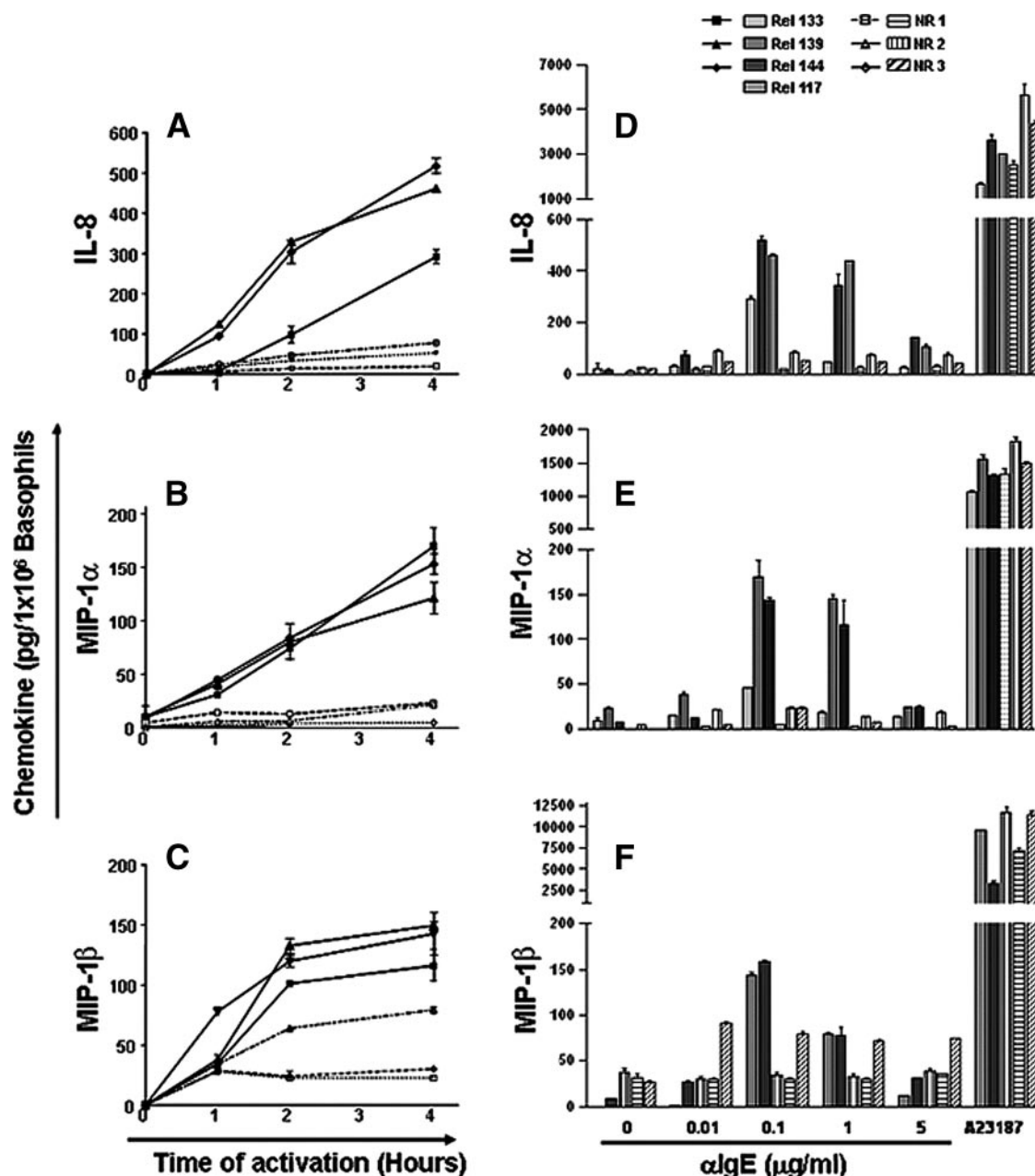


FIGURE 5. Time course and dose-response studies of anti-IgE-induced basophil chemokine release. In A–C, releaser (solid lines) or nonreleaser (dashed lines) basophils were incubated for 1, 2, or 4 h with 0.1 μg/ml anti-IgE. In D–F, releaser (solid bars) or nonreleaser (striped bars) basophils were incubated with 0.01, 0.1, 1, and 5 μg/ml anti-IgE or 500 ng/ml A23187 for 4 h at 37°C. Levels of IL-8 (A), MIP-1α (B), and MIP-1β (C) in the culture supernatants were analyzed by ELISA.

in concentrated serum samples from five releaser and five nonreleaser donors yielded values that ranged from nondetectable to 10 pg/ml and showed no obvious correlation with releaser status (data not shown). Having thus failed to find an obvious explanation for nonreleaser and cyclophosphamide-treated basophils, we undertook microarray analyses to try to better understand these phenotypes.

Detection of basophil-specific transcripts

We purified the RNA for microarray analysis from the basophils of eight subjects, three nonreleasers and five releasers. A small number of basophils from each preparation were set aside to confirm their “releasability” on the same day (Fig. 3). Table I documents the constitutive expression in unstimulated releaser basophils of transcripts encoding FcεRI subunits, granule proteins and chemo-

kines, cytokines, and their receptors. Transcripts related to translationally controlled tumor protein (TCTP), also known as IgE-dependent histamine-releasing factor (HRF), were also detected consistently. This combination of genes is predicted only in basophils and validates the experimental and analytical techniques used here.

A review of tyrosine kinase expression revealed transcripts for the Src family members, Lyn, Yes, and Fyn, and for Fak. Transcripts for Syk were not detected, even though this the FcεRI-associated tyrosine kinase is detected readily by RT-PCR (6, 8).

Effects of FcεRI cross-linking on gene expression in releaser basophils

Fig. 4A shows a heat map of gene expression for freshly isolated basophils (>99% purity) that were cultured for 2 h in medium with

or without 1 $\mu\text{g/ml}$ anti-IgE. Cells were from three different subjects with releaser basophils. Pairwise replicate comparisons were very similar between the three different donors, further validating our procedures and demonstrating the similarity in gene expression between different preparations of releaser basophils. Using two methods of filtering (basic and Anova), a total of 253 genes was found to be regulated 2-fold or greater up or down in response to Fc ϵ RI cross-linking for 2 h (previously shown to be the optimum for cytokine expression in releaser basophils (6)). Of these, 109 genes were common to both filtering lists (Venn diagram in Fig. 3A), and only 19 genes were down-regulated.

In Table II, a subset of these genes was assigned to 1 of 12 categories based on known functions. Stimulated basophils show substantial increases in transcripts for the chemokines RANTES and MIP-1 α , for the mitogen, vascular endothelial growth factor (VEGF), and for proteins involved in signaling, cytoskeletal organization, and the regulation of transcription, translation, and apoptosis. They also show increases in the expression of heat shock proteins. This raised the possibility that prolonged exposure of basophils to their own released products may induce stress responses through autocrine signals that are independent of signals through Fc ϵ RI.

To address this concern, cells from two separate releaser donors were incubated for 30 min with and without anti-IgE, then the medium was replaced with fresh medium (again with or without anti-IgE), and incubation continued for a total of either 2 or 4 h. Data are again summarized in a heat map and a Venn diagram (Fig. 4B) and a gene list (Table III). Replacing the medium reduced the expression of stress response genes while revealing other up-regulated genes, including genes for receptors (the Fc ϵ RI α and β subunits, the histamine H4 receptor, and the chemokine (C-C motif) receptor 1); kinases (Lyn); chemokines (IL-8, RANTES, MIP-1 α , and MIP-1 β); transcription factors (early growth response (Egr)-1, Egr-3, FOS-B, and AP-1); and pro- and antiapoptotic proteins (caspase-9 and Bcl2-A1). The down-regulated genes included G protein-coupled receptor kinase 5 and Checkpoint suppressor 1.

Genes common to activated cells, with and without medium exchange, included Egr-1 and -3, FosB, MIP-1 α , and Lyn (all more up-regulated in cells with medium exchange) and heat shock 70 kDa protein 1B (more down-regulated in cells with medium exchange).

Gene expression in unstimulated nonreleaser basophils

The heat map in Fig. 4C compares the gene expression profiles of unstimulated basophils from two nonreleaser donors with those from five releaser donors. Using the Anova incorporating filter, 59 genes were found to be transcribed at a lower level in nonreleaser basophils, whereas 37 were at a higher level. Genes that could be assigned a signaling-related function are presented in Table IV. All the molecules of interest, including Lyn, were expressed at lower levels in the nonreleaser cells with the exceptions of TNF- α -induced protein 6, histamine receptor 4, and one serine/threonine kinase.

Effects of Fc ϵ RI cross-linking on gene expression in nonreleaser basophils

The heat map and the Venn diagram in Fig. 4D compare the gene expression profiles of nonreleaser basophils from two donors that were incubated for 2 and 4 h with or without 1 $\mu\text{g/ml}$ anti-IgE. Eighty-five genes were differentially expressed in nonreleaser basophils following Fc ϵ RI cross-linking. The list of genes up-regulated in activated nonreleaser basophils (Table V) includes two heat shock proteins, Lyn and dual specificity phosphatase 6, previously identified as up-regulated in activated releaser basophils

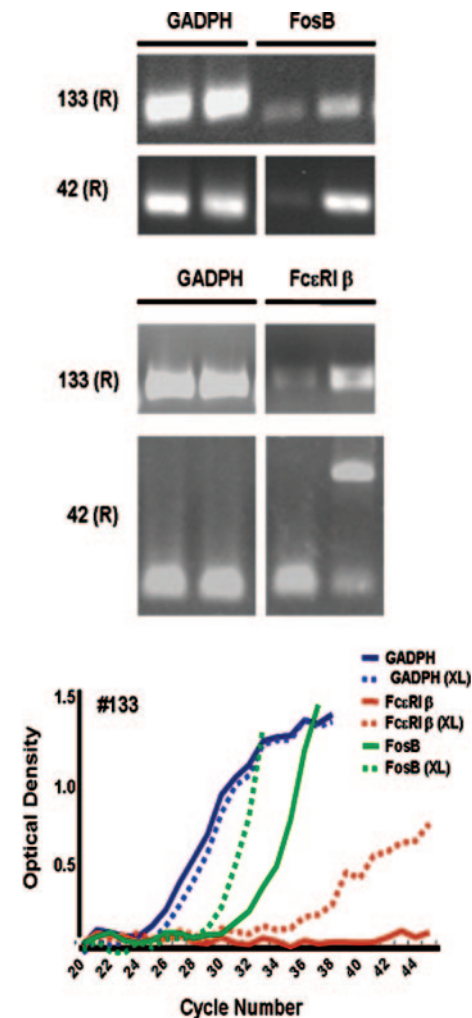


FIGURE 6. PCR verification of microarray data. The expression levels of three genes were compared between resting and activated releaser basophils by both semiquantitative (upper panels) and real-time (lower panel) PCR. GADPH expression was unchanged, whereas expression levels of FosB and Fc ϵ RI β both increased in response to anti-IgE (0.1 $\mu\text{g/ml}$, 2 h).

(Table IV). In contrast to the releaser cells, no chemokines or cytokines were found among the up-regulated genes in activated nonreleaser basophils.

The entire profile of basophil gene expression, including further information on differentially expressed sequence tag/cDNA in resting and activated releaser and nonreleaser cells, may be accessed online at <http://hsc.unm.edu/som/micro/Genomics>.

ELISA verification of microarray data

The Fc ϵ RI-mediated release of IL-8, MIP-1 α , and MIP-1 β protein could be readily measured in culture supernatants from releaser basophils within 1 h of anti-IgE challenge, which is consistent with low levels of constitutive expression (Fig. 5, A–C). Levels of IL-8, MIP-1 α , or MIP-1 β were 10- to 20-fold greater after 4 h of stimulation, most likely reflecting up-regulation of their gene expression in releaser basophils (solid lines, Fig. 5, A–C). Consistent with the unchanged gene expression revealed by microarray, nonreleaser basophils released very little IL-8, MIP-1 α , or MIP-1 β in response to a wide range of anti-IgE concentration (dashed lines, Fig. 5, A–C), even though they could generate these chemokines in response to stimulation with the calcium ionophore, A23187 (Fig. 5, D–F).

PCR verification of microarray data

To further validate the microarray data, we compared the levels of several genes before and after Fc ϵ RI cross-linking by both semi-quantitative (Fig. 6, *upper panels*) and real-time (Fig. 6, *lower panel*) PCR. Donor 133 (typical of five) showed higher levels of transcripts for the transcription factor, FosB and for the Fc ϵ RI β subunit, in both assays. Intriguingly, donor 42 (typical of two) was similar, except that the amplification product for Fc ϵ RI β was larger in the anti-IgE-stimulated basophils (Fig. 6, *upper panel*). The result is not due to DNA contamination because amplifying the same RNA using other primer pairs does not result in a size difference. Sequencing data (data not shown) confirmed that the extended sequence is found within the gene sequence, indicating that it is also not due to nonspecific amplification. The Fc ϵ RI β gene sequence contains one large intron with splice sites at bp 512 and 1380. We propose that a polymorphism resulting in failure to excise this intron is responsible for increasing the size of the expected product from the small size (173 bp) to the large product (868 + 173 = 1041 bp) in some basophils.

Discussion

Within minutes of Fc ϵ RI cross-linking, basophils from most donors release preformed inflammatory mediators contributing to smooth muscle contraction, vascular permeability, and other symptoms of allergic disorders. Immediate responses are followed by the synthesis of Th2 cytokines like IL-4 and IL-13 that stimulate and amplify T and B cell responses. As an exception, it has been known since 1973 that occasional preparations of human basophils express normal levels of the high-affinity IgE receptor Fc ϵ RI, but release little or no histamine in response to Fc ϵ RI cross-linking (2). Several groups have documented properties that are the same between normal (releaser) and signaling-impaired (nonreleaser) basophils, including the numbers and composition of their membrane Fc ϵ RI, their histamine levels, and their signaling responses to other stimuli, including calcium ionophores and the formyl peptide receptor (3–5). We were the first to demonstrate that the nonreleaser phenotype can be explained by the loss of the critical tyrosine kinase Syk (5). Syk is absent or barely detectable in all nonreleaser basophils identified since these initial publications (6–8, 20) (and including those studied here). Based on the observation that Syk mRNA levels are normal and that treating nonreleaser basophils with proteasome inhibitors restores Syk expression, we concluded that the signaling defect is primarily the result of excess Syk degradation (6, 8).

Our original goal was to determine whether the granule releasability of peripheral blood basophils is unaltered, reduced, or increased in individuals with mild allergic asthma. Previous groups have reported both increases and decreases in Fc ϵ RI-mediated degranulation when basophils are isolated from asthmatics (21–24). These data have contributed to the longstanding controversy over the role of basophils in asthma (reviewed in Ref. 11). In this study, statistical analysis of initial histamine release assays using basophils from 61 well-characterized atopic asthmatics and 68 nonatopic nonasthmatics shows clearly that freshly isolated basophils from asthmatics release more histamine both spontaneously and in response to Fc ϵ RI cross-linking with anti-IgE than basophils from control subjects. Our data also reveal substantial overlap between the asthmatic and control groups and large variability between individuals and, indeed, between different assays in the same individual, all factors likely to have obscured differences, except when many measurements are available for analysis.

Continued histamine release assays over 4 years revealed a strikingly higher proportion of nonreleasers in the population than

was previously anticipated: 28% of the control group and 13% of the asthmatic group were nonreleasers for all or part of our study. Clearly, the presence of nonreleaser basophils does not preclude the diagnosis of asthma. Supporting this conclusion, there was no correlation between basophil releaser status and methacholine sensitivity (a test with high specificity for asthma) in two asthmatic “cyclers.” Previously, we had only encountered one donor whose basophils cycled between releaser and nonreleaser status (6). Remarkably, 8 (of 8) asthmatic nonreleasers and 16 (of 23) nonasthmatic nonreleasers cycled at least once to releaser status during the course of our studies, indicating that cycling between releaser and nonreleaser status is also much less rare than previously appreciated.

Direct measurements of serum IL-3 did not support the earlier hypothesis (6, 9) that levels of this low abundance cytokine determine basophil releasability. Therefore, we undertook gene expression profiling studies to better understand differences between resting and stimulated releaser and nonreleaser basophils.

Unstimulated basophils contained transcripts for Fc ϵ RI receptor subunits, the Fc ϵ RI-associated tyrosine kinase, Lyn, and granule proteins. A transcript related to the TCTP, also known as IgE-dependent HRF (25) or P23, was also expressed abundantly and consistently in all basophil preparations. The presence of these characteristic basophil transcripts validates the technology and analyses used in this study.

In releaser basophils, Fc ϵ RI cross-linking with anti-IgE caused a strong transcriptional activation that peaked after 2 h of stimulation and returned toward resting levels by 4 h. Transcripts for both the α and β subunits of Fc ϵ RI were up-regulated, suggesting that receptor activity can affect receptor levels. The Fc ϵ RI α result complements previous evidence that local intranasal allergen challenge increases the expression of Fc ϵ RI α mRNA and protein (26). Unexpectedly, activated basophils from some donors express a larger transcript of Fc ϵ RI β , most likely reflecting a splice variation. Work is in progress to characterize this isoform, to establish its consequences for Fc ϵ RI β protein levels and function, and to determine whether the truncated Fc ϵ RI β isoform (β_{-}) previously described (27) is also differentially synthesized in activated cells. Other up-regulated transcripts in Ag-activated basophils included the H4 histamine receptor, implicated in the development of allergy (28) and the chemokine receptor-1 (CCR1), that binds MIP-1 α and MIP-1 β . We also found increased expression of components of the AP-1 transcriptional complex in the activated cells. In vitro studies have shown that gene expression for many inflammatory mediators requires transcriptional activation of both AP-1 and NF- κ B. Moreover, the promoter regions of multiple inflammatory cytokines and chemokines, including IL-8, RANTES, TNF- α , IL-1 α , MCP, and IL-6, contain AP-1 binding sites. Transcript levels for IL-4 and IL-13 did not change significantly, even though protein levels increase substantially for these two cytokines after Fc ϵ RI cross-linking (6).

Ninety-six genes were differentially expressed between releaser and nonreleaser basophils, with about two-thirds being transcribed at a lower level in the nonreleaser cells. The overall higher basal gene expression in releaser cells may reflect a modest level of constitutive activation through Syk-dependent pathways. The reduced expression of Lyn in the nonreleasers was unexpected because our previous biochemical studies had not revealed consistent differences in Lyn protein levels between releaser and nonreleaser cells (5, 6). However, another group has reported reduced levels of Lyn protein in nonreleaser basophils (20). The loss of Syk protein in nonreleaser basophils has been attributed in part to excess proteasome-mediated degradation (8). Our data analysis to date has

not revealed any obvious patterns of transcription that could account for this protein loss. Continuing studies will target two genes not represented on the U133A gene chip: OCA-B, a transcriptional coactivator recently shown to interact with and stabilize Syk (29), and CIN85, an adaptor protein implicated in regulating the stability of FcεRI and, potentially, of the Syk-FcεRI complex (30).

It is significant that incubation with anti-IgE induced a substantial number of transcriptional changes, including increased Lyn expression, in nonreleaser basophils. The time course of these changes was similar to the time course of the more extensive changes that occur when releaser basophils are stimulated. Thus, the absence of detectable Syk does not necessarily mean the complete absence of signaling. Consistent with this, Rivera and colleagues (31) have described signaling responses to FcεRI cross-linking in RBL-2H3 mast cells that are mediated by Fyn and Gab2 and are independent of Syk.

In summary, we show through repeated measures over 4 years that basal and FcεRI-stimulated histamine release is higher on average in peripheral blood basophils from mild asthmatics than in basophils from nonasthmatic controls. We also show that donors with nonreleaser and cyclical basophils are quite common in the general population and that these donors are more likely to be nonasthmatic than those with releaser basophils. However, the presence of nonreleaser basophils does not rule out a diagnosis of asthma. In releaser basophils, FcεRI cross-linking up-regulates the expression of many genes, including those coding for receptors, signaling proteins, transcription factors, and chemokines. Although gene expression levels are generally lower in nonreleaser basophils than in releaser basophils, a characteristic subset of genes is up-regulated in these cells following Ag stimulation. Further study may reveal the molecular mechanisms by which basophils cycle between the releaser and nonreleaser state and the clinical relevance of this process.

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Disclosures

The authors have no financial conflict of interest.

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