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Mast cells, which interact with *Escherichia coli*, up-regulate genes associated with innate immunity and become less responsive to FcεRI-mediated activation

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Abstract: Mast cells, which are associated with T helper cell type 2-dependent inflammation, have now been implicated in the innate immune response. To further characterize how mast cells are programmed to respond to infectious organisms, we used expression profiling using DNA microarray analysis of gene expression by human mast cells (huMC) during ingestion of *Escherichia coli* and examined immunoglobulin E (IgE)-mediated degranulation. Analysis of data revealed that specific groups of genes were modulated, including genes encoding transcription factors, cell signaling molecules, cell cycle regulators, enzymes, cytokines, novel chemokines of the CC family, adhesion molecules, and costimulatory molecules. Enzyme-linked immunosorbent assay analysis confirmed the production of tumor necrosis factor and the chemokines CC chemokine ligand (CCL)-1/I-309, CCL-19/macrophage-inflammatory protein-3β (MIP-3β), and CCL-18/MIP-4; flow cytometry confirmed the up-regulation of carcinoembryonic antigen-related cell adhesion molecule 1, the integrin CD49d, and CD80. Coincubation with *E. coli* down-regulated Fc receptor for IgE I (FcεRI) expression and FcεRI-mediated huMC degranulation. These data are consistent with the concept that bacterial exposure directs mast cell responses toward innate immunity and away from IgE-mediated effects. *J. Leukoc. Biol.* 79: 339–350; 2006.

Key Words: *IgE · MIP-3β · CCL-19 · CCL-18*

INTRODUCTION

In the effort to understand innate immunity and the means by which protection against infectious organisms is accomplished, recent attention has been directed to mast cells, which occupy tissues that interface the external environment, including the respiratory tract, skin, and gastrointestinal tract, placing them in a unique position to encounter invading organisms, including bacteria, and orchestrate a response.

To date, mast cells have been reported to respond to bacteria through pattern recognition receptors [1] and through phagocytosis, the latter, first reported to occur in mast cells as early as 1923 [2]. The recognition and ingestion of bacteria by mast cells have now been well-documented and occur through complement receptors [3] and immunoglobulin G (IgG) [4] and via direct interaction with type 1 fimbriated bacteria [5], which when ingested by human mast cells (huMC), undergo a significant loss of viability, and mast cells respond to this interaction by secreting tumor necrosis factor (TNF) and interleukin (IL)-6 [6–8]

To further explore how huMC focus their response to bacteria and whether this process directs mast cells away from IgE-mediated events, we examined the expression of 13,971 genes in mast cells internalizing *Escherichia coli*, comparing the pattern of gene expression with that of resting mast cells. As will be shown, the response of mast cells to this bacteria is global and includes up-regulation of genes involved in transcription, cell signaling, the cell cycle, enzyme pathways, adhesion, and surface molecule expression, and huMC simultaneously become less responsive to IgE-mediated activation. These biologic changes in mast cells are an interesting adjunct to the hygiene hypothesis, which says that bacterial exposure directs the immune system toward T helper cell type 1 (Th1)- and away from Th2-mediated inflammation.

MATERIALS AND METHODS

E. coli

The *E. coli* K12 strain, purchased from American Type Culture Collection (Manassas, VA), was cultured in 8 g/l trypton (Fisher Scientific, Fair Lawn, NJ) and 0.5 g/l NaCl (Sigma-Aldrich, St. Louis, MO). This media (20 μL), in a sterilized, 50 ml tube, was inoculated with *E. coli*, incubated at 37°C for 16 h with constant shaking at 250 rotations per minute (rpm), and spread on a 1.5%

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agar plate containing Luria-Bertani (LB) broth (Molecular Biologicals, Inc., Columbia, MD) using a platinum loop. Single colonies of bacteria were picked and placed in 50 ml sterilized tubes containing 20 ml bacteria culture media. These cultures were incubated at 37°C for 16 h and then centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and the *E. coli* pellet was resuspended in 20 ml phosphate-buffered saline (PBS) and centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and the concentration of *E. coli* was adjusted to 5×10^9 bacteria/ml with PBS. Additionally, Alexa Fluor 488-labeled *E. coli* (K12 strain, Molecular Probes, Inc., Eugene, OR) was used in some confocal experiments.

Cell cultures

Laboratory of Allergic Diseases Cell 3 (LAD3) huMC [9], which have similar characteristics to its sister cell lines (LAD1 and LAD2 cells) and primary cultured mast cells, were maintained in StemPro-34 serum-free medium (SFM; Invitrogen Corp., Carlsbad, CA), supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin, 100 IU/ml penicillin (Biosource International, Camarillo, CA), and 100 ng/ml recombinant human stem cell factor (rhSCF; PeproTech, Inc., Rocky Hill, NJ).

Human peripheral blood-derived $CD34^+$ cells were cultured in StemPro-34 SFM supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin, 100 IU/ml penicillin, 100 ng/ml SCF, and 100 ng/ml rhIL-6 (PeproTech, Inc.). rhIL-3 (30 ng/ml) was added for the first week. Half of the culture medium was replaced every 7 days. Cultures at 8–10 weeks consisted of greater than 99% huMC.

E. coli internalization by mast cells

huMC were centrifuged at 1000 rpm for 5 min, the supernatant discarded, and the cells resuspended in cell culture media adjusted to 5×10^5 cells/ml. The cell suspension (5 ml) was then added to a 25-cm^2 culture flask and incubated for 30 min at 37°C in a 5% CO_2 incubator, and 5 μl 5×10^9 bacteria/ml was added (bacteria-to-cell ratio of 10:1). The cell-bacteria suspension was then maintained at 37°C in a 5% CO_2 incubator for times specified. After incubation, the cell-bacteria suspension was centrifuged at 1000 rpm for 5 min, and supernatant was discarded. The pellet was resuspended in 20 ml cell culture media containing antibiotics and centrifuged at 1000 rpm for 5 min. The procedure was repeated, and the pellet was resuspended in 2.5 ml culture media containing antibiotics and incubated for times indicated.

Confocal scanning microscopy

After culture of huMC with *E. coli* for 5, 15, and 30 min, the cells were fixed and permeabilized using IntraPrep permeabilization reagent (Immunotech, Marseille, France) as per the manufacturer's instructions. Permeabilized cells were then incubated with rabbit anti-*E. coli* K12 strain antiserum (a kind gift of Denka Seiken, Tokyo, Japan) for 15 min, the cell suspension was centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. Cells were next resuspended with 1 ml PBS and centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. This procedure was repeated, the pellet suspended in 1 ml PBS, incubated with Alexa Fluor 488-conjugated anti-rabbit IgG (Molecular Probes Inc.) for 15 min, suspended in 0.5% paraformaldehyde in PBS, and placed on poly-L-lysine-coated chambered coverglass slips (Nalgene Nunc International, Naperville, IL), which were examined by a confocal-scanning microscopy Leica TCS-NT/SP laser-scanning confocal microscope. For characterization of live cells, cells were incubated with anti-Fc receptor for IgE I (FcεRI)-fluorescein isothiocyanate (FITC) or anti-Kit-phycoerythrin (PE) monoclonal antibody (mAb; from BD Biosciences, San Jose, CA) in PBS/0.1% bovine serum albumin (BSA) for 1 h, washed twice with PBS/0.1% BSA, and then analyzed by confocal microscopy.

Microarray analysis

The array chips are custom-made by National Institute of Allergy and Infectious Diseases [Bethesda, MD; human sequence chip series "sa" (Hssa)] and consist of 13,971 oligonucleotides, each of which represents a unit gene cluster. All of these elements are 70 mer oligonucleotides synthesized by Qiagen Operon Inc. (Valencia, CA), which hybridize human cDNA synthesized from a human mRNA library.

Total RNA was extracted from three separate phagocytosis experiments for each time-point. RNA was then purified using an RNeasy mini-kit (Qiagen Inc., Valencia, CA). For probe generation, RNA was converted to double-stranded cDNA by reverse transcription (RT) and Cy3- or Cy5-labeled. Oligo dT 20-mer was first annealed to the RNA, and then RT was performed using Superscript II RT (Invitrogen Corp.). Cy3-labeled deoxyuridine triphosphate (dUTP; Amersham Biosciences AB, Uppsala, Sweden) was added along with unlabeled deoxy-unspecified nucleoside 5′-triphosphates (dNTPs) to make Cy3-labeled probe for *E. coli*-nonexposed samples (control), and Cy5-labeled dUTP (Amersham Biosciences AB) was added along with unlabeled dNTPs to make Cy5-labeled probe for *E. coli*-exposed samples. The probes were then purified using a Vivaspin 30K centrifugal filter device (Vivascience AG, Hannover, Germany) in Tris-EDTA buffer and quantitated at 550 nm for Cy3 or at 650 nm for Cy5. The microarray chip was incubated with blocking mixture containing 5× saline sodium citrate (SSC), 1% BSA, and 0.1% sodium dodecyl sulfate (SDS) at 42°C for 1 h, and the hybridization was performed by adding 50 pmol-labeled probes to a reaction mix, which included 10 μg human Cot-1 DNA, 1 μg Poly dA40–60, 4 μg yeast t-RNA (Invitrogen Corp.), 5× SSC, and 0.1% SDS in 25% formamide solution. The mixture was heated at 98°C for 2 min, applied to a microarray slip after cooling, and incubated overnight at 42°C. The arrays were then sequentially washed in 1× SSC and 0.05% SDS and 0.1× SSC. Next, the arrays were centrifuged for 5 min at 500 rpm for drying and scanned with a GenePix 4000A microarray scanner (Axon Instruments, Inc., Union City, CA). Data were analyzed using a mAdb program (located at http://nciarray.nci.nih.gov/), and the data, with $P \leq 0.02$ compared with control and a signal-to-noise ratio 2.0 or greater, were selected for further analysis.

For determination of gene induction following FcεRI activation, huMC were sensitized overnight with 100 ng/mL biotin-IgE and then stimulated with 100 ng/mL streptavidin for 3 h. Total RNA was isolated from each preparation by the RNeasy mini-kit (Qiagen Inc.) The cDNA probes were generated and hybridized to the Affymetrix gene chip human genome U133 (HG-U133) according to the manufacturer's protocol (Affymetrix, Santa Clara, CA; http://www.affymetrix.com/support/technical/datasheets/human_datasheet.pdf). Global scaling was done by all probes and analyzed using Affymetrix GCOS 1.2. Genes with $P \leq 0.02$, compared with control and a signal-to-noise ratio 2.0 or greater, were selected for further analysis.

Real-time polymerase chain reaction (PCR)

The microarray results were validated using real-time PCR on an ABI7500 SDS system. The cDNA templates were prepared independently from cDNA templates, which were used for the microarray hybridizations. At 10 ng, an equivalent of cDNA was used in each quantitative PCR assay. Primer sets for PCR amplifications were designed using the Primer Express software (Perkin-Elmer Applied Biosystems, Boston, MA). All reactions were performed in triplicate for 40 cycles as per the manufacturer's recommendation. Relative quantitation was performed using standard curves for each primer set. Samples are normalized using the geometric mean of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene, and all data are reported as the fold induction over untreated control cells using the comparative threshold cycle method.

Cytokine, chemokine, and β-hexosaminidase assays

Human I-309 [CC chemokine ligand 1 (CCL1)], macrophage-inflammatory protein-4 (MIP-4)/pulmonary and activation-regulated chemokine (PARC; CCL18), MIP-3β (CCL19), and TNF were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits or commercial ELISA development sets (R&D Systems, Minneapolis, MN). Lowest limits of detection for the ELISA kits were 0.71 pg/mL (I-309), 10 pg/mL (MIP-4), 10 pg/mL (MIP-3β), and 0.12 pg/mL (TNF). MIP-1α, MIP-1β, monocyte chemoattractant protein-1 (MCP-1), and regulated on activation, normal T expressed and secreted (RANTES) were measured using the BD™ cytometric bead array (CBA) human chemokine kit II and analyzed on a FACSarray (BD Biosciences). Lowest limits of detection for the CBA assay are 0.05 pg/mL (RANTES), 4.08 pg/mL (MIP-1α), 3.27 pg/mL (MIP-1β), and 0.79 pg/mL (MCP-1). For determination of degranulation, 1×10^6 cells were incubated with 10×10^6 bacteria (2 μl 5×10^9 bacteria/mL) or 2 μl LB broth (as control) for 8 h at 37°C in antibiotic-free media, washed with media containing antibiotics, and then sensitized overnight with 1 μg/mL IgE-biotin. Cells were washed and resuspended in Hepes buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.38 mM $Na_2HPO_4.7H_2O$, 5.6 mM glucose, 1.8 mM $CaCl_2.2H_2O$, 1.3 mM $MgSO_4.7H_2O$,

0.04% BSA, pH 7.4) and then stimulated with streptavidin and incubated at 37°C for 30 min. The β-hexosaminidase in the supernatants and cell lysates was quantified by hydrolysis of p-nitrophenyl N-acetyl-β-D-glucosamide (Sigma-Aldrich) in 0.1 M sodium citrate buffer (pH 4.5) for 90 min at 37°C. The percentage of β-hexosaminidase release was calculated as percent of total content. Chemokine content was measured using the CBA assay as above.

Flow cytometry

For analysis of expression of surface molecules, cells were incubated with anti-human leukocyte antigen (HLA)-DR, -DP, and -DQ FITC-conjugated and anti-CD49d PE-conjugated antibody (BD Biosciences) for 30 min on ice and analyzed by fluorescein-activated cell sorter. In the case of CD80 and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), cells were incubated with anti-CD80 (BD Biosciences) or anti-CEACAM1 (CD66a; Axxora LLC, San Diego, CA) and then anti-mouse IgG PE-conjugated antibody (Southern Biotech, Birmingham, AL). For intracellular analysis of tryptase and chymase content, huMC were fixed with 4% paraformaldehyde for 5 min, permeabilized with PBS/0.1% saponin, blocked with PBS/5% milk/0.1% saponin, and incubated with rabbit antitryptase, mouse antichymase-biotin, or appropriate isotype antibody (all from Chemicon International, Temecula, CA) for 1 h at 4°C. Cells were washed twice with PBS/0.1% saponin and incubated with anti-rabbit-allophycocyanin or streptavidin-PE (both from BD Biosciences) before analysis by flow cytometry.

Mast cell bactericidal assay

Viability of internalized bacteria was determined by measuring colony-forming units (CFU) as described [10]. huMC (0.3×10^6/ml) were grown overnight in StemPro medium (antibiotic-free) at 37°C with 5% CO, then exposed to 3.0×10^7 bacteria for 30 min at 37°C. huMC were washed twice with StemPro (antibiotic-free) to remove adherent or noninternalized bacteria. Cells were rapidly frozen and thawed to release internalized bacteria, and viable bacteria were quantitated by determining the number of CFU from serial dilutions of the cell lysates plated on MacConkey agar. The viability of the huMC during the incubation period was monitored periodically by Trypan blue dye exclusion assay and determined to be consistently >90%.

Statistical analysis

Data in the text and figures are expressed as the mean ± SEM. Statistical comparisons were carried out using ANOVA and the Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Binding and internalization of *E. coli* by huMC

LAD huMC are sister cell cultures (1–3) obtained from a patient with severe systemic mast cell disease [9]. They have many characteristics in common with primary $CD34^+$ progenitor-derived, cultured huMC. Specifically, these cells stain metachromatically with toluidine blue (**Fig. 1A**); contain histamine (5.3±1.2 pg/cell), tryptase (Fig. 1B), and chymase (Fig. 1C); express surface Kit and FcεRI (Fig. 1, D–F); require SCF for growth; and degranulate in response to FcεRI cross-linking. LAD3 mast cells were used in the studies below.

Murine and cord blood-derived huMC are reported to attach to and internalize bacteria [5–7]. Therefore, we first verified that LAD huMC were capable of internalizing bacteria and then characterized the process. As can be seen in **Figure 2**, confocal scanning microscopy shows that *E. coli* (green) adhered to the surface of and were internalized into mast cells (Fig. 2, A and B). Adherence and internalization could be inhibited by pretreatment with cytochalasin D or mannose (Fig. 2, A and C). A time course of the interaction between mast cells and live *E. coli*, which starts with adhesion of the bacteria to the cell surface, followed by internalization, is shown in Figure 2D. Taken together, these results indicate that huMC recognize and attach to *E. coli.* On average, and by 30 min, four bacteria were internalized by each huMC.

The viability of the internalized bacteria was measured to determine if adhesion and internalization were microbicidal. After allowing bacteria to be internalized for 30 min in the presence of mannose or cytochalasin D (see Materials and Methods), mast cells were lysed, and bacteria were plated onto agar plates. Although each huMC internalized 3.8 + 0.2 bacteria/huMC (Fig. 2D), only 1.9 + 0.4 (34%) of those bacteria were able to grow and form colonies. If huMC had been

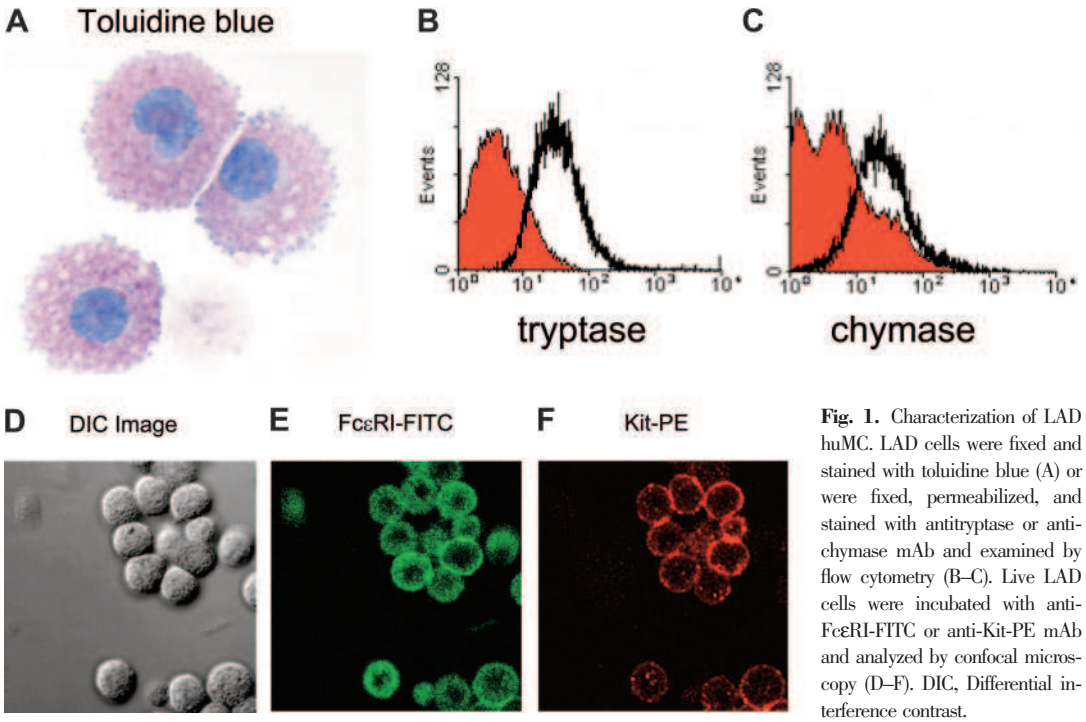

Fig. 1. Characterization of LAD huMC. LAD cells were fixed and stained with toluidine blue (A) or were fixed, permeabilized, and stained with antitryptase or anti-chymase mAb and examined by flow cytometry (B–C). Live LAD cells were incubated with anti-FcεRI-FITC or anti-Kit-PE mAb and analyzed by confocal microscopy (D–F). DIC, Differential interference contrast.

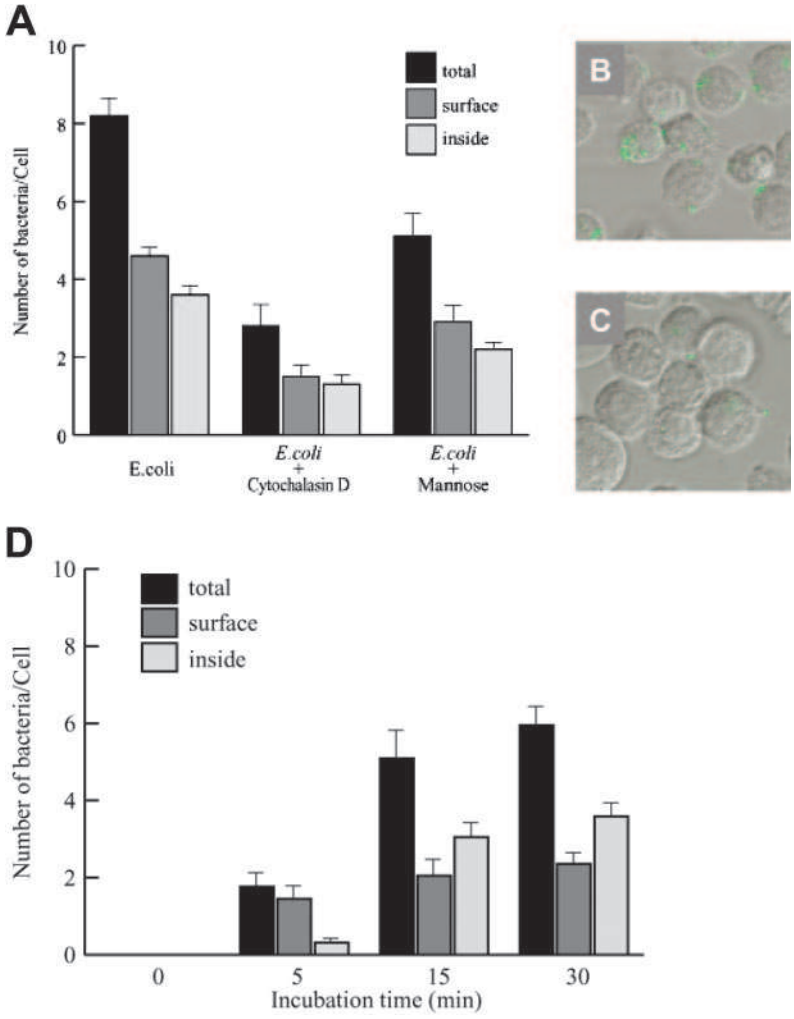

Fig. 2. Confocal scanning microscopy analysis of the effects of cytochalasin D and mannose on huMC internalization of bacteria. (A) The effects of 4 μM cytochalasin D and 10 mM mannose pretreatment on adhesion and internalization of fluoro-labeled *E. coli* by mast cells. (B) Mast cells incubated with fluoro-labeled *E. coli*. (C) Cytochalasin D-treated (4 μM) mast cells incubated with fluoro-labeled *E. coli*. In all the above, mast cells were incubated with tenfold bacterial particles for 30 min. (D) Time course of internalization of live *E. coli* by huMC, as analyzed by confocal scanning microscopy. Mast cells were exposed to live *E. coli*, and cells and bacteria were fixed and stained with FITC-labeled anti-*E. coli* antibody.

preincubated with cytochalasin D, only 0.3 + 0.1 (8%) bacteria/huMC formed colonies. Mannose did not have a significant effect, as 1.0 + 0.5 (26%) bacteria/huMC formed colonies. These data suggest that once internalized, huMC are able to destroy the *E. coli*.

Gene expression by huMC following exposure to *E. coli*

A critically important outcome of the interaction of opsonized bacteria with mast cells would be the up-regulation of families

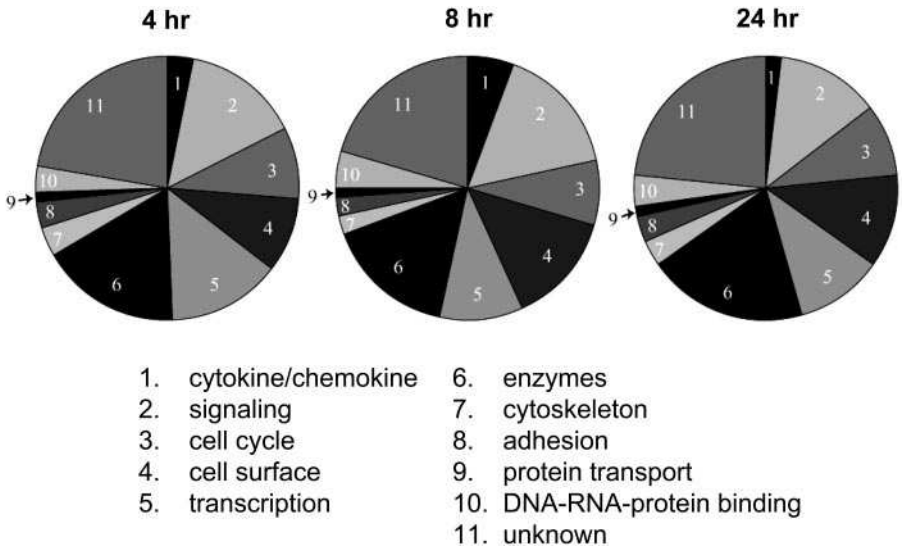

Fig. 3. Annotation distribution of activated genes at 4, 8, and 24 h of incubation of live *E. coli* with huMC, which were elevated at least twofold at $P < 0.02$. Definitions of the gene families are based on the Gene Bank annotation for each gene, which at each time-point, was averaged (n=3 separate phagocytosis experiments) and selected for display if greater than twofold up-regulated at $P < 0.02$.

TABLE 1. Genes Up-Regulated following *E. coli* Exposure

Gene	Protein description	Fold induction 4 h	8 h	24 h
Antigen presentation				
CD80	CD80 antigen (B7-1)	2.36	1.81	2.74
CD86	CD86 antigen (B7-2)	6.32	1.05	1.30
HLA-DMB	MHC-II	3.01	1.58	2.65
CRTAM	MHC-I-restricted molecule	2.30	1.75	2.59
HKE4	HLA class II region-expressed gene KE4	2.79	1.66	1.24
Adhesion				
CDH1	cadherin, type 1, E-cadherin	1.49	1.28	4.52
ITGA4	CD49d integrin, α 4	2.20	0.99	1.42
ITGA3	CD49c integrin, α 3	−1.27	−1.35	−2.34
ITGAV	CD51 integrin, α V (vitronectin receptor)	−2.13	−1.32	0.99
ITGB1	CD29 integrin, β 1	−1.30	−1.95	−2.59
ITGB4	integrin, β 4	2.01	1.49	2.81
ITGB5	integrin, β 5	1.23	1.19	3.04
ICAM1	intercellular adhesion molecule (CD54)	1.08	0.88	2.49
ICAM5	intercellular adhesion molecule 5	2.38	2.11	1.28
FBLN2	fibulin 2	2.09	2.12	3.07
CEACAM1	cell adhesion molecule 1	1.15	1.04	3.62
CEACAM3	cell adhesion molecule 3	2.05	1.25	1.67
CEACAM4	cell adhesion molecule 4	1.73	1.41	2.18
CEACAM6	cell adhesion molecule 6	2.11	1.52	2.25
CEACAM8	cell adhesion molecule 8	2.44	1.97	2.05
ARHGDIA	ρ GDP dissociation inhibitor (GDI) α	2.43	1.50	1.24
Transcription and signaling				
PDE4A	phosphodiesterase 4A, cAMP-specific	5.18	4.85	1.23
SH2D2A	SH2 domain protein 2A	6.39	4.62	1.41
MT1X	metallothionein 1X	3.56	3.48	1.95
MT2A	metallothionein 2A	3.67	4.93	2.18
MT1G	metallothionein 1G	3.45	3.81	1.76
MT1A	metallothionein 1A	3.03	3.20	1.77
MT1F	metallothionein 1F	3.33	3.38	1.74
HEY1	hairy/enhancer-of-split related with YRPW	7.40	3.77	1.10
CREM	cAMP-responsive element modulator	4.23	6.64	1.46
IRF1	interferon regulatory factor 1	1.00	1.00	2.82
IRF7	interferon regulatory factor	3.78	1.70	10.68
Cytokines, chemokines, and growth factors				
SCYA1	chemokine ligand 1 (I-309)	2.52	1.61	1.27
SCYA2	chemokine ligand 2 (MCP-1)	2.69	1.64	1.06
SCYA15	chemokine ligand 15 (MIP-1δ)	0.85	1.05	4.30
SCYA18	chemokine ligand 18 (PARC or MIP-4)	1.38	2.20	3.87
SCYA19	chemokine ligand 19 (MIP-3β)	8.27	1.58	3.92
SCYA23	chemokine ligand 23 (CKβ8)	2.46	2.31	2.05
SCYA24	chemokine ligand 24 (Eotaxin-2)	2.56	2.73	2.73
ISG15	interferon-stimulated protein	2.27	1.95	1.42
MIF	macrophage migration inhibitory factor	2.78	1.92	1.27
TNF	tumor necrosis factor	2.73	2.20	3.19
Surface receptors				
IFNAR1	interferon (α, β, and ω) receptor 1	2.38	1.65	2.52
IL4R	IL-4 receptor	3.22	2.77	1.18
IL8RA	IL-8 receptor, α	3.44	1.45	2.20
IL9R	IL-9 receptor	2.53	2.26	1.16
IL10RB	IL-10 receptor, β	2.25	1.18	1.07
CD22	CD22 antigen, member of siglec family	0.86	1.05	2.15
CD37	CD37 antigen, tetraspanin	1.05	1.23	2.10
CD48	CD48 antigen, bacterial recognition	0.77	0.93	2.44
CD68	CD68 antigen, gram-neg bacterial recognition	1.05	1.18	2.45
C5R1	complement component 5 receptor 1	1.91	2.36	1.16
FCER1A	FcεRI, IgE high-affinity receptor, α chain	−1.47	−1.48	−1.13
FCGR1A	FcγRIa, IgG high-affinity receptor (CD64)	−1.45	−0.80	−0.90

TABLE 1. (Continued)

Gene	Protein description	Fold induction 4 h	8 h	24 h
Enzymes				
TPSG1	tryptase γ 1	2.33	1.31	2.30
CTSL	cathepsin L	1.28	2.20	2.71
CTSD	cathepsin D	1.17	0.90	2.31
RNASE3	ribonuclease, RNase A family, 3 (ECP)	0.74	2.18	1.49
OAS3	2′-5′-oligoadenylate synthetase 3, 100 kDa	1.84	2.98	12.32
PLAA	phospholipase A2-activating protein	0.98	−2.13	−2.38
HEXB	hexosaminidase B (β polypeptide)	−1.59	−2.04	−1.35

Red = Over two-fold induction and significant when compared with control ($P \leq 0.02$); blue = over two-fold reduction and significant when compared with control ($P \leq 0.02$); green = over two-fold induction or reduction but not significant. MHC, Major histocompatibility complex; GDP, guanosine 5′-diphosphate; cAMP, cyclic adenosine monophosphate; SH2, src homology 2; Ckβ8, chemokine β 8; ECP, eosinophil cationic protein.

of genes whose products facilitate the ability of mast cells to respond to the presence of microorganisms and in turn, would contribute to the initiation of an innate immune response. We thus examined the global gene expression program engaged by the interaction of huMC with live *E. coli*. Using microarray technology, we selected genes for further study, which were up-regulated at least twofold in mast cells exposed to *E. coli* versus resting cells at the $P < 0.02$ level and at 4, 8, and 24 h (n=3–6). By this process, we identified 251 genes at 4 h, 88 genes at 8 h, and 158 genes at 24 h for further analysis by grouping as to function.

Significantly up-regulated genes were categorized into 11 groups according to the known functions of their corresponding proteins: cytokines/chemokines, signaling, cell cycle, cell surface, transcription, enzyme, cytoskeleton, adhesion, protein transport, DNA/RNA/protein-binding, and unknown (**Fig. 3**). The overall pattern of gene up-regulation appeared rather consistent through 24 h. The largest group of transcripts up-regulated (16–20%) coded for enzymes. These genes included the respiratory chain-related enzymes such as reduced nicotinamide adenine dinucleotide dehydrogenase α and β (fivefold induction), three isoforms of 2′-5′-oligoandenylate synthetase (two- to 12-fold induction), cytochrome C oxidase subunits (threefold induction), and RNase 3 (eosinophil cationic protein; **Table 1**). These genes represent an increase in cell metabolism.

The next largest group of genes up-regulated following mast cell exposure to *E. coli* included signaling molecules, accounting for 14–16% of the total number of induced genes. These included the cAMP responsive element modulator (eightfold induction), SH2 domain protein 2A (fivefold induction), phosphodiesterase 4A (3.5-fold induction), the TNF receptor-associated factor 1 (3.4-fold induction), and the interferon (IFN) regulatory factors 1 and 7 (IRF1 and IRF7; three- to 10-fold induction; Table 1).

The cytokine/chemokine group accounted for 2–6% of all selected genes. In this group, the most strongly induced genes were TNF (threefold induction), CCL1 (or I-309; 2.5-fold induction), CCL19 (MIP-3β; eightfold induction), CCL18 (MIP-4; fourfold induction), CCL24 (eotaxin-2; threefold induction), CCL15 (MIP-1δ; fourfold induction), and MIF (threefold induction; Table 1). IL-5, IL-7, and IL-16 were up-regulated two- to threefold, but this induction was not significant when compared with control (Table 1). These cytokines and chemokines are associated with innate responses involving dendritic cells (DC) and lymphocytes [11].

Genes encoding cell-surface receptors accounted for 8–12% of up-regulated genes. These receptors included IFNAR1 (2.5-fold induction), IL-4R (threefold induction), IL-8RA (threefold induction), IL-9R (2.5-fold induction), IL-10RB (twofold induction), CD22, CD37, CD48, CD68, and complement component 5 receptor 1 (Table 1). Expression of the FcεRI (FCER1A) and FcγRI (FCGR1A) receptors was down-regulated slightly (onefold reduction).

The gene profile induced in response to bacteria differs from that generated by IgE-dependent activation of mast cells. FcεRI-mediated activation resulted in the expression of proinflammatory cytokines and chemokines such as IL-1, IL-3, granulocyte macrophage-colony stimulating factor (GM-CSF), CCL5, RANTES, CCL2, MCP-1, CCL3, or MIP-1α and CCL4 or MIP-1β (**Table 2**). MCP-1, MIP-1β, MIP-1α, and RANTES protein production was confirmed in supernatants from huMC stimulated with IgE/anti-IgE for 24 h (**Fig. 4**). Although FcεRI activation also induced expression of I-309 and TNF, it did not induce expression of MIP-3 and MIP-4.

TABLE 2. Genes Up-Regulated following FcεRI Activation

Gene	Protein description	Fold induction
SCYA5	CCL5, RANTES	68.59
MCP-1	monocyte chemotactic protein	45.25
SCYA4	CCL4, MIP-1β	25.99
SCYA1	CCL1, I-309	22.63
TNFRSF1B	TNF receptor 1β	13.93
GM-CSF	colony-stimulating factor 2 (granulocyte-macrophage)	10.56
SCYA3	CCL3, MIP-1α	6.96
NFATC1	NF-ATc transcription factor	6.96
TNF	tumor necrosis factor	6.06
IL3	interleukin-3	5.66
FYN	FYN oncogene related to SRC	4.59
IL1A	interleukin-1α	2.00

NF-ATC, Nuclear factor of activated T cell, cytoplasmic.

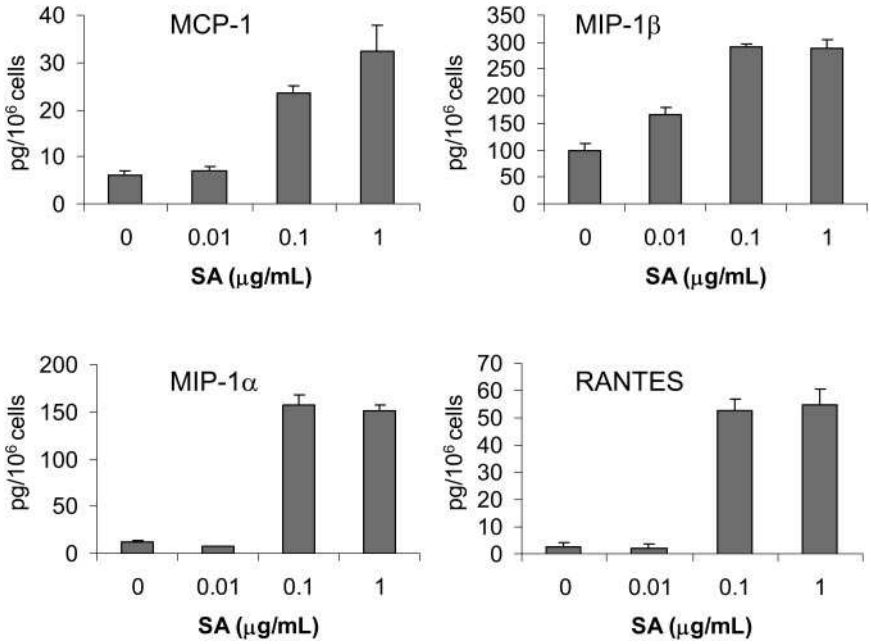

Fig. 4. MCP-1, MIP-1α, MIP-1β, and RANTES release following FcεRI activation of huMC. Mast cells were sensitized with 1 μg/mL IgE and then stimulated with 100 ng/mL streptavidin for 24 h, and chemokine levels were measured by the CBA assay. SA, .

huMC secrete TNF and chemokines after contact with *E. coli*

Microarray results showing up-regulation of I-309, MIP-4, and MIP-3 by primary huMC derived from human peripheral blood $CD34^+$ cells in response to *E. coli* were first validated by real-time PCR (**Fig. 5**). There was at least a fivefold induction of mRNA expression of TNF, I-309, MIP-3β, and MIP-4 at 8–24 h after the mast cells had been exposed to *E. coli* (Fig. 5). The protein products of the chemokine/TNF genes were then measured in the supernatants of LAD huMC, which had been exposed to *E. coli* for 30 min. Although each mediator was found to be released in a different time course, all were significantly elevated 24 h after encountering *E. coli* (**Fig. 6A**). For comparison, huMC derived from human peripheral blood $CD34^+$ cells were similarly incubated with *E. coli*, and TNF, I-309, MIP-3β, and MIP-4 were measured in the supernatants. Similar to the LAD huMC, peripheral blood $CD34^+$-derived mast cells also released these mediators in a time-dependent manner (Fig. 6B). Thus, these observations indicate that huMC respond to contact with *E. coli* by releasing certain chemokines, as well as TNF, validating array data and consistent with a response that would further innate immunity.

Coincubation with *E. coli* up-regulates expression of adhesion and antigen-presenting molecules

A novel finding of the microarray analysis was that several adhesion molecules were up-regulated or down-regulated following *E. coli* coincubation (Table 1). These molecules included the β1 integrins and the newly characterized family of CEACAM molecules. CEACAM1, a receptor known to bind bacteria in other cell types [12], was up-regulated threefold at

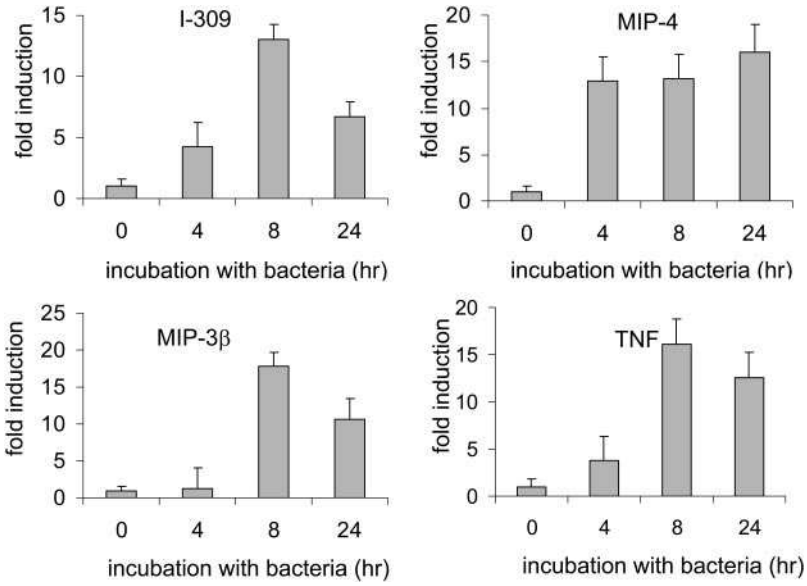

Fig. 5. TNF and chemokine mRNA expression induced by mast cell phagocytosis of *E. coli* particles. Total RNA isolated from peripheral blood $CD34^+$ progenitor-derived mast cells after phagocytosis for analysis of TNF and chemokine expression by real-time PCR. GAPDH served as a control for constitutive gene expression.

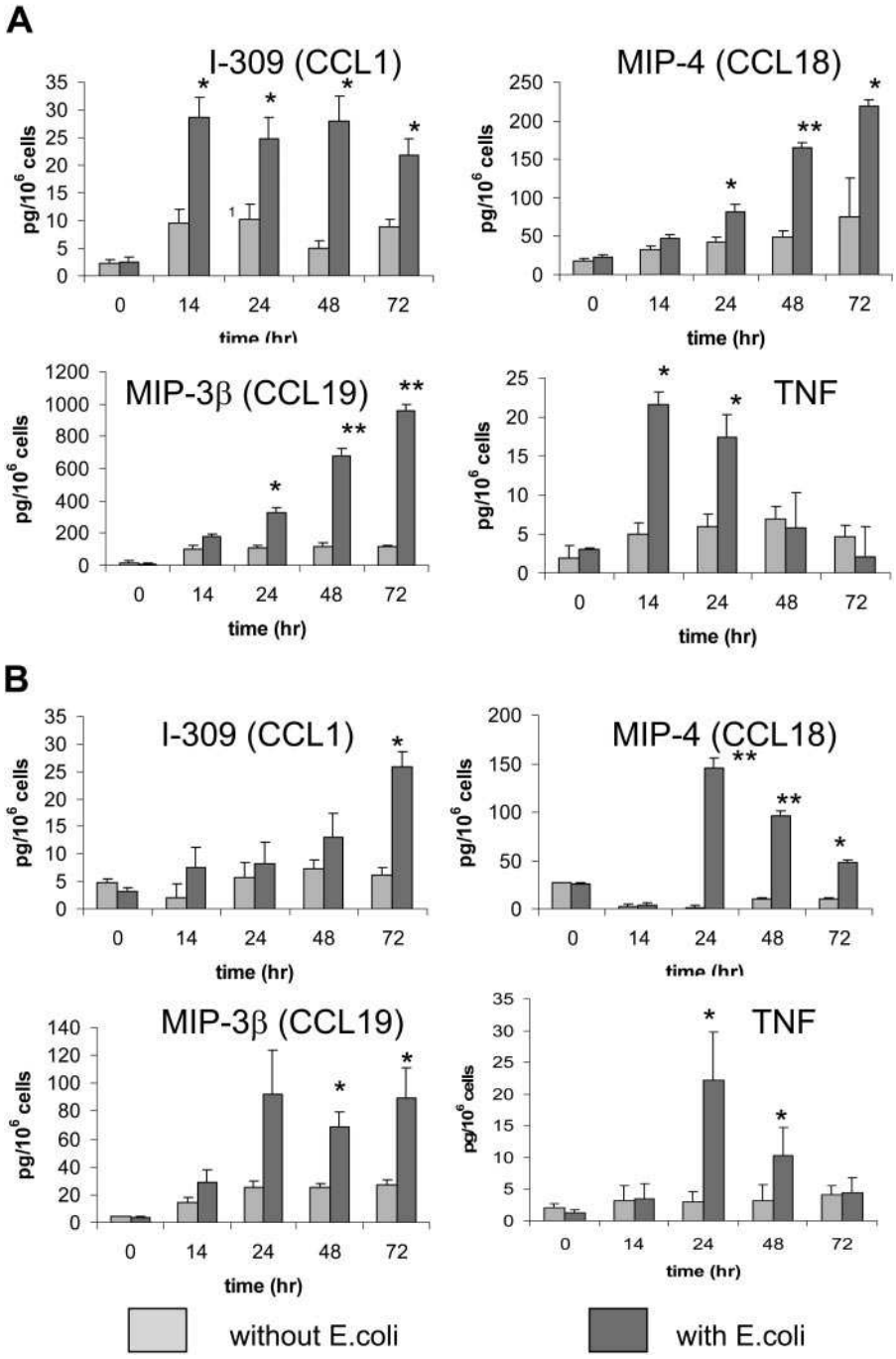

Fig. 6 . TNF and chemokine release from huMC after phagocytosis of *E. coli*. LAD mast cells (A) and peripheral blood $CD34^+$ progenitor-derived mast cells (B) were incubated with tenfold live *E. coli* particles for 30 min. The cells were then incubated for several time periods following the removal of *E. coli* by washing with PBS. Figures represent protein concentrations as measured by ELISA. Data are shown as mean ± SEM, n = 3 separate experiments. *, $P < 0.05$; **, $P < 0.01$.

24 h, and CD49d was up-regulated twofold at 4 h. These data are diagramed in **Figure 7**. Flow cytometric analysis confirmed that CEACAM1 (Fig. 7E) and CD49d (Fig. 7F) expression is up-regulated after 24 h.

Rodent and huMC are known to express antigen-presenting molecules such as MHC-II [13–15] and CD80 (B7-1) [16]. Moreover, mouse mast cells are known to present intracellular antigens to T cells [17, 18]. Our microarray analysis shows that several of these antigen-presenting molecules are up-regulated following *E. coli* coincubation, including CD80 and less so for MHC-II (Table 1 and Fig. 7), which would enhance a subsequent response leading to augmented immunity. Flow cytometric analysis of CD80 (Fig. 7G) and MHC-II (Fig. 7H) confirms that surface expression of CD80 is up-regulated at 24 h, and MHC-II expression is relatively unchanged.

Coincubation with *E. coli* down-regulates expression of FcεRI and degranulation

To examine whether huMC alter their ability to respond to FcεRI-mediated signals after coincubation with *E. coli*, we exposed huMC to *E. coli*, activated these mast cells via FcεRI with IgE-biotin/streptavidin, and measured degranulation. Mast cells exposed to *E. coli*, then activated via FcεRI, released significantly less β-hexosaminidase (**Fig. 8A**). Using the initial analysis criteria of greater-than-twofold induction/reduction, the microarray did not show any change in FcεRI

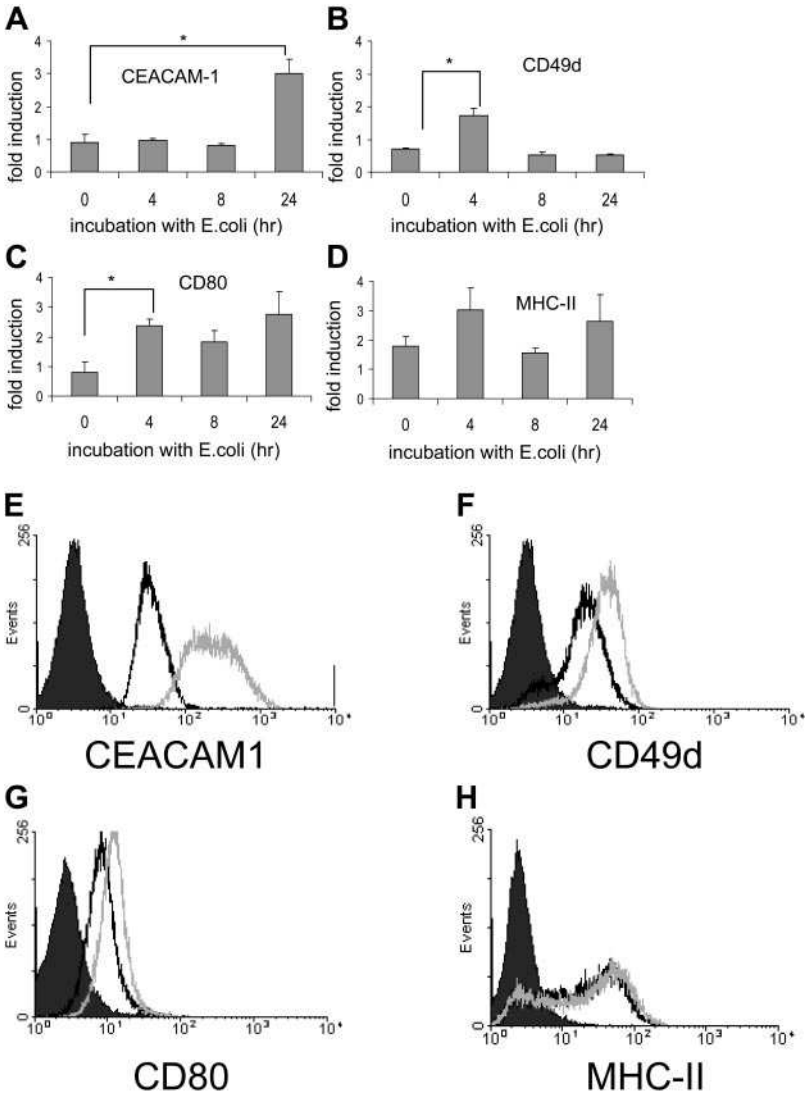

Fig. 7. Incubation with *E. coli* modifies huMC surface molecule expression. CEACAM1 (A), CD49d (B), CD80 (C), and MHC-II (D) mRNA expression was assessed by microarray analysis. Surface protein expression of CEACAM1 (E), CD49d (F), CD80 (G), and MHC-II (H) by untreated huMC (black line) or mast cells coincubated (24 h) with *E. coli* (shaded line) as compared with isotype control (solid curve; n=3; *, $P<0.01$).

gene expression. However, when the microarray data were reanalyzed using a greater-than-onefold induction/reduction criteria, FcεRI mRNA expression was down-regulated at 4 h and 8 h of coincubation with *E. coli* (Table 1 and Fig. 7B). Flow cytometry analysis confirmed that huMC expressed less surface FcεRI following coincubation with *E. coli* (Fig. 8C). Furthermore, when huMC were preincubated with *E. coli* for 24 and then stimulated via FcεRI, they released less MIP-1α and MIP-1β compared with untreated cells (Figs. 4 and 8D).

DISCUSSION

Coincubation of huMC with *E. coli* resulted in bacterial internalization, as assessed by confocal microscopy (Fig. 2), in agreement with previous findings [5, 6, 19]. Internalized bacteria could not form CFU and were presumably destroyed by the huMC phagolysosomal system. Furthermore, pretreatment of huMC with mannose and cytochalasin D inhibited *E. coli* internalization. FimH, a mannose-binding subunit, located preferentially at the type 1 fimbrial tips, is the specific determinant recognized by CD48 on mast cells, and FimH-negative *E. coli* mutants exhibit limited mast cell binding [7, 8]. Therefore, pretreatment of huMC with mannose inhibited the FimH-CD48 interaction and subsequently prevented bacterial adhesion to huMC. Cytochalasin D is a cell-permeable fungal toxin, which inhibits filamentous actin assembly and mast cell membrane ruffling [20] and has been used to inhibit macrophage phagocytosis [21].

Microarray analysis showed that the pattern of gene induction in response to *E. coli* is prolonged, continuing through at least 24 h of coincubation with bacteria (Table 1). Transcripts up-regulated include those coding for enzymes, surface molecules, components of the cytoskeleton, and molecules associated with signaling, the cell cycle, adhesion, and protein transport (Fig. 3).

One group of important up-regulated genes included chemokines such as CCL1/I-309, CCL18/MIP-4, and CCL19/MIP-3β (Table 1, Figs. 4 and 5). Mast cells have heretofore not been known to produce CCL18 and CCL19. CCL1/I-309 binds CC chemokine receptor 8 on activated Th2 cells, monocytes, and immature DC and recruits them to target tissues during inflammation [22]. CCL1/I-309 also binds to human umbilical vein endothelial cells and induces their chemotaxis, invasion,

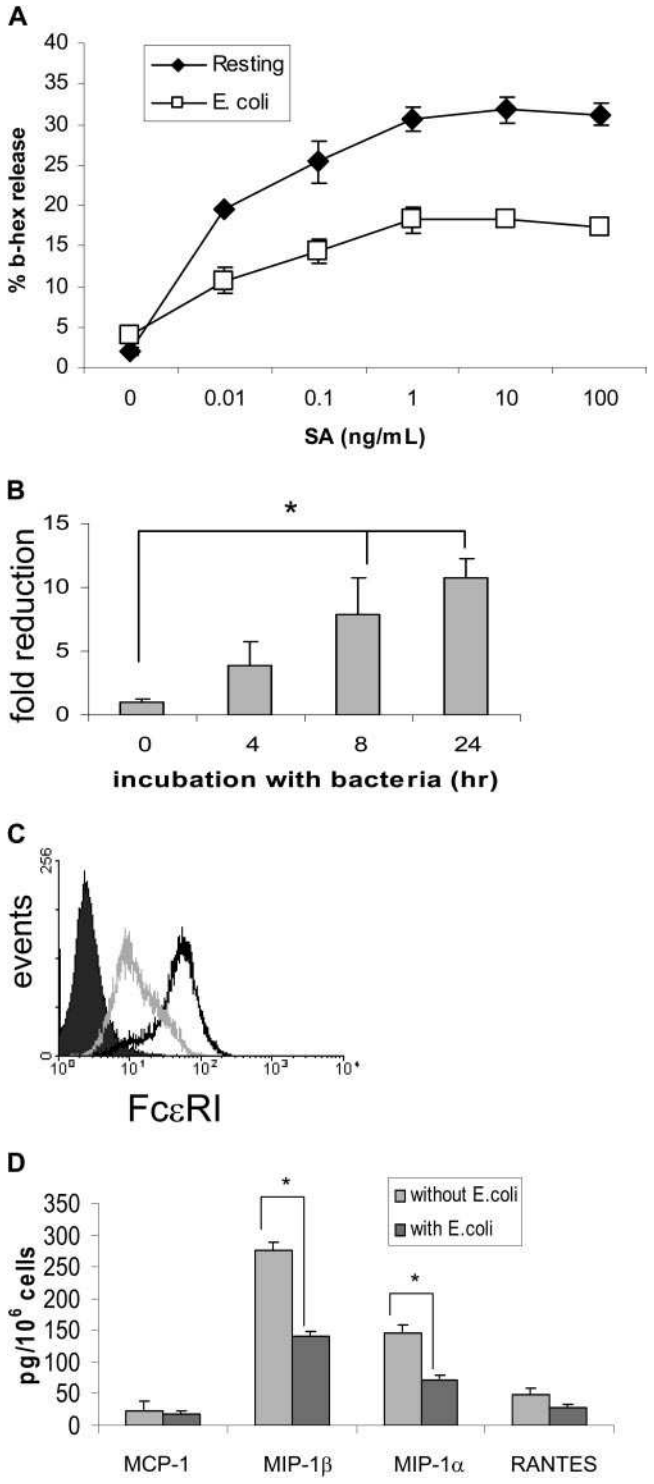

Fig. 8. Coincubation down-regulates huMC degranulation via FcεRI. (A) Untreated huMC and huMC coincubated with *E. coli* (24 h) were stimulated via FcεRI (see Materials and Methods), and β-hexosaminidase (b-hex) was measured to determine degranulation. (B) FcεRI mRNA expression following exposure to *E. coli* was validated by real-time PCR (*, $P<0.01$). (C) Untreated huMC (solid line) or mast cells coincubated (24 h) with *E. coli* (shaded line) were assessed for expression of surface FcεRI protein by flow cytometry (solid curve, isotype control; representative of two experiments). (D) Mast cells (1.0×10^6) were incubated with 1.0×10^8 *E. coli* or LB broth (as control) for 8 h at 37°C in antibiotic-free media, washed with media containing antibiotics, and then sensitized overnight with 1 μg/mL IgE-biotin. Cells were washed and stimulated with 10 ng/mL streptavidin and incubated at 37°C for 24 h, and chemokine production was measured by the CBA assay (*, $P<0.01$).

and differentiation [23]. Therefore, production of CCL1/I-309 by huMC during bacterial infection in the lung and other tissues would recruit DC and further the immune response.

CCL18/MIP-4, also called PARC, is a new, recently described CC chemokine, originally identified as a T cell chemoattractant produced by DC [24]. Unlike other chemokines, CCL18/MIP-4 is thought to be produced mainly by monocytes and DC [25] and is involved in innate immune responses to staphylococcal enterotoxins [26], pulmonary fibrosis in scleroderma [27], and hypersensitivity pneumonitis [28], perhaps by inducing collagen production by lung fibroblasts [29].

CCL19/MIP-3β, also called Epstein-Barr virus-induced gene 1-ligand chemokine or CKβ11, is a member of the CC chemokine family and is constitutively expressed in thymus, lymph nodes, spleen, and small intestines, and expression in the bone marrow is inducible by IFN-γ, TNF, and lippolysaccharide (LPS) [30]. In addition to recruiting macrophage progenitors, MIP-3β is essential in recruiting immature DC and memory T cells to secondary lympoid organs [31]. Therefore, during *E. coli* infection, huMC-derived CCL19/MIP-3β would recruit DC and memory T cells and initiate an adaptive immune response. Similarly, our study shows that huMC coincubated with *E. coli* up-regulated expression of TNF [32], which promotes local inflammation, activates endothelial cells, and is involved in T cell recruitment to draining lymph nodes during bacterial infection [1, 33].

The microarray analysis also indicated a profile of cytokine production in response to bacteria, which noticeably lacked evidence of up-regulation of cytokines/chemokines including IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IFN-γ, CCL3, and CCL4. This pattern differs from FcεRI-mediated mast cell activation, where among other cytokines and chemokines, message for IL-1, IL-3, GM-CSF, CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES) is up-regulated (Table 2). The gene profile induced by *E. coli* also differs from the gene profile induced by selective pathogen ligands such as LPS. In a study of cord blood-derived mast cells, Okumura et al. [34] found that LPS up-regulated mRNA for cytokines IL-1, IL-10, IL-15, CCL5, and CCL8. The majority of genes induced by LPS was members of the NF-κB pathway and did not include I-309, MIP-3β, or MIP-4, suggesting that whole, live *E. coli* activates different pathways from LPS alone.

Microarray analysis also showed a previously unknown profile of adhesion molecule up-regulation. The family of novel adhesion molecules, CEACAMs, in particular, was up-regulated following *E. coli* exposure (Fig. 6). CEACAMs mediate binding to and engulfment of *Neisseria gonorrhoeae* [12] and *E. coli* [35] by epithelial cells. We show that huMC express CEACAM1 and may be involved in internalization and engulfment of *E. coli*. Similarly, CD49d, a β1 integrin, is also up-regulated following *E. coli* exposure (Fig. 6). In the mouse, CD40d mediates mast cell adhesion to endothelium and is important for mast cell immigration into the small intestine during nematode infection [36]. huMC up-regulated genes involved in antigen presentation such as CD80 (Fig. 6). Although huMC express CD86 and MHC-II, surface protein expression of these molecules is not up-regulated following *E. coli* exposure (Fig. 6 and data not shown). However, these data suggest in total that huMC direct their activation toward responses that

favor those beneficial to the host in addressing an infectious insult.

Consistent with the hypothesis that huMC reprogram themselves following exposure to bacteria, receptor expression was modified significantly following *E. coli* exposure. For example, mast cells up-regulated receptors IL-4, IL-9, and IL-10, which are known to modulate mast cell differentiation, degranulation, and cytokine release (Table 1). IL-4 and IL-9 activate mast cells to proliferate and potentiate their release of proinflammatory cytokines [37–39], and IL-10 inhibits FcεRI expression and mediator release [40–42]. As expected, huMC up-regulate CD48, which binds *E. coli* and aids in internalization of bacteria [7]. Mast cells retained their ability to degranulate after exposure to bacteria but less efficiently, possibly as a result of a decrease in surface expression of FcεRI (Fig. 7, A–C).

In this report, we thus examine the mRNA profile of gene expression in huMC, associated with exposure to and internalization of intact bacteria, in this case, *E. coli*. The up-regulation of genes was global and included genes involved in a diversity of mast cell functions. Among the genes up-regulated were chemokines, adhesion molecules, costimulatory molecules, and surface receptors, suggesting a concerted reprogramming of the ability of mast cells to respond to a bacterial insult. Taken together, these data support the concept of a specific and vigorous mast cell-dependent response to bacteria to further host defense. IgE-dependent events, which are critical to the induction of any allergic responses, appear down-regulated. This direction of mast cell responses to a more Th1-type inflammation and away from a Th2 allergic response is an interesting support of the hygiene hypothesis, which states that exposure to infectious organisms and their products skews away from atopy.

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REFERENCES

1. Kulka, M., Alexopoulou, L., Flavell, R. A., Metcalfe, D. D. (2004) Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *J. Allergy Clin. Immunol.* **114,** 174–182.
2. Tsuda, S. (1923) Experimentelle Untersuchungen uber die entzundliche reaktion der subcutis in beziehung zum individuellen immunitatszustand. *Virchows Arch. Pathol.* **247,** 123.
3. Sher, A., Hein, A., Moser, G., Caulfield, J. P. (1979) Complement receptors promote the phagocytosis of bacteria by rat peritoneal mast cells. *Lab. Invest.* **41,** 490–499.
4. Talkington, J., Nickell, S. P. (2001) Role of Fc γ receptors in triggering host cell activation and cytokine release by *Borrelia burgdorferi*. *Infect. Immun.* **69,** 413–419.
5. Malaviya, R., Ross, E. A., MacGregor, J. I., Ikeda, T., Little, J. R., Jakschik, B. A., Abraham, S. N. (1994) Mast cell phagocytosis of FimH-expressing enterobacteria. *J. Immunol.* **152,** 1907–1914.
6. Arock, M., Ross, E., Lai-Kuen, R., Averlant, G., Gao, Z., Abraham, S. N. (1998) Phagocytic and tumor necrosis factor α response of human mast cells following exposure to gram-negative and gram-positive bacteria. *Infect. Immun.* **66,** 6030–6034.
7. Malaviya, R., Gao, Z., Thankavel, K., van der Merwe, P. A., Abraham, S. N. (1999) The mast cell tumor necrosis factor α response to FimH-expressing *Escherichia coli* is mediated by the glycosylphosphatidylinositol-anchored molecule CD48. *Proc. Natl. Acad. Sci. USA* **96,** 8110–8115.
8. Malaviya, R., Ikeda, T., Ross, E., Abraham, S. N. (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α. *Nature* **381,** 77–80.
9. Kirshenbaum, A. S., Akin, C., Wu, Y., Rottem, M., Goff, J. P., Beaven, M. A., Rao, V. K., Metcalfe, D. D. (2003) Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRI. *Leuk. Res.* **27,** 677–682.
10. Malaviya, R., Ross, E. A., MacGregor, J. I., Ikeda, T., Little, J. R., Jakschik, B. A., Abraham, S. N. (1994) Mast cell phagocytosis of FimH-expressing enterobacteria. *J. Immunol.* **152,** 1907–1914.
11. Rossi, D., Zlotnik, A. (2000) The biology of chemokines and their receptors. *Annu. Rev. Immunol.* **18,** 217–242.
12. McCaw, S. E., Liao, E. H., Gray-Owen, S. D. (2004) Engulfment of *Neisseria gonorrhoeae*: revealing distinct processes of bacterial entry by individual carcinoembryonic antigen-related cellular adhesion molecule family receptors. *Infect. Immun.* **72,** 2742–2752.
13. Dimitriadou, V., Mecheri, S., Koutsilieris, M., Fraser, W., Al Daccak, R., Mourad, W. (1998) Expression of functional major histocompatibility complex class II molecules on HMC-1 human mast cells. *J. Leukoc. Biol.* **64,** 791–799.
14. Warbrick, E. V., Taylor, A. M., Botchkarev, V. A., Coleman, J. W. (1995) Rat connective tissue-type mast cells express MHC class II: up-regulation by IFN-γ. *Cell. Immunol.* **163,** 222–228.
15. Raposo, G., Tenza, D., Mecheri, S., Peronet, R., Bonnerot, C., Desaymard, C. (1997) Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. *Mol. Biol. Cell* **8,** 2631–2645.
16. Yu, C. K., Chen, C. L. (2003) Activation of mast cells is essential for development of house dust mite *Dermatophagoides farinae*-induced allergic airway inflammation in mice. *J. Immunol.* **171,** 3808–3815.
17. Tkaczyk, C., Villa, I., Peronet, R., David, B., Mecheri, S. (1999) FcεRI-mediated antigen endocytosis turns interferon-γ-treated mouse mast cells from inefficient into potent antigen-presenting cells. *Immunology* **97,** 333–340.
18. Frandji, P., Oskeritzian, C., Cacaraci, F., Lapeyre, J., Peronet, R., David, B., Guillet, J. G., Mecheri, S. (1993) Antigen-dependent stimulation by bone marrow-derived mast cells of MHC class II-restricted T cell hybridoma. *J. Immunol.* **151,** 6318–6328.
19. Malaviya, R., Navara, C., Uckun, F. M. (2002) Augmentation of mast cell bactericidal activity by the anti-leukemic drug, 4-(3′bromo-4'-hydroxylphenyl)-amino-6,7-dimethoxyquinazoline. *Leuk. Lymphoma* **43,** 1329–1332.
20. Vosseller, K., Stella, G., Yee, N. S., Besmer, P. (1997) c-kit receptor signaling through its phosphatidylinositide-3′-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion, and membrane ruffling. *Mol. Biol. Cell* **8,** 909–922.
21. Miller, B. H., Fratti, R. A., Poschet, J. F., Timmins, G. S., Master, S. S., Burgos, M., Marletta, M. A., Deretic, V. (2004) Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect. Immun.* **72,** 2872–2878.
22. Miller, M. D., Krangel, M. S. (1992) The human cytokine I-309 is a monocyte chemoattractant. *Proc. Natl. Acad. Sci. USA* **89,** 2950–2954.
23. Bernardini, G., Spinetti, G., Ribatti, D., Camarda, G., Morbidelli, L., Ziche, M., Santoni, A., Capogrossi, M. C., Napolitano, M. (2000) I-309 binds to and activates endothelial cell functions and acts as an angiogenic molecule in vivo. *Blood* **96,** 4039–4045.
24. Adema, G. J., Hartgers, F., Verstraten, R., de Vries, E., Marland, G., Menon, S., Foster, J., Xu, Y., Nooyen, P., McClanahan, T., Bacon, K. B., Figdor, C. G. (1997) A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* **387,** 713–717.
25. Kodelja, V., Muller, C., Politz, O., Hakij, N., Orfanos, C. E., Goerdt, S. (1998) Alternative macrophage activation-associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-1 α with a Th2-associated expression pattern. *J. Immunol.* **160,** 1411–1418.
26. Schutyser, E., Struyf, S., Wuyts, A., Put, W., Geboes, K., Grillet, B., Opdenakker, G., Van Damme, J. (2001) Selective induction of CCL18/PARC by staphylococcal enterotoxins in mononuclear cells and enhanced levels in septic and rheumatoid arthritis. *Eur. J. Immunol.* **31,** 3755–3762.
27. Atamas, S. P., White, B. (2003) Cytokine regulation of pulmonary fibrosis in scleroderma. *Cytokine Growth Factor Rev.* **14,** 537–550.

28. Pardo, A., Smith, K. M., Abrams, J., Coffman, R., Bustos, M., McClanahan, T. K., Grein, J., Murphy, E. E., Zlotnik, A., Selman, M. (2001) CCL18/DC-CK-1/PARC up-regulation in hypersensitivity pneumonitis. *J. Leukoc. Biol.* **70,** 610–616.
29. Atamas, S. P., Luzina, I. G., Choi, J., Tsymbalyuk, N., Carbonetti, N. H., Singh, I. S., Trojanowska, M., Jimenez, S. A., White, B. (2003) Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* **29,** 743–749.
30. Kim, C. H., Pelus, L. M., White, J. R., Broxmeyer, H. E. (1998) Macrophage-inflammatory protein-3 β/EBI1-ligand chemokine/CK β-11, a CC chemokine, is a chemoattractant with a specificity for macrophage progenitors among myeloid progenitor cells. *J. Immunol.* **161,** 2580–2585.
31. Kim, C. H., Broxmeyer, H. E. (1999) Chemokines: signal lamps for trafficking of T and B cells for development and effector function. *J. Leukoc. Biol.* **65,** 6–15.
32. Galli, S. J. (2000) Mast cells and basophils. *Curr. Opin. Hematol.* **7,** 32–39.
33. McLachlan, J. B., Hart, J. P., Pizzo, S. V., Shelburne, C. P., Staats, H. F., Gunn, M. D., Abraham, S. N. (2003) Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nat. Immunol.* **4,** 1199–1205.
34. Okumura, S., Kashiwakura, J., Tomita, H., Matsumoto, K., Nakajima, T., Saito, H., Okayama, Y. (2003) Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcεRI. *Blood* **102,** 2547–2554.
35. Berger, C. N., Billker, O., Meyer, T. F., Servin, A. L., Kansau, I. (2004) Differential recognition of members of the carcinoembryonic antigen family by Afa/Dr adhesins of diffusely adhering *Escherichia coli* (Afa/Dr DAEC). *Mol. Microbiol.* **52,** 963–983.
36. Pennock, J. L., Grencis, R. K. (2004) In vivo exit of c-kit+/CD49d(hi)/β7+ mucosal mast cell precursors from the bone marrow following infection with the intestinal nematode *Trichinella spiralis*. *Blood* **103,** 2655–2660.
37. Matsuzawa, S., Sakashita, K., Kinoshita, T., Ito, S., Yamashita, T., Koike, K. (2003) IL-9 enhances the growth of human mast cell progenitors under stimulation with stem cell factor. *J. Immunol.* **170,** 3461–3467.
38. Lorentz, A., Bischoff, S. C. (2001) Regulation of human intestinal mast cells by stem cell factor and IL-4. *Immunol. Rev.* **179,** 57–60.
39. Tachimoto, H., Ebisawa, M., Hasegawa, T., Kashiwabara, T., Ra, C., Bochner, B. S., Miura, K., Saito, H. (2000) Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN-γ. *J. Allergy Clin. Immunol.* **106,** 141–149.
40. Gillespie, S. R., DeMartino, R. R., Zhu, J., Chong, H. J., Ramirez, C., Shelburne, C. P., Bouton, L. A., Bailey, D. P., Gharse, A., Mirmonsef, P., Odom, S., Gomez, G., Rivera, J., Fischer-Stenger, K., Ryan, J. J.(2004) IL-10 inhibits Fc ε RI expression in mouse mast cells. *J. Immunol.* **172,** 3181–3188.
41. Lin, T. J., Befus, A. D. (1997) Differential regulation of mast cell function by IL-10 and stem cell factor. *J. Immunol.* **159,** 4015–4023.
42. Royer, B., Varadaradjalou, S., Saas, P., Gabiot, A. C., Kantelip, B., Feger, F., Guillosson, J. J., Kantelip, J. P., Arock, M. (2001) Autocrine regulation of cord blood-derived human mast cell activation by IL-10. *J. Allergy Clin. Immunol.* **108,** 80–86.