Journal of Cell Science 110, 771-780 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS1315

Increased degranulation and phospholipase A_2, C, and D activity in RBL cells stimulated through FcεR1 is due to spreading and not simply adhesion

John R. Apgar

Department of Molecular and Experimental Medicine, SBR-4, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

SUMMARY

Rat basophilic leukemia cells will adhere to and spread out on fibronectin coated surfaces in an integrin dependent manner. Adhesion and spreading on fibronectin leads to increased degranulation, inositol phosphate production, phospholipase D activation, and increased production of prostaglandin D_2 and leukotriene C_4 when the cells are activated through the high affinity IgE receptor. Rat basophilic leukemia cells will also adhere to surfaces coated with anti-rat class I antibodies, poly-L-lysine, and a lectin purified from *Tetragonolobus purpureas*. In all cases, antigen activated cells, which were adherent, displayed increased signaling, degranulation and eicosanoid production as compared to cells which were non-adherent. Cells which adhere to either anti-rat class I antibodies or poly-L-lysine also spread even though this is not mediated through integrins. In contrast, adhesion to the lectin from *Tetragonolobus* did not cause any appreciable spreading unless the cells were also triggered through the IgE receptor. Cells were also able to bind to fibronectin immobilized on polystyrene beads which mimics adhesion but does not allow spreading. However, these cells exhibited no increased signaling, degranulation, or eicosanoid production. Furthermore, rat basophilic leukemia cells can be modified by incubating them in the presence of biotinylated-phosphatidylserine which becomes incorporated into the membrane. These modified cells will adhere to streptavidin coated plates while unmodified cells will not. However, these modified cells do not spread, even after activation with antigen, and they show no increased degranulation or production of eicosanoids. These results indicate that adhesion itself is not sufficient for upregulation of the cells in response to antigen and that spreading of the cells may be the critical component.

Key words: RBL cell, Adhesion, Spreading, IgE, Degranulation, Phospholipase, Integrin

INTRODUCTION

Adherence of cells to the extracellular matrix or to each other is of vital importance to the development and regulation of these cells and is involved in growth, gene expression, morphology, migration, and differentiation (Hemler, 1990; Hynes, 1992). Adhesion to extracellular matrix proteins such as fibronectin generally occurs through the integrins which are heterodimeric, transmembrane cell surface proteins. Binding of the integrins to extracellular matrix proteins causes clustering which in turn leads to an association of the integrin cytoplasmic domains with cytoskeletal proteins such as α-actinin, vinculin, and talin (Burridge et al., 1988). These proteins in turn interact either directly or indirectly with actin microfilaments. The integrins are therefore functioning as a bridge which connects the cytoskeleton with the extracellular matrix. Although originally thought to be involved solely in adhesion, it is now quite clear that the integrins are also able to transduce signals across the plasma membrane (Clark and Brugge, 1995; Juliano and Haskill, 1993; Richardson and Parsons, 1995). Changes in tyrosine phosphorylation, intracellular Ca^{2+} levels, intracellular pH, activation of phospholipase A_2 (PLA_2), activation of PKC, and increases in phosphatidylinositol bisphosphate levels have all been reported. In addition to this type of chemical signaling, it has been hypothesized that integrins are involved in mechanical signaling and that the shape of the cell itself may be crucial for the proper functioning of the cell (Ingber, 1993; Sims et al., 1992; Wang et al., 1993).

Mast cells have been shown to express several integrins including VLA4 and VLA5 (Valent and Bettelheim, 1992). Mouse bone marrow derived mast cells have receptors for both fibronectin (Dastych et al., 1991) and laminin (Thompson et al., 1990). These receptors are in a low affinity state and can be induced into a high affinity state by crosslinking of the high affinity IgE receptor (FcεR1). In addition, these cells contain an integrin which recognizes vitronectin and which is normally in a high affinity state (Bianchine et al., 1992). Rat basophilic leukemia (RBL) cells are of mucosal mast cell origin and are a major model for studying immediate hypersensitivity reactions (Beaven and Metzger, 1993; Holowka and Baird, 1990; Metzger, 1992; Metzger et al., 1986; Oliver et al., 1988). It has been shown that these cells adhere to fibronectin in an RGD dependent manner in the absence of any stimulation and that a β1 integrin is involved (Hamawy et al., 1992, 1993a). Adhesion of RBL cells to fibronectin causes tyrosine phosphorylation of several proteins including $pp125^{FAK}$ while stim-

ulation of RBL cells through FcεR1 causes increased tyrosine phosphorylation of pp105-115, pp125^{FAK,} and paxillin when the cells are adherent (Hamawy et al., 1993a,b, 1994). In addition, although adhesion itself does not cause any degranulation, it is able to enhance degranulation induced by either multivalent antigen or ionophore.

RBL cells express FcεR1 which binds IgE in a 1:1 ratio (Beaven and Metzger, 1993; Holowka and Baird, 1990; Metzger, 1992; Metzger et al., 1986; Oliver et al., 1988). Crosslinking of the receptors through multivalent antigen initially leads to the activation of several src family tyrosine kinases such as lyn and syk. This in turn leads to the activation of phospholipase C, A_2, and D. Phospholipase C (PLC) mediated hydrolysis of the polyphosphoinositides leads to the production of inositol phosphates and diacylglycerol which in turn cause an increase in intracellular Ca^{2+} and the activation of protein kinase C (PKC), respectively (Beaven and Cunha-Melo, 1988). Both of these signals are important in degranulation and the release of pre-formed mediators such as histamine, serotonin, and neutral proteases. Phospholipase D (PLD) hydrolyzes phosphatidylcholine thus producing phosphatidic acid which is converted into diacylglycerol. It is believed that this pathway is the major source of diacylglycerol and is responsible for the continued activation of PKC in both RBL cells (Lin et al., 1991) and mast cells (Kennerly, 1990). Activation of phospholipase A_2 leads to the production of arachidonic acid which is further metabolized into the leukotrienes and the prostaglandins. All of these mediators are important in the inflammatory response.

Although the cytoskeleton is certainly involved in adhesion and spreading of cells, its exact role in the degranulation process is not well understood. Upon adhesion and spreading, there is significant reorganization of both microfilaments and microtubules in RBL cells (Hamawy et al., 1992). Activation of these spread RBL cells through FcεR1 causes further polymerization and reorganization of the actin cytoskeleton (Apgar, 1991; Pfeiffer and Oliver, 1994; Pfeiffer et al., 1985). The purpose of this study was to investigate further the role of adhesion and spreading in the activation of RBL cells. It was found that adhesion increases both PLC and PLD activity as well as degranulation and eicosanoid production when the cells are activated through FcεR1. Furthermore, adhesion alone does not support these augmented responses while the ability of the cells to spread on a surface appears to be the critical factor leading to increased activation.

MATERIALS AND METHODS

Cells

RBL-2H3 cells (Barsumian et al., 1981) were grown as a monolayer in EMEM (M.A. Bioproducts, Walkersville, MD) supplemented with 20% FCS, 2 mM glutamine, 10 mM Hepes, 1 mM sodium pyruvate, and penicillin-streptomycin. Cells were passaged twice every week and were harvested by incubation with 5 mM EDTA in PBS for 10 minutes at 37°C. The cells were sensitized by resuspending them in complete EMEM at a concentration of 3×10^6 cells/ml, adding IgE to yield a final concentration of 1 μg/ml, and rotating the cells at 37°C for 2 hours.

Reagents

Human fibronectin was purchased from Collaborative Biomedical. The 120 kDa α-chymotryptic fragment from fibronectin was obtained from Gibco/BRL Products while BSA, poly-L-lysine, polystyrene latex beads (3.2 μm), biotinamidocaproate *N*-hydroxysuccinimide ester, and *Tetragonolobus purpureas* lectin were from Sigma. Streptavidin was obtained from the Pierce Chemical Company. The mouse monoclonal anti-rat class I antibody (6.40.2) was kindly provided by Dr Don Bellgrau (Barbara Davis Center, University of Colorado) and the mouse monoclonal IgE that is specific for DNP was affinity purified from an ascities fluid according to established procedures (Liu et al., 1980). DNP-BSA containing at least 40 moles of DNP per mole of BSA was purchased from Calbiochem. Lipid standards such as phosphatidylethanol were obtained from Avanti Polar Lipids (Alabaster, Alabama). [^{3}H]5-hydroxytryptamine binoxalate ([^{3}H]5-HT), [^{3}H]myoinositol, [^{3}H]myristic acid, and [^{3}H]leucine were purchased from New England Nuclear/DuPont.

Preparation of biotinylated phosphatidylserine (biotin-PS) and modification of the cells

The method outlined here is based on a technique by Bayer et al. (1979). Phosphatidylserine (2 mg) was biotinylated by adding it to biotinamidocaproate *N*-hydroxysuccinimide ester (2 mg) in a volume of 300 μl (chloroform/methanol, 2:1) for 1 hour at room temperature. The mixture was then dried down in a stream of N_2 and resuspended in 1 ml of 0.5% sodium deoxycholate for 15 minutes. This material was dialyzed for 5 days against 3 changes of 150 mM NaCl, 20 mM Tris-HCl, pH 8.0, and against 2 changes of PBS. The material was spun in a microfuge for 5 minutes before use. RBL cells were resuspended in Hanks' buffered saline solution (HBSS) containing 0.2% BSA at a concentration of 6×10^6 cells/ml and were incubated with 100 μg of biotin-PS at 37°C for 1.5 hours. The cells were then washed 5 times with PBS before use.

Adhesion assay

Non-tissue culture treated 96-well and 24-well plates (Falcon) were used in all assays. Fibronectin, poly-L-lysine, anti-class I antibody, streptavidin, and *Tetragonolobus* lectin were added to the wells for 2 hours at 37°C. The wells were washed twice with PBS and blocked with BSA (20 mg/ml) for 1.5 hours at 37°C. The wells were then washed 3 times before the addition of cells. Tissue culture flasks containing adherent RBL cells were rinsed twice with PBS and the cells were incubated for 1 hour in leucine free-MEM with Earle's salts containing 1.0 μCi/ml of [^{3}H]leucine. The cells were harvested and then washed 3 times before being resuspended in HBSS containing 1.5 mM $CaCl_2$ and 0.2% BSA at a concentration of 1.5×10^6 cells/ml. In assays using 96-well plates, 50 μl (75,000 cells) of cells were used while 300 μl (450,000 cells) were used in 24-well plate assays. Cells were allowed to adhere for the times indicated before the supernatant was removed, and the wells were washed 3 times with HBSS containing 1.5 mM $CaCl_2$ and 0.2% BSA. Adherent cells were lysed with 3% SDS and cpm determined by liquid scintillation counting. A sample of cells was used to determine total cpm added, and the results are expressed as % cells bound ± s.e.m.

In the experiments in which polystyrene latex beads were used, a 0.5% suspension of beads (3.2 μm) was coated with 50 μg/ml of fibronectin for 2 hours at 37°C. In some experiments, the 120 kDa α-chymotryptic fragment of fibronectin was used in order to minimize aggregation of the beads while they were being coated. The beads were washed by centrifugation at 2,500 rpm for 10 minutes, and remaining sites were blocked with BSA (20 mg/ml) for 1.5 hours at 37°C. An equal volume of cells (3×10^6 cells/ml) and beads (0.5% suspension) were mixed together and then 300 μl samples were immediately added to BSA coated wells for 45 minutes at 37°C.

Spreading assay

Non-tissue culture treated 24-well plates were coated with fibronectin, poly-L-lysine, anti-class I antibody, streptavidin, and *Tetragonolobus* lectin followed by blocking with BSA as described above. RBL cells

were added to the wells and allowed to attach and spread for 45 minutes. The wells were washed three times and the bound cells were fixed with 3.7% formaldehyde containing 2% sucrose for 10 minutes at room temperature. The wells were then washed twice and the cells were stained for 30 minutes with 0.5% Crystal Violet in 20% methanol. The wells were washed three times, a layer of mineral oil was added, and random fields were photographed using a Nikon N2000 camera attached to a Nikon Diaphot inverted microscope. The photographs were scanned and the images digitized using a Microtek ScanMaker IIG scanner. NIH Image, an image processing and analysis program, was used to calculate the relative areas of the spread cells. Cells were considered spread if their area was greater than 3 standard deviations above the average area of a non-adherent cell.

[^{3}H]5HT assay

RBL cells were resuspended in complete EMEM medium at a concentration of 3×10^6 cells/ml and were incubated with 1 μg of IgE/ml and 3 μCi/ml of [^{3}H]5HT for 2 hours at 37°C. The cells were then washed three times with PBS and resuspended in HBSS containing 1.5 mM $CaCl_2$ and 0.2% BSA. Cells were added to 24-well plates and allowed to adhere for 45 minutes before the addition of DNP-BSA (25 ng/ml final) for another 45 minutes. The supernatants were centrifuged to pellet any detached cells, and a sample was added to scintillation fluid to determine released [^{3}H]5HT. SDS extracts of the cells were used to determine the unreleased material, and the results are expressed as % specific release ± s.e.m.

Assay for inositol phosphate production

The polyphosphoinositides were radiolabeled by growing RBL cells in Eagle's basal medium containing 10% dialyzed FCS, 2 mM glutamine, 1 mM sodium pyruvate, 1% (v/v) each of Eagle's essential and non-essential amino acids, 20 mM Hepes, penicillin-streptomycin, and 3-5 μCi/10^6 cells of [^{3}H]myoinositol for 24 hours. The cells were harvested, washed, and sensitized with IgE for 2 hours at 37°C. After sensitization, the cells were washed three times to remove unbound IgE and then resuspended in HBSS containing 0.1% BSA at a concentration of 2×10^6 cells/ml. Aliquots containing 6×10^5 cells (300 μl) were added to the coated wells and allowed to adhere for 45 minutes. For the final 10 minutes of the adhesion phase, LiCl was added to a give a final concentration of 10 mM. The cells were activated with DNP-BSA for 20 minutes at 37°C. The supernatant was removed and placed in glass 12 mm × 75 mm tubes. Methanol (0.5 ml) was added to the wells, and the cells were detached by scrapping. The methanol was removed, added to the glass tubes, and 1 ml of chloroform/methanol (1:1 v/v) was added to the wells. The chloroform/methanol extract was removed, added to the tubes, followed by the addition of 500 μl of chloroform and 500 μl of 0.044 M HCl. The phases were separated by centrifugation at 1,000 rpm for 5 minutes. The aqueous phase was removed and saved, while the organic phase was re-extracted with 1 ml of water, and the phases were separated by centrifugation. The two aqueous phases were combined, and the pH returned to neutrality by the addition of NaOH. Dowex 1-X8 in the formate form was used to isolate the total [^{3}H]inositol phosphates as well as individual [^{3}H]inositol phosphates using a modification (O'Rourke and Mescher, 1988) of the method of Berridge et al. (1982).

Phospholipase D activation

PLD activity was assessed by measuring the production of phosphatidylethanol when cells were activated in the presence of ethanol (Lin and Gilfillan, 1992). Briefly, cells were labeled overnight in complete medium containing 1 μCi/ml of [^{3}H]myristic acid. The cells were washed, sensitized with IgE as described previously, and allowed to adhere to coated wells for 45 minutes. Ethanol was then added to a final concentration of 0.5% for 10 minutes before the cells were activated with DNP-BSA for an additional 45 minutes. The cells were extracted with chloroform and methanol as described above except that the organic layer was retained and the aqueous layer was discarded. The organic layer was dried down under N_2 and the sample was solubilized in chloroform containing a phosphatidylethanol standard. The lipids were separated using a double one-dimensional thin layer chromatography system (Gruchalla et al., 1990). The samples were spotted 12 cm from the bottom of a 20 cm polyester backed silica gel plate and developed in hexane/ether/acetic acid (30:70:1) until the solvent front reached the top. The plate was then dried and cut 13 cm from the bottom. The 13 cm piece was rotated 180° and developed in chloroform/methanol/ammonium hydroxide (65:35:5). After drying, the lipids were visualized by exposure to iodine vapor, and the phosphatidylethanol spot was cut out. The samples were allowed to sit for 24 hours in order to remove the iodine, Scintiverse II was added, and the cpm were determined using a liquid scintillation counter.

Determination of leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2) secretion

Production of LTC_4 and PGD_2 was determined by an enzyme immunoassay (Cayman Chemical, Ann Arbor, MI). The assays were performed according to the manufacturer's directions.

RESULTS

RBL cells were added to wells containing different concentrations of fibronectin, anti-class I, poly-L-lysine, or *Tetragonolobus* lectin for 45 minutes (Fig. 1). Background binding to BSA coated wells was usually less than 1% and the cells retained a spherical shape. Approximately 60-80% of the cells bound to immobilized fibronectin when used at concen-

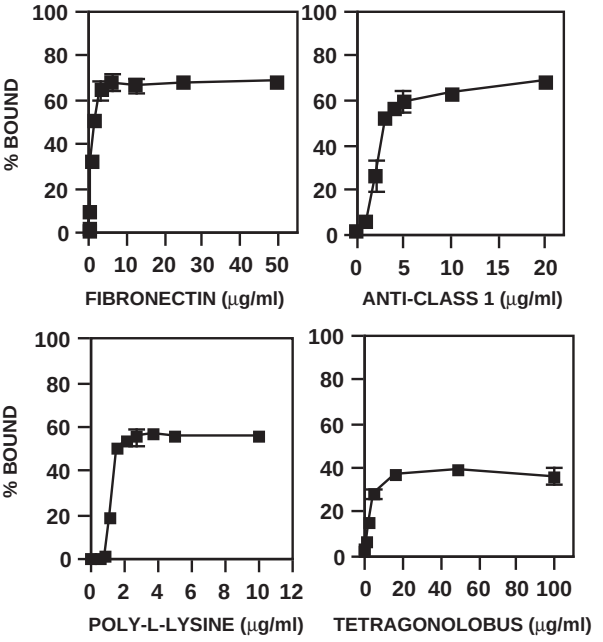

Fig. 1. Adhesion of RBL cells to wells coated with different substrates. RBL cells, metabolically labelled with [^{3}H]leucine, were added to fibronectin, anti-class I, poly-L-lysine, or *Tetragonolobus* coated wells for 45 minutes at 37°C. The wells were washed three times, adherent cells were solubilized in detergent, and cpm bound were determined by liquid scintillation counting. A sample of cells was used to determine total cpm added, and the results are expressed as % cells bound ± s.e.m.

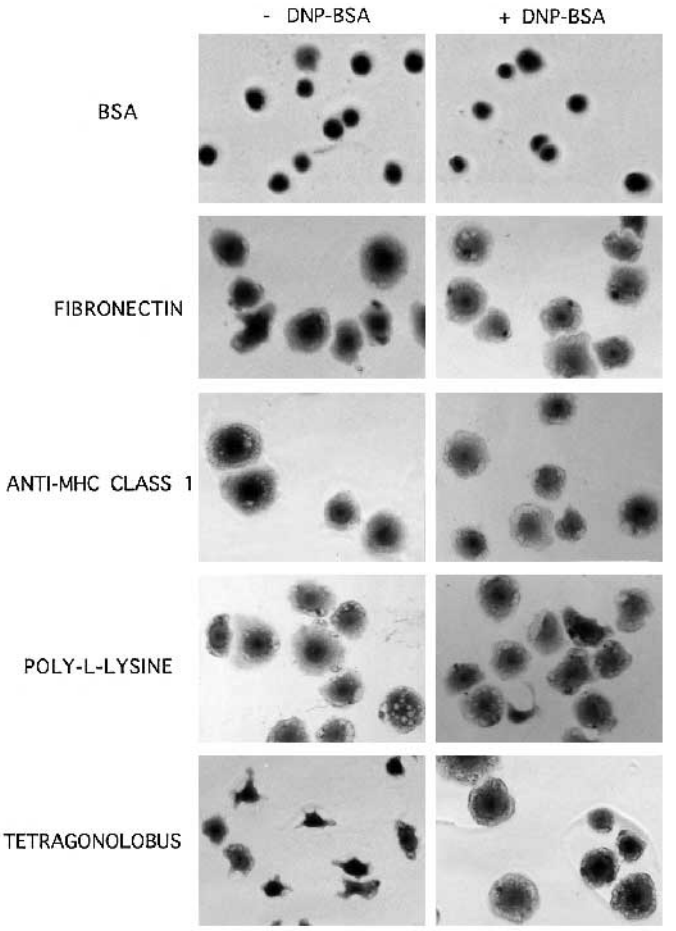

Fig. 2. Effect of cell activation on spreading to different substrates. IgE sensitized RBL cells were allowed to adhere to coated wells for 45 minutes before the addition of either buffer or DNP-BSA for an additional 30 minutes. The wells were coated with BSA (20 mg/ml), fibronectin (25 μg/ml), anti-class I (20 μg/ml), poly-L-lysine (5 μg/ml), or *Tetragonolobus* lectin (50 μg/ml). All of the wells, except those coated with BSA, were then washed 3 times and the adherent cells were fixed with formaldehyde and stained with Crystal Violet. Cells added to the BSA coated wells are not adherent. They were collected, fixed, stained, and added back to the wells for comparison.

trations above 5 μg/ml. A similar level of binding was seen in wells coated with poly-L-lysine as well as a monoclonal antisera directed against the rat class I major histocompatibility antigen, RI. Maximal binding to *Tetragonolobus* lectin required a concentration of over 20 μg/ml and only 30-40% of the cells bound. This lectin was chosen because it is known that it does not bind to the IgE receptor nor does it activate mast cells (Shibasaki et al., 1992).

Cells added to BSA coated wells remain non-adherent and spherical. However, adhesion to fibronectin caused considerable flattening and spreading of the cells (Fig. 2). Similar changes were seen when the cells adhered to anti-class I antibody and to poly-L-lysine. In contrast, adhesion to *Tetragonolobus* lectin caused relatively little spreading although the cells often appeared elongated with long, thin processes extending outward from the cell body. Addition of DNP-BSA to IgE sensitized cells caused little change, at this magnification, in the appearance of non-adherent cells or on cells spread on fibronectin, anti-class I, and poly-L-lysine. IgE activated cells added to BSA coated wells also remained unchanged and spherical. However, activation of cells through the IgE receptor on cells which were adherent on *Tetragonolobus purpureas* caused considerable spreading.

Photographs, such as in the preceeding figure, were scanned and the area of the cells was determined. Fig. 3A shows the distribution of the areas of the cells attached to various proteins. As can be seen, there is considerable variation in the area of the spread cells. Cells added to BSA coated wells were non-adherent, had a relative size of 1.00±0.17, and none of the cells were spread. The cells did adhere and spread on fibronectin (relative area=3.66±0.59, 100% spread), anti-class 1 (relative area=3.38±0.73, 100% spread), poly-L-lysine (relative area=2.78±0.55, 100% spread), and *Tetragonolobus* lectin (relative area=1.27±0.31, 35% spread). Fig. 3B shows

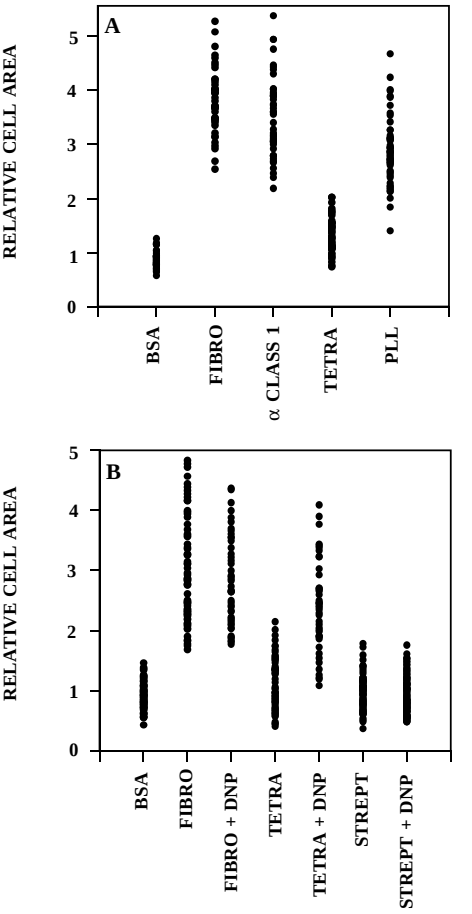

Fig. 3. Quantitation of cells spreading on different substrates. Photographs of random fields of adherent cells, such as is seen in Fig. 2, were scanned, digitized, and the area of individual cells was quantitated. In all cases between 100 and 200 cells were measured and each cell was plotted to show the distribution of sizes. The results have been normalized so that 1.00 is the average area for a non-adherent (BSA) cell. (A) The size distribution of cells spread on fibronectin, anti-class 1, *Tetragonolobus* lectin, and poly-L-lysine as compared to non-adherent cells (BSA). (B) The effects of cell activation on the area of cells spread on fibronectin and *Tetragonolobus* lectin as well as the area of activated and unactivated biotin-PS cells spread on Streptavidin.

Table 1. Degranulation of RBL cells on different adhesive substrates*

Substrate	% Adherent	% Degranulation (–DNP-BSA)	% Degranulation (+DNP-BSA)	% Specific degranulation
BSA	1.6	5.5	21.5	16.0
Fibronectin	53.6	5.9	39.9	34.0
Anti-class I	65.4	5.8	36.0	30.2
Tetragonolobus lectin	21.3	5.7	28.0	22.3
Poly-L-lysine	51.5	7.0	37.0	30.0

*IgE sensitized RBL cells were allowed to adhere to BSA (20 mg/ml), fibronectin (25 μg/ml), anti-class I (20 μg/ml), poly-L-lysine (5 μg/ml), or *Tetragonolobus* lectin (50 μg/ml) coated wells for 30 minutes at 37 °C. [^{3}H]5HT release was determined after a 45 minute incubation with either DNP-BSA or buffer at 37 °C. The maximum error in all cases was less than 5%. In this particular experiment, [^{3}H]5HT was also used to determine the percentage adherence.

the distribution of the area of the cells spread on fibronectin and *Tetragonolobus* lectin after activation through FcεR1. There is no change seen in the area of cells spread on fibronectin but activated cells do tend to spread when attached to *Tetragonolobus* (relative area=2.30±0.90, 70% spread).

Previous studies had shown that adhesion to fibronectin caused increased degranulation when the cells were triggered through FcεR1 (Hamawy et al., 1992). In order to determine if increased degranulation was specifically due to adhesion to fibronectin or whether adhesion and spreading itself was responsible, sensitized cells were allowed to adhere to fibronectin, anti-class I, poly-L-lysine, and *Tetragonolobus* lectin for 45 minutes before being triggered with DNP-BSA. Table 1 shows that adherence to any of the substrates did not cause any degranulation on its own but did lead to increased degranulation when the cells were activated through FcεR1. Degranulation was the greatest when cells were bound to fibronectin, followed by anti-class I, poly-L-lysine, and *Tetragonolobus* lectin.

The previous results suggested that there might be two classes of cells, with adherent cells degranulating very well while non-adherent cells degranulate relatively poorly. In Fig. 4A, cells were added to wells containing increasing concentrations of fibronectin. The cells were allowed to adhere for 45 minutes and then were triggered with DNP-BSA for an additional 45 minutes. There is a close correlation between the degree of adhesion and the level of degranulation as the concentration of fibronectin increases. In Fig. 4B, the cells were allowed to adhere for 45 minutes but non-adherent cells were removed before the addition of DNP-BSA. Thus, degranulation was measured in adherent cells only. Increasing concentrations of fibronectin led to increasing numbers of cells adhering but the level of degranulation from all of these adherent cells was approximately the same. This result supports the hypothesis that there are two groups of cells in terms of their degranulation capacity. Similar results were seen with cells attached and spread on anti-class I, poly-L-lysine, and *Tetragonolobus* lectin (data not shown).

In addition to degranulation, activated mast cells secrete leukotrienes and prostaglandins which are also very important in the inflammatory response. In order to determine whether adhesion also affects eicosanoid production, sensitized cells were allowed to adhere to increasing concentrations of fibronectin. The cells were then triggered with DNP-BSA and the levels of LTC_4 and PGD_2 produced were determined. In the absence of antigen, extremely low levels of PGD_2 and LTC_4 were present regardless of whether the cells were adherent or not. Non-adherent RBL cells which had been triggered by antigen produced 1,500 pg of $LTC_4/10^6$ cells while adherent cells released almost 3,000 pg (Fig. 5A). A similar increase was seen in the production of PGD_2 (Fig. 5B). Thus, both degranulation and the production of LTC_4 and PGD_2 increased as a function of the number of cells bound to fibronectin. Similarly, adhesion to anti-rat class I antibodies, poly-L-lysine, and the lectin from *Tetragonolobus purpureas* also caused increased release of PGD_2 (Fig. 6). Thus, PLA_2 activity is also

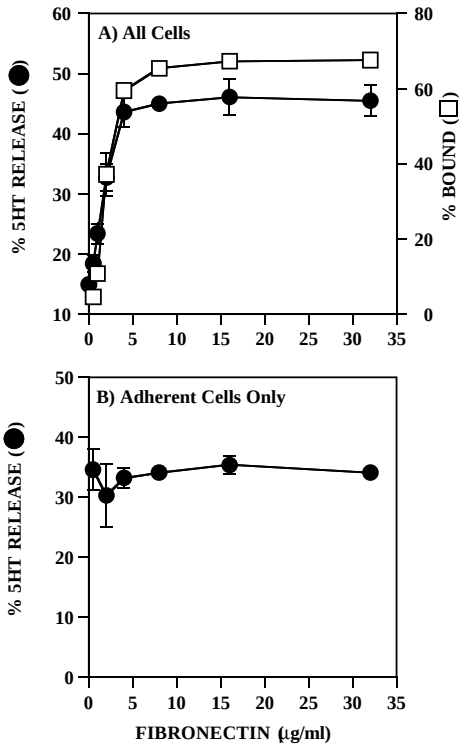

Fig. 4. Effect of adhesion to fibronectin coated wells on degranulation of RBL cells. RBL cells, sensitized with IgE, were added to wells coated with different concentrations of fibronectin. The cells were allowed to adhere for 30 minutes at 37°C. (A) Cells were triggered for 45 minutes with DNP-BSA and [^{3}H]5HT release from all of the cells added, both adherent and non-adherent, was determined. (B) After allowing the cells to adhere, the wells were washed three times to remove unbound cells. The adherent cells were then activated with DNP-BSA for 45 minutes. Degranulation of adherent cells only was assayed. SDS extracts of the cells in each well were used to determine the unreleased material in order to calculate percentage release.

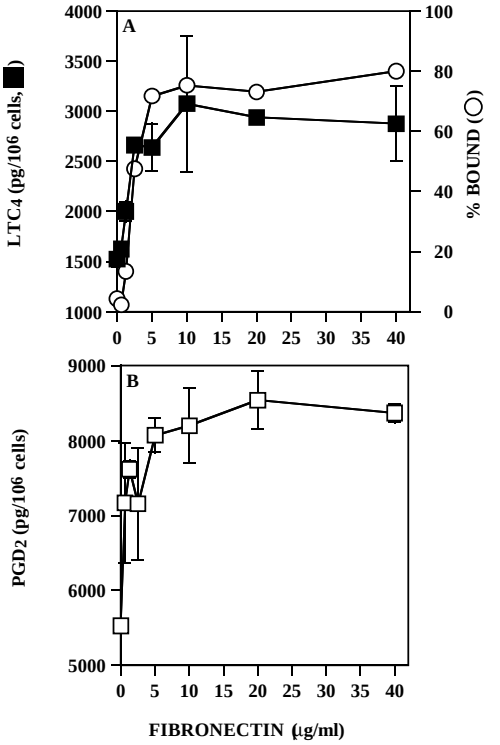

Fig. 5. Eicosanoid production from cells bound to fibronectin coated wells. IgE sensitized RBL cells were added to wells coated with different concentrations of fibronectin for 30 minutes at 37°C. The cells were activated with DNP-BSA for 45 minutes, and the supernatants were tested for LTC_4 content (A) or PGD_2 content (B).

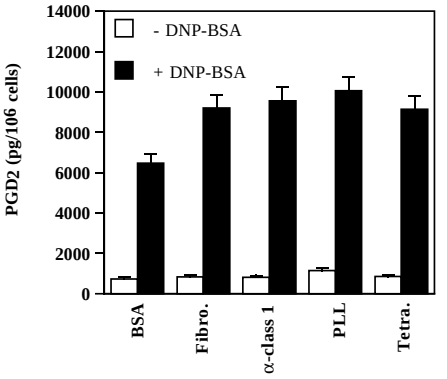

Fig. 6. Effect of adhesion to different substrates on PGD_2 production. IgE sensitized RBL cells were added to wells which had been coated with BSA (20 mg/ml), fibronectin (25 μg/ml), anti-class I (20 μg/ml), poly-L-lysine (5 μg/ml), or *Tetragonolobus* lectin (50 μg/ml). The cells were allowed to adhere for 30 minutes at 37°C followed by activation with DNP-BSA for 45 minutes. The supernatants were assayed for PGD_2 content.

upregulated by adhesion to these different substrates although adhesion itself does not trigger eicosanoid production.

It is not known why adhesion and spreading of RBL cells causes increased degranulation although increased tyrosine phosphorylation has also been reported (Hamawy et al., 1993a). Activation of PLCγ1, which leads to PKC activation and an increase in intracellular Ca^{2+}, is a critical event leading to IgE mediated degranulation in RBL cells (Beaven and Cunha-Melo, 1988). Another important signaling pathway in these cells is mediated by PLD. In order to determine whether these pathways are also upregulated, cells were allowed to adhere to the various substrates and were triggered with antigen. PLC activity was assessed by measuring the generation of inositol phosphates. The production of phosphatidylethanol, which is produced when the assay is performed in the presence of 0.5% ethanol, is a measure of PLD activity. Adhesion and spreading of RBL cells on the different substrates had no effect on basal levels of either the inositol phosphates or phosphatidylethanol thus indicating that adhesion and spreading does not by itself activate these phospholipases (Table 2). When the cells were stimulated through FcεR1 by the addition of the multivalent antigen DNP-BSA, there was a large increase in both inositol phosphate production and phosphatidylethanol production. As in the case of degranulation, cells which were adherent on the various substrates showed much greater PLC and PLD activity than non-adherent cells which were in BSA coated wells.

Adhesion of the cells to the various adhesive substrates that have been tested all involve binding through integral membrane proteins. Although it is not known whether binding of any of these membrane proteins other than the fibronectin receptor induces any signaling, it is a possibility, and this may lead to increased degranulation. Therefore, cells were incubated with liposomes containing biotinylated phosphatidylserine. This modified lipid was incorporated into the plasma membrane of the cells which allowed them to bind to streptavidin coated wells. Although 50-70% of the cells bound (Fig. 7A), they did not spread to any appreciable extent (Fig. 8). Thus, binding can occur in the absence of detectable spreading. However, it took 90-120 minutes for maximal binding to occur which is in contrast to the binding to fibronectin coated wells which occurs

Table 2. Inositol phosphate and phosphatidylethanol production in RBL cells on different adhesive substrates*

	Inositol phosphate production†			Phosphatidylethanol production‡		
Substrate	−DNP-BSA	+DNP-BSA	Specific	−DNP-BSA	+DNP-BSA	Specific
BSA	9,985	26,883	16,898	485	863	378
Fibronectin	9,601	44,965	35,364	410	1,011	601
Anti-class I	9,144	50,055	40,911	428	1,112	684
Poly-L-lysine	10,347	37,996	27,649	498	983	485

*IgE sensitized RBL cells were allowed to adhere to the different substrates for 30 minutes at 37 °C before activation with DNP-BSA for 45 minutes. Wells were coated with BSA (20 mg/ml), fibronectin (25 μg/ml), anti-class I (20 μg/ml), or poly-L-lysine (5 μg/ml).

†Adherent cells were pre-incubated with LiCl (10 mM) which was also present during activation. The error in all cases was less than 8%.

‡Adherent cells were pre-incubated with 0.5% ethanol for 10 minutes before the addition of antigen. The error in all cases is less than 11%.

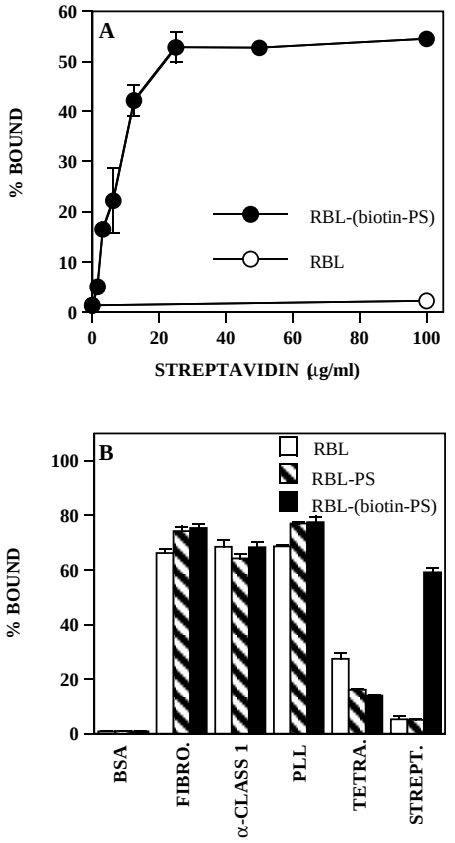

Fig. 7. Binding of biotin-PS modified cells to different substrates including streptavidin. The wells were coated with BSA (20 mg/ml), fibronectin (25 μg/ml), anti-class I (20 μg/ml), poly-L-lysine (5 μg/ml), or *Tetragonolobus* lectin (50 μg/ml). (A) Control cells and RBL cells modified with biotin-PS were added for 90 minutes at 37°C to wells coated with different concentrations of streptavidin. (B) Control cells, cells modified with PS and cells modified with biotin-PS were added to wells coated with different substrates for 90 minutes at 37°C.

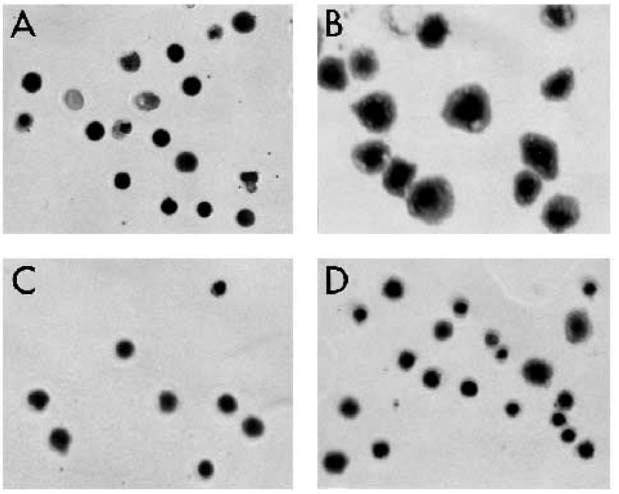

Fig. 8. Adhesion and spreading of biotin-PS modified cells on different substrates. Biotin-PS modified RBL cells were added to (A) BSA coated wells, (B) fibronectin coated wells, and (C,D) streptavidin coated wells for 90 mintues at 37°C. The cells which had been added to the streptavidin coated wells were then stimulated with either buffer (C) or DNP-BSA (D) for an additional 30 minutes. All of the wells, except the one coated with BSA, were then washed 3 times and the adherent cells were fixed and stained. Cells added to the BSA coated well are not adherent. They were collected, fixed, stained, and added back to the wells for comparison.

in 20 minutes. Cells which were not modified or modified with just phosphatidylserine alone did not bind to the streptavidin coated wells at any concentration (Fig. 7A). In addition, modification of the cells with biotin-PS did not have any effect on the binding of these cells to other substrates. As can be seen in Fig. 7B, control cells, cells modified with phosphatidylserine, and cells modified with biotin-PS were allowed to adhere to fibronectin, anti-class I, poly-L-lysine, *Tetragonolobus* lectin, and streptavidin. The binding was virtually identical except in the case of streptavidin. Over 60% of the biotin-PS modified cells bound while binding of the other cell types was less than 5%. Thus adhesion to streptavidin coated surfaces is specific for biotin-PS modified cells.

The effect of adhesion of biotin-PS modified cells to streptavidin coated surfaces on degranulation was tested. Unmodified cells and biotin-PS modified cells were allowed to adhere to BSA, fibronectin, or streptavidin. Unmodified cells did not bind to BSA or streptavidin and less than 20% specific degranulation was seen (Table 3). However, these cells did bind to fibronectin and showed increased degranulation on this substrate. Biotin-PS cells also did not bind to BSA, and the level of degranulation was the same as for unmodified cells. However, although these cells bound to both fibronectin and streptavidin, only fibronectin promoted increased degranulation. Over 50% of the cells bound to streptavidin, but there was no increase in specific degranulation as compared to non-adherent cells. As seen in Fig. 8, biotin-PS modified cells also did not spread on streptavidin even after they were triggered with DNP-BSA. Fig. 3B shows that biotin-PS modified cells bound to streptavidin had a relative area of 1.03±0.30 as compared to non-adherent cells and only 3% of the cells were spread. Biotin-PS modified cells activated through FcεR1 had a relative area of 1.01±0.37 and 4% were spread. However, these cells were able to spread on fibronectin thus indicating that modification with biotin-PS had not impaired their ability to spread. These results indicate that adherence itself is not sufficient to trigger augmented degranulation and that the ability to spread may be crucial to this phenomenon.

The previous results using biotin-PS modified cells suggested that it is spreading which may be important in increased FcεR1 mediated degranulation. In order to determine further whether adhesion due to engagement of the integrins is important in increased degranulation or whether it is the subsequent spreading of the cells, 3 μm polystyrene beads were coated with either BSA or fibronectin. Fibronectin coated beads adhere to RBL cells and cover the surface while BSA coated beads do not. This size of beads was used because they are much smaller than the cells and are not large enough to allow spreading. As can be seen in Table 4, fibronectin coated beads did not cause increased degranulation when the cells were activated by antigen. The level of degranulation was only increased when the cells were able to spread on fibronectin coated wells. Furthermore, cells which were activated and had

Table 3. Degranulation of biotin-PS modified RBL cells on fibronectin and streptavidin*

	Unmodified cells		Biotin-PS modified cells	
Substrate	% Adherent	% Specific [^{3}H]5HT release	% Adherent	% Specific [^{3}H]5HT release
BSA	0.5±0.02	18.6±1.8	0.2±0.01	16.9±0.4
Fibronectin	75.0±4.69	31.6±1.8	74.2±1.47	31.6±1.2
Streptavidin	0.5±0.25	17.3±0.6	54.7±1.01	17.4±0.6

*Biotin-PS and unmodified cells were allowed to adhere for 60 minutes before activation with DNP-BSA for 45 minutes at 37 °C. The wells were coated with BSA (20 mg/ml), fibronectin (20 μg/ml), or streptavidin (50 μg/ml). Specific degranulation is the % released in the presence of DNP-BSA minus the % released in the presence of buffer. In this experiment, [^{3}H]5HT was also used to determine the percentage adherence.

bound fibronectin coated beads show no increased inositol phosphate production or phosphatidylethanol production as compared to activated cells incubated with BSA coated beads. In these experiments, the number of 3 μm beads added provided an equal surface area to that of the wells. The beads were coated with fibronectin to give a concentration 5 times greater than that immobilized on the wells. Under these conditions, the cells had 30-50% of their surface area coated with beads and very little increase in degranulation was seen. However, very high densities of fibronectin on the 3 μm beads did cause increased degranulation although they also caused massive clumping of the cells and beads into very large aggregates (data not shown). Small beads (1 μm) never caused any increased degranulation even at high fibronectin concentrations (data not shown). Beads coated with the 120 kDa α-chymotryptic fragment of fibronectin gave similar results. Only when large aggregates of cells and beads occurred did augmented degranulation occur. These results support the conclusions drawn from the degranulation experiments involving biotin-PS modified cells bound to streptavidin and indicate that spreading of the cells is important in increased PLC and PLD activity.

Table 4. Degranulation of RBL cells attached to fibronectin coated wells and beads*

	% Specific degranulation†	Specific inositol phosphate production (CPM)‡	Specific phosphatidyethanol production (CPM)§
BSA coated wells	14.8	7,613	446
Fibronectin coated wells	23.7	10,417	754
BSA beads	16.8	7,134	402
Fibronectin beads	16.0	7,397	426

*IgE sensitized RBL cells were added either to BSA (20 mg/ml) or fibronectin (20 μg/ml) coated wells or were mixed with either fibronectin or BSA coated beads and added to BSA coated wells. After 30 minutes, either buffer or 25 ng/ml DNP-BSA was added to the wells.

†[^{3}H]5HT release was determined after a 45 minute incubation with either DNP-BSA or buffer at 37 °C. Background release, which has been subtracted, was 6.5%, 4.9%, 6.6%, and 7.1%, respectively, for the four conditions listed. The maximum error in all cases was less than 6%.

‡[^{3}H]Inositol phosphate production was determined by activating the cells for 45 minutes with DNP-BSA in the presence of LiCl. Background production, which has already been subtracted, was 10,623 cpm for BSA coated wells, 11,314 cpm for fibronectin coated wells, 10,019 cpm for BSA coated beads, and 10,406 cpm for fibronectin coated beads. The error in all cases was less than 9% in all cases.

§[^{3}H]Phosphatidylethanol production was measured by activating the cells with DNP-BSA in the presence of 0.5% ethanol for 45 minutes at 37 °C. Background production, which has already been subtracted, was 294 cpm for BSA coated wells, 270 cpm for fibronectin coated wells, 276 cpm for BSA coated beads, and 314 cpm for fibronectin coated beads. The error in all cases was less than 10%.

DISCUSSION

It has been shown here and by other investigators (Hamawy et al., 1992) that RBL cells adhere and spread on fibronectin coated wells. These cells will also adhere to other non-physiological substrates such as anti-class I, poly-L-lysine, and the lectin from *Tetragonolobus purpureas*. Surprisingly, the cells are also able to spread on all of these substrates except *Tetragonolobus purpureas*. However, in the case of *Tetragonolobus purpureas*, the cells are able to spread if they are activated through FcεR1. Upon activation, adherent cells degranulate to a much greater degree than non-adherent cells. This seems to occur regardless of whether the cells are bound to a physiological substrate such as fibronectin or a non-physiological substrate such as anti-class I, poly-L-lysine, or *Tetragonolobus* lectin. Similar findings have also been reported by Siraganian and colleagues (Hamawy et al., 1993a) using antibodies against RBL cell surface proteins in order to bind the cells to the plate. We have also found that in addition to degranulation, adherent cells also show much greater PLC activity, PLD activity and eicosanoid production. Thus, adherent cells which have been triggered with multivalent antigen seem to display increased levels of activation as measured by a number of parameters. However, the adherent cells do show a wide distribution in area as well as shape. Because degranulation cannot be measured on individual cells, it is not known whether there is a size threshold for this effect or whether a large, spread cell degranulates to a greater extent than another spread cell which is smaller in area. Similarly, there is no way to quantitate or manipulate the shapes of the cells so that the importance of this parameter is also unknown.

Integrins form a bridge which links the extracellular matrix to the cytoskeleton and in the process they may form a signal transduction center (Clark and Brugge, 1995; Juliano and Haskill, 1993; Richardson and Parsons, 1995). Certainly a number of signaling pathways are activated when cells adhere to extracellular matrix proteins. In other cell types increases or changes have been seen in intracellular Ca^{2+}, pH, phosphatidylinositol-3-kinase activity, tyrosine kinase activity, arachidonic acid metabolites, MAP kinase activity, PKC activity, cAMP, and phosphatidylinositol bis-phosphate (Clark and Brugge, 1995; Juliano and Haskill, 1993; Richardson and Parsons, 1995). In many cell types FAK becomes autophos-

phorylated and associated with the clustered integrins. This tyrosine kinase is then partly responsible for the tyrosine phosphorylation cascade which ensues. Although RBL cells do not form classical focal adhesion plaques (Pfeiffer and Oliver, 1994), there is increased phosphorylation of FAK upon adhesion to fibronectin (Hamawy et al., 1993b). This phosphorylation is further increased if the adherent cells are activated through FcεR1. Furthermore, adhesion and spreading of RBL cells on antibody coated plates did not cause phosphorylation of FAK indicating that it is integrin specific. The conclusion from their work was that phosphorylation of FAK was not necessary for the increased degranulation nor was it necessary for adhesion and spreading.

The results presented here show that neither adherence to fibronectin nor the other substrates causes any changes in the basal levels of PLC activity, PLD activity, or eicosanoid production which is controlled by PLA_2 activity. It is not known what is responsible for the increased degranulation seen in adherent RBL cells, but if it is a signal then it is not short-lived since the cells can be spread for hours before being triggered and still show an augmented response. In addition, the data suggest that if a specific signal is sent, then adhesion to several non-physiologic substrates is also able to transduce the signal. Both PLC activity and PLD activity are critical for degranulation but since both are upregulated when the cells are adherent, it is impossible to tell if one single pathway is important in increased degranulation. Since tyrosine phosphorylation also seems to be upregulated, it would not be surprising if other pathways also showed increased activity.

The fact that increased degranulation and signaling was seen when the cells were adherent and spread on non-physiological substrates indicates that it is not the integrins themselves which are critical but adhesion and spreading. In addition, several lines of evidence also support the conclusion that it is spreading which is important and not simply adhesion. First, RBL cells can be modified with biotinylated-PS which becomes incorporated into the plasma membrane. These cells are able to bind to the other substrates and spread. Under these conditions they also exhibit augmented degranulation. They are also able to adhere to streptavidin coated wells, through the biotinylated phospholipid, although they remain round and do not spread even after being activated through FcεR1. They also did not exhibit increased signaling or degranulation under these conditions. Second, fibronectin coated small polystyrene beads bind quite effectively to cells thus mimicking adhesion but they do not support spreading of the cells. Binding of fibronectin coated beads followed by activation of the cells does not cause increased degranulation, PLC activity, PLD activity, or eicosanoid production. There is, however, no positive control for determining whether the integrins were activated under these conditions or whether enough receptors were engaged. Given this reservation, in these two cases, adherent cells which have not spread do not show any increased activity.

It is not known why RBL cells are able to spread on non-physiological substrates such as anti-class I and poly-L-lysine although it may have to do with the fact that these are tumor cells. It is also not known why cells modified with biotin-PS do not spread on streptavidin coated plates. The fact that they can spread on fibronectin as well as the other non-physiological substrates indicates that the modified cells are still able to spread. All of these non-physiological substrates which lead to spreading bind to transmembrane proteins which could potentially be involved in signaling or could be attached to the cytoskeleton thus allowing the cells to spread. In most cell types, spreading occurs primarily through integrin mediated adhesion (Hemler, 1990; Hynes, 1992). Clustering of the integrins results in the polymerization of actin and rearrangement of the cytoskeleton which in turn is involved in cell spreading. Many cell types form focal adhesion plaques which contain clusters of integrins, actin binding proteins such as talin, vinculin, and α-actinin, and microfilaments (Burridge et al., 1988). RBL cells adhere and spread on a variety of coated surfaces and this does not require activation of the cells. However, upon activation through FcεR1, there is re-distribution of actin into actin rich plaques, membrane ruffles, and stress fibers (Pfeiffer and Oliver, 1994). In addition, there is redistribution and co-localization of vinculin and talin (Kawasugi et al., 1995). Adhesion of these cells is known to cause tyrosine phosphorylation of several proteins and spreading is dependent on both activation of tyrosine kinases and PKC (Pfeiffer and Oliver, 1994; J. R. Apgar, unpublished observations).

In several cell systems, adhesion and spreading on extracellular matrix proteins has been shown to augment receptor mediated events. Previous studies in RBL cells demonstrated increased antigen induced degranulation when the cells were adherent on various substrates (Hamawy et al., 1992). In addition, the cytokine induced respiratory burst in neutrophils (Nathan, 1987) and phagocytosis of opsonized material in macrophages is augmented when the cells are adherent (Newman and Tucci, 1990). The studies presented here show that in addition to degranulation, PLC activity, PLD activity, and the generation of eicosanoids are all increased when adherent and spread RBL cells are triggered with antigen. It was also shown that it is spreading of the cells and not adhesion alone which is important. Thus the physical shape of the cell may be crucial to its responses to external stimuli. An interesting hypothesis in this regard has been proposed by Ingber and colleagues (Ingber, 1993; Schwartz and Ingber, 1994). They believe that binding of integrins to the extracellular matrix organizes the cytoskeleton thus allowing mechanical signaling. Thus, in addition to being involved in chemical signaling, integrins may also be acting as mechanoreceptors. In fact, they have proposed that it is through the integrins that the cell can coordinate both chemical and mechanical signals. The fact that integrins could sense mechanical forces was shown by coating small magnetic beads with peptides containing the RGD sequence (Wang et al., 1993). These beads were allowed to bind to endothelial cells and then a magnetic force was applied which caused the beads to twist. This in turn led to focal adhesion formation and a stiffening response thus demonstrating that the integrins were sensing a mechanical force. It has further been found that in many cell types such as endothelial cells and hepatocytes, growth only occurs when the cells are adherent. Hansen et al. (1994) found that primary rat hepatocytes will adhere to surfaces coated with either fibronectin or a synthetic RGD peptide but the cells will only spread on fibronectin. Furthermore, in the presence of growth factors, only the cells which have spread are able to enter S phase. Thus, in several cell types including RBL cells, cell shape is critical to the functioning of the cell. Further research will be necessary in order to determine the mechanism by which spreading of RBL cells leads to increased activation.

I thank Dr Belen Ybarrondo and Dr Fu-Tong Liu for their helpful discusions and Dr Don Bellgrau for the monoclonal anti-rat class I antibody. This work was supported by National Institutes of Health grant RO1-GM42388. This is manuscript number 10291-MEM from the Department of Molecular and Experimental Medicine.

REFERENCES

Apgar, J. R. (1991). Regulation of the antigen-induced F-actin response in rat basophilic leukemia cells by protein kinase C. *J. Cell Biol.* **112**, 1157-1163.

Barsumian, E. L., Isersky, C., Petrino, M. G. and Siraganian, R. P. (1981). IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur. J. Immunol.* **11**, 317-323.

Bayer, E. A., Rivnay, B. and Skutelsky, E. (1979). On the mode of liposome-cell interactions. Biotin-conjugated lipids as ultrastructural probes. *Biochim. Biophys. Acta* **550**, 464-473.

Beaven, M. A. and Cunha-Melo, J. R. (1988). Membrane phosphoinositide-activated signals in mast cells and basophils. *Prog. Allergy* **42**, 123-184.

Beaven, M. A. and Metzger, H. (1993). Signal transduction by Fc receptors: the FcεR1 case. *Immunol. Today* **14**, 222-226.

Berridge, M. J., Downes, C. P. and Hanley, M. R. (1982). Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**, 587-595.

Bianchine, P. J., Burd, P. R. and Metcalfe, D. D. (1992). IL-3-dependent mast cells attach to plate-bound vitronectin. *J. Immunol.* **149**, 3665-3671.

Burridge, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988). Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* **4**, 487-525.

Clark, E. A. and Brugge, J. S. (1995). Integrins and signal transduction pathways: the road taken. *Science* **268**, 233-239.

Dastych, J., Costa, J. J., Thompson, H. L. and Metcalfe, D. D. (1991). Mast cell adhesion to fibronectin. *Immunology* **73**, 478-484.

Gruchalla, R. S., Dinh, T. T. and Kennerly, D. A. (1990). An indirect pathway of receptor-mediated 1,2-diacylglycerol formation in mast cell. I. IgE receptor-mediated activation of phospholipase D. *J. Immunol.* **144**, 2334-2342.

Hamawy, M. M., Oliver, C., Mergenhagen, S. E. and Siraganian, R. P. (1992). Adherance of rat basophilic leukemia (RBL-2H3) cells to fibronectin-coated surfaces enhances secretion. *J. Immunol.* **149**, 615-621.

Hamawy, M. M., Mergenhagen, S. E. and Siraganian, R. P. (1993a). Cell adherence to fibronectin and the aggregation of the high affinity immunoglobulin E receptor synergistically regulate tyrosine phosphorylation of 105-115-kDa proteins. *J. Biol. Chem.* **268**, 5227-5233.

Hamawy, M. M., Mergenhagen, S. E. and Siraganian, R. P. (1993b). Tyrosine phosphorylation of $pp125^{FAK}$ by the aggregation of high affinity immunoglobulin E receptors requires cell adherence. *J. Biol. Chem.* **268**, 6851-6854.

Hamawy, M. M., Swaim, W. D., Minoguchi, K., de Feijter, A. W., Mergenhagen, S. E. and Siraganian, R. P. (1994). The aggregation of the high affinity IgE receptor induces tyrosine phosphorylation of paxillin, a focal adhesion protein. *J. Immunol.* **153**, 4655-4662.

Hansen, L. K., Mooney, D. J., Vacanti, J. P. and Ingber, D. E. (1994). Integrin binding and cell spreading on extracellular matrix act at different points in the cell cycle to promote hepatocyte growth. *Mol. Biol. Cell* **5**, 967-975.

Hemler, M. E. (1990). VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.* **8**, 365-400.

Holowka, D. and Baird, B. (1990). Structure and function of the high-affinity receptor for immunoglobulin E. *Cell. Mol. Mech. Inflam.* **1**, 173-197.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signalling in cell adhesion. *Cell* **69**, 11-25.

Ingber, D. E. (1993). Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *J. Cell Sci.* **104**, 613-627.

Juliano, R. L. and Haskill, S. (1993). Signal transduction from the extracellular matrix. *J. Cell Biol.* **120**, 577-585.

Kawasugi, K., French, P. W., Penny, R. and Ludowyke, R. I. (1995). Focal adhesion formation is associated with secretion of allergic mediators. *Cell Motil. Cytoskel.* **31**, 215-224.

Kennerly, D. A. (1990). Phosphatidylcholine is a quantitatively more important source of increased 1,2-diacylglycerol than is phosphatidylinositol in mast cells. *J. Immunol.* **144**, 3912-3919.

Lin, P. Y., Wiggan, G. A. and Gilfillan, A. M. (1991). Activation of phospholipase D in a rat mast (RBL 2H3) cell line. A possible unifying RBL-2H3 mechanism for IgE-dependent degranulation and arachidonic acid metabolite release. *J Immunol.* **146**, 1609-1616.

Lin, P. and Gilfillan, A. M. (1992). The role of calcium and protein kinase C in the IgE-dependent activation of phosphatidylcholine-specific phospholipase D in a rat mast (RBL-2H3) cell line. *Eur. J. Biochem.* **207**, 163-168.

Liu, F. T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R. and Katz, D. H. (1980). Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* **124**, 2728-2737.

Metzger, H., Alcaraz, G., Hohman, R., Kinet, J. P., Pribluda, V. and Quarto, R. (1986). The receptor with high affinity for immunoglobulin E. *Annu. Rev. Cell Biol.* **4**, 419-470.

Metzger, H. (1992). The receptor with high affinity for IgE. *Immunol. Rev.* **125**, 37-48.

Nathan, C. F. (1987). Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J. Clin. Invest.* **80**, 1550-1560.

Newman, S. L. and Tucci, M. A. (1990). Regulation of human monocyte/macrophage function by extracellular matrix. Adherence of monocytes to collagen matrices enhances phagocytosis of opsonized bacteria by activation of complement receptors and enhancement of Fc receptor function. *J. Clin. Invest.* **86**, 703-714.

O'Rourke, A. M. and Mescher, M. F. (1988). T cell receptor-mediated signalling occurs in the absence of inositol phosphate production. *J. Biol. Chem.* **263**, 18594-18597.

Oliver, J. M., Seagrave, J. C., Stump, R. F., Pfeiffer, J. R. and Deanin, G. G. (1988). Signal transduction and cellular response in RBL-2H3 mast cells. *Prog. Allergy* **42**, 185-245.

Pfeiffer, J. R., Seagrave, J. C., Davis, B. H., Deanin, G. G. and Oliver, J. M. (1985). Membrane and cytoskeletal changes associated with IgE-mediated serotonin release from rat basophilic leukemia cells. *J. Cell Biol.* **101**, 2145-2155.

Pfeiffer, J. R. and Oliver, J. M. (1994). Tyrosine kinase-dependent assembly of actin plaques linking FcεR1 cross-linking to increased cell substrate adhesion in RBL-2H3 tumor mast cells. *J. Immunol.* **152**, 270-279.

Richardson, A. and Parsons, J. T. (1995). Signal transduction through integrins: a central role for focal adhesion kinase. *BioEssays* **17**, 229-236.

Schwartz, M. A. and Ingber, D. E. (1994). Integrating with integrins. *Mol. Biol. Cell* **5**, 389-393.

Shibasaki, M., Sumazaki, R., Isoyama, S. and Takita, H. (1992). Interaction of lectins with human IgE: IgE-binding property and histamine-releasing activity of twelve plant lectins. *Inter. Archives Aller. Immunol.* **98**, 18-25.

Sims, J. R., Karp, S. and Ingber, D. E. (1992). Altering the cellular mechanical force balance results in integrated changes in cell, cytoskeletal and nuclear shape. *J. Cell Sci.* **103**, 1215-1222.

Thompson, H. L., Burbelo, P. D. and Metcalfe, D. D. (1990). Regulation of adhesion of mouse bone marrow-derived mast cells to laminin. *J. Immunol.* **145**, 3425-3431.

Valent, P. and Bettelheim, P. (1992). Cell surface structures on human basophils and mast cells: biochemical characterization. *Advan. Immunol.* **52**, 333-423.

Wang, N., Butler, J. P. and Ingber, D. E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260**, 1124-1127.

(Received 28 August 1996 – Accepted, in revised form, 8 January 1997)