

Pivotal Advance: IgE accelerates in vitro development of mast cells and modifies their phenotype

Jun-ichi Kashiwakura,* Wenbin Xiao,* Jiro Kitaura,*¹ Yuko Kawakami,* Mari Maeda-Yamamoto,[†] Janet R. Pfeiffer,[‡] Bridget S. Wilson,[‡] Ulrich Blank,[§] and Toshiaki Kawakami*²

*Division of Cell Biology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA; [†]National Research Institute of Vegetables and Tea Science, National Agriculture Research Organization, Shizuoka, Japan;

[‡]Department of Pathology and Cancer Research and Treatment Center, University of New Mexico School of Medicine, Albuquerque, New Mexico, USA; and [§]Inserm U699 and Université Paris 7-Diderot, Faculté de Médecine, Site Xavier Bichat, Paris, France

Abstract: Antigen-dependent activation of IgE-bound mast cells is critical for immediate hypersensitivity and other allergic disorders. Recent studies have revealed the effects of monomeric IgEs on mast cell survival and activation. Furthermore, IgE molecules exhibit a wide range of heterogeneity in the ability to induce mast cell activation in the absence of antigen. Highly cytokinergic (HC) IgEs can induce a variety of activation events including cell survival, degranulation, cytokine production, and migration, whereas poorly cytokinergic (PC) IgEs can do so inefficiently. Here, we show that culture of bone marrow cells in the presence of monomeric IgEs results in an increased number of mast cells compared with cultures grown without IgE. Furthermore, time in culture required to generate $\geq 80\%$ pure mast cells is decreased. IgE molecules can directly influence mast cell progenitors to differentiate into mast cells. mRNA expression of several mast cell proteases and mast cell-related transcription factors is higher in mast cells cultured with a HC IgE than those cultured with a PC IgE or without IgE. Expression of early growth response factor-1, a transcription factor that is involved in the production of TNF- α in mast cells, is enhanced in cultures containing high and low concentrations of HC IgE and a high concentration of PC IgE. Consistent with this, expression of TNF- α is higher in mast cells cultured with HC IgE than PC IgE. Therefore, our results suggest that monomeric IgEs, especially HC IgEs, not only promote mast cell development but also modulate the mast cell phenotype. *J. Leukoc. Biol.* 84: 000–000; 2008.

Key Words: highly cytokinergic · poorly cytokinergic · differentiation · TNF

INTRODUCTION

Mast cells play a pivotal role in allergic inflammatory reactions and the defense against certain bacteria and parasites [1, 2]. In

addition to these well-characterized areas, recent studies have revealed the roles of mast cells in innate and adaptive immunity and implicated them in the pathogenesis of autoimmune diseases, chronic heart failure, cancer, and tolerance [3, 4]. Mast cells differentiate from hematopoietic progenitor cells in the bone marrow (BM) after progenitor cells migrate into local tissues [5]. Three recent studies identified mouse mast cell progenitors (mMCPs) using various combinations of cell surface markers [6–8]. The stem cell factor (SCF)/c-Kit system is essential for the differentiation, proliferation, and survival of mast cells [9]. Loss-of-function mutations in the *Sl* locus encoding SCF [10] and the *W* locus encoding c-Kit, the SCF receptor [11], lead to severe defects in mast cell development.

Properties of mast cells exhibit heterogeneity, depending on tissues and species from which they are derived. For example, in mice, mucosal mast cells (MMC) are located in the intestine and lung, and connective tissue mast cells (CTMCs) are located in the skin [12, 13]. These different types of cells exhibit differences in lifespan, morphology, development, expression pattern of mMCPs and proteoglycans, and sensitivity to immunologic and nonimmunologic stimuli: MMCs predominantly express mMCP-1 and -2, whereas CTMCs preferentially express mMCP-4, -5, -6, and -7 and carboxypeptidase A [14–19].

Aggregation of the high-affinity IgE receptor (Fc ϵ RI) on IgE-bound mast cells with multivalent antigen induces their activation. Activated mast cells release a variety of preformed and de novo-synthesized chemical and protein mediators, such as histamine, proteases, leukotrienes, PGs, and various cytokines/chemokines [2]. In addition to this traditional mechanism for mast cell activation, survival and other outcomes of mast cell activation can be induced by monomeric IgE in the absence of multivalent antigen [20, 21]. Our recent study showed that mouse IgE molecules display a vast heterogeneity

¹ Current address: Division of Cellular Therapy, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

² Correspondence: Division of Cell Biology, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037, USA. E-mail: toshi@liai.org

Received December 18, 2007; revised April 11, 2008; accepted April 14, 2008.

doi: 10.1189/jlb.1207841

in their ability to induce survival and activation events in mouse mast cells [22]: On the one hand, highly cytokinergic (HC) IgEs induce survival, degranulation, proliferation, adhesion, migration, and expression of cytokines/chemokines such as IL-6 and TNF- α ; at the other end of the spectrum, poorly cytokinergic (PC) IgEs do so inefficiently [23].

Here, we show that IgE molecules, particularly HC IgEs, have the ability to facilitate mast cell differentiation from BM cells and purified MCPs. IgEs do not simply accelerate mast cell differentiation but affect the phenotype of resulting mast cells.

MATERIALS AND METHODS

Reagents

Anti-DNP IgE mAb [clone H1 DNP- ϵ -206 (abbreviated as 206), clone H1 DNP- ϵ -26 (abbreviated as 26), clone 27-74, and clone SPE-7] were described previously [22]. DNP conjugated with human serum albumin (HSA), DNP₂₃-HSA, was a gift from Teruko Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA). Recombinant (r)mSCF was a gift from Kirin Brewery (Tokyo, Japan). rmlL-3 was purchased from PeproTech (Rocky Hill, NJ, USA). Anti-Syntaxin-2, -3, and -4, anti-vesicle-associated membrane protein (VAMP)-8, and anti-Munc18-2 have been described [24, 25]. Anti-VAMP-2 and anti-soluble N-ethylmaleimide sensitive factor attachment protein (SNAP)-23 were purchased from Synaptic Systems (Goettingen, Germany). Anti-mouse β -actin and p38 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Culture of BM cells and MCPs

BM cells were cultured in the presence of an optimal concentration (5 ng/ml) of IL-3 with various concentrations of different IgEs, with or without antigen, from the initiation of culture. MCPs were isolated from BM cells as defined by Chen et al. [7]. Lin⁻Sca-1⁺Ly6c⁻Fc ϵ RI⁻c-Kit⁺ β 7⁺CD27^{low} MCPs were sorted into 96-well plates using a FACSVantage cell sorter (BD Biosciences, San Jose, CA, USA) and cultured in IL-3-containing medium with or without IgEs. Mouse studies were approved by the La Jolla Institute for Allergy and Immunology Review Board. Histamine contents of the resulting mast cells [BM-derived mast cells (BMMCs)] were measured as described previously [22].

Flow cytometry

For the measurement of surface expression of Fc ϵ RI and c-Kit, BMMCs were incubated first with 10 μ g/ml 2.4G2 mAb (BD Biosciences Pharmingen, San Diego, CA, USA) at 4°C for 10 min and then with 20 μ g/ml 206 IgE at room temperature for 30 min. The cells were incubated with FITC-conjugated anti-mouse IgE (BD Biosciences Pharmingen) and PE-conjugated anti-c-Kit mAb (BD Biosciences Pharmingen) for 30 min. Flow cytometric analysis of the stained cells was performed with FACScan or FACSCalibur (BD Biosciences) equipped with CellQuest software.

Electron microscopy

BMMCs were postfixed in 2% glutaraldehyde in PBS, washed in PBS, and then stained with 1% OsO₄ in 0.1 M cacodylate buffer, 1% tannic acid, and 1% uranyl acetate. Samples were examined using a Hitachi 600 transmission electron microscope [26].

Quantitative RT-PCR analysis

An equal amount of total RNA (1 μ g) was used for RT. Real-time RT-PCR was performed as follows: cDNA (5 ng) was amplified in a 20- μ l reaction volume containing a LightCycler[®] 480 SYBR Green I Master mixture (Roche Diagnostics GmbH, Mannheim, Germany) and 0.5 μ M forward and reverse primers purchased from Integrated DNA Technologies (Coralville, IA, USA), using a LightCycler[®] 480 (Roche Diagnostics GmbH) as follows: 95°C for 5 min, followed by 45 amplification cycles (95°C, 10 s; 55°C, 10 s; 72°C, 10 s).

Melting-curve analysis was performed for the identification of PCR products. All amplified PCR products yielded a single melting peak. Relative expression levels using 18S RNA as a control were determined using the $\Delta\Delta$ -comparative threshold method. The sequence of each primer set is described in **Table 1**.

Immunoblotting

BMMCs were lysed in 1% Nonidet P-40 (NP-40)-containing lysis buffer (20 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin, 25 μ M *p*-nitrophenyl *p*'-guanidinobenzoate, 1 μ M pepstatin, and 0.1% sodium azide). In some experiments, BMMCs were directly lysed and boiled in SDS sample buffer (0.1 M Tris-HCl, pH 8.0, 2% SDS, 0.01 M DTT, 10% glycerol). Cell lysates were analyzed by SDS-PAGE, followed by immunoblotting. Proteins reactive with primary antibodies were visualized with a HRP-conjugated secondary antibody and Western Lightning[™] chemiluminescence reagents (PerkinElmer Life Sciences, Boston, MA, USA).

RESULTS

Mast cell numbers increase in the presence of IgE

HC and PC IgEs affect various aspects of mast cell biology in the absence of specific antigen [23]. However, it is not yet known whether IgE can affect mast cell differentiation. To evaluate the effect of IgE on mast cell differentiation, BM cells from 129/SvJ mice were cultured in the presence of an optimal concentration (5 ng/ml) of IL-3, with or without 206 IgE (a typical PC IgE) or SPE-7 IgE (a typical HC IgE), and total and mast cell numbers were monitored over time. When BM cells were cultured with IgE, total live cell numbers were higher as compared with cells cultured with IL-3-containing medium alone or PBS in place of IgE. Moreover, the numbers of SPE-7 IgE-incubated BM cells were greater than those of 206 IgE-incubated cells (**Fig. 1A**). As a comparison, BM cells were cultured with 206 IgE plus increasing concentrations of antigen. Under such conditions, total live cell numbers increased in an antigen concentration-dependent manner (**Fig. 1B**). Addition of SCF increased cell numbers dramatically as expected (data not shown).

When BM cells were cultured in IL-3-containing medium, the proportion of Fc ϵ RI⁺/c-Kit⁺ mast cells gradually increased, and these cultures consisted of >85% mast cells after 5 weeks (**Fig. 1C**). In the presence of either IgE, the mast cell purity reached $\geq$ 80% faster than without IgE. Furthermore, the purity of the cells cultured with SPE-7 IgE rose to $\geq$ 80% faster than the cells cultured with 206 IgE (**Fig. 1C**). When BM cells were cultured with 206 IgE plus antigen, the mast cell purity increased in an antigen concentration-dependent manner (**Fig. 1D**). We also analyzed Fc ϵ RI expression levels on the surface of the cells cultured with or without IgE. In the absence of IgE, MFI of Fc ϵ RI progressively increased to 181 ± 53.74 after 5 weeks of culture. In BM cells cultured with HC or PC IgE, Fc ϵ RI expression was higher than without IgE, and the expression was increased dramatically in cultures containing a high concentration of IgE (**Fig. 1, E and F**), probably as a result of receptor stabilization [27, 28]. In contrast, Fc ϵ RI expression was changed according to the antigen concentrations in cultures containing 206 IgE plus antigen. Suppression of Fc ϵ RI levels at a high antigen concentration was likely a result of

TABLE 1. Primer Set Sequences

Gene	Forward primer	Reverse primer
Proteases		
mMCP-1	GCACTTCTCTTGCCTTCTGG	TAAGGACGGGAGTGTGGTCT
mMCP-2	GCACTTCTTTTGCCTTCTGG	TAAGGACGGGAGTGTGGTTT
mMCP-4	TGGCACTTCTCTTGCCTTCT	TAAGGGCGAGAATGTTGGTCT
mMCP-5	TGCATCTTCTTACTCTTCATCTGC	CGTGCCTCCAATGATCTCTC
mMCP-6	TGCTGTGTGCTGGAAATACC	CCCTTCACTTTGCAGACCAG
mMCP-7	GTGCTGGGAATGAAGGACAT	TGCAGCCAGGTGTCTTCTAC
mMCP-8	GGATGTTCTGCTCCTCGGT	TGGGGTTGGACTCTGTACC
mMCP-9	TGGCACTTCTCTTGCCTTCT	TCGGGAGTGTGGTTCAGAC
mMCP-10	TGGCACTTCTCTTGCCTTCT	TTGCCACATAACCTTTCCTTC
CPA	GCTATTAATTCCTTATGGCTACACATT	GTGGCAATCCTTGCAACTTT
Transcription factors		
GATA-1	GAATCCTCTGCATCAACAAGC	GGGCAAGGGTTCTGAGGT
GATA-2	GCTTCACGCCCTAAGCAGAGA	TGGCACCAGAGTTGACACA
GATA-3	TTATCAAGCCCAAGCGAAG	TGGTGGTGGTCTGACAGTTC
MITF	GGTCTGCGGTGTCTCCTG	TTCCAGGTGGGTCTGCAC
PIAS3	CGTGTACCTGGTGAGGCAAT	GCGGTCAAGTTTCTCCTTGAT
Egr-1	CCCTATGAGCACCTGACCAC	TCGTTTGGCTGGGATAACTC
YY1	GCAAAGCGTTCGTTGAGAGC	CCAATCACACTCCTGAAGAGGC
Cytokines		
TNF- α	TCTTCTCATTCCTGCTTGTGG	GGTCTGGGCCATTGAACTGA
IL-13	CCTCTGACCCCTTAAGGAGCTTAT	CGTTGCACAGGGGAGTCT
TGF- β	TGGAGCAACATGTGGAATC	GTCAGCAGCCGGTTACCA
SNAREs		
Munc18-2	TCTTGTATCCTGTTGTAAATGTC	CCGTTTGTGATGTCTTCCA
SNAP-23	GGAAGTAGTCAGCGGGACAG	GATTATCCATGGTGAGTCGTCA
VAMP-2	GCACCTCCTCCAAACCTTACT	CAGCTCCGACAACCTTCTGGT
VAMP-8	CTTTGAGAGGCCCAAGTGC	GCAGGTTCCCTAACTCGGTCA
syntaxin-2	CATCCTGTCTGCTCCAAACC	GCAGTTTCTTGATCTCTTGTCC
syntaxin-3	GCCTTCATGGACGAGTTCTT	TCATGTGAGCTCAATCTCAGGA
syntaxin-4	GCTCCATGAGATCTTACGTTTC	TTCTTCTCGATGCGGTTGAT
Control		
18S RNA	ATGGTAGTCGCCGTGCCTAC	CCGGAATCGAACCCTGATT

CPA, ●●●; GATA, ●●●; MITF, microphthalmia-associated transcription factor; PIAS3, protein inhibitor of activated stat3; Egr-1, early growth response factor-1; yy1, Yin-Yang 1; SNAREs, soluble N-ethylmaleimide-sensitive fusion protein attachment receptors.

receptor internalization [29]. Collectively, HC and PC IgEs accelerated IL-3-dependent in vitro mast cell development. Increased mast cell development was also observed using 26 (another HC IgE) and 27–74 (another PC IgE) IgEs, and higher effects were shown by HC IgEs (data not shown). Similar promoting effects of 206 and SPE-7 IgEs on mast cell development were also observed with BM cells cultured in a sub-optimal concentration (1 ng/ml) of IL-3 (data not shown). Similar IgE-mediated acceleration of mast cell development was observed with BM cells derived from C57BL/6 mice as well (data not shown).

SPE-7 but not 206 IgE elicits substantial degranulation

When BMMCs are stimulated with IgE in the absence of antigen SPE-7 but not 206, IgE can induce mast cell activation events such as degranulation and production of cytokines [20–22]. To examine the effect of monomeric IgE on degranulation in long-term, cultured BM cells, we analyzed the granularity of mast cells cultured with or without IgE by toluidine blue staining and transmission electron microscopy. BMMCs cultured in IL-3-containing medium had lobulated nuclei and characteristic granules (**Fig. 2A**). 206 IgE

did not induce any discernible changes in the morphology of mast cells. In contrast, BMMCs cultured with SPE-7 IgE exhibited degranulation (Fig. 2, A and B, upper panel). The percentage of degranulated cells was increased in BMMCs that had been incubated with 206 IgE plus antigen in an antigen concentration-dependent manner (Fig. 2, A and B, upper panel). BMMC cultured with SPE-7 but not 206 IgE had increased histamine contents (Fig. 2B, lower panel). These results therefore indicate that a HC but not PC IgE can induce degranulation and histamine production in long-term, cultured BM cells, similar to short-term stimulation of BMMCs with SPE-7 IgE [22].

HC and PC IgEs affect the generation of mast cells from MCPs

Recent studies identified MCPs in mouse BM and intestines using certain cell surface markers [6–8]. Our observed effects of IgE on mast cell development from BM cells could be a result of direct effects of IgE on MCPs or indirect effects via other IgE receptor-expressing cell types. To clarify whether IgE can directly affect the MCP population, we purified MCPs from BM cells by FACS sorting (**Fig. 3A**) and cultured them

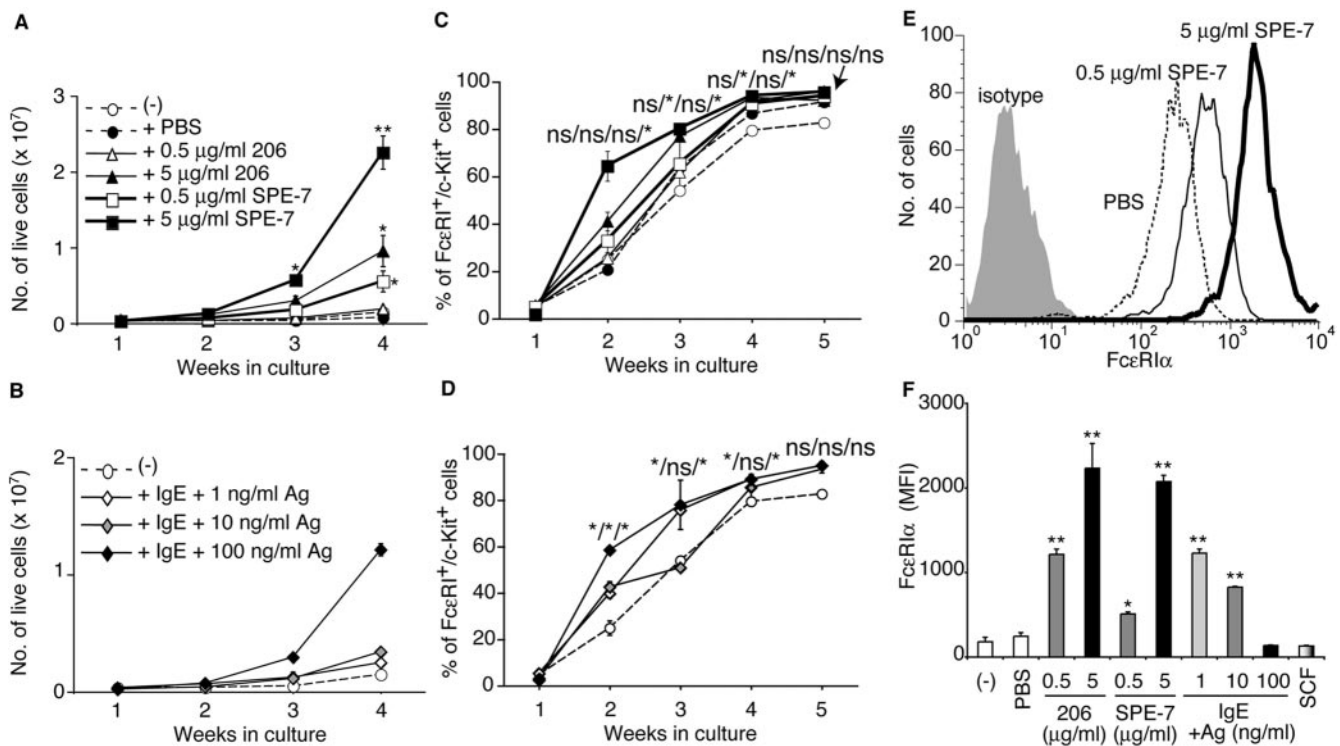


Fig. 1. Development of mast cells from BM cells cultured with or without IgE. BM cells from 129/SvJ mice were cultured in IL-3-containing medium supplemented with the following agents: PBS alone; 0.5 or 5 μ g/ml 206 IgE; 0.5 or 5 μ g/ml SPE-7 IgE; 0.5 μ g/ml 206 IgE plus 1, 10, or 100 ng/ml DNP₂₃-HSA; or 50 ng/ml SCF. Culture media were changed weekly, and live cells were counted (A, B) and checked for expression of FcεRI and c-Kit (C, D). (E, F) Five weeks after culture, FcεRI expression was measured by flow cytometry. Data are presented as mean $\pm$ SD, representative of three independent experiments with all data points measured in triplicate. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with PBS control by Student's *t*-test. (C) Statistical significance is indicated in the order of 0.5 μ g/ml 206 IgE/5 μ g/ml 206 IgE/0.5 μ g/ml SPE-7 IgE/5 μ g/ml SPE-7 IgE. (D) 1 ng/ml antigen/10 ng/ml antigen/100 ng/ml. ns, Not significant ($P > 0.05$); MFI, mean fluorescence intensity.

with or without IgE. The number of live cells was counted during each medium change, and after 20 days, cells were collected and analyzed for surface expression of FcεRI and c-Kit. Incubation with SPE-7 and 206 IgEs resulted in increased mast cell numbers over those cultured in PBS instead of IgE, and this increase was IgE concentration-dependent (Fig. 3B). The purity of FcεRI⁺/c-Kit⁺ cells after 20 days was >90% in all conditions (Fig. 3C). The kinetics and extent of the HC versus PC IgE effects on mast cell development were similar when BM cells and MCPs were used as starting materials (compare Fig. 1A with Fig. 3B). Therefore, it is likely that IgE exerts its effects directly on MCPs in long-term BM cell cultures.

Expression and detergent solubility of SNARE proteins are affected by IgE

Degranulation involves secretory granule fusion with the mast cell plasma membrane. This membrane fusion reaction is mediated by integral membrane proteins termed SNAREs [30]. As a HC but not PC IgE could induce degranulation in long-term, cultured BM cells, we determined the expression levels of SNARE proteins and the fusion accessory protein Munc18-2 in mast cells generated in the presence or absence of IgE by real-time RT-PCR and immunoblot analyses. In this experiment, we analyzed the expression of SNARE proteins solubilized in NP-40 or SDS sample buffer,

as some SNAREs were shown to be localized in the NP-40-insoluble fraction in FcεRI-stimulated mast cells [31]. VAMP-2 transcript levels tended to be low in mast cells cultured with SPE-7 IgE, and its levels were increased insignificantly in mast cells cultured with 206 IgE (Fig. 4A). The VAMP-2 protein levels detected in SDS lysates correlated well with the trend of mRNA expression (Fig. 4B), suggesting that VAMP-2 expression is regulated by IgE, at least partly at mRNA (transcriptional and/or post-transcriptional) levels. mRNA and protein (detected in SDS lysates) levels of the other tested SNARE proteins (i.e., SNAP-23, VAMP-8, and Syntaxin-2, -3, and -4) were comparable under our conditions. Interestingly, SNAP-23 and VAMP-8 protein levels (and VAMP-2 levels to a lesser extent) were drastically decreased in NP-40 lysates of mast cells cultured with SPE-7 IgE and in those of mast cells cultured with a high (5 μ g/ml) concentration of 206 IgE (Fig. 4B). However, when mast cells were cultured with a low concentration of 206 IgE, expression levels of Munc18-2, SNAP-23, and VAMP-2, and -8 were increased in NP-40 lysates (Fig. 4B). These changes likely reflect changes in subcellular location of these proteins. Similarly, low expression levels of Munc18-2, SNAP-23, and VAMP-2 and -8 (and Syntaxin-2 to a lesser extent) were observed in NP-40 lysates of the cells cultured in 206 IgE plus antigen, compared with PBS control.

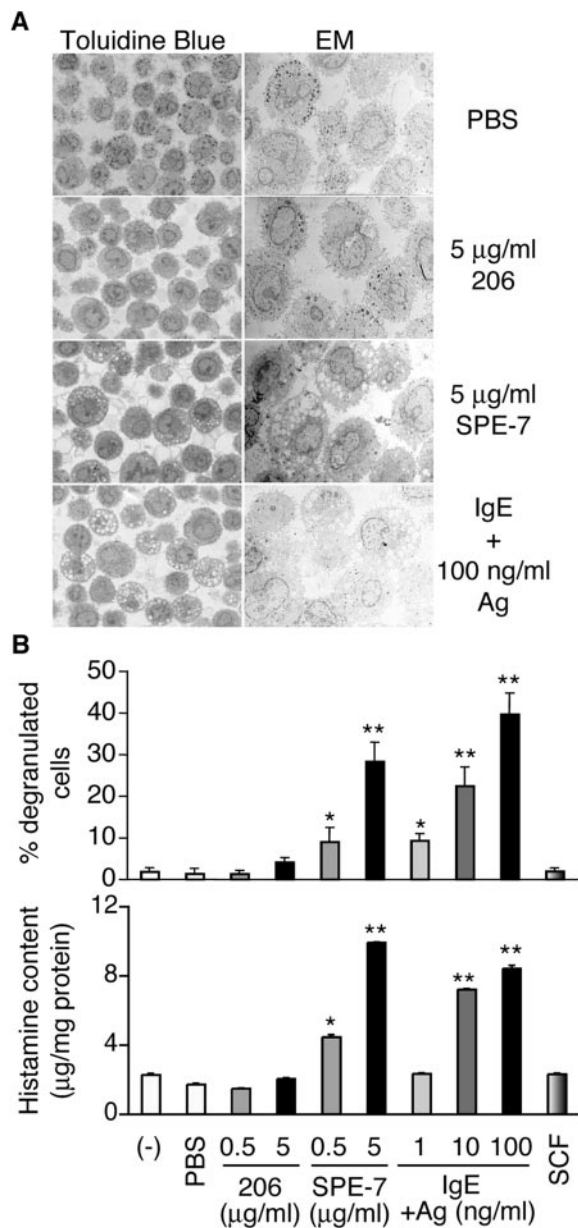


Fig. 2. Granularity of mast cells cultured in the presence or absence of IgE. BM cells were cultured in IL-3-containing medium, with or without IgE. Some cells were incubated with 0.5 μ g/ml 206 IgE plus 1, 10, or 100 ng/ml DNP₂₃-HSA. As another control, aliquots of cells were incubated in 50 ng/ml SCF plus IL-3. (A) After 5 weeks of culture, cells were harvested, and their morphology was analyzed by toluidine blue staining (left panels) or electron microscopy (EM; right panels, 2500 $\times$). (B) Total and degranulated mast cells were counted, and percentages of degranulated cells are plotted (upper panel). Histamine contents of the resulting BMMCs were measured (lower panel). Data are presented as mean $\pm$ SD, representative of two independent experiments. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with PBS control by Student's t -test.

SPE-7 but not 206 IgE increases the expression of some mMCPs

mMCPs and other proteases are major constituents of mast cell granules, and their expression is regulated by cytokines such as TGF- β , IL-10, and IL-3 [31–37]. However, it is not known whether monomeric IgE affects expression of mMCPs in mast cells. To test this possibility, we extracted total RNA from

BMMCs cultured with or without IgE and analyzed the expression pattern of a number of proteases by real-time RT-PCR. Expression of mMCP-2 and -4 was up-regulated in BMMCs cultured with SPE-7 but not 206 IgE, and mMCP-1 expression also showed the same tendency (Fig. 5). Expression of mMCP-6 and mMCP-7, which are mouse tryptases [38], was found to be low in BMMCs cultured with IL-3 alone. However, when BMMCs were generated in the presence of SPE-7 but not 206 IgE, their expression levels were increased dramatically. Culturing in SCF also led to a remarkable increase in mRNAs encoding mMCP-1 and -4, and the same tendency was seen for mMCP-6 and -7 mRNAs. mMCP-8 expression was suppressed in mast cells cultured with a high concentration of 206 IgE or SPE-7 IgE. In contrast, mMCP-9 and mMCP-10 expression levels were unchanged in BMMCs cultured with or without IgE. Carboxypeptidase A mRNA was up-regulated by SPE-7 but not 206 IgE. These results collectively indicate that certain mMCPs are up-regulated by HC IgE in long-term BM cell cultures.

Expression of some mast cell-related transcription factors increases in BMMCs cultured with SPE-7 IgE

A number of transcription factors are required for mast cell differentiation [39]. To determine whether the inclusion of IgE in BM cell cultures affects expression of these factors, we analyzed expression of a number of transcription factors in BMMCs cultured with or without IgE. GATA-1 and -2 are known to be required for mast cell differentiation [40–42]. GATA-1 expression was insignificantly increased in BMMCs cultured with SPE-7 IgE, 206 IgE plus a high concentration of antigen, or SCF (Fig. 6A). GATA-2 expression was comparable in the BMMCs generated in the presence of SPE-7 and 206 IgEs. Although it has been reported that GATA-3 is not expressed in BMMCs [43], we detected GATA-3 mRNA expression in BMMCs. However, GATA-3 expression levels were unchanged by IgEs.

We next analyzed expression of Stat transcription factors, as Stat5a and Stat5b are critical regulators of in vitro and in vivo mast cell development and survival [44]. However, expression of all known Stat family proteins (i.e., Stat1, -2, -3, -4, -5a, -5b, and -6) was not affected by IgEs (data not shown).

MITF-deficient mice exhibit mast cell deficiency [45]. MITF also regulates expression of mMCP-5 and -6 and c-Kit [46–48]. However, expression of MITF was unaltered by IgE. The granulocyte-related transcription factor C/EBP α plays a primary role in the fate decision of bipotent basophil/MCPs, expressed in basophil progenitors but not in MCPs [6]. Consistent with this, we did not detect its expression in mast cells cultured with or without IgE (data not shown). PIAS3 is a repressor protein that regulates MITF and Stat3 activity [49–51]. Expression of PIAS3 was increased in BMMCs cultured with 206 IgE, SPE-7 IgE, and 206 IgE plus antigen. Egr-1 is a transcription factor that mediates allergen-induced airway inflammation and reactivity [52] and also regulates expression of some cytokines including TNF- α in mast cells [53]. We observed that Egr-1 expression was up-regulated in BMMCs cultured with SPE-7 IgE and to a lesser extent, with 206 IgE (Fig. 6). We did not observe any remarkable changes among

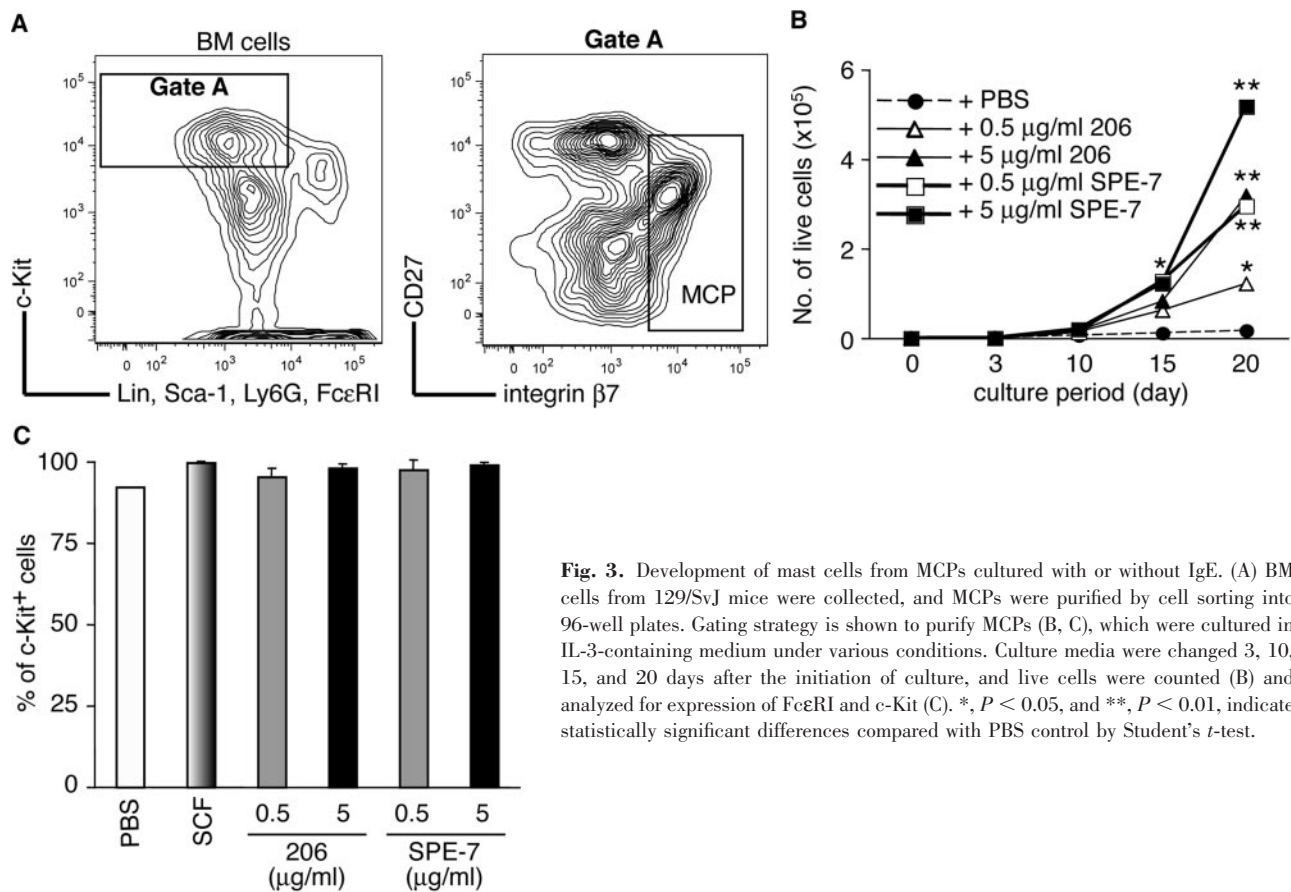


Fig. 3. Development of mast cells from MCPs cultured with or without IgE. (A) BM cells from 129/SvJ mice were collected, and MCPs were purified by cell sorting into 96-well plates. Gating strategy is shown to purify MCPs (B, C), which were cultured in IL-3-containing medium under various conditions. Culture media were changed 3, 10, 15, and 20 days after the initiation of culture, and live cells were counted (B) and analyzed for expression of FcεRI and c-Kit (C). *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with PBS control by Student's *t*-test.

other transcription factors that were analyzed [PU.1, Oct-1, upstream stimulatory factor 2 (USF2), YY-1, myeloid zinc finger-1 (MZF-1), friend of GATA-1 (FOG-1), and histidine triad nucleotide-binding protein 1 (Hint-1); Fig. 6, and data not shown]. Therefore, the results in this section indicate that HC IgE can selectively affect the expression of several transcription factors such as PIAS3 and Egr-1. Expression of these transcription factors is also affected by 206 IgE plus antigen.

TNF-α expression is up-regulated in mast cells incubated with SPE-7 IgE

TNF-α plays an important role in the induction of mast cell-mediated inflammatory reactions [52, 54, 55] and mediates IL-3-dependent differentiation of mast cells [56]. HC IgE can induce the production and secretion of IL-3, which is largely responsible for IgE-dependent mast cell survival [57]. We therefore examined the expression of TNF-α in BMMCs in the presence or absence of either IgE. Expression of TNF-α mRNA was increased substantially in BMMCs cultured with SPE-7 IgE and to a lesser extent in those with a high concentration of 206 IgE (Fig. 7). However, levels of TNF-α protein in culture supernatants were under the detection limit of ELISA, and membrane-bound TNF-α protein in BMMCs cultured with SPE-7 IgE was barely detectable (data not shown). We also analyzed the expression levels of other cytokines. IL-13, which is involved in allergic airway inflammation [58], is expressed in BMMCs. TGF-β is an important cytokine that modulates expression of mMCP-1, -2, -6, and -7 in mast cells [32, 33, 37,

59]. However, expression of IL-13 and TGF-β was comparable in all samples.

DISCUSSION

MCPs in the BM presumably migrate into the circulation and shortly thereafter settle in tissues, where further differentiation takes place to generate mature mast cells [5]. This homeostatic mast cell differentiation appears to be controlled by SCF, as loss-of-function mutations of SCF and its receptor c-Kit result in mast cell deficiency [60, 61]. However, numerous cytokines, growth factors, and other factors are known to affect the development, proliferation, survival, and differentiation of mast cells [39]. Although such factors might affect the homeostasis of mast cell generation and turnover, they may be more important in the regulation of mast cell numbers and functions in various immune settings. For instance, mast cell numbers and expression patterns of mMCPs change dynamically during parasite infection [62], in which serum IgE levels become several orders of magnitude higher over homeostatic levels. However, there have been no systematic studies about the effect of IgE on mast cell differentiation. Our present study demonstrates that levels of IgEs, which can be found in parasite infections and atopic mice and humans [23], can promote differentiation of mast cells in IL-3-dependent cultures of mouse BM cells.

Our interpretation of IgE-dependent promotion of mast cell differentiation was based on the accelerated development of

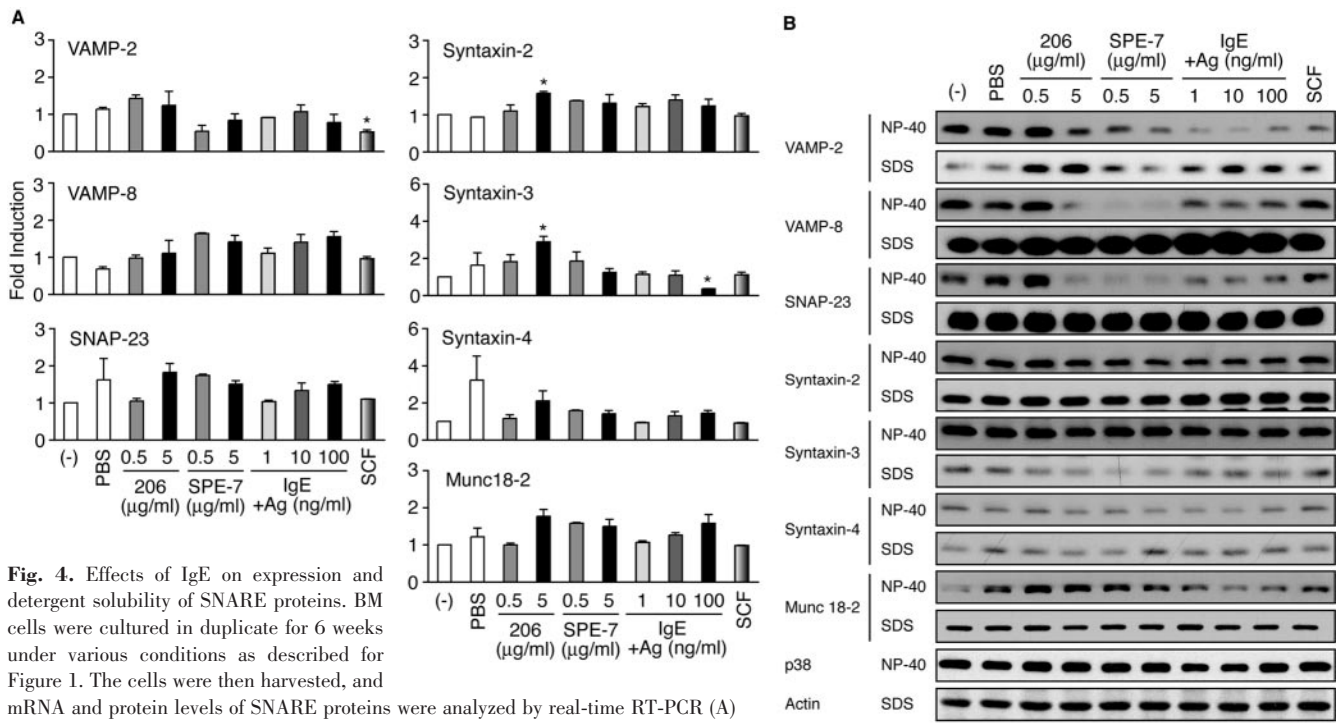


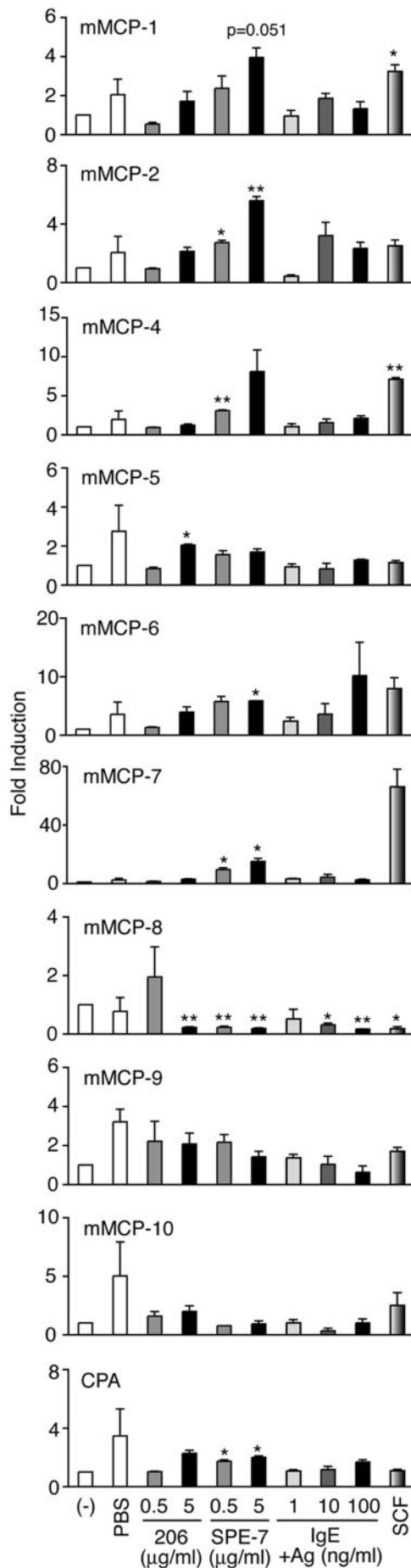
Fig. 4. Effects of IgE on expression and detergent solubility of SNARE proteins. BM cells were cultured in duplicate for 6 weeks under various conditions as described for Figure 1. The cells were then harvested, and mRNA and protein levels of SNARE proteins were analyzed by real-time RT-PCR (A) and immunoblotting (B), respectively. RNA analysis was performed separately for each of duplicate cultures, but protein analysis was performed for the combined samples of duplicate cultures. (B) For immunoblotting analysis, 15 μ g cell lysates were run on 10% SDS-PAGE gels. NP-40 and SDS indicate the detergents used to prepare cell lysates. p38 MAPK and β -actin were probed for loading control of NP-40 and SDS lysates, respectively. Data are presented as mean $\pm$ SEM. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with nontreated control by Student's t -test.

Fc ϵ RI⁺/c-Kit⁺ cells in the presence of IgE, particularly HC IgE, in cultures of BM cells (Fig. 1C) and the increased, absolute numbers of mast cells at the end of 5–6 weeks of culture (Fig. 1A). Similar results were obtained with cultures of MCPs (Fig. 3). Under BM cell cultures, it is not clear whether IgEs directly affect the differentiation of MCPs, as these progenitor cells do not express Fc ϵ RI [7], and the known IgE effects are mediated through the Fc ϵ RI [23]. In contrast, once MCPs commit to the expression of Fc ϵ RI, their fate will be regulated by HC and PC IgEs: Both IgEs can promote cell survival [20–22]; HC IgEs can induce mast cell proliferation as well. Furthermore, the survival and proliferation effects by HC IgEs are mediated by a large amount of IL-3 secreted by mast cells themselves, and autocrine IL-3 suppresses apoptosis by inducing Bcl-x_L and Bcl-2 expression [57]. In the future, it will be necessary to study whether MCPs or earlier progenitors express IgE-binding receptors such as Fc ϵ RII, galectin-3, or Fc γ RIV and if so, whether the engagement of these receptors affects mast cell differentiation at MCP or earlier hematopoietic stages.

This study provides the first morphological evidence of HC IgE-induced degranulation (Fig. 2A). Our data also appear to be in partial agreement with dynamic changes in the membrane fusion machinery responsible for degranulation. SNARE proteins have been categorized based on their locations: Vesicle SNAREs (such as VAMP/synaptobrevin family proteins) and target SNAREs (such as proteins of syntaxin and SNAP-23 families) are present on vesicle and target membranes, respectively [30]. Three cognate SNARE proteins such as SNAP-23/syntaxin/VAMP on opposing membranes assemble upon regu-

lated secretion. Some SNARE proteins are localized in sphingolipid/cholesterol-rich lipid raft domains of the plasma membrane, and the integrity of these domains is important for exocytosis [63]. Ternary complexes composed of SNAP-23/syntaxin-4/VAMP-2 are enriched in lipid rafts in rat RBL-2H3 mast cells, and syntaxin-4 and VAMP-2 levels in lipid rafts are increased after IgE stimulation [31]. Our data indicate that expression of certain SNARE proteins is decreased in NP-40 lysates of mast cells cultured with HC IgE, whereas their protein levels are unchanged in SDS lysates. As lipid rafts are insoluble in nonionic detergents, but soluble in SDS, we interpret that the decrease in SNARE protein levels in NP-40 lysates may be a result of a shift of their address to lipid rafts induced by IgE. Alternatively, HC IgE may also mobilize SNARE proteins to cytoskeleton-bound pools, as for example, SNAP-23 has been described to associate with vimentin and serve as a reservoir of supply [64]. Although the changes in solubility of the SNARE proteins in NP-40 might suggest that they are associated with degranulation in mast cells generated in SPE-7 IgE or 206 IgE plus antigen, the same changes were observed in cells cultured in 5 μ g/ml 206 IgE, in which no degranulation was detected. This result might suggest that even subthreshold stimuli induce relocation of some SNARE proteins.

Expression patterns of mast cell proteases are a useful indicator to distinguish different types of mast cells: MMC versus CTMC in mice and three types of human mast cells, depending on expression of tryptases and chymases (MC_T, MC_T + c, and MC_C). We showed that expression of mMCP-1, -2, -4, -6, and -7 is up-regulated in mast cells cultured with



HC IgE. This result indicates that HC IgE cannot only promote mast cell differentiation but also modulate the cellular phenotype; however, HC IgE does not skew differentiation simply toward a specific type of mast cell. The latter phenotypic change could be a result of the fact that HC IgE induces degranulation and release of various chemical, lipid, and protein mediators. One molecule that could be responsible for those modifications is TGF- β , as TGF- β affects expression of some mMCPs such as mMCP-1 in mast cells [32, 33, 37, 59, 64]. However, our data suggest that TGF- β expression is not affected in mast cells cultured with IgE (Fig. 7). Taken together, we hypothesize that IgE stimulation modifies the expression of mMCPs through direct or indirect mechanisms.

Mouse mast cell development and survival are largely controlled by IL-3 and SCF. TNF- α was also reported to play a role in the process [56]: When BM cells from TNF- α -deficient mice were cultured in the presence of IL-3, the resulting mast cells were drastically decreased, which could be reversed by rTNF- α . IL-3-dependent TNF- α secretion from membrane-activated complex-1⁺ BM cells suppressed apoptosis of developing mast cells. Consistent with the role of TNF- α in IL-3-dependent mast cell survival, our data indicate that TNF- α is strongly expressed in mast cells cultured with HC IgE in the presence of IL-3 but weakly expressed in those with PC IgE. However, SCF does not affect TNF- α expression. This suggests that IgE-induced mast cell differentiation may differ mechanistically from SCF-induced differentiation. Egr-1 is required to produce TNF- α in mast cells [53]. Consistent with this report, we show that Egr-1 mRNA expression is strongly enhanced in mast cells cultured with HC IgE but weakly enhanced in those with PC IgE.

Several phenotypic changes (i.e., degranulation, increased histamine content, shift in location of several SNARE proteins, and expression of several mMCPs and transcription factors) observed in mast cells generated in the presence of HC IgE, compared with the cells generated without IgE, were also seen in those generated in the presence of IgE plus antigen. This could be a result of the strong ability of typical HC IgEs to induce receptor aggregation, just like the canonical Fc ϵ RI-aggregating stimulus IgE plus antigen [22]. Although it is not known under what condition or by what mechanism strongly receptor-aggregating HC IgEs similar to SPE-7 IgE are synthesized *in vivo*, such IgEs will promote more rapid differentiation of mast cells and more strongly affect the phenotype of mast cells than PC IgEs will. Therefore, it will be important to determine what factor(s) constitute HC versus PC IgEs. However, it may be worthy of note that HC and PC properties

Fig. 5. Expression of proteases in mast cells cultured with or without IgE. BM cells were cultured in duplicate for 6 weeks under various conditions as described for Figure 1. The resulting cells were harvested, and total RNAs were extracted. cDNA was synthesized from RNA, and the expression of mouse mast cell proteases was analyzed by real-time RT-PCR. RNA analysis was performed separately for each of duplicate cultures. Results shown are representative of three independent experiments. Data are presented as mean $\pm$ SEM. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with nontreated control by Student's *t*-test.

represent extreme opposite ends of a spectrum in character [23].

In summary, we demonstrate that IgE can induce mast cell differentiation and proliferation in the absence of multivalent

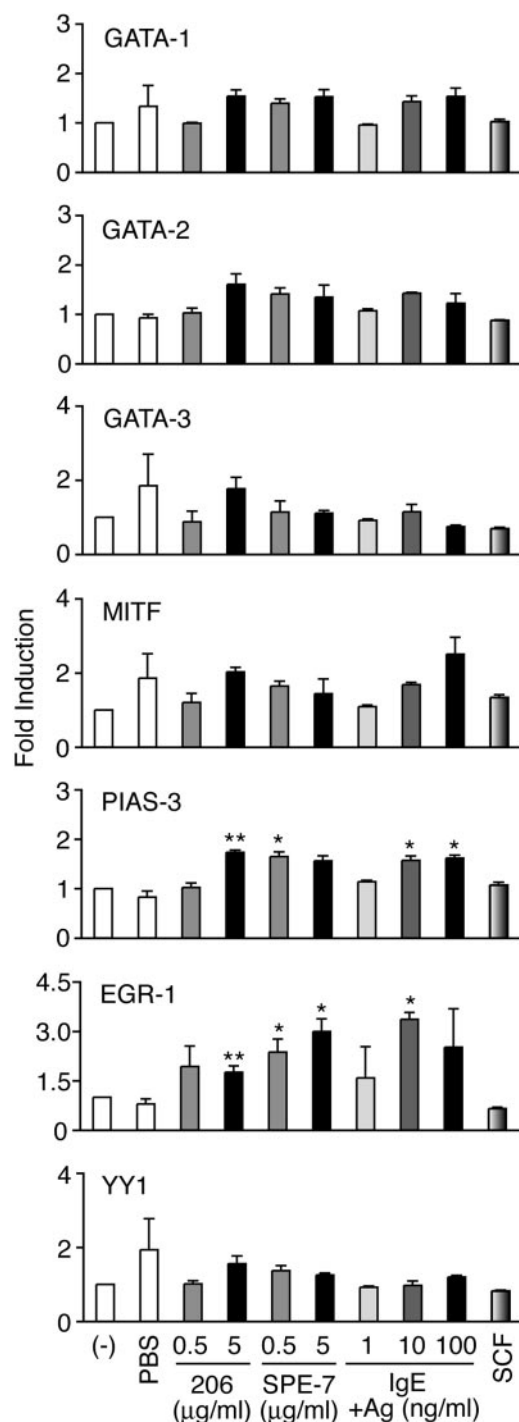


Fig. 6. Expression of transcription factors in mast cells cultured with or without IgE. BM cells were cultured in duplicate for 6 weeks under various conditions as described for Figure 1. Real-time PCR was performed. Shown are levels of GATA family and other transcription factors related to mast cell differentiation. Results shown are representative of three independent experiments. Data are presented as mean $\pm$ SEM. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with nontreated control by Student's t -test.

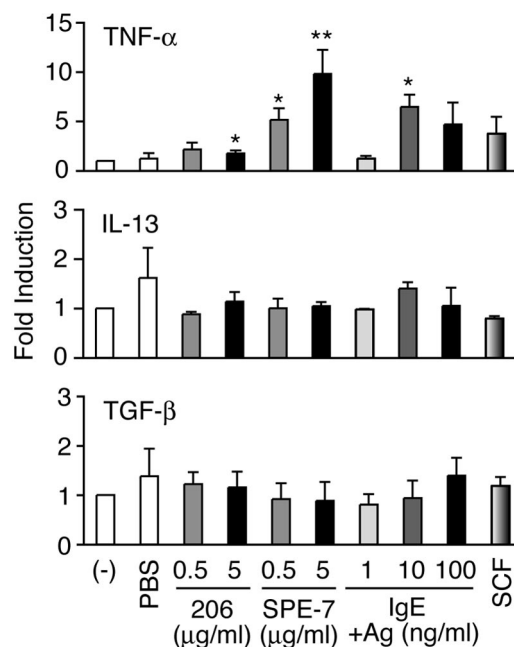


Fig. 7. Increased TNF- α expression in mast cells cultured with SPE-7 IgE. BM cells were cultured in duplicate for 6 weeks under various conditions as described for Figure 1. Real-time RT-PCR analysis was performed. 18S RNA was used as a control. Results are representative of three independent experiments. Data are presented as mean $\pm$ SEM. *, $P < 0.05$, and **, $P < 0.01$, indicate statistically significant differences compared with nontreated control by Student's t -test.

antigen. HC IgE is more effective at inducing mast cell differentiation than PC IgE. HC IgE also induces degranulation, which is associated with the expression and changes in sub-cellular localization of some SNARE proteins and alterations in the expression of certain mMCPs and transcription factors including Egr-1. Consistent with the increased Egr-1 mRNA expression, mast cells cultured with HC IgE show an increased TNF- α expression. Understanding how IgE regulates mast cell differentiation will be useful in the development of new therapeutic strategies to treat allergic asthma, atopic dermatitis, and other allergic diseases.

ACKNOWLEDGMENTS

This study was supported in part by grants from the National Institutes of Health AI50209 and AI38348 (T. K.). This is Publication 932 from the La Jolla Institute for Allergy and Immunology. The authors have no financial conflict of interest. We are grateful to Dr. Michael Poderycki for critical reading of the manuscript.

REFERENCES

- Galli, S. J., Maurer, M., Lantz, C. S. (1999) Mast cells as sentinels of innate immunity. *Curr. Opin. Immunol.* **11**, 53–59.
- Metcalfe, D. D., Baram, D., Mekori, Y. A. (1997) Mast cells. *Physiol. Rev.* **77**, 1033–1079.
- Galli, S. J., Kalesnikoff, J., Grimbaldeston, M. A., Piliponsky, A. M., Williams, C. M., Tsai, M. (2005) Mast cells as “tunable” effector and

- immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* **23**, 749–786.
4. Galli, S. J., Nakae, S., Tsai, M. (2005) Mast cells in the development of adaptive immune responses. *Nat. Immunol.* **6**, 135–142.
5. Kitamura, Y., Ito, A. (2005) Mast cell-committed progenitors. *Proc. Natl. Acad. Sci. USA* **102**, 11129–11130.
6. Arinobu, Y., Iwasaki, H., Gurish, M. F., Mizuno, S., Shigematsu, H., Ozawa, H., Tenen, D. G., Austen, K. F., Akashi, K. (2005) Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl. Acad. Sci. USA* **102**, 18105–18110.
7. Chen, C. C., Grimaldeston, M. A., Tsai, M., Weissman, I. L., Galli, S. J. (2005) Identification of mast cell progenitors in adult mice. *Proc. Natl. Acad. Sci. USA* **102**, 11408–11413.
8. Jamur, M. C., Grodzki, A. C., Berenstein, E. H., Hamawy, M. M., Siraganian, R. P., Oliver, C. (2005) Identification and characterization of undifferentiated mast cells in mouse bone marrow. *Blood* **105**, 4282–4289.
9. Galli, S. J., Zsebo, K. M., Geissler, E. N. (1994) The kit ligand, stem cell factor. *Adv. Immunol.* **55**, 1–96.
10. Copeland, N. G., Gilbert, D. J., Cho, B. C., Donovan, P. J., Jenkins, N. A., Cosman, D., Anderson, D., Lyman, S. D., Williams, D. E. (1990) Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. *Cell* **63**, 175–183.
11. Chabot, B., Stephenson, D. A., Chapman, V. M., Besmer, P., Bernstein, A. (1988) The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature* **335**, 88–89.
12. Barrett, K. E., Metcalfe, D. D. (1984) Mast cell heterogeneity: evidence and implications. *J. Clin. Immunol.* **4**, 253–261.
13. Bienenstock, J., Befus, A. D., Pearce, F., Denburg, J., Goodacre, R. (1982) Mast cell heterogeneity: derivation and function, with emphasis on the intestine. *J. Allergy Clin. Immunol.* **70**, 407–412.
14. McNeil, H. P., Austen, K. F., Somerville, L. L., Gurish, M. F., Stevens, R. L. (1991) Molecular cloning of the mouse mast cell protease-5 gene. A novel secretory granule protease expressed early in the differentiation of serosal mast cells. *J. Biol. Chem.* **266**, 20316–20322.
15. Reynolds, D. S., Gurley, D. S., Austen, K. F., Serafin, W. E. (1991) Cloning of the cDNA and gene of mouse mast cell protease-6. Transcription by progenitor mast cells and mast cells of the connective tissue subclass. *J. Biol. Chem.* **266**, 3847–3853.
16. Reynolds, D. S., Stevens, R. L., Gurley, D. S., Lane, W. S., Austen, K. F., Serafin, W. E. (1989) Isolation and molecular cloning of mast cell carboxypeptidase A. A novel member of the carboxypeptidase gene family. *J. Biol. Chem.* **264**, 20094–20099.
17. Serafin, W. E., Reynolds, D. S., Rogelj, S., Lane, W. S., Conder, G. A., Johnson, S. S., Austen, K. F., Stevens, R. L. (1990) Identification and molecular cloning of a novel mouse mucosal mast cell serine protease. *J. Biol. Chem.* **265**, 423–429.
18. Serafin, W. E., Sullivan, T. P., Conder, G. A., Ebrahimi, A., Marcham, P., Johnson, S. S., Austen, K. F., Reynolds, D. S. (1991) Cloning of the cDNA and gene for mouse mast cell protease 4. Demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral proteases of the mouse and rat. *J. Biol. Chem.* **266**, 1934–1941.
19. Trong, H. L., Newlands, G. F., Miller, H. R., Charbonneau, H., Neurath, H., Woodbury, R. G. (1989) Amino acid sequence of a mouse mucosal mast cell protease. *Biochemistry* **28**, 391–395.
20. Asai, K., Kitaura, J., Kawakami, Y., Yamagata, N., Tsai, M., Carbone, D. P., Liu, F. T., Galli, S. J., Kawakami, T. (2001) Regulation of mast cell survival by IgE. *Immunity* **14**, 791–800.
21. Kalesnikoff, J., Huber, M., Lam, V., Damen, J. E., Zhang, J., Siraganian, R. P., Krystal, G. (2001) Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* **14**, 801–811.
22. Kitaura, J., Song, J., Tsai, M., Asai, K., Maeda-Yamamoto, M., Mocsai, A., Kawakami, Y., Liu, F. T., Lowell, C. A., Barisas, B. G., Galli, S. J., Kawakami, T. (2003) Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the FcεRI. *Proc. Natl. Acad. Sci. USA* **100**, 12911–12916.
23. Kawakami, T., Kitaura, J. (2005) Mast cell survival and activation by IgE in the absence of antigen: a consideration of the biologic mechanisms and relevance. *J. Immunol.* **175**, 4167–4173.
24. Martin-Verdeaux, S., Pombo, I., Iannascoli, B., Roa, M., Varin-Blank, N., Rivera, J., Blank, U. (2003) Evidence of a role for Munc18–2 and microtubules in mast cell granule exocytosis. *J. Cell Sci.* **116**, 325–334.
25. Paumet, F., Le Mao, J., Martin, S., Galli, T., David, B., Blank, U., Roa, M. (2000) Soluble NSF attachment protein receptors (SNAREs) in RBL-2H3 mast cells: functional role of syntaxin 4 in exocytosis and identification of a vesicle-associated membrane protein 8-containing secretory compartment. *J. Immunol.* **164**, 5850–5857.
26. Wilson, B. S., Steinberg, S. L., Liederman, K., Pfeiffer, J. R., Surviladze, Z., Zhang, J., Samelson, L. E., Yang, L. H., Kotula, P. G., Oliver, J. M. (2004) Markers for detergent-resistant lipid rafts occupy distinct and dynamic domains in native membranes. *Mol. Biol. Cell* **15**, 2580–2592.
27. Borkowski, T. A., Jouvin, M. H., Lin, S. Y., Kinet, J. P. (2001) Minimal requirements for IgE-mediated regulation of surface FcεRI. *J. Immunol.* **167**, 1290–1296.
28. Kubo, S., Matsuoka, K., Taya, C., Kitamura, F., Takai, T., Yonekawa, H., Karasuyama, H. (2001) Drastic up-regulation of FcεRI on mast cells is induced by IgE binding through stabilization and accumulation of FcεRI on the cell surface. *J. Immunol.* **167**, 3427–3434.
29. Kitaura, J., Xiao, W., Maeda-Yamamoto, M., Kawakami, Y., Lowell, C. A., Kawakami, T. (2004) Early divergence of Fcε receptor I signals for receptor up-regulation and internalization from degranulation, cytokine production, and survival. *J. Immunol.* **173**, 4317–4323.
30. Blank, U., Cyprien, B., Martin-Verdeaux, S., Paumet, F., Pombo, I., Rivera, J., Roa, M., Varin-Blank, N. (2002) SNAREs and associated regulators in the control of exocytosis in the RBL-2H3 mast cell line. *Mol. Immunol.* **38**, 1341–1345.
31. Puri, N., Roche, P. A. (2006) Ternary SNARE complexes are enriched in lipid rafts during mast cell exocytosis. *Traffic* **7**, 1482–1494.
32. Funaba, M., Ikeda, T., Murakami, M., Ogawa, K., Abe, M. (2005) Up-regulation of mouse mast cell protease-6 gene by transforming growth factor-β and activin in mast cell progenitors. *Cell. Signal.* **17**, 121–128.
33. Funaba, M., Ikeda, T., Murakami, M., Ogawa, K., Tsuchida, K., Sugino, H., Abe, M. (2003) Transcriptional activation of mouse mast cell Protease-7 by activin and transforming growth factor-β is inhibited by microphthalmia-associated transcription factor. *J. Biol. Chem.* **278**, 52032–52041.
34. Ghildyal, N., McNeil, H. P., Gurish, M. F., Austen, K. F., Stevens, R. L. (1992) Transcriptional regulation of the mucosal mast cell-specific protease gene, MMCP-2, by interleukin 10 and interleukin 3. *J. Biol. Chem.* **267**, 8473–8477.
35. Ghildyal, N., McNeil, H. P., Stechschulte, S., Austen, K. F., Silberstein, D., Gurish, M. F., Somerville, L. L., Stevens, R. L. (1992) IL-10 induces transcription of the gene for mouse mast cell protease-1, a serine protease preferentially expressed in mucosal mast cells of *Trichinella spiralis*-infected mice. *J. Immunol.* **149**, 2123–2129.
36. Gurish, M. F., Ghildyal, N., McNeil, H. P., Austen, K. F., Gillis, S., Stevens, R. L. (1992) Differential expression of secretory granule proteases in mouse mast cells exposed to interleukin 3 and c-kit ligand. *J. Exp. Med.* **175**, 1003–1012.
37. Miller, H. R., Wright, S. H., Knight, P. A., Thornton, E. M. (1999) A novel function for transforming growth factor-β1: upregulation of the expression and the IgE-independent extracellular release of a mucosal mast cell granule-specific β-chymase, mouse mast cell protease-1. *Blood* **93**, 3473–3486.
38. Miller, H. R., Pemberton, A. D. (2002) Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut. *Immunology* **105**, 375–390.
39. Okayama, Y., Kawakami, T. (2006) Development, migration, and survival of mast cells. *Immunol. Res.* **34**, 97–115.
40. Harigae, H., Takahashi, S., Suwabe, N., Ohtsu, H., Gu, L., Yang, Z., Tsai, F. Y., Kitamura, Y., Engel, J. D., Yamamoto, M. (1998) Differential roles of GATA-1 and GATA-2 in growth and differentiation of mast cells. *Genes Cells* **3**, 39–50.
41. Migliaccio, A. R., Rana, R. A., Sanchez, M., Lorenzini, R., Centurione, L., Bianchi, L., Vannucchi, A. M., Migliaccio, G., Orkin, S. H. (2003) GATA-1 as a regulator of mast cell differentiation revealed by the phenotype of the GATA-1low mouse mutant. *J. Exp. Med.* **197**, 281–296.
42. Tsai, F. Y., Orkin, S. H. (1997) Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood* **89**, 3636–3643.
43. Zon, L. I., Gurish, M. F., Stevens, R. L., Mather, C., Reynolds, D. S., Austen, K. F., Orkin, S. H. (1991) GATA-binding transcription factors in mast cells regulate the promoter of the mast cell carboxypeptidase A gene. *J. Biol. Chem.* **266**, 22948–22953.
44. Shelburne, C. P., McCoy, M. E., Piekorz, R., Sexl, V., Roh, K. H., Jacobs-Helber, S. M., Gillespie, S. R., Bailey, D. P., Mirmansh, P., Mann, M. N., Kashyap, M., Wright, H. V., Chong, H. J., Bouton, L. A., Barnstein, B., Ramirez, C. D., Bunting, K. D., Sawyer, S., Lantz, C. S., Ryan, J. J. (2003) Stat5 expression is critical for mast cell development and survival. *Blood* **102**, 1290–1297.
45. Moore, K. J. (1995) Insight into the microphthalmia gene. *Trends Genet.* **11**, 442–448.
46. Isizaki, K., Tsujimura, T., Nomura, S., Morii, E., Koshimizu, U., Nishimune, Y., Kitamura, Y. (1994) Cell type-specific deficiency of c-kit gene

- expression in mutant mice of mi/mi genotype. *Am. J. Pathol.* **145**, 827–836.
47. Morii, E., Jippo, T., Tsujimura, T., Hashimoto, K., Kim, D. K., Lee, Y. M., Ogiwara, H., Tsujino, K., Kim, H. M., Kitamura, Y. (1997) Abnormal expression of mouse mast cell protease 5 gene in cultured mast cells derived from mutant mi/mi mice. *Blood* **90**, 3057–3066.
 48. Morii, E., Tsujimura, T., Jippo, T., Hashimoto, K., Takebayashi, K., Tsujino, K., Nomura, S., Yamamoto, M., Kitamura, Y. (1996) Regulation of mouse mast cell protease 6 gene expression by transcription factor encoded by the mi locus. *Blood* **88**, 2488–2494.
 49. Levy, C., Lee, Y. N., Nechushtan, H., Schueler-Furman, O., Sonnenblick, A., Hacohen, S., Razin, E. (2006) Identifying a common molecular mechanism for inhibition of MITF and STAT3 by PIAS3. *Blood* **107**, 2839–2845.
 50. Levy, C., Sonnenblick, A., Razin, E. (2003) Role played by microphthalmia transcription factor phosphorylation and its Zip domain in its transcriptional inhibition by PIAS3. *Mol. Cell. Biol.* **23**, 9073–9080.
 51. Sonnenblick, A., Levy, C., Razin, E. (2004) Interplay between MITF, PIAS3, and STAT3 in mast cells and melanocytes. *Mol. Cell. Biol.* **24**, 10584–10592.
 52. Silverman, E. S., De Sanctis, G. T., Boyce, J., Maclean, J. A., Jiao, A., Green, F. H., Grasmann, H., Faunce, D., Fitzmaurice, G., Shi, G. P., Stein-Streilein, J., Milbrandt, J., Collins, T., Drazen, J. M. (2001) The transcription factor early growth-response factor 1 modulates tumor necrosis factor- α , immunoglobulin E, and airway responsiveness in mice. *Am. J. Respir. Crit. Care Med.* **163**, 778–785.
 53. Li, B., Power, M. R., Lin, T. J. (2006) De novo synthesis of early growth response factor-1 is required for the full responsiveness of mast cells to produce TNF and IL-13 by IgE and antigen stimulation. *Blood* **107**, 2814–2820.
 54. 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.
 55. 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.
 56. Wright, H. V., Bailey, D., Kashyap, M., Kepley, C. L., Drutskaya, M. S., Nedospasov, S. A., Ryan, J. J. (2006) IL-3-mediated TNF production is necessary for mast cell development. *J. Immunol.* **176**, 2114–2121.
 57. Kohno, M., Yamasaki, S., Tybulewicz, V. L., Saito, T. (2005) Rapid and large amount of autocrine IL-3 production is responsible for mast cell survival by IgE in the absence of antigen. *Blood* **105**, 2059–2065.
 58. Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T. Y., Karp, C. L., Donaldson, D. D. (1998) Interleukin-13: central mediator of allergic asthma. *Science* **282**, 2258–2261.
 59. Wright, S. H., Brown, J., Knight, P. A., Thornton, E. M., Kilshaw, P. J., Miller, H. R. (2002) Transforming growth factor- β 1 mediates coexpression of the integrin subunit α E and the chymase mouse mast cell protease-1 during the early differentiation of bone marrow-derived mucosal mast cell homologues. *Clin. Exp. Allergy* **32**, 315–324.
 60. Kitamura, Y., Go, S. (1979) Decreased production of mast cells in S1/S1d anemic mice. *Blood* **53**, 492–497.
 61. Kitamura, Y., Go, S., Hatanaka, K. (1978) Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood* **52**, 447–452.
 62. Friend, D. S., Ghildyal, N., Austen, K. F., Gurish, M. F., Matsumoto, R., Stevens, R. L. (1996) Mast cells that reside at different locations in the jejunum of mice infected with *Trichinella spiralis* exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J. Cell Biol.* **135**, 279–290.
 63. Salaun, C., James, D. J., Chamberlain, L. H. (2004) Lipid rafts and the regulation of exocytosis. *Traffic* **5**, 255–264.
 64. Faigle, W., Colucci-Guyon, E., Louvard, D., Amigorena, S., Galli, T. (2000) Vimentin filaments in fibroblasts are a reservoir for SNAP23, a component of the membrane fusion machinery. *Mol. Biol. Cell* **11**, 3485–3494.