The Role of Actin Microfilaments in the Down-Regulation of the Degranulation Response in RBL-2H3 Mast Cells

Luciano Frigeri and John R. Apgar

*J Immunol* 1999; 162:2243-2250; ;
http://www.jimmunol.org/content/162/4/2243


---

**Why The JI?**

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

---

**References**

This article cites 48 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/162/4/2243.full#ref-list-1

---

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

---

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

---

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Role of Actin Microfilaments in the Down-Regulation of the Degranulation Response in RBL-2H3 Mast Cells

Luciano Frigeri and John R. Apgar

Cross-linking of FceRI on rat basophilic leukemia (RBL) cells initiates a signaling cascade leading to degranulation of the cells and the release of inflammatory mediators. Inhibitors that disrupt microfilaments, such as latrunculin and cytochalasin D, do not cause any degranulation on their own, but they do enhance FceRI-mediated degranulation. Dose-response studies show a good correlation between inhibition of actin polymerization and increased degranulation. In RBL cells, latrunculin causes a decrease in basal levels of filamentous actin (F-actin), while cytochalasin D does not. This is particularly evident in the Triton-insoluble pool of F-actin which is highly cross-linked and associated with the plasma membrane. A concentration of 500 nM latrunculin decreases the basal level of Triton-insoluble F-actin by 60–70% and total F-actin levels by 25%. Latrunculin increases both the rate and extent of Ag-induced degranulation while having no effect on pervanadate-induced degranulation. Pervanadate activates the signaling pathways directly and bypasses the cross-linking of the receptor. RBL cells, activated through FceRI in the presence of latrunculin, show increased phospholipase activity as well as increased tyrosine phosphorylation of Syk and increased tyrosine phosphorylation of the receptor itself by the tyrosine kinase Lyn. This indicates that the very earliest signaling events after receptor cross-linking are enhanced. These results suggest that actin microfilaments may interact, either directly or indirectly, with the receptor itself and that they may regulate the signaling process at the level of receptor phosphorylation. Microfilaments may possibly act by uncoupling Lyn from the cross-linked receptor. The Journal of Immunology, 1999, 162: 2243–2250.

Received for publication May 4, 1998. Accepted for publication November 5, 1998.

Address correspondence and reprint requests to Dr. John R. Apgar, Scripps Research Institute, La Jolla, CA 92037.

1 This work was supported by National Institutes of Health Grants RO 1GM42388 and U19 AI4224-1. This is manuscript 11613-MEM from the Department of Molecular and Experimental Medicine at the Scripps Research Institute.

2 Abbreviations used in this paper: RBL, rat basophilic leukemia; FcεRI, high affinity immunoglobulin E receptor; ITAM, immunoreceptor tyrosine-based activation motif; PKC, protein kinase C; F-actin, filamentous actin; NBD-phallacidin, 7-nitrobenz-2-oxa-1,3-diazole-phallacidin.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
correlation between inhibition of actin polymerization and increases in phospholipase activity, tyrosine kinase activity, and degranulation. Microfilaments appear to down-regulate the response by affecting the level of receptor tyrosine phosphorylation, thus affecting all the signaling pathways involved.

Materials and Methods

Cells

RBL-2H3 cells were grown in Eagle’s MEM supplemented with 20% FCS, 4 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate, MEM nontoxic essential amino acids, and penicillin-streptomycin. The cells were harvested by incubating them in PBS containing 5 mM EDTA for 10 min at 37°C. RBL-2H3 cells were pretreated by resuspending them in complete PBS at a concentration of 3 × 10^6 cells/ml. IgE that is specific for DNP (21) was added to a concentration of 1 µg/ml, and the cells were rotated at 37°C for 2 h. The cells were then washed three times in PBS before resuspension in HBSS containing 0.2% BSA.

Reagents

Latrunculin was purchased from Biomol (Plymouth Meeting, PA). 7-Nitrobenz-2-oxa-1,3-diazole-phallacidin (NBD-phallacidin) was obtained from Molecular Probes (Eugene, OR), while DNP-BSA, containing at least 40 µl of DNP/mg of protein, was purchased from Calbiochem (La Jolla, CA). Protease inhibitor mixture tablets were purchased from Boehringer Mannheim (Indianapolis, IN). [H]mioinositol was purchased from Amersham (Arlington Heights, IL), while [H]arachidonic acid, [H]myristic acid, and the Renaissance chemiluminescence kit were obtained from New England Nuclear-DuPont. (Boston, MA). Rabbit anti-Syk was obtained from both Santa Cruz Biotechnology (Santa Cruz, CA) and Dr. Reuben Siraganian (National Institutes of Health, Bethesda, MD). PY20 (anti-phosphotyrosine) was from Transduction Laboratories (Lexington, KY), and 4G10 (anti-phosphotyrosine) was obtained from Upstate Biotechnology (Lake Placid, NY). The mouse mAb, JRK, which reacts with the first 23 amino acids of the N-terminal cytoplasmic domain of the β-chain, was purchased from Dr. Leandra Rivera (National Institutes of Health). Protein A was purchased from Pierce (Rockford, IL). All secondary Abs and conjugates were obtained from Zymed (South San Francisco, CA). All other reagents were purchased from Sigma (St. Louis, MO).

Pervanadate was prepared according to previously published methods (22). Equal volumes of 10 mM sodium orthovanadate in HBSS containing 50 mM HEPES (pH 7.2) and 10 mM H_2O_2, in the same buffer were mixed together at room temperature for 15 min. Any remaining H_2O_2 was removed by adding catalase (1000 U/ml, final concentration) for 5 min. The pervanadate solution was then used immediately. 

F-actin assay

A slightly modified version of the assay developed by Howard and his colleagues (23) has been used. IgE-sensitized RBL cells were added to BSA-coated 12- × 75-mm polystyrene tubes in 200 µl (5 × 10^6 cells/tube). The cells were preincubated with latrunculin for 15 min at 37°C before the addition of DNP-BSA (50 ng/ml). To measure total F-actin, the reaction was stopped by the addition of formaldehyde to a final concentration of 3.7% for 15 min at room temperature. The cells were permeabilized by the addition of ice-cold solubilizing buffer (10 mM imidazole (pH 7.2), 40 mM KCl, 10 mM EGTA, 1% Triton X-100, 1 mM PMSF, and protease inhibitor mixture tablet). After a single wash with PBS at 2200 g for 10 min, the supernatant was tested with chloroform/methanol. Radiolabeled inositol phosphates were isolated using Dowex chromatography (25, 26). Phospholipase A activity was assayed by following the release of [H]arachidonic acid and its metabolites. Cellular phospholipids were labeled with [H]arachidonic acid. The cells were washed, suspended in buffer in tubes, and activated with DNP-BSA, and [H]arachidonic acid released into the supernatant was determined by liquid scintillation counting. Phospholipase D activity was monitored by the production of phosphatidylethanol (24, 27). Briefly, cellular phospholipids were labeled by growing the cells overnight in medium containing [H]myristic acid. The cells were sensitized with IgE, washed, resuspended in buffer containing 0.5% ethanol, and activated with DNP-BSA. Phospholipids were extracted using chloroform/methanol, and [H]phosphatidylethanol was isolated by TLC using a double one-dimensional system developed by Kennedy and colleagues (28).

Immunoprecipitations and immunoblotting

IgE-sensitized RBL cells in suspension were activated with 50 ng/ml DNP-BSA at 37°C. The reaction was stopped by the addition of ice-cold solubilizing buffer (1% Triton-X-100, 100 mM NaCl, 120 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2 mM sodium orthovanadate, 100 mM sodium fluoride, 50 mM sodium pyrophosphate, PMSF, and protease inhibitor tablets) for 30 min at 4°C. Intracellular Ca^2+ was measured overnight in medium containing 2 × 10^6 cells/ml. IgE that is specific for DNP (21) was added to BSA-coated tubes and preincubated with 4G10, an anti-phosphotyrosine Ab, followed by a 1-h incubation with either goat anti-mouse IgG or goat anti-rabbit IgG bound to Sepharose beads. The beads were washed three times, and bound proteins were eluted by adding SDS sample buffer and placing the tubes in a boiling water bath for 3 min. The proteins were resolved on a 12.5% SDS-PAGE gel and then transferred to an Immobilon P membrane using a semidry transfer apparatus. The samples were immunoblotted with 4G10, an anti-phosphotyrosine Ab, followed by goat anti-mouse horseradish peroxidase. The New England Nuclear Renaissance chemiluminescence kit was used to visualize the results.

Results

Latrunculin is a compound isolated from Red Sea sponges that disrupts the actin cytoskeleton (29). It is believed that latrunculin binds to monomeric actin, thus preventing actin polymerization (30). The effects of different concentrations of latrunculin on the basal levels of F-actin in RBL cells in suspension are shown in Fig. 1. Latrunculin reduces total F-actin levels only moderately in unstimulated cells even at concentrations as high as 1 µM. At 500 nM, total F-actin levels are reduced by approximately 20%. Howard and colleagues (23, 31, 32) have determined that there are at least two different types of F-actin, which can be distinguished by their solubility in Triton-containing buffers. Triton-soluble F-actin consists of short oligomers that are associated with gelsolin and are found throughout the cytoplasm of neutrophils. Triton-insoluble F-actin forms a cross-linked web of actin that is found in corollary between inhibition of actin polymerization and increases in phospholipase activity, tyrosine kinase activity, and degranulation. Microfilaments appear to down-regulate the response by affecting the level of receptor tyrosine phosphorylation, thus affecting all the signaling pathways involved.
association with tropomyosin, filamin, and α-actinin. Triton-insoluble F-actin also tends to be associated with the plasma membrane. In RBL cells, approximately 60–70% of the basal level of F-actin is Triton soluble. In unstimulated RBL cells, incubated with 500 nM latrunculin, there is no change in Triton-soluble F-actin levels, but there is a 60% decrease in the Triton-insoluble pool. Thus, latrunculin does not affect all forms of F-actin equally. It appears to be exerting its effects primarily on the Triton-insoluble fraction of F-actin.

Activation of RBL cells through FcεRI causes a rapid increase in the level of total F-actin. Most of this change occurs in the Triton-insoluble pool of F-actin (data not shown). As shown in Fig. 2, after activation of the cells with Ag, the level of Triton-insoluble F-actin rises to between 150 and 200% of the basal level in 3–5 min. The F-actin level peaks and then slowly declines to basal levels over 30–45 min (data not shown). In the presence of 500 nM latrunculin, the basal level is much lower than the control value, as also shown in Fig. 1. After the addition of DNP-BSA, there is almost complete inhibition of actin polymerization in treated cells. Fig. 2A shows the total level of Triton-insoluble F-actin. In Fig. 2B, the basal level of Triton-insoluble F-actin has been subtracted from both sets of samples so that only Ag-induced actin polymerization is seen.

Cytochalasin D inhibits actin polymerization by capping the barbed end of actin microfilaments and preventing elongation (33). In contrast to latrunculin, cytochalasin D has relatively little effect on the basal level of Triton-insoluble F-actin (Fig. 3A). However, preincubation of the cells with 500 nM cytochalasin D does effectively inhibit Ag-induced actin polymerization. In Fig. 3B, the basal level of F-actin has been subtracted so that only the Ag-induced F-actin response is seen. As in the case with latrunculin, cytochalasin D is more effective in inhibiting the early rise of F-actin.

It has been hypothesized that microfilaments might be involved in the down-regulation of the degranulation response (9). Therefore, inhibition of microfilament formation should increase degranulation. Latrunculin and cytochalasin D have completely different modes of action, but both inhibit Ag-induced actin polymerization as shown in Figs. 2 and 3. To determine their effects on cell activation, IgE-sensitized RBL cells in suspension were preincubated with either latrunculin (Fig. 4A) or cytochalasin D (Fig. 4B) before activation with DNP-BSA. Neither latrunculin nor cytochalasin D caused any degranulation in the absence of Ag. However, upon addition of Ag, there was increased degranulation, in a dose-dependent manner, in cells that had been preincubated with either cytoskeletal inhibitor. In both cases, the peak response occurred at approximately 500 nM, and degranulation was increased by two- to threefold. In addition, the amount of Ag-induced F-actin was monitored in cells preincubated with the different concentrations of latrunculin and cytochalasin D. As can be seen, inhibition of actin polymerization occurred in the same dose range as did the increase in degranulation for both inhibitors.

Fig. 5 shows the degranulation responses at different concentrations of Ag. As can be seen, latrunculin and cytochalasin D were able to increase degranulation at all concentrations of DNP-BSA.
All these experiments were performed using RBL cells in suspension. This was done to avoid any effects that these microfilament inhibitors may have on adhesion and spreading, which is also known to affect degranulation. If latrunculin was added to RBL cells that were attached and spread, there was relatively little change seen in the number of adherent cells. Changes were seen in the shape of the cells, although most remained spread. Adherent and nonadherent cells were preincubated with latrunculin before activation with DNP-BSA (Fig. 6). Latrunculin caused increased degranulation from both adherent and nonadherent cells, although the level of release in nonadherent cells was considerably less than in adherent cells.

RBL cells can be activated to degranulate through FceRI or by directly activating the signaling pathways, thus bypassing the receptor. As shown in Table I, latrunculin caused increased degranulation in nonadherent RBL cells stimulated through FceRI but not with pervanadate. Pervanadate inhibited certain phosphatases, thus causing increased tyrosine phosphorylation, which activated the signaling pathways leading to degranulation. This mechanism bypassed the cross-linking of the receptor. However, in contrast to latrunculin, cytochalasin D also increased degranulation when the cells were stimulated with pervanadate. Degranulation could also be induced, in a nonreceptor manner, with a combination of A23187 and PMA. In nonadherent cells, latrunculin and cytochalasin D had their optimal effects on Ag-induced degranulation at a concentration of 500 nM. At this concentration, increased degranulation was seen when the cells were preincubated with cytochalasin D and activated with A23187 and PMA. Augmented degranulation was also seen using latrunculin, although the increase was considerably less than with cytochalasin D. In adherent cells, 150 nM was the optimal concentration for increased degranulation when the cells were activated with DNP-BSA. Using adherent cells and 150 nM cytochalasin D, increased degranulation was

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Adherent</th>
<th>Control</th>
<th>Latrunculin</th>
<th>Cytochalasin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>–</td>
<td>2.8</td>
<td>2.7</td>
<td>3.7</td>
</tr>
<tr>
<td>DNP-BSA</td>
<td>–</td>
<td>16.6</td>
<td>38.1</td>
<td>35.1</td>
</tr>
<tr>
<td>Pervanadate</td>
<td>–</td>
<td>20.3</td>
<td>22.5</td>
<td>40.0</td>
</tr>
<tr>
<td>A23187 + PMA</td>
<td>–</td>
<td>22.9</td>
<td>30.3</td>
<td>43.1</td>
</tr>
<tr>
<td>Buffer</td>
<td>+</td>
<td>2.8</td>
<td>3.1</td>
<td>2.7</td>
</tr>
<tr>
<td>DNP-BSA</td>
<td>+</td>
<td>44.2</td>
<td>73.1</td>
<td>76.4</td>
</tr>
<tr>
<td>Pervanadate</td>
<td>+</td>
<td>53.3</td>
<td>52.4</td>
<td>71.2</td>
</tr>
<tr>
<td>A23187 + PMA</td>
<td>+</td>
<td>43.8</td>
<td>44.7</td>
<td>79.3</td>
</tr>
</tbody>
</table>

* IgE-sensitized RBL cells were added to BSA-coated wells or to fibronectin-coated wells. The cells added to BSA-coated wells remain nonadherent and were preincubated with either 500 nm latrunculin or 500 nM cytochalasin D for 15 min. The cells added to fibronectin-coated wells were allowed to adhere and spread for 45 min before being pre-incubated with 150 nM latrunculin or cytochalasin D. In both cases, the cells were activated with DNP-BSA (50 ng/ml), pervanadate (200 μM), or A23187 (300 nM) plus PMA (50 nM) for 45 min at 37°C. Degranulation is expressed as %β-hexosaminidase released. The error in all cases was < 7%.
The increased activity of all the phospholipases, when the cells were activated in the presence of latrunculin, indicated that an upstream signal such as a tyrosine kinase was responsible. Since both Lyn and Syk are known to associate with the cross-linked receptor, this is consistent with the conclusion that latrunculin may be acting at the level of the receptor itself. When IgE-sensitized RBL cells were stimulated with DNP-BSA, there was a fairly rapid increase in the tyrosine phosphorylation of Syk (Fig. 8). In the presence of latrunculin, increased tyrosine phosphorylation of Syk was observed.

The previous experiments demonstrated that latrunculin causes increased tyrosine phosphorylation of Syk in Ag-stimulated cells. Syk has been shown to be tyrosine phosphorylated by the Src family tyrosine kinase, Lyn. Lyn is associated with the β-chain of the receptor and is known to phosphorylate both the β- and γ-chains of the receptor upon activation. This is the earliest known event to occur after receptor cross-linking. Therefore, to determine whether microfilaments regulate degranulation by decreasing Lyn-mediated phosphorylation of the β-chain, cells were preincubated with 500 nM latrunculin and activated with DNP-BSA. Fig. 9 shows an increase in β-chain tyrosine phosphorylation in untreated cells after activation. Pretreatment of the cells with latrunculin,
Culmin is exerting its effect at the level of receptor phosphorylation. Phosphorylation of the FcεRI was noprecipitated using a mouse monoclonal anti-FcεRIβ Ab and immunoblotted with an anti-phosphotyrosine Ab. This experiment was performed six times.

Followed by activation through FcεRI, caused increased tyrosine phosphorylation of the β-chain. These results suggest that latrunculin is exerting its effect at the level of receptor phosphorylation.

Discussion

The results reported here show that there is a good correlation between inhibition of actin polymerization and increased degranulation in Ag-activated RBL cells. In these studies, nonadherent cells were used, although latrunculin will also increase the degranulation from activated, adherent cells. Nonadherent cells were used to avoid any complications that might result from the actions of latrunculin on adhesion and spreading. Increased degranulation due to inhibition of actin polymerization has been shown using two inhibitors, latrunculin and cytochalasin D, which have very different modes of action. Latrunculin, which sequesters monomeric actin (30), caused a considerable decrease in the basal level of Triton-insoluble actin. This was unexpected, since this pool of F-actin is cross-linked and is thought to be more stable than the Triton-soluble pool of F-actin (23, 32, 34). Latrunculin also very effectively inhibited Ag-induced actin polymerization, although the inhibition was greater at early time points. Cytochalasin D had similar effects on Ag-induced actin polymerization, but it had virtually no effect on the basal level of Triton-insoluble F-actin. The fact that cytochalasin D also increases degranulation would indicate that it is not a decrease in basal levels of F-actin that is important for this effect on degranulation. However, it is not known how these inhibitors affect the structure of pre-existing microfilament structures. Therefore, it is difficult to determine whether it is the disruption of microfilaments that existed before activation of the cells or whether it is the inhibition of Ag-induced actin polymerization that is important. If it is a pre-existing structure that is important, then it exists in both adherent and nonadherent cells. If it is inhibition of microfilaments formed as a consequence of receptor cross-linking that is involved, then the signaling pathways that are activated and that lead to actin polymerization are, in turn, down-regulated by these very same actin filaments in a negative feedback loop.

One of the problems in using cytochalasin D is that it also enhances degranulation triggered by substances that bypass the receptor, such as pervanadate or calcium ionophore. This indicates that cytochalasin may be acting at more than one point in the signaling pathway. Cytochalasin D also increases receptor tyrosine phosphorylation, thus indicating that it is acting at a very early stage (data not shown). However, the degranulation results with pervanadate indicate that it is also acting at a downstream stage. Latrunculin only causes increased degranulation when the cells are activated through FcεRI and not when the receptor is bypassed with pervanadate. The effect with latrunculin and A23187 plus PMA is concentration dependent. Latrunculin is, therefore, generally more specific than cytochalasin D. These results indicate that the effect of latrunculin is mainly at the level of the receptor itself and that microfilaments may interact, either directly or indirectly, with cross-linked receptors. In addition to degranulation, the activities of all the phospholipases as well as tyrosine phosphorylation of Syk were also increased in latrunculin-treated cells that had been activated with Ag. Increased phosphorylation of the β-chain of FcεRI, which is the earliest known event after receptor cross-linking, was also seen in activated cells treated with latrunculin. In some experiments, although not all, preincubation with latrunculin also caused a small increase in the basal level of FcεRIβ tyrosine phosphorylation. Although much more pronounced in activated cells, it is possible that microfilaments are also regulating the level of receptor tyrosine phosphorylation in resting cells. However, increases in none of the other signaling pathways, including tyrosine phosphorylation of Syk, was ever seen in unactivated cells treated with latrunculin. Since the tyrosine kinases associate with the cross-linked receptors, this further supports the idea that microfilaments are acting on the receptors themselves and not on a downstream event. Our current hypothesis is that microfilaments are uncoupling Lyn from the cross-linked receptors, thus shutting down the response. If actin polymerization is inhibited with either cytochalasin D or latrunculin, then no uncoupling takes place, and the receptors remain active, thus leading to increased signaling and degranulation.

In RBL cells, the addition of monovalent DNP-lysine to cells activated with multivalent Ag, such as DNP-BSA or DNP-phycocyanin, causes immediate cessation of all signaling and degranulation. Oliver and co-workers (35) coupled DNP to the fluorescent protein phycocyanin so that they could monitor the displacement of this multivalent Ag. What was found was that the addition of DNP-lysine stopped all further degranulation, but a considerable amount of DNP-phycocyanin remained on the cell surface. This indicated that some of the receptors on the cell surface remained cross-linked, but they were no longer actively signaling. With increasing time of exposure to DNP-phycocyanin, an increasing number of receptors became DNP-lysine resistant, as the multivalent Ag could not be displaced by the monovalent Ag. Furthermore, this rate of conversion to DNP-lysine resistance was affected if the cells were preincubated with cytochalasin D. Thus, some cross-linked receptors are actively signaling while others have been turned off, and this process appears to be dependent on microfilaments. In neutrophils, it has also been suggested that actin microfilaments might be involved in down-regulation (36–38). Neutrophils can be activated through the f-Met-Leu-Phe receptor, which can exist in a high affinity or a low affinity state. The different receptor states can be isolated from different plasma membrane fractions using sucrose density centrifugation. Desensitized receptors are found in a fraction that is rich in actin but contains no G proteins, while the active receptors are found in a fraction that contains G proteins but no actin. The conclusion from these studies was that actin microfilaments physically separated receptors from G proteins, thus turning off the response.

In the present study we found that in cells treated with latrunculin, increased tyrosine phosphorylation of the β-chain of FcεRI was observed. FcεRI has no intrinsic kinase activity, and therefore, initiation of the signaling cascade is dependent on Lyn (5, 6, 7). Studies in RBL cells have shown that some receptors have Lyn constitutively associated with the β-chain (39, 40) and that after receptor cross-linking, there is transphosphorylation of the ITAM regions in the β- and γ-chains (8). Studies have shown that the β-chain is not absolutely necessary for signaling to occur but that it acts as an amplifier that increases the response five- to sevenfold...
Tyrosine phosphorylation of the ITAM regions leads to the recruitment of more Lyn as well as Syk. Exactly how Lyn becomes associated with the cross-linked receptors is not known, but recent work indicates that detergent-resistant membrane domains may be important. The fact that plasma membrane domains exist in RBL cells has been shown both biochemically (42, 43) as well as by confocal fluorescent microscopy (44). FceRI normally has a uniform distribution on the cell surface, indicating that it is not pre-localized to these domains. Cross-linking of the receptors leads to a rapid, although transient, relocalization into these domains that are enriched in Lyn. This leads to tyrosine phosphorylation of the receptors and the recruitment and activation of Syk and phospholipase Cγ1. The conclusion from these studies was that signal transduction mediated through FceRI is compartmentalized and spatially restricted.

It is not known how actin microfilaments help to regulate the responses triggered by FceRI cross-linking, although the experiments reported here indicate that it occurs at a very early stage. However, it should be pointed out that although the experiments presented here show an excellent correlation between the inhibition of the F-actin response and increased degranulation, they do not definitively prove that there is a connection between these two events. Microfilaments could regulate tyrosine phosphorylation of the receptors by physically restricting the entry of cross-linked receptors into the detergent-resistant membrane domains. Inhibition of actin polymerization by latrunculin would increase the likelihood of the cross-linked receptors entering the domains and becoming tyrosine phosphorylated by Lyn. Another possibility is that actin microfilaments do not control the entry into these lipid domains but that they are involved in separating the receptors from the lipid domains. If this is the case, then all the cross-linked receptors potentially become activated, and the microfilaments are involved in deactivate them. Latrunculin would presumably function by inhibiting actin polymerization, thus keeping the receptors in an activated state for a longer period of time. In any case, signaling in these cells is compartmentalized, and the role of actin microfilaments may be to regulate whether cross-linked receptors are in an active compartment.

Activation of mast cells through FceRI leads to the release of a variety of inflammatory mediators such as histamine, proteases, leukotrienes, PGs, and cytokines. Control of the release of these mediators is important, and mast cells have developed several potential ways of regulating the signaling pathways leading to their release. The work reported here details one mechanism in which microfilaments are involved in controlling the extent of the reaction, possibly by uncoupling the cross-linked receptors from the tyrosine kinases. There are, however, several other potential mechanisms. Activation of phosphatases, which dephosphorylate the cross-linked receptors, has been reported, and it is known that even stably cross-linked receptors rapidly undergo rounds of phosphorylation and dephosphorylation (45). It has also been found that several receptors containing immunoreceptor tyrosine-based inhibition motifs leads to down-regulation if they are co-cross-linked with FceRI (46–48). Phosphorylation of immunoreceptor tyrosine-based inhibition motifs recruits and serves as a docking site for phosphatases that can turn off the response by dephosphorylating the FceRI. Finally, it has been reported that the amount of Lyn that associates with the cross-linked receptors is a limiting factor and that cross-linked receptors compete for this kinase (49). Thus, it is believed that Lyn shuttles from one cross-linked receptor complex to another. It is not known what controls the available pool of Lyn, but that is another potential source of regulation.

Finally, the experiments reported here focus on the potential role of actin microfilaments in the down-regulation of the signaling pathways leading to the degranulation response. However, previous studies have shown that adhesion and spreading, which are dependent on microfilament assembly and rearrangement, are involved in up-regulation of these same signaling pathways (23, 50). Spreading of the cells on extracellular matrix proteins seems to prime the cells for increased degranulation after activation with Ag. It is apparent that actin filaments have a very complex interaction with the signaling machinery leading to degranulation and that some microfilaments are involved in up-regulating the response, while others may be involved in down-regulation.

Acknowledgments

We thank Dr. Juan Rivera (National Institutes of Health) for his generous contribution of the mouse monoclonal anti-FceRIβ antisera (JRK), and Dr. Reuben Siraganian for kindly providing rabbit anti-Syk antisera.

References


