Mast cells are critical effector cells mediating immunoglobulin E (IgE)–dependent allergic responses. Binding of an allergen to IgE, already bound to its high-affinity receptor Fc\(\varepsilon\)RI on mast cells, leads to aggregation and subsequent activation. This initiates signalling events that typically result in degranulation, changes in gene expression, and the release of inflammatory mediators, contributing to acute and late-phase allergic responses.\(^1\text{-}^3\) Fc\(\varepsilon\)RI consists of a tetrameric protein complex, the IgE-binding amplifying \(\alpha\) chain, a signalling \(\beta\) chain, and two \(\gamma\) chains.\(^4\) The \(\beta\) and \(\gamma\) subunits of the Fc\(\varepsilon\)RI each contain an immunoreceptor tyrosine-based activation motif (ITAM), which is phosphorylated upon Fc\(\varepsilon\)RI aggregation and which is both necessary and sufficient for receptor-induced signal transduction.\(^5\)

Mast cells also express other Fc receptors, either constitutively or upon stimulation; among these, Fc\(\gamma\)RI (CD64), Fc\(\gamma\)RIIB (CD32), and Fc\(\gamma\)RIII (CD16) are receptors for immunoglobulin G (IgG). Fc\(\gamma\)RI (high-affinity IgG receptor) and Fc\(\gamma\)RIII (low-affinity IgG receptor) are activating receptors, both containing ITAM, that initiate signalling upon aggregation.\(^6\text{-}^7\) Fc\(\gamma\)RIIB is a low-affinity receptor containing an immunoreceptor tyrosine-based inhibitory motif (ITIM),\(^8\) which negatively regulates the activating signal when coaggregated with activating receptors bearing an ITAM.\(^8\) The coaggregation results in the
recruitment of the inhibitory signalling molecule SHIP, leading to the abrogation of the ITAM-induced activation.\textsuperscript{2,10,11}

IgE-induced mast cell activation (ie, FcεRI aggregation) is negatively regulated by coaggregation of FcεRI with FcγRIIB.\textsuperscript{9,12} The release of mediators and cytokines is inhibited in a process in which FcεRI contributes to the ITIM-dependent inhibition of its own intracellular signalling. This is achieved by the FcεRI-associated tyrosine kinase Lyn, which phosphorylates the FcγRIIB ITIM that recruits SHIP1, thus leading to FcεRI signal abrogation.\textsuperscript{11,13,14} The receptors interact with the F-actin skeleton that enables FcγRIIB to recruit SHIP1, which is provided by filamin-1. FcγRIIB is believed to negatively regulate FcεRI signalling in two ways: by facilitating the translocation of FcεRI into the F-actin skeleton but also by concentrating SHIP1 at the site close to FcεRI.\textsuperscript{15} Investigations of the mechanism by which SHIP mediates its FcγRIIB inhibitory function have also suggested p62 dok as a possible mediator of FcγRIIB inhibition of FcεRI signalling downstream of SHIP in mast cells.\textsuperscript{16}

FcεRI-mediated degranulation and release of mediators are inhibited when FcεRI is coaggregated with FcγRIIB.\textsuperscript{12} In addition to elucidating the impact of coaggregation on mast-cell degranulation, this study has elucidated the effect on the activation of downstream signalling pathways involved in the regulation of mast-cell survival. The aggregation of FcεRI induces rapid but transient phosphorylation of the signalling protein Akt and the forkhead transcription factor Foxo3a, known to regulate Bim expression at the transcriptional level.\textsuperscript{17} Phosphorylated Akt phosphorylates and thereby inactivates Foxo3a, which in its unphosphorylated state is located in the nucleus and acts as a transcription factor for Bim. Bim is a proapoptotic protein of the Bcl-2 family, involved in the regulation of mast-cell apoptosis.\textsuperscript{18,19} Another Bcl-2 family member of crucial importance for FcεRI-mediated activation-induced mast-cell survival is A1.\textsuperscript{20} Mast cells lacking A1 do not survive IgE receptor aggregation.\textsuperscript{20}

In this study, we investigated if FcεRI-mediated activation/expression of Akt, Foxo3a, Bim, and A1 are inhibited when FcεRI is coengaged with FcγRIIB. We report here that although mast-cell degranulation is inhibited and the phosphorylation of Akt is attenuated by the coaggregation of FcεRI with FcγRIIB, Foxo3a and Bim are still phosphorylated and up-regulated, respectively. We also demonstrate that the level of A1 messenger ribonucleic acid (mRNA) induced by FcεRI is not significantly altered upon coaggregation with FcγRIIB. Altogether, this indicates that only certain signalling pathways are affected by the coaggregation of FcεRI with FcγRIIB whereas others, closely related to cell survival, remain largely unaffected.

Materials and Methods

Mast-Cell Cultures

The murine mast cell line C57\textsuperscript{21} (kindly provided by Dr. S.J. Galli, Stanford University, Stanford, CA) was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM of L-glutamine, 100 µg/mL of penicillin/streptomycin, and 50 µM of 2-mercaptoethanol. All culture reagents were obtained from Sigma Chemical Co. (St. Louis, MO). The C57 mast cell line has previously been characterized for FcγRII/FcγRIII expression.\textsuperscript{22}

Antibodies and Reagents

AffiniPure rabbit anti-mouse IgG (RAM IgG), AffiniPure RAM IgG F(ab’\textsuperscript{2}) fragment (RAM F(ab’\textsuperscript{2})), and AffiniPure mouse anti-rat IgG (H+L) F(ab’\textsuperscript{2}) fragment (MAR F(ab’\textsuperscript{2})) were all purchased from Jackson ImmunoResearch Laboratories, Inc., Baltimore, MD. Purified RAM CD16/CD32 (FcγRIII/II receptor) monoclonal antibody (2.4G2 rat Ab) was obtained from BD Biosciences, Heidelberg, Germany. Anti-rabbit IgE horseradish peroxidase–linked donkey anti-rabbit antibody was obtained from Amersham Biosciences, Uppsala, Sweden. LumiGLO reagent and peroxide, 10× cell lysis buffer, antibodies directed against phospho-Akt (serine [Ser] 473 and threonine [Thr] 308), and Akt were purchased from Cell Signaling Technology, Beverly, MA. Antibodies directed against phospho-Foxo3a (Thr 32 and Ser 253) and Foxo3a were
obtained from Upstate Biotechnology, Lake Placid, NY. Anti-Bim antibody was purchased from Affinity Bioreagents, Inc., Golden, CO. 4× NuPAGE LDS Sample Buffer and 10× NuPAGE Sample Reducing Agent were obtained from Invitrogen, Carlsbad, CA; TriPure Isolation Reagent was purchased from Boehringer Mannheim, Mannheim, Germany; and Tween 20 was obtained from Merck Schuchardt, Hohenbrunn, Germany. All other reagents were purchased from Sigma Chemical Co.

Antibody Conjugation

MAR F(\(\text{ab}'\))\(_2\) was trinitrophenylated by incubation for 2 hours at room temperature with picrylsulfonic acid (2,4,6-trinitrobenzene sulfonic acid in borate-buffered saline, pH 8.0). The TNP\(_\gamma\)F(\(\text{ab}'\))\(_2\) MAR obtained was purified on a prepacked disposable PD-10 column containing Sephadex G-25 medium (Amersham Biosciences).

Mast-Cell Activation

Mast cells to be used for ribonuclease (RNAse) protection assay and \(\alpha\)-hexosaminidase release assay were resuspended in RPMI-1640 medium supplemented with 0.2% bovine serum albumin, 2 mM of L-glutamine, and 100 µg/mL of penicillin/streptomycin. The cells were sensitized for 90 minutes at 37°C by the addition of 0.1 µg/mL of monoclonal anti-dinitrophenyl (anti-DNP) clone SPE-7 IgE mouse antibody (anti-DNP IgE). After washing, the cells were activated by the addition of either 45 µg/mL of RAM IgG (coaggregation of Fc\(\varepsilon\)RI with Fc\(\gamma\)RIIB) or 30 µg/mL of RAM F(\(\text{ab}'\))\(_2\) (aggregation of Fc\(\varepsilon\)RI) at 37°C for the time periods indicated. Mast cells to be used for Western blot analysis were resuspended in the previously mentioned medium. The cells were sensitized for 90 minutes at 37°C by the addition of 0.1 µg/mL of the same IgE as previously mentioned or 0.1 µg/mL of the same IgE together with 5 µg/mL of 2.4G2 rat Ab. After being washed, the cells were activated by the addition of 10 µg/mL of TNP7-F(\(\text{ab}'\))\(_2\) mouse anti-rabbit (MAR) at 37°C, causing either coaggregation of Fc\(\gamma\)RIIB with Fc\(\varepsilon\)RI or aggregation of Fc\(\varepsilon\)RI, for the time periods indicated. The conjugated antibody, TNP7-MAR F(ab')\(_2\), functions as a multivalent antigen recognized by the Fc\(\varepsilon\)RI-bound IgE but also recognizing bound 2.4G2 rat Ab.\(^{13}\) Aggregation with 2.4G2 rat Ab together with TNP7-MAR F(ab')\(_2\) does not cause degranulation, which indicates that expression of Fc\(\gamma\)RII (an activating low-affinity receptor for IgG) on C57 cells does not interfere with our system (data not shown). In experiments in which the phosphorylation pattern of Akt and Foxo3a as well as the total amount of these two proteins were measured, the mast cells were starved for approximately 24 hours at 37°C in RPMI-1640 medium supplemented with 0.5% FBS before sensitization and activation. For Bim expression experiments, the mast cells were resuspended in RPMI-1640 medium supplemented with 10% filtered FBS, 2 mM of L-glutamine, 100 µg/mL of penicillin/streptomycin, and 50 µM of 2-mercaptoethanol during both sensitization and activation, which lasted for 24 hours.

N-Acetyl-\(\beta\)-D-Hexosaminidase Release Assay

For detection of the granular enzyme \(\beta\)-hexosaminidase, an enzymatic colorimetric assay was used.\(^{23}\) After 30 minutes of activation, 60 µL of supernatant were transferred to a 96-well plate and mixed with an equal volume of substrate solution (7.5 mM of p-nitrophenyl-N-acetyl-\(\beta\)-D-glucosaminide dissolved in 80 mM of citric acid, pH 4.5). The mixture was incubated on a rocker platform for 2 hours at 37°C. After incubation, 120 µL of glycine (0.2 M, pH 10.7) was added to each well, and the absorbance was measured with an Emax Precision Microplate Reader (Molecular Devices, Sunnyvale, CA).

Western Blot Analysis

The cells were lysed in SDS sample buffer (125 mM of tris-hydrochloric acid [pH 6.8], 4% w/v SDS, 20% glycerol, 0.02% w/v bromphenol blue, and 50 mM of dithiothreitol, added just before use) or in cell lysis buffer (1× cell lysis buffer, 1 mM of phenylmethylsulfonyl fluoride [PMSF]) before
being sonicated on ice. The phosphorylation and/or the total amount of proteins of interest were studied by Western blot with a NuPAGE Bis-Tris Western gel (Invitrogen, Carlsbad, CA). After electrophoresis, the proteins were electrically transferred to a nitrocellulose membrane (Hybond ECL, Amersham Biosciences). All was performed according to the manufacturers’ instructions. The membrane was incubated overnight at 4°C with the primary antibody and thereafter incubated with horseradish peroxidase–conjugated secondary antibody for 1 hour at room temperature. The proteins were visualized with an enhanced chemiluminescence system (LumiGLO) and exposure to a Hybond ECL film.

**RNAse Protection Assay**

TriPure isolation reagent was used for isolation of total cellular ribonucleic acid (RNA). An RNAse protection assay (RPA) was performed (according to RiboQuant System protocol) with an mAPO-2 multiprobe set (PharMingen, San Diego, CA). The gel was dried and exposed on Kodak film (Eastman Kodak Company, Rochester, NY) with intensifying screens at –70°C. Expression of RNA was detected with a phosphoimager device, and levels of expression were quantified with MacBAS 2.2 software (Fuji Photo Film Co., Ltd., Tokyo, Japan).

**Statistical Analysis**

We used an analysis of variance, followed by multiple comparison with the Wilcoxon matched-pairs test.

**Results**

**Coaggregation of FceRI with FcγRIIB Inhibits IgE-Dependent Mast-Cell Degranulation**

To analyze the effect of FcγRIIB-mediated inhibition of mast-cell activation, we used murine C57 mast cells known to express the receptors FceRI and FcγRIIB. C57 cells were sensitized with murine IgE and challenged with polyclonal RAM F(ab’)_2 to aggregate FceRI or with RAM IgG to coaggregate FceRI and FcγRIIB. The RAM F(ab’)_2 induced activation of mast cells, leading to degranulation as measured by β-hexosaminidase release (Figure 1A). When FceRI was coaggregated with FcγRIIB by the addition of RAM IgG, the release of β-hexosaminidase was inhibited (see Figure 1A).

In addition to the activation system whereby RAM IgG or RAM F(ab’)_2 was added, we also used another system for Western blot analysis, one by which each receptor can be aggregated separately or coaggregated. C57 cells were sensitized with murine anti-DNP IgE and incubated with or without 2.4G2 rat Ab before challenge with TNP-MAR F(ab’)_2, TNP-conjugated F(ab’)_2 fragments of mouse anti-rat IgG. The conjugated antibody, TNP7-MAR F(ab’)_2, functions as a multivalent antigen recognized by the FceRI-bound IgE but also recognizing FcγRII-bound 2.4G2 rat Ab.13 The addition of TNP7-MAR F(ab’)_2 will aggregate FceRI in cells sensitized with IgE, aggregate FcγRIIB in cells sensitized with 2.4G2 rat Ab, and (as a consequence) coaggregate FceRI and FcγRIIB in cells sensitized with both IgE and 2.4G2 rat Ab. Since aggregation using 2.4G2 rat Ab together with TNP7-MAR F(ab’)_2 does not cause degranulation, this indicates that expression of FcγRIII (an activating low-affinity receptor for IgG) on C57 cells does not interfere with our system (data not shown). Although not as sufficient as the other system for causing degranulation, this system induced the activation of mast cells, causing degranulation, and showed inhibition upon coaggregation of FceRI with FcγRIIB (see Figure 1B).

**Phosphorylation of Akt Is Attenuated by Coaggregation of FceRI with FcγRIIB**

To assess the effects of coaggregating FceRI with FcγRIIB on signals transduced downstream of FceRI, the phosphorylation pattern of Akt protein was investigated. Akt is a signal-transducing protein downstream of PI3-kinase, involved in a variety of cellular functions such as survival and
metabolism.\textsuperscript{24,25} Via 3-phosphoinositide–dependent protein kinases, the PI3-kinase can activate Akt by phosphorylation at three different sites, two of which—threonine 308 (Thr 308) and serine 473 (Ser 473)—were investigated in this report. We compared the pattern of Akt phosphorylation at the Thr 308 and Ser 473 sites in cell lysates after FcεRI aggregation or coaggregation of FcεRI with FcγRIIB. FcεRI aggregation induced rapid phosphorylation of Akt at Thr 308; the maximum phosphorylation stage was reached within 1 minute, and phosphorylation decreased at 5 minutes. The phosphorylation of Akt after FcεRI aggregation at Ser 473 was achieved within 1 minute and remained at a comparable level for 10 minutes before decreasing at 30 minutes (Figure 2). Considerable reductions of Akt phosphorylation at Thr 308 and Ser 473 were observed as early as 1 minute after coaggregation of FcεRI with FcγRIIB (see Figure 2).

**Coaggregation of FcεRI with FcγRIIB Does Not Affect the Phosphorylation of Transcription Factor Foxo3a**

Phosphorylated Akt phosphorylates and thereby inactivates the forkhead protein Foxo3a.\textsuperscript{26} The phosphorylation of Foxo3a prevents its translocation into the nucleus, where it acts as a transcription factor for certain genes. We investigated the phosphorylation of Foxo3a at sites Ser 253 and Thr 32. Phosphorylation of Foxo3a at Ser 253 occurred within 1 minute but reached background phosphorylation level again after 30 minutes (Figure 3). However, after rapid phosphorylation at site Thr 32 within 1 minute after FcεRI aggregation, phosphorylation remained constant until 30 minutes had elapsed (see Figure 3). In contrast to the effect on Akt phosphorylation, coengagement of FcεRI with FcγRIIB did not affect either the levels of phosphorylation or the duration of the FcεRI-induced Foxo3a phosphorylation (see Figure 3).
FcεRI-Induced Expression of Bim Is Not Inhibited by Coaggregation with FcγRIIB

A key regulator of apoptosis is the Bcl-2 family of proteins, which consists of proapoptotic and antiapoptotic proteins. Bim, one of the proapoptotic members, is transcriptionally regulated by Foxo3a, and we recently showed that Bim is involved in the regulation of mast-cell apoptosis. Furthermore, Bim is induced upon aggregation of FcεRI. Therefore, we next investigated if coaggregation of FcεRI with FcγRIIB would have an effect on Bim expression. After FcεRI aggregation and coaggregation of FcεRI and FcγRIIB, respectively, the two isoforms of Bim (BimEL and BimL) were up-regulated to similar levels (Figure 4). BimEL consisted of two bands, owing to a shift in band motility; this shift of the BimEL band is probably the result of phosphorylation. The results herein demonstrate that Bim induced by FcεRI aggregation is not affected by coaggregation with FcγRIIB (see Figure 4).

Coaggregation of FcεRI with FcγRIIB Does Not Affect the Induction of A1

Apoptosis is regulated by members of the Bcl-2 family. A1, one of the antiapoptotic Bcl-2 family members, is described as being important for the survival of mast cells during allergic reactions. To determine whether the coaggregation of FcεRI with FcγRIIB affects the induced transcriptional regu-
translation of A1, an RPA was performed. A1 was absent in cells incubated only with IgE but was substantially up-regulated after FcγRI aggregation, as well as in cells where FcγRI had been coaggregated with FcγRII for 6 hours (Figure 5). The A1 mRNA level in cells activated by FcγRI aggregation had increased 12-fold, and coaggregation of FcγRII with FcγRI led to a ninefold increase when the signal was compared to control cells incubated with IgE alone (see Figure 5). Thus, although A1 up-regulation is slightly reduced after the coaggregation of FcγRI with FcγRII when compared to FcγRI aggregation, the induction of A1 in cells after either coaggregation of FcγRI with FcγRII or FcγRI aggregation (as compared to resting cells) was consistent in several experiments.

**Discussion**

Although coaggregation of FcγRI with FcγRII is known to inhibit mast-cell degranulation, the effect of coaggregation on other signaling pathways in mast cells has not been investigated previously. In this study, we found that even though coaggregation of FcγRI with FcγRII inhibits degranulation and decreases the phosphorylation of Akt, we observed no effect on Foxo3a phosphorylation or Bim expression (see Figures 2, 3, and 4). Results from RPAs showed that the mRNA of A1 (an antiapoptotic Bcl-2 family member) was up-regulated both when mast cells were activated through FcγRI aggregation and when they were activated through coaggregation of FcγRI with FcγRII (see Figure 5). Thus, FcγRII inhibits some but not all signaling pathways downstream of FcγRI.

One pathway affected by FcγRI aggregation is the PI3-K pathway, where PI3-K is phosphorylated and thereby activated. Activated PI3-K can, via 3-phosphoinositide-dependent protein kinases or specific lipid products, phosphorylate the protein Akt. Phosphorylation of Ser 473 and/or Thr 308 enables Akt to carry out its multifunctional activities, which are involved in a variety of cellular functions such as survival and metabolism. Akt became rapidly phosphorylated at the two sites that were investigated after FcγRI aggregation. The phosphorylation at Thr 308 was clearly diminished already after 5 minutes whereas the phosphorylation of Ser 473 remained for at least 20 minutes. This difference in phosphorylation between the two sites might reflect a strict regulation of phosphorylation of Akt.
After coaggregation of FcεRI with FcγRIIB, the phosphorylation of Akt was attenuated when compared to FcεRI aggregation. Akt is more heavily phosphorylated after FcεRI aggregation, but the duration of the phosphorylation does not change after coaggregation of FcεRI with FcγRIIB. These data are in line with data from earlier studies showing that coaggregation of FcγRIIB and the B-cell receptor (as well as coaggregation with the receptor for stem-cell factor Kit, present on mast cells) affects the PI3-K pathway and thereby inhibits the activation of Akt.\textsuperscript{33,34}

Members of the transcription factor forkhead family, such as Foxo3a, can be inactivated through phosphorylation by activated Akt.\textsuperscript{26} We found that FcεRI aggregation and FcεRI coaggregation with FcγRIIIA result in the same phosphorylation pattern of Foxo3a. This is an interesting observation because one might expect the phosphorylation of Foxo3a to decrease in response to less phosphorylated Akt being available. A possible explanation is that because the phosphorylation of Akt is not totally abrogated, there might still be enough to phosphorylate Foxo3a to the same extent. Another interesting feature is that phosphorylated Foxo3a is present in cells that are not activated by either FcεRI aggregation or coaggregation of FcγRIIB with FcεRI. This suggests a natural equilibrium between phosphorylated and unphosphorylated Foxo3a in the cells, which is shifted toward phosphorylation upon activation. Akt is a major effector protein, and although the phosphorylation of Foxo3a by Akt does not seem to be affected, a pathway (or pathways) other than the one investigated might be where the inhibition of Akt phosphorylation plays a more crucial role.

A protein known to be under the transcriptional control of the forkhead transcription factor Foxo3a is Bim.\textsuperscript{27} We previously found Bim to be strongly increased upon FcεRI aggregation.\textsuperscript{18} After coaggregation of FcγRIIB with FcεRI or after FcεRI aggregation, the two isoforms of Bim (BimEL and BimL) were up-regulated in comparison to unactivated control cells. The results demonstrate that FcεRI-induced Bim up-regulation is not affected upon coaggregation with FcγRIIB. Bim\textsubscript{EL} consisted of two bands, probably due to phosphorylation. We have previously seen that stem-cell factor (SCF) promotes the survival of mast cells through inactivation of Foxo3a, preventing the up-regulation of Bim and leading to increased phosphorylation of Bim. Those results show that inhibition of Foxo3a and (consequently) Bim provides an important mechanism by which SCF acts to prevent apoptosis in mast cells.\textsuperscript{19}

Antiapoptotic members of the Bcl-2 family are needed for cell survival. One of the murine pro-survival Bcl-2 family members is A1, which plays a prominent role in preventing apoptosis in a variety of cell systems.\textsuperscript{35,36} Previously, we demonstrated that mRNA levels for A1 are increased after FcεRI aggregation and that A1 is critical for the activation-induced survival of mast cells.\textsuperscript{20} Similarly, the human homologue bfl-1 is up-regulated in human mast cells upon FcεRI aggregation.\textsuperscript{37} We examined the mRNA induction of the antiapoptotic A1 protein after coaggregation of FcεRI with FcγRIIB; we found that A1 mRNA was up-regulated both when mast cells are activated through FcεRI aggregation and when FcεRI is coaggregated with FcγRIIB. Our finding that both antiapoptotic A1 and proapoptotic Bim proteins are up-regulated as a result of FcεRI aggregation could be an explanation of why this activation results in cell death or survival in some experimental settings, since the fate of cells is likely to be influenced by the relative balance of these molecules.

The only treatment of allergic diseases that leads to long-lasting effects is allergen-specific immunotherapy. The immunologic mechanisms responsible for a successful treatment are still not fully defined. One hypothesis is that the antigen-specific IgG that increases in serum during treatment blocks antibodies,\textsuperscript{38} leading to possible coaggregation of FcεRI with FcγRIIB. The finding that allergic activity is inhibited by coaggregating FcεRI with FcγRIIB by using a human Fcγ-Fcε fusion protein highlights a new promising therapeutic approach to immunomodulation.\textsuperscript{39} The fusion protein showed antiallergic effects both in vitro and in vivo and was shown to inhibit IgE-mediated activation of blood basophils and cord blood–derived mast cells.\textsuperscript{40}

Furthermore, evidence for negative regulation of allergic responses by FcγRIIB has been...
demonstrated by the use of FcγRIIB-deficient mice. These mice produce more immunoglobulin than wild-type mice in response to immunization, in which this increase is partly due to the increase in IgG1. The negative regulation of IgG production by FcγRIIB probably decreases the production of IgE. This would work in favour of reduced FcεRI expression on the cells and less IgE being available for activation. FcγRIIB-deficient mice also display more vascular permeability in the IgG-dependent passive cutaneous anaphylaxis reaction than do wild-type mice, indicating mast-cell activation of a greater extent than that seen in wild-type mice. During IgE- and IgG-dependent passive systemic anaphylaxis, the FcγRIIB-deficient mice undergo increased hypothermia and death. These findings indicate an important role for FcγRIIB on mast cells in down-regulating immediate hypersensitivity reactions as a result of anaphylactic mast-cell activation.

This report shows that although mast-cell degranulation is inhibited by coaggregation of FcεRI with FcγRIIB, other downstream signalling proteins that are closely related to cell survival remain largely unaffected. Figure 6 presents a schematic overview of how these processes could be separated in the cell. Our previous finding that both proapoptotic and antiapoptotic proteins are up-regulated as a result of FcεRI aggregation suggests that the fate of cells is likely to be based on the balance between these proteins.

Acknowledgements

The authors would like to thank Dr. Marc Daëron for helpful discussions and advice, Prof. Birgitta Heyman and Mrs. Imma Brogren for help in the production of conjugated antibody, and Prof. Stephen Galli for C57 cells.

This work was supported by the Swedish Research Council-Medicine; the Swedish Cancer Foundation; the Bror Hjerpstedts Foundation; the Consul Th. C. Berghs Foundation; the Swedish Cancer and Allergy Fund; Ollie and Ehol Ericsson’s Foundation; King Gustav V’s 80 Years Foundation; the Ellen, Walter, and Lennart Hesselmans Foundation; and the Karolinska Institutet.

References

1. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;77:1033–79.
2. Daeron M. Fc receptor biology. Annu Rev Immunol 1997;15:203–34.
3. Ott VL, Cambier JC. Activating and inhibitory signaling in mast cells: new opportunities for therapeutic intervention? J Allergy Clin Immunol 2000;106:429–40.
4. Blank U, Ra C, Miller L, et al. Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 1989;337:187–9.
5. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. Nature 1999;402(6760 Suppl):B24–30.
6. Hulett MD, Hogarth PM. Molecular basis of Fc receptor function. Adv Immunol. 1994;57:1–127.
7. Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell 1994;76:263–74.
8. Bolland S, Ravetch JV. Inhibitory pathways triggered by ITIM-containing receptors. Adv Immunol 1999;72:149–77.
9. Daeron M, Latour S, Malbec O, et al. The same tyrosine-based inhibition motif, in the intracy-
toplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 1995;3:635–46.

10. Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001;19:275–90.

11. Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. Nature 1996;383:263–6.

12. Daeron M, Malbec O, Latour S, et al. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. J Clin Invest 1998;106:1647–58.

13. Malbec O, Fong DC, Turner M, et al. Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. J Immunol 1998;160:1647–58.

14. Huber M, Helgason CD, Damen JE, et al. Regulation of high-affinity IgE receptor-mediated mast cell activation by the receptor Fc(gamma)RIIB. Nature 1996;383:263–6.

15. Lesourne R, Fridman WH, Daeron M. Dynamic interactions of Fc gamma receptor IIB with filamentous actin: requirement for the common FcR gamma-chain. J Immunol 2001;167:173–81.

16. Ott VL, Tamir I, Niki M, et al. Downstream of kinase, p62(dok), is a mediator of Fc gamma receptor IIB inhibition of Fc epsilon receptor I signaling. J Immunol 2002;168:4430–9.

17. Alfredsson J, Moller C, Nilsson G. IgE-receptor activation of mast cells regulates phosphorylation and expression of forkhead and Bcl-2 family members. Scand J Immunol 2006;63:1–6.

18. Alfredsson J, Puthalakath H, Martin H, et al. Proapoptotic Bcl-2 family member Bim is involved in the control of mast cell survival and is induced together with Bcl-2 upon IgE-receptor activation. Cell Death Differ 2005;12:136–44.

19. Möller C, Alfredsson J, Engström M, et al. Stem cell factor promotes mast cell survival via activation of FOXO3a-mediated transcriptional induction and MEK-regulated phosphorylation of the proapoptotic protein Bim. Blood 2005;106:1330–6.

20. Xiang Z, Ahmed AA, Möller C, et al. Essential role of the prosurvival bcl-2 homologue A1 in mast cell survival after allergic activation. J Exp Med 2001;194:1561–9.
33. Aman MJ, Lamkin TD, Okada H, et al. The inositol phosphatase SHIP inhibits Akt/PKB activation in B cells. J Biol Chem 1998; 273:33922–8.

34. Malbec O, Schmitt C, Bruhns P, et al. Src homology 2 domain-containing inositol 5-phosphatase 1 mediates cell cycle arrest by FcgammaRIIB. J Biol Chem 2001;276:30381–91.

35. Noble KE, Wickremasinghe RG, DeCornet C, et al. Monocytes stimulate expression of the Bcl-2 family member, A1, in endothelial cells and confer protection against apoptosis. J Immunol 1999;162:1376–83.

36. Lin EY, Orloffsky A, Wang HG, et al. A1, a Bcl-2 family member, prolongs cell survival and permits myeloid differentiation. Blood 1996;87:983–92.

37. Xiang Z, Möller C, Nilsson G. IgE-receptor activation induces survival and expression of the prosurvival gene bfl-1 in human mast cells but not in basophils. Allergy 2006. [DOI]

38. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. Int Arch Allergy Immunol 2003;132:13–24.

39. Zhu D, Kepley CL, Zhang M, et al. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. Nat Med 2002;8:518–21.

40. Saxon A, Zhu D, Zhang K, et al. Genetically engineered negative signaling molecules in the immunomodulation of allergic diseases. Curr Opin Allergy Clin Immunol 2004;4:563–8.

41. Takai T, Ono M, Hikida M, et al. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature 1996;379:346–9.

42. Yamaguchi M, Lantz CS, Oettgen HC, et al. IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 1997;185:663–72.

43. MacGlashan D Jr, McKenzie-White J, Chichester K, et al. In vitro regulation of FcepsilonRIalpha expression on human basophils by IgE antibody. Blood 1998;91:1633–43.

44. Ujike A, Ishikawa Y, Ono M, et al. Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. J Exp Med 1999;189:1573–9.