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RELATIONS BETWEEN $Fc_{\varepsilon}RI$ CROSSLINKING-INDUCED MAST CELL ACTIVATION AND ADHESION TO FIBRONECTIN

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Demonstration of murine mast cell adhesion to fibronectin (FN) following PMA-mediated cell activation raised the question whether crosslinking of high affinity IgE receptors on mouse mast cells might induce changes in adhesiveness of these cells to FN. Murine mast cells of line MCP5/L were used to investigate the effect of antigenic stimulation on cell adhesion to FN and mediator secretion. Adhesion assays were performed using sensitized radiolabeled cells and FN- or BSA-coated 96-well plates. The presence of antigen in the concentrations up to 10 ng/ml resulted in concentration-dependent adhesion potentiation, which was detectable after 5 min, reached maximum at 30 min and persisted or decreased over the next 30 min. Adhesion potentiation decreased at antigen excess and was abolished by heat inactivation of IgE in the antiserum prior to cell treatment. External calcium ion and temperature dependence of adhesion together with the observation that RGD (Arg, Gly, Asp) — containing peptide blocked cell binding to FN suggests that $FC_{\varepsilon}RI$ crosslinking-induced adhesion potentiation involves an integrin type receptor on cell surface. Sensitized mast cells allowed to adhere spontaneously to FN released more histamine and β-hexosaminidase upon antigen challenge. Hence, the results show the relations between IgE-induced mast cell activation, adhesion to FN and mediator secretion.

Key words: *Adhesion to FN, mast cells, IgE, cell activation, mediator secretion.*

INTRODUCTION

The response to tissue injury in allergic and other inflammatory diseases is accompanied by an infiltration of mast cells into the damaged tissues. Interactions between mast cells and their surrounding extracellular matrix appear to play an important role in the biological functions of these cells. Cell activation-dependent attachment of mast cells from primary mouse bone marrow-derived mast cells (BMMC) to laminin, a major component of basement membrane, has been demonstrated (1). Both BMMC and the cells of MCP5 murine mast cells line (2) have been recently shown to adhere to

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fibronectin (FN), another ubiquitous extracellular matrix protein, following activation of the cells with phorbol myristate acetate (3) or Stem Cell Factor (4). Because of the effector role of mast cells in anaphylaxis and the possibility that specific cellular responses are generated in vivo as mast cells adhere to multiple extracellular matrix components either simultaneously or in sequence, we chose to examine whether crosslinking of high affinity IgE receptors on mouse mast cells might potentiate adhesion of these cells to FN and whether adhesion of these to FN can affect their IgE receptor crosslinking-induced mediator secretion. Therefore, in the present study, murine mast cells of line MCP5/L were used to investigate the effect of antignic stimulation on mast cell adhesion to FN as well as the effect of mast cell adhesion on antigen-induced mediator release.

MATERIALS AND METHODS

Materials

Human fibronection, foetal calf serum (FCS), bovine albumin (BSA), ovalbumin grade V (OA), RPMI 1640, sodium pyruvate , sodium bicarbonate 7.5%, EGTA, Triton X-100, phorbol myristate acetate(PMA), glycine, p-nitrophenyl N-acethyl-β-D-glucosaminide and RGDS peptide (Sigma Chemical Company, St. Louis, MO, USA), Cellex P (Bio-Rad Laboratories, Richmont, California, USA), L-glutamine, nonessential amino acids and penicillin/streptomycin (Gibco Ltd, Paisley, U.K.), HEPES (Serva Feinbiochemica, Heidelberg), 2-mercaptoethanol (Merck, Darmstadt, Germany), thymidine [methyl-^{3}H], 15.7 Ci/mmol (Amersham International plc., Aylesbury, Buckinghamshire, England), histamine enzyme immunoassay kits (Immunotech International, Marseille, France), and 96-well flat bottom polystyrene plates (Corning Laboratory Sciences Company, Vernon, N.Y., USA) were purchased from manufacturers. WEHI-3 cell line and MCP5 murine cell line were obtained from Mast Cell Physiology Section, Laboratory of Clicical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA). The MCP5 cells maintained in our laboratory were named MCP5/L.

Mast cell cultures

MCP5/L cells were maintained as previously decribed (4), in RPMI-1640 media supplemented with 25 mM HEPES, 50 μM 2-mercaptoethanol, 4 mM L-glutamine, 100 μg/ml penicillin/streptomycin, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate, 10% heat inactivated foetal calf serum (FCS) and 10% WEHI-3 supernatant as a source of IL-3 (complete RPMI). Cells were harvested weekly, with remaining cells resuspended in fresh media at 5×10^5 cells/ml. To initiate primary bone marrow cultures, bone marrow was obtained from femurs of 6 week old female BALB/c mice and cells were suspended in complete RPMI. The cells were centrifuged at $400 \times g$ for 10 min and resuspended in coplete RPMI medium three times. Cell number was then adjusted to 2×10^5 cells/ml and cells were cultured at 37°C in a CO_2 incubator. At weekly intervals, cells were centrifuged and the cell pellets were resuspended in fresh media. In 4 to 5 week old cultures, mast cell number was assessed after toluidine blue staining. BMMC used in experiments consisted of greater than 95% mast cells and were of 96% or greater viability as determined by trypan blue dye exclusion.

Production of mouse reaginic serum

Female BALB/c mice 8—10 weeks old were immunized by four subcutaneous injections of 1 μg OA adsorbed on 1 mg $Al(OH)_3$ gel, given 2—4 weeks apart, as previously described (5). One week after the last injection the blood for serum was collected. IgE titre of the serum was 2560, as determined by Passive Cutaneous Anaphylaxis in Wistar rats (6). Incubation of the serum at 56°C (2 h) abolished its ability to sensitize mast cells.

Cell sensitization and adhesion assay

Mast cells were sensitized with mouse reaginic serum containing anti-OA IgE antibodies. Cells at the concentration 1×10^6 cells/ml in complete RPMI medium were incubated with mouse reaginic serum (or normal mouse serum) in final dilution 1 : 10 and with [^{3}H] thymidine in a final concentration of 10 μCi/ml for 18 h in a CO_2 incubator. Cells were then separated from free [^{3}H] thymidine by centrifugation at $400 \times g$ for 10 min at room temperature, resuspended in complete RPMI medium (without FCS and WEHI-3 conditioned medium), centrifuged once more and resuspended at 4×10^5 cells/ml prior to use in adhesion experiments. The adhesion assay was performed as described (3). Briefly, rows A and B of 96-well plates were coated with human FN for 18 h at 4°C using FN diluted in PBS at concentration 10 μg/ml (100 μl of FN solution/well). Control wells were incubated with PBS alone. PBS (0.1 ml) containing 4% BSA was then added to each well and the plates were incubated for 2 h at 37°C to inhibit non-specific binding to polystyrene (7). The wells were then gently rinsed twice with 0.1 ml complete RPMI (without FCS and WEHI-3 conditioned medium) and 0.1 ml of cell suspension (containing 4×10^4 cells) were added to each well. To determine the effect of IgE receptor crosslinking the specific antigen (OA) dissolved in RPMI at concentrations indicated was added to selected wells. RPMI for rinsing of the assay wells was located in rows E, F, G, and H. Rows C and D remained empty. The plates were then placed in CO_2 incubator for 30 min unless otherwise indicated. When adhesion to FN was stimulated by PMA, non-sensitized mast cells were used and adhesion was measured after 90 min of incubation of the cells in FN-coated wells (3). After incubation, cell suspensions containing nonadherent cells were transferred from assay wells to rows C and D using a 12-channel pipette. The assay wells were then rinsed twice with RPMI from rows E, F, G and H to remove remaining nonadherent cells and rinsing media were returned to the original wells. The adherent cells in the wells A and B as well as the nonadherent cells in wells C to H were then harvested using SKATRON cell harvester, Norway. Adherent cells from every well was harvested on one filter and nonadherent cells from two corresponding wells together on a second filter. The filter-bound radioactivity wa measured in a liquid scintillation counter (WALLAC 1410, LKB, Pharmacia, Sweden) using Omnifluor (Bio-Rad) as a scintillation fluid. The radioactivity in the adherent and nonadherent cells was considered as an index of cell number and the percentage of cell adhesion was calculated by dividing the radioactivity associated with adherent cells by the total radioactivity associated with both adherent and nonadherent cells.

Inhibition of cell adhesion

To determine the effect of EGTA on mast cell adhesion, various concentrations of EGTA, dissolved in RPMI were added in a volume of 10 μl just before addition of the antigen: 10 μl of RPMI were added to control wells. The adhesion assay was performed as described above. To test whether RGDS peptide inhibits mast cell adhesion to FN, 10 μl RGDS diluted in RPMI at concentrations ranging from 100 to 1500 μg/ml was added after addition of the cells to FN-coated wells, before addition of the antigen. Then the adhesion experiment was performed as described above.

Mast cell adhesion to FN was also determined in the presence of antibodies specific for MCP5/L cells. The polyclonal anti-MCP5/L antiserum was produced in rabbits by subcutaneous injections of 5×10^7 MCP5/L cells suspended in 0.5 ml of physiological saline and mixed with 0.5 ml of complete Freund adjuvant. After four weeks two booster immunizations were performed in two week interval. One week after the last injection the antisera were obtained.

The effect of an anti-MCP5/L antiserum on mast cell adhesion to FN was determined by addition of 4×10^4 mast cells to various diluions of this antiserum (as specified in the Results) in a total volume of 100 µl. The cells were then placed in FN-coated wells (10µg/ml), stimulated with PMA for 90 min (3) and adhesion was assessed as described above.

Mediator release

BMMC and MCP5/L cells were tested for antigen-induced histamine and β-hexosaminidase secretion. In parallel experiments, duplicate 180 µl aliquots of cell suspensions containing 10^6 cells and 5×10^5 cells for histamine and β-hexosaminidase assay respectively, were preincubated 5 min at 37°C, antigen (OA) at various concentrations was added (20 µl), secretion was allowed to proceed to an appropriate time point and the reaction terminated by the addition of ice-cold medium (1.8 ml). Spontaneous histamine release was measured for each experiment by the same procedure in the absence of an antigen. Cells and supernatants were separated by centrifugation (5 min, 400 × g). The isolation of histamine was carried out by the method of May et al. (8), and then the samples were passed through Cellex P columns (9). Histamine was determined fluorometrically at 360/450 nm after condensation with O-phtaldialdehyde. β-hexosaminidase activity in supernatants and pellets was assayed by quantitative spectrophotometry (change in absorbance at 420 nm) which measures the release of 4-p-nitrophenol due to cleavage of the synthetic substrate p-nitrophenol-β-D-glucosaminide (10). Histamine as well as β-hexosaminidase secretion is expressed as net mediator release and calculated according to the following formula:
induced mediator release

$$(\%) = \frac{\text{mediator in supernatant}}{\text{mediator in pellt} + \text{mediator in supernatant}} \times 100$$

where net mediator release is defined as:

induced mediator release — spontaneous mediator release

When the effect of adhesion to FN on secretion from MCP5/L cells was studied, mediator release was compared in adherent and nonadherent cells handled in identical ways. FN-coated wells were prepared and adhesion experiments performed as described above. Duplicate samples to those used in adhesion experiments were analyzed for histamine secretion; after 30 min incubation of sensitized cells in FN-coated wells different concentrations of the antigen were added to the wells and incubation continued for indicated periods of time. The reaction was stopped by cooling the plates. Cells and supernatants were separated by centrifugation (5 min, 400 × g). Histamine was determined using an enzyme immunoassay that uses mAb raised against acylated histamine (11). β-hexosaminidase was determined by the method described (10), adapted for 96-well microplates. Briefly, 50 µl of supernatant from each well, except wells for total, was transferred to corresponding well on the second plate. Triton X-100 (1.2%), was then added to each well for total in the original plate (20 µl) and to each supernatant and blank in the second plate (10 µl). Totals were mixed 5 min and 60 µl aliquots transferred to corresponding wells in second plate. Substrate/citric buffer solution (60 µl) was added to each well in second plate and the plate was incubated at 37°C for 2 h. Glycine (0.2 M, pH 10.7, 120 µl) was used to terminate enzymatic reaction. The plate was read using Dynatech MR 5000 microplate reader. The reading filter was 410 nm and the reference filter was 490 nm.

RESULTS

It is well known that BMMC are able to secrete mediators after $Fc_{\varepsilon}RI$ crosslinking mediated activation (12). Mast cells of MCP5 line developed from murine BMMC are known to secrete histamine upon $Fc_{\varepsilon}RI$ crosslinking--mediated activation as well (2). To determine whether the secretion of another preformed mast cell granule mediator β-hexosaminidase is parallel to histamine release from MCP5/L variant of these cells, we compared antigen-induced secretion of both mediators from BMMC and MCP5/L cells upon antigen-mediated activation. The patterns of histamine and β-hexosaminidase release were compared in dose response experiments (*Fig. 1 A, C*). Both populations of mast cells released β-hexosaminidase in similar to histamine dose-dependent fashion. In order to determine whether the kinetics of release are similar as well, the secretory response to the antigen in concentration of 10 μg/ml was examined at different time points after the challenge (*Fig. 1 B, D*). In BMMC as well as in MCP5/L cells the release of both mediators was detected by 5 sec and reached maximal levels between 45 and 60 sec. These results show that both mast cell populations secrete histamine and β-hexosaminidase in parallel dose-dependent fashion and the tine course of histamine and β-hexosaminidase secretion is similar. So we used MCP5/L cells in subsequent adhesion and secretion experiments. *Fig. 2* demonstrates, that when sensitized cells were placed in polystyrene wells coated with FN and increasing amounts of antigen was added, antigen concentration-dependent increase of cell adhesion to FN was observed. The stimulation of cell adhesion reached a maximum at 10 ng/ml of antigen and further increase in antigen concentration (up to 100 ng/ml) caused the diminishion of cell adhesion (data not shown). The increase of cell adhesion was detectable by 5 min, reached a maximum at 30 min and persisted or decreased over the next 30 min (*Fig. 2*). The concentrations of antigen required to induce potentiation of cell adhesion to FN were lower than those required to induce significant mediator secretion.

We next sought to determine whether IgE receptor crosslinking was responsible for the potentiation of mast cell adhesion. As can be seen in *Fig. 3*, incubation of the reaginic serum at 56°C for 2 h (the treatment known to inactivate IgE) prior to cell treatment resulted in the abolishion of antigen-induced adhesion potentiation. When the cells were pretreated with normal mouse serum (instead of IgE antibody containing serum) their subsequent adhesion to FN was comparable to the adhesion observed in the cells pretreated with heat-inactivated serum. These results strongly suggested that antigen-induced adhesion potentiation was linked with IgE receptor crosslinking.

In order to test if MCP5/L cells possess specific proteins responsible for their adhesion to FN we tested whether polyclonal anti-MCP5/L antibody is

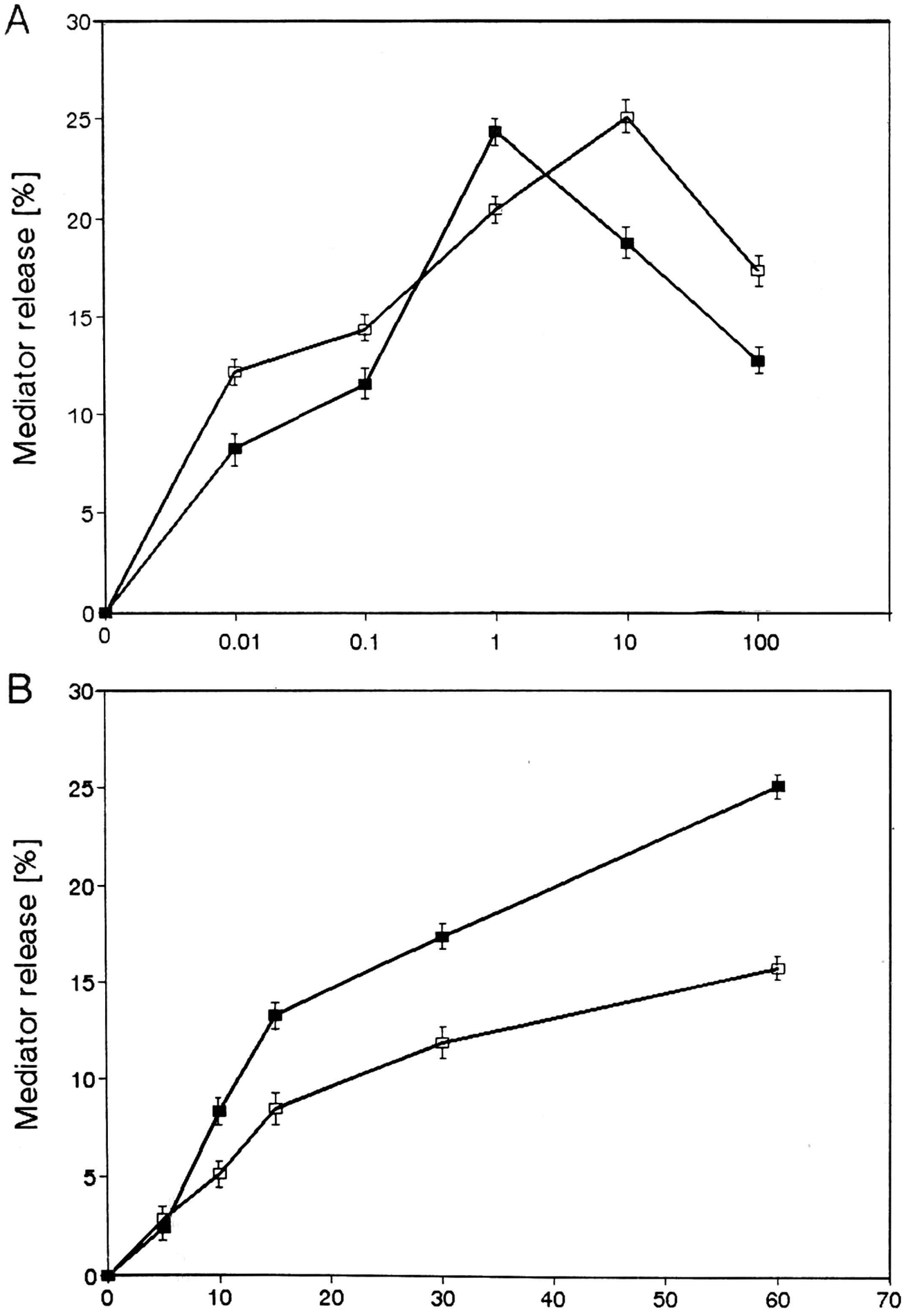
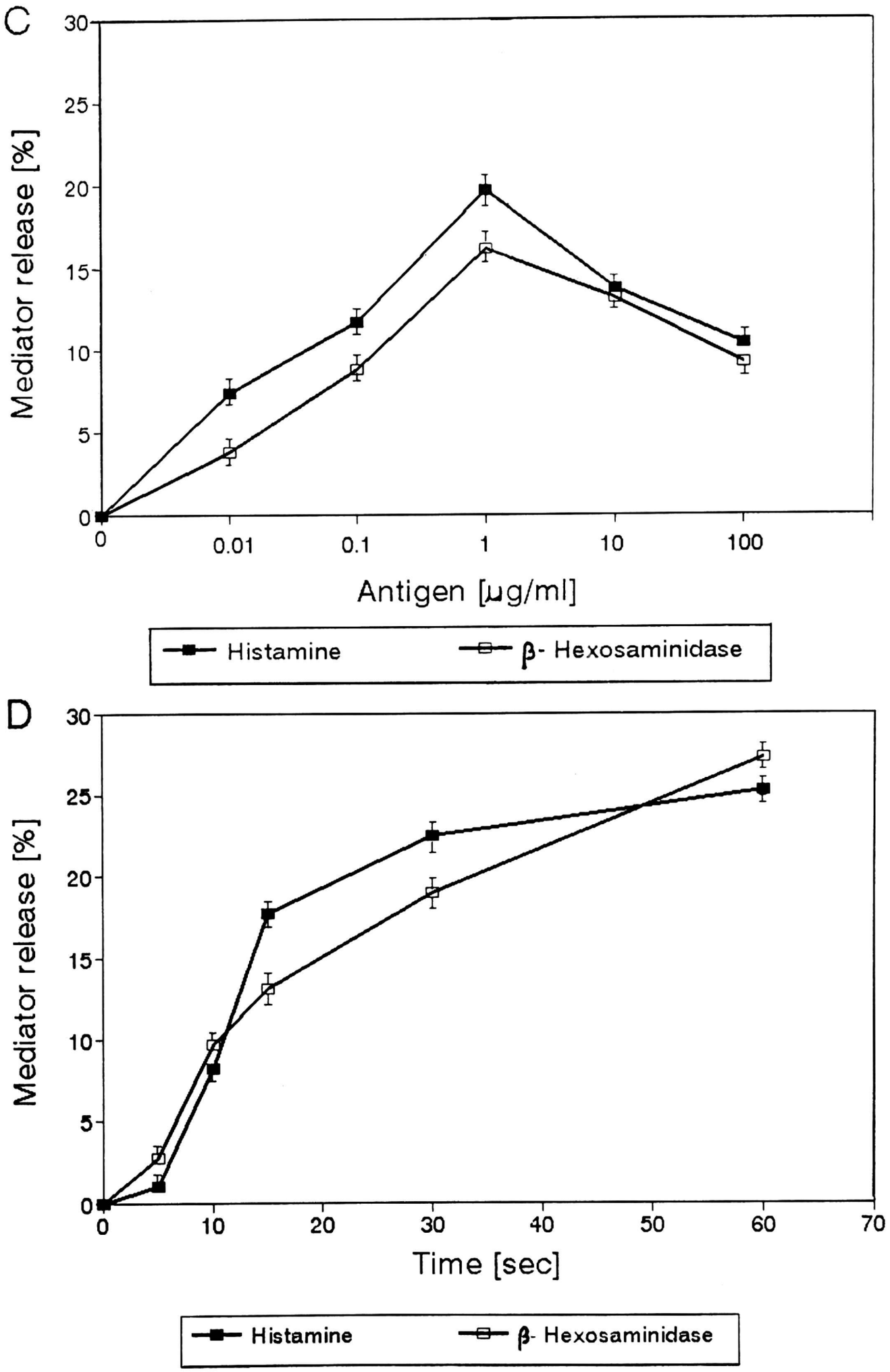


Fig. 1. Antigen-induced mediator (histamine and β-hexosaminidase) secretion from sensitized BMMC (panels A and B) and MCP5/L cells (panels C and D). Cells were sensitized with mouse anti-OA reaginic serum in dilution 1:10 at 37°C for 24 hours, washed and resuspended in fresh medium. After 5 min preincubation cells were challenged with antigen (OA).



Panels A and C — antigen concentration-dependent secretion measured after 60 sec of reaction. Panels B and D — time course of secretion from cells challenged with OA (10 µg/ml). Each value represents mean ±SEM from four independent experiments, each performed in duplicate.

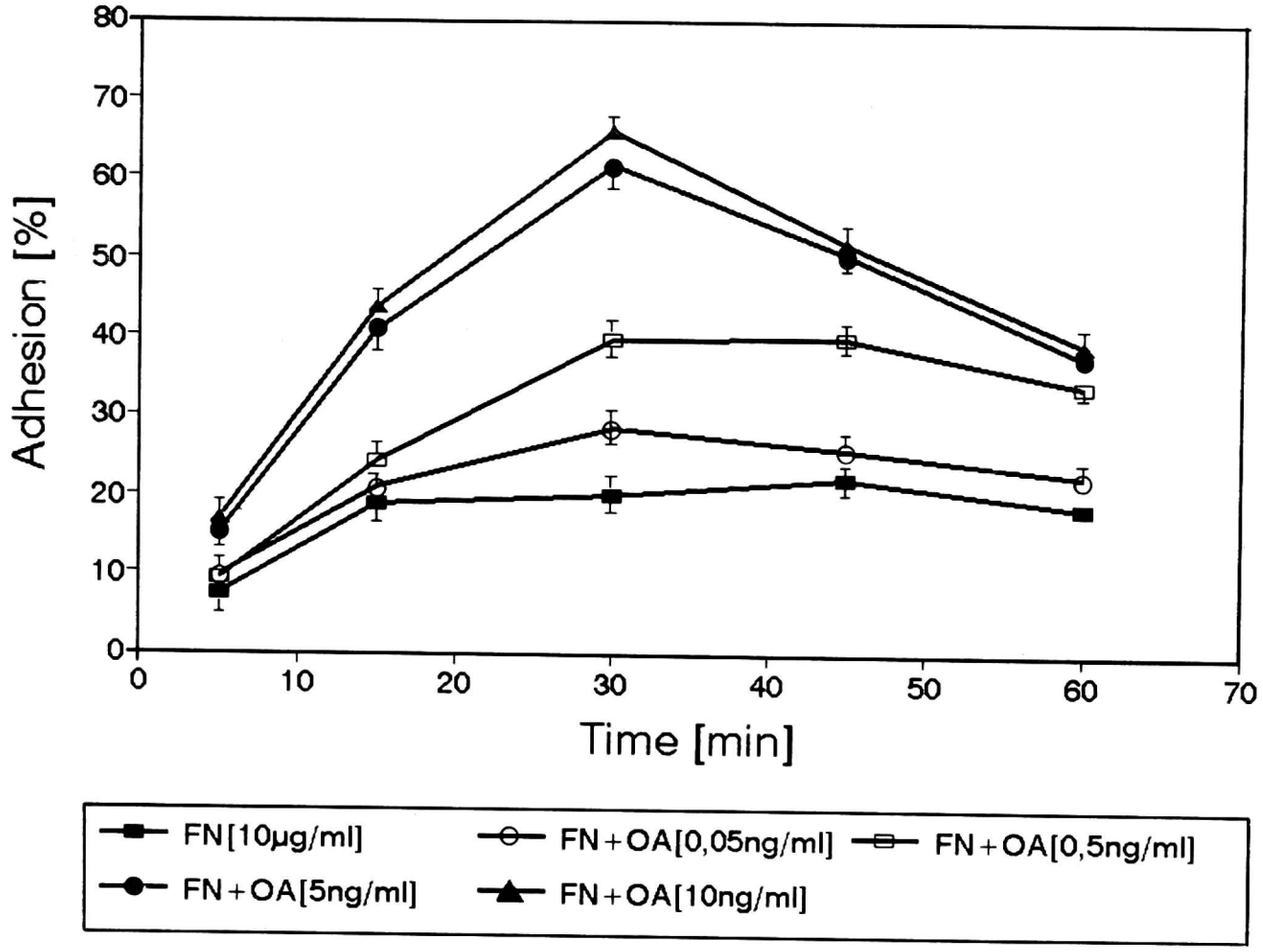


Fig. 2. Time course and dose-dependence of antigen-induced stimulation of mast cell adhesion to FN. MCP5/L cells were sensitized with mouse reaginic serum and placed in plastic wells coated with FN (10 µg/ml). Cells were incubated without antigen (solid rectangles) or with antigen (OA concentrations indicated on the graphs) for different periods of time. Each value represents the mean ±SEM from four independent experiments, each performed in duplicate.

able to inhibit mast cell adhesion to FN. As shown in *Fig. 4*, these antibodies were effective in inhibiting mast cell adhesion induced by PMA in dose-dependent manner and were more effective in inhibiting adhesion of MCP5/L cells as compared with BMMC. The results suggested that mast cells may possess on their membranes specific proteins responsible for adhesion of these cells to FN.

To explore whether antigen-induced potentiation of adhesion of sensitized mast cells to FN might be mediated through an integrin receptor we examined the calcium dependency of this process. As is shown in *Fig. 5*, addition of EGTA in different concentrations into the cell suspensions before addition of the antigen resulted in the decrease of antigen-induced adhesion potentiation in a dose-dependent manner, with complete abolishion in the presence of 1 mM EGTA. The RGDS peptide has been previously reported to inhibit PMA-induced mast cell adhesion to FN, which is in agreement with the possible involvement of integrin receptor in this process (3, 4). We tested if IgE receptor crosslinking-induced potentiation of mast cell adhesion to FN

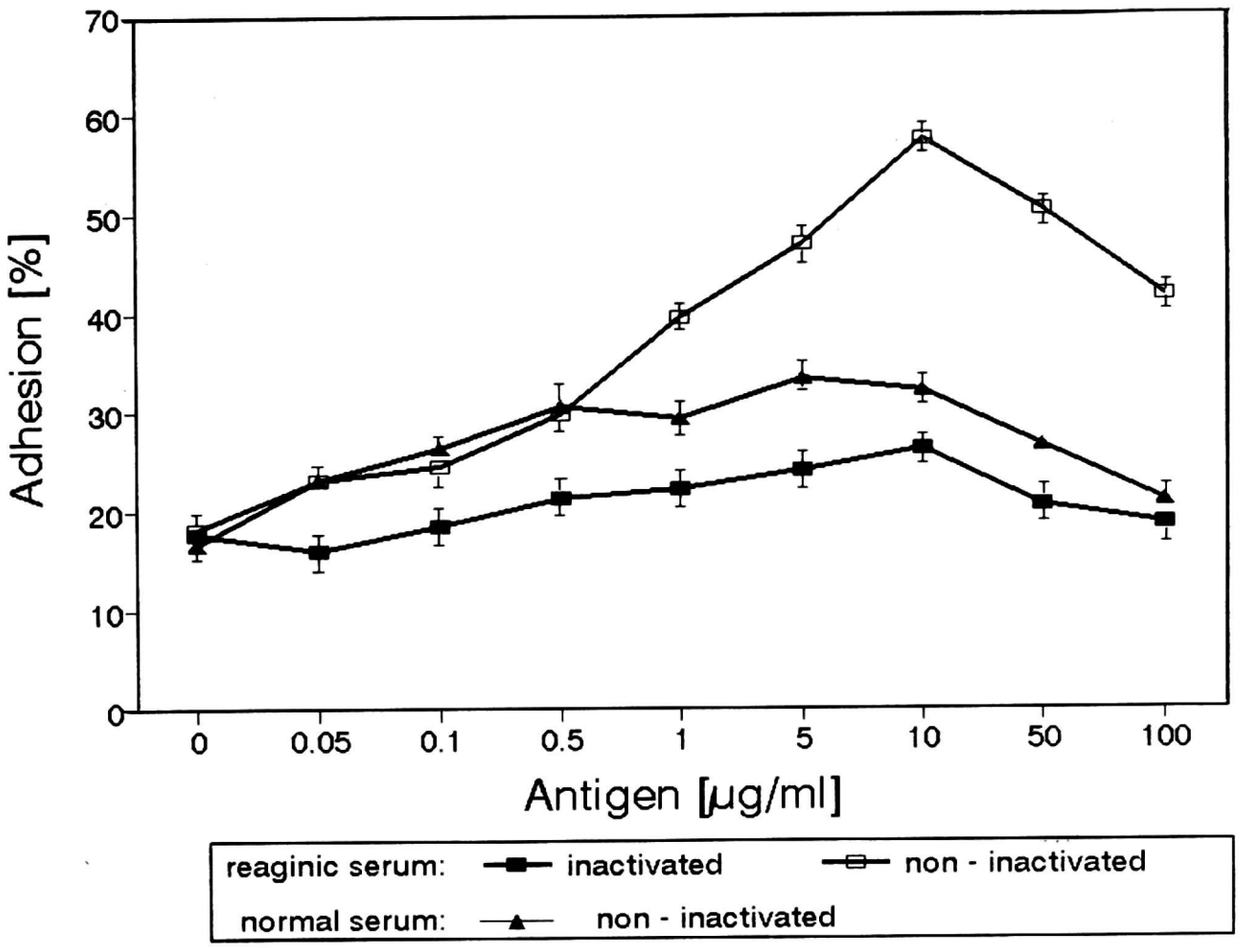


Fig. 3. The effect of heat-induced inactivation of IgE in mouse reaginic serum (prior to cell treatment) on adhesion of serum treated cells to FN. One part of reaginic serum was incubated at 56°C for two hours before 24h cell treatment. Normal mouse serum non-inactivated was used as a control. After cell treatment the sera were washed out and antigen-induced adhesion experiment was performed using 30 min incubation period. Each value represent the mean ±SEM from four independent experiments, each performed in duplicate.

is inhibited by RGDS peptide. Peptide concentration-dependent inhibition of adhesion potentiation was observed with 93% inhibition at 1000 µg/ml of RGDS peptide (data not shown). Temperature dependence of MCP5/L cells adherence to FN was also tested. Adhesion was maximal at 37°C (55.4±3.2%), diminished at 22°C (44.6±4.1%) and was significantly inhibited at 4°C (15.7±2.8%).

Spontaneous adhesion to FN did not by itself induce mediator release. Spontaneous histamine and β-hexosaminidase secretion during the incubation of the cells in FN-coated wells (up to 30 min) did not exceed 7.6±1.5% and 8.7±1.5% for histamine and β-hexosaminidase respectively. Similar spontaneous mediator release was observed when the cells were incubated in the wells coated with BSA only (8.4±1.0% and 12.0±1.3% for histamine and β-hexosaminidase respectively). Incubation of the cells in the presence of antigen in final concentrations ranging from 0.05 to 10 ng/ml resulted in

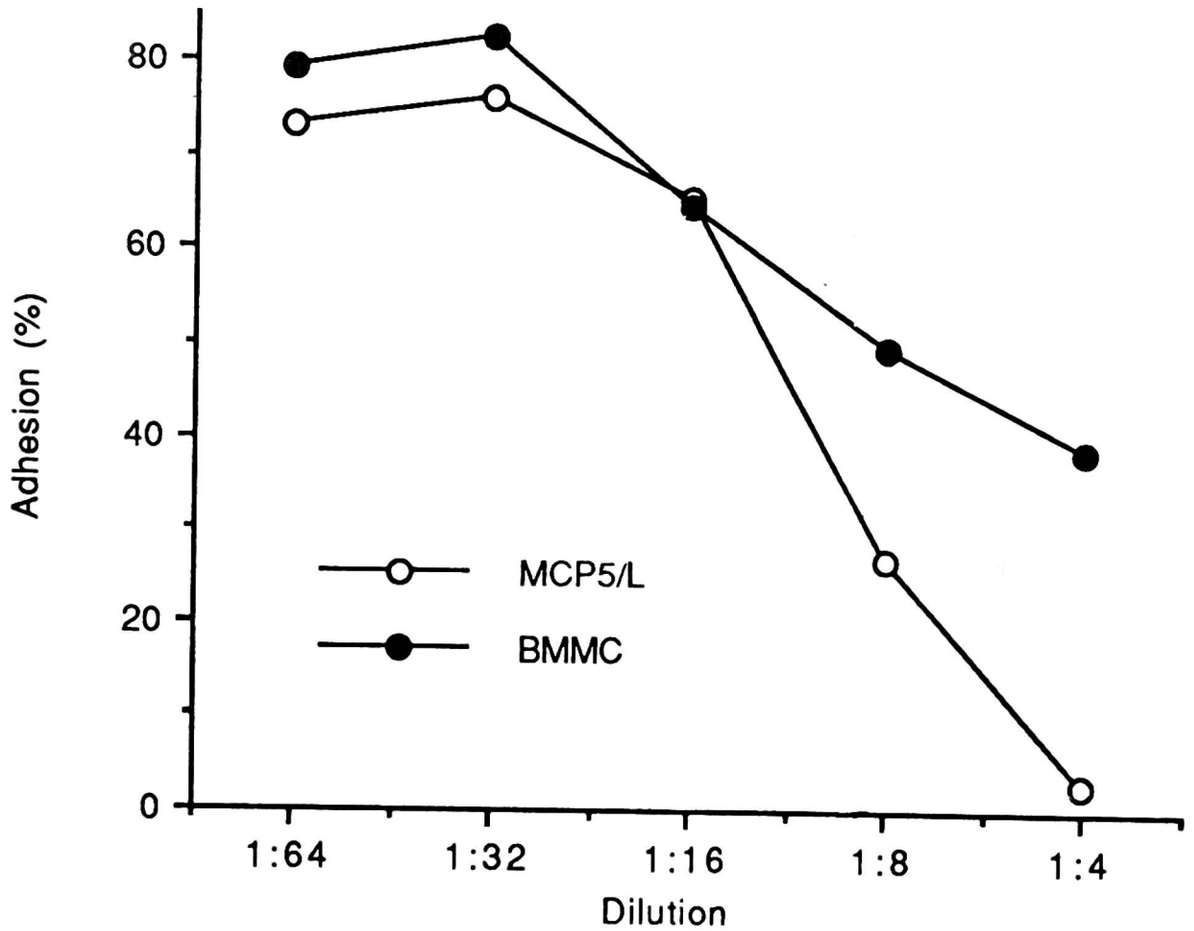


Fig. 4. Effect of anti-MCP5/L serum on adhesion of MCP5/L and BMMC to FN. The cells were added to different dilutions of anti-MCP5/L serum and cell suspensions were then placed in FN-coated wells, PMA (50 μg/ml) was added to selected wells and adhesion was measured after 90 min of incubation in CO_2 incubator. Each value represents the result of a single experiment performed in duplicate.

adhesion potentiation (*Fig. 2*), however these antigen concentrations were too low to induce significant mediator release (data not shown). To examine the possibility that adhesion to FN-coated surface changed the secretory response of sensitized mast cells to the stimulation with antigen in optimal concentrations, we investigated the effect of non-stimulated adhesion to FN on subsequent antigen-induced mediator release from passively sensitized mast cells. As shown in *Fig. 6*, sensitized mast cells allowed to adhere to FN-coated wells without stimulation (30 min) and then challenged with antigen in increasing concentrations released more histamine as compared to the cells incubated in wells coated with BSA only. The enhancement of histamine release was observed in antigen concentrations inducing well pronounced mediator secretion (*Fig. 6A*) and at all time points in time-course experiments (*Fig. 6B*). Similar results were obtained when under the same experimental conditions antigen-induced secretion of β-hexosaminidase was measured (*Fig. 7*). The results show, that non stimulated adhesion of sensitized MCP-5/L cells to FN-coated curfaces enhances antigen-induced secretion of histamine and β-hexosaminidase from these cells.

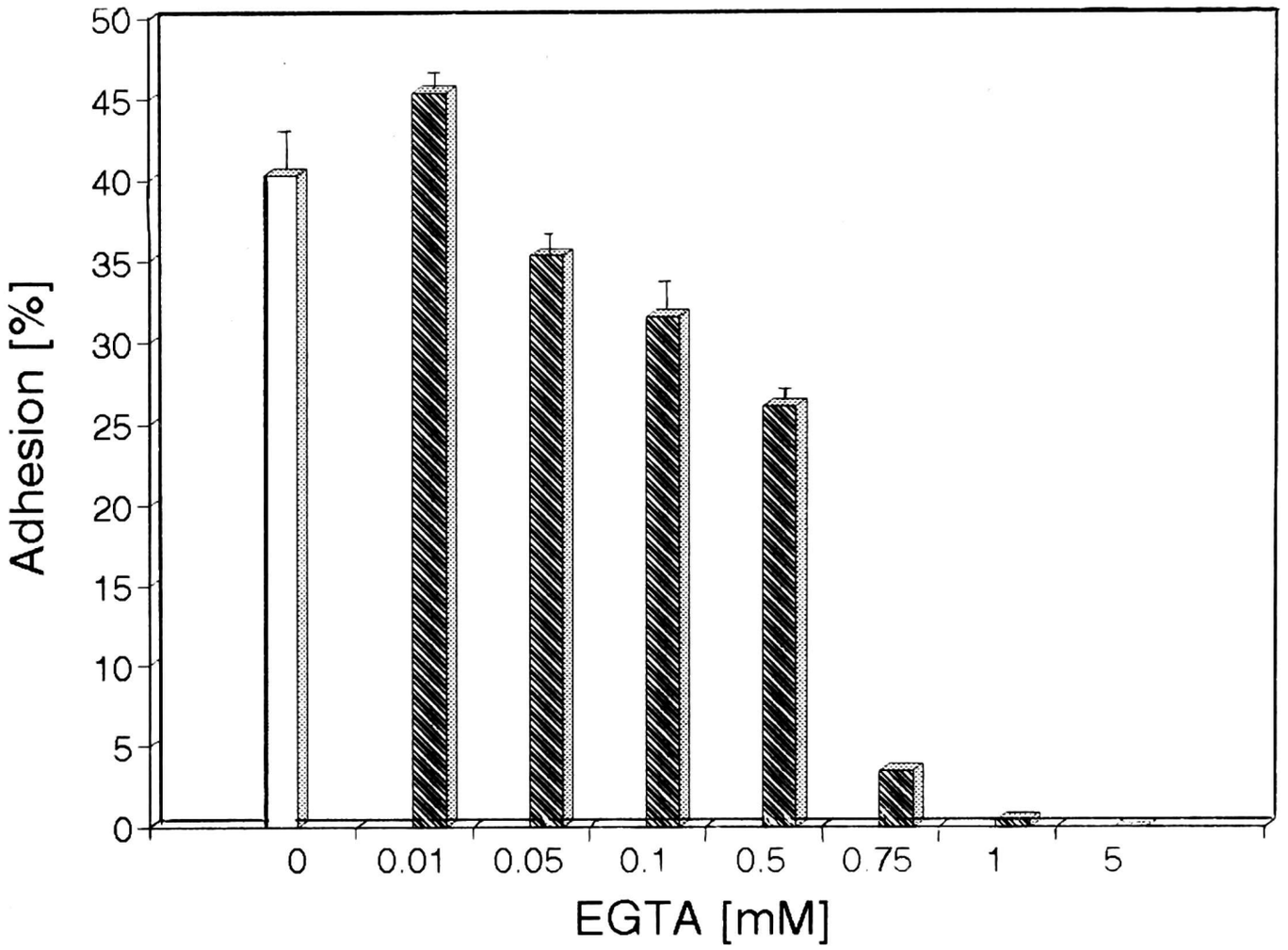


Fig. 5. Effect of EGTA on antigen-induced potentiation of MCP5/L cell adhesion to FN. Sensitized cells were placed in FN-coated wells and EGTA at different concentrations was added just before addition of the antigen (10 μg/ml) and adhesion was measured after 30 min incubation period. Each value represent the mean ±SEM from four independent experiments, each performed in duplicate.

DISCUSSION

Mast cells are well recognized as the principal effector cells in immediate hypersensitivity and are involved in inflammatory reactions. They are widely distributed throughout the tissues, where they execute specific biological functions. Stimulation of mast cells following antigen-mediated $Fc_{\varepsilon}RI$ crosslinking has been shown to promote their adherence to laminin (1). Demonstration of mast cell adhesion to FN (3) raised the question whether antigen-induced $Fc_{\varepsilon}RI$ crosslinking on sensitized mast cells may affect their adhesion to FN as well. MCP5/L cells were used in the present experiments since they appear similar in morphology, biochemical properties and biological responssiveness to the primary BMCMC from which they are derived (2, 4). Our comparative study of the responsiveness of passively sensitized BMCMC and MCP5/L cells to antigenic stimulation showed similar dose-dependent

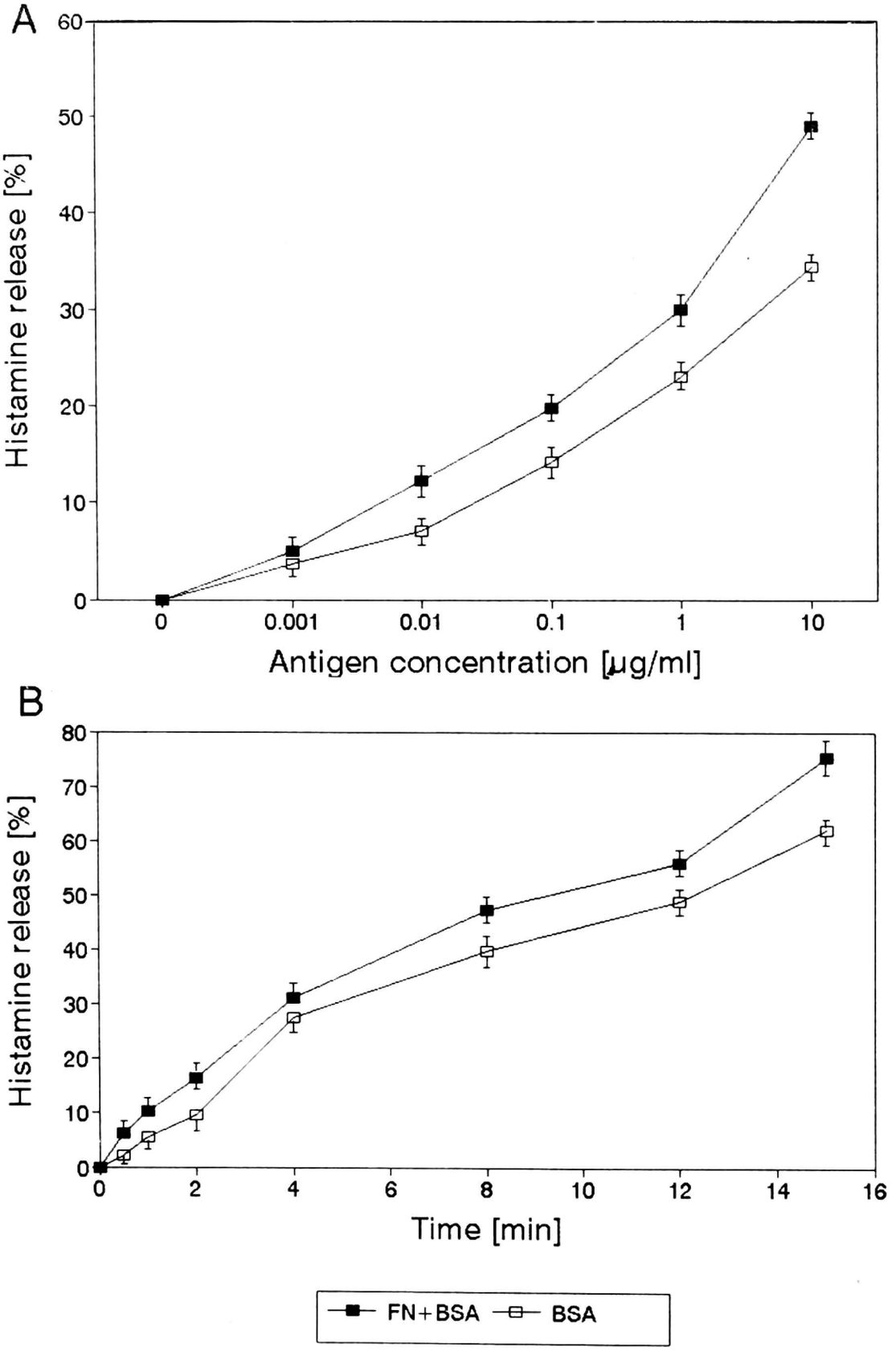


Fig. 6. The effect of adhesion to FN on the level of antigen-induced histamine release from sensitized MCP5/L cells. The cells were allowed to adhere to FN spontaneously for 30 min, antigen in different concentrations was then added and reaction of histamine release was stopped after 8 min (panel A). In time course experiment (panel B) spontaneously adhered cells were challenged with OA in concentration of 10 µg/ml and histamine release was terminated at different time points of the reaction. Mean spontaneous adhesion of the cells to FN after 30 min of incubation reached 23%. Each value represents the mean ±SEM from four independent experiments, each performed in duplicate.

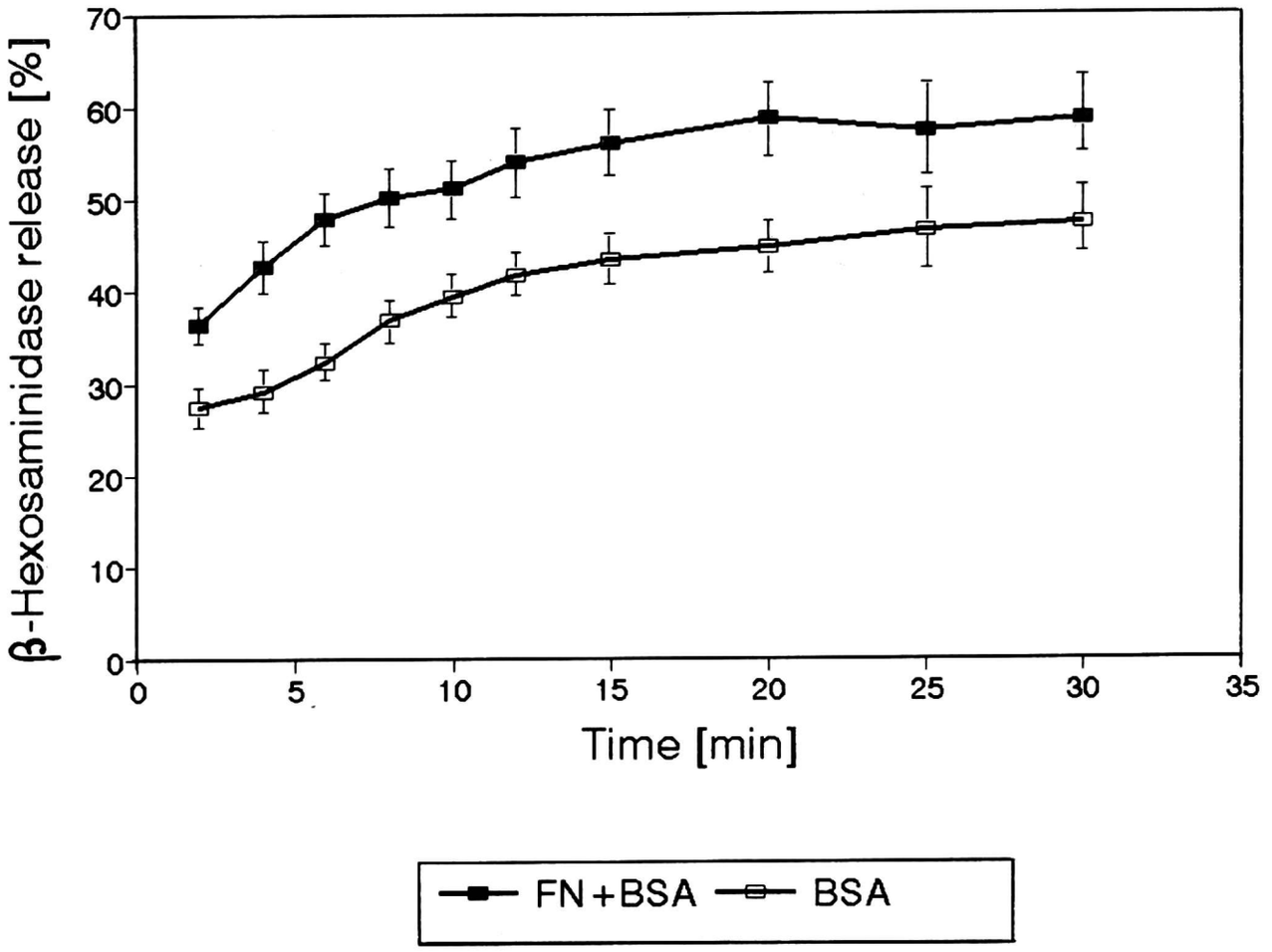


Fig. 7. Effect of adhesion to FN on time course of antigen-induced β-hexosaminidase secretion from sensitized MCP5/L cells. The cells were allowed to adhere to FN spontaneously for 30 min, OA (10 μg/ml) was then added and the reaction was terminated at different time points. Mean spontaneous adhesion of the cells to FN after 30 min of incubation reached 20±2%. Each value represents the mean±SEM from four independent experiments, each performed in duplicate.

fashion and time course of mediator release in the two cell populations (*Fig. 1*). It has been previously demonstrated, that both primary BMCMC and MCP5 cells adhere to FN-coated surfaces (3). However, in these studies BMCMC adhered to FN only after activation with PMA, whereas in MCP5 cells some extent of adhesion to FN was observed without PMA, suggesting the possibility of slight activation of MCP5 cells in culture (3).

The data presented in this paper demonstrate that MCP5/L cells sensitized with mouse IgE adhere to FN-coated surfaces spontaneously (*Fig. 2*). The adhesion is greatly enhanced after stimulation of the cells with low concentrations of antigen, insufficient for significant mediator release. These results suggest that mast cell adhesion to FN is not directly linked with mediator secretation, although antigen-induced $Fc_{\varepsilon}RI$ crosslinking on sensitized mast cells seems to be responsible for transient adhesion potentiation (*Fig. 3*). Although crosslinking of cell surface IgE receptors is necessary for antigen-induced mediator release, the observation that extremely low concentrations of antigen are sufficient to cause changes in mast cell adhesion

in the absence of detectable mediator release suggests that mast cell adhesion reactions have lower threshold for activation than does mediator release. Similar results were obtained in the studies of IgE receptor crosslinking-mediated stimulation of mast cell adherence to laminin (13) and in the studies of IgE-mediated adherence of human basophils to vascular endothelium (14). A diminished threshold of response for adhesion process may have important implications *in vivo*, where antigen concentrations may be extremely low. Our data are analogous to the studies demonstrating that up-regulation of CD18 molecules on neutrophils can occur at subnanomolar concentrations, in the nanomolar range a chemotactic effect occurs, whereas at higher concentrations this same stimulus induces secretion (15). Time course of MCP5/L mast cell adhesion to FN after addition of antigen demonstrated the transient character of such activation (*Fig. 2*). It is known that sensitized mast cells after antigen-induced activation enter a refractory period (16, 17). The possibility that transient character of antigen-induced potentiation of mast cell adhesion to FN depends on such refractory period have to be considered.

The external calcium ion requirement (*Fig. 5*) and temperature dependence of antigen-mediated adhesion potentiation are in accordance with the studies of IgE receptor crosslinking-mediated mast cell adherence to laminin (13). Such adherence of cells usually involves specific cell membrane receptors (18, 19). Calcium ion and temperature dependence together with the observation that RGD sequence-containing peptide blocked cell binding to FN suggests that IgE-induced enhancement of adhesion of MCP5/L cells to FN involves an integrin type receptor on cell surface (18—20).

Adhesion of mast clls to FN has been shown to induce spreading of the cells over the FN-coated surfaces (3). Our observation that sensitized MCP5/L cells allowed to adhere to FN-coated surface release more histamine and β-hexosaminidase on the challenge with antigen in optimal concentrations is consistent with these results. The enhanced mediator release from adhered MCP5/L cells could be the result of such spreading and its consequences, as it has has been suggested in the study of rat basophilic leukemia cell adherence to FN (21). The mechanism by which adhesion of sensitized non-stimulated mast cells to FN results in enhanced mediator secretion after antigenic stimulation requires further investigation. If the effects described in the present paper occur *in vivo*, an $Fc_{\varepsilon}RI$-derived signaling may be one of the mechanisms that regulates interactions between mast cells and FN.

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REFERENCES

1. Thompson HL, Burbelo PD, Segui-Real B, Yamada Y. Metcalfe DD. Laminin promotes mast cell attachment. *J Immunol* 1989; 143: 2323—2327.
2. Arora N, Min K-U, Costa JJ, Rhim JS, Metcalfe DD. Immortalization of mouse bone marrow-derived mast cells with Ad12-SV40 virus. *Int Arch Allergy Immunol* 1993; 100: 319—327.
3. Dastych J, Costa JJ, Thompson HL, Metcalfe DD. Mast cell adhesion to fibronectin. *Immunology* 1991; 73: 478—484.
4. Dastych J, Metcalfe DD. Stem Cell Factor induces mast cell adhesion to fibronectin. *J Immunol* 1994; 152: 213—219.
5. Wyczółkowska J, Brzezińska-Błaszczyk E, Maśliński C. Kinetics of specific IgE antibody and total IgE responses in mice: the effect of immunosuppressive treatment. *Int Archs Allergy appl Immun* 1983; 72: 16—21.
6. Mota I, Wong D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci* 1969; 8: 813—820.
7. Curtis ASG, Forrester JV, McInnes C, Lawrie F. Adhesion of cells to polystyrene surfaces. *J Cell Biol* 1983; 97: 1500—1506.
8. May CD, Lyman M, Alberto R, Cheng J. Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J Allerg* 1970; 46: 12—20.
9. Kremzner LT, Pfeiffer CC. Identification of substances interfering with the fluorometric determination of brain histamine. *Biochem Pharmacol* 1966; 15: 197—200.
10. Schwartz LB, Austen KF, Wasserman SI. Immunologic release of β-hexosaminidase and β-glukuronidase from purified rat serosal mast cells. *J Immunol* 1979; 123: 1445—1450.
11. Morel AM, Delaage MA. Immunoanalysis of histamine through a chemical derivatization. *J Allergy Clin Immunol* 1988; 82: 646—654.
12. Razin E, Mencia-Huerta J, Stevens RL et al. IgE-mediated release of leukotriene C_4, chondroitin sulphate E proteoglycan, beta-hexosaminidase, and histamine from cultured bone marrow-derived mouse mast cells. *J Exp Med* 1983; 157: 189—201.
13. Thompson HL, Burbelo PD, Metcalfe DD. Regulation of adhesion of mouse bone marrow-derived mast cells to laminin. *J Immunol* 1990; 145: 3425—3431.
14. Bochner BS, MacGlashan DW, Marcotte GV, Schleimer RP. IgE-dependent regulation of human basophil adherence to vascular endothelium. *J Immunol* 1989; 142: 3180—3186.
15. Charo IF, Yuen C, Perez HD, Goldstein LM. Chemotactic peptides modulate adherence of human polymorphonuclear leukocytes to monolayers of cultured endothelial cells. *J Immunol* 1986; 136: 3412—3419.
16. Ishizaka T, Sterk AR, Daeron M, Becker EL, Ishizaka K. Biochemical analysis of desensitization of mouse mast cells. *J Immunol* 1985; 135: 492—501.
17. Maeyama K, Hohman RJ, Ali H, Cunha-Melo JR, Beaven MA. Assessment of IgE-receptor function through measurement of hydrolysis of membrane inositol phospholipids. New insights on the phenomena of biphasic antigen concentration-response curves and desensitization. *J Immunol* 1988; 140: 3919—3927.
18. Yamada M. Adhesive recognition sequences. *J Biol Chem* 1991; 226: 12809—12812.
19. Pytela R, Pierschbacher MD, Rouslahti E. Identification and isolation of 140 Kd cell surface glycoprotein with properties expected of fibronectin receptor. *Cell* 1985; 40: 191—198.

20. Hynes RO. Integrins: versatility, modulation and signaling in cell adhesion. *Cell* 1992; 69: 11—25.
21. Hamawy MM, Oliver C, Mergenhagen SE, Siraganian RP. Adherence of rat basophilic leukemia (RBL-2H3) cells to fibronectin-coated surfaces enhances secretion. *J Immunol* 1992; 149: 615—621.

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