Protein Kinase C Regulates Proliferation of Mast Cells and the Expression of the mRNAs of \textit{fos} and \textit{jun} Proto-oncogenes During Activation by IgE-Ag or Calcium Ionophore A23187

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Short-term stimulation (up to 16 hours) of interleukin-3 (IL-3)–dependent mouse bone marrow-derived mast cells, Abelson transformed mouse liver-derived mast cells, or rat basophilic leukemia cells by either IgE-Ag or calcium ionophore A23187 resulted in inhibition of their proliferation as measured by \textsuperscript{3}H-thymidine incorporation and MTT (tetrazolium) assays, and in accumulation of the mRNAs of \textit{c-fos}, \textit{c-jun}, \textit{junB} and slightly of \textit{junD} proto-oncogenes. The involvement of protein kinase C (PKC) in these responses was investigated by using several approaches of enzyme activity regulation. Direct activation of the PKC was achieved by short-term exposure of the cells to the PKC-specific activator phorbol 12-myristate-13-acetate (PMA). Inhibition of PKC activity was obtained by either prolonged treatment of the cells with PMA or by exposure of the cells to the PKC inhibitors H-7 and staurosporine. The results showed the following: (1) Short-term exposure of mast cells to PMA was sufficient to induce inhibition of proliferation. (2) An increase in PKC activity was associated with a decrease in the proliferation of IgE-dinitrophenol (DNP) or calcium ionophore A23187–stimulated cells. (3) A direct correlation was found between the increase in PKC activity and the increase in the level of the mRNAs of the \textit{jun} proto-oncogenes in cells activated by both stimuli mentioned. (4) While an increase in PKC activity was associated with the upregulation of the level of \textit{c-fos} mRNA during calcium ionophore A23187 stimulation, it showed the opposite effect on the expression of the mRNA of this proto-oncogene when the cells were triggered by IgE-DNP. Therefore, we concluded that PKC plays various roles in the expression of the mRNA of \textit{c-fos} in activated mast cells depending on the stimulus involved. In addition, the expression of the mRNA of \textit{c-jun} and \textit{junB} proto-oncogenes is not coordinately regulated with that of \textit{c-fos} during immunologic stimulation. This discordancy, which is associated with the increase in PKC activity in mast cells, may play a role in the regulation of the transcription of AP-1–responsive genes, and therefore could be associated with the regulation of proliferation of these cells.

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regulated. The expression of jun and c-fos proto-oncogenes in mast cells has not yet been characterized, nor is it known whether they play any role in mast cell proliferation.

Protein kinase C (PKC) seems to be of importance in cell proliferation, as activation of this enzyme by phorbol esters promotes growth in many cell systems. However, such enzyme activation can also exert negative effects on cellular events associated with mitogenesis. Recently, it was reported that PKC is a negative regulator of IL-3- and IL-4-mediated mast cell proliferation. In addition to the role of PKC in cell proliferation, it was reported that AP-1 and two genes encoding AP-1–associated proteins, c-fos and c-jun, are known to be regulated by phorbol esters, which are potent activators of PKC. However, the involvement of this enzyme in regulation of junB and junD is still not clear.

In light of this information, we investigated the role played by PKC in the proliferative process of mast cells due to exocytotic stimuli, and its association to the expression of the mRNA of the above-mentioned proto-oncogenes. Our data showed that stimulation of mast cells by the calcium ionophore A23187, or by cross-linking of the FcRI receptors, caused inhibition of proliferation and elevation in the mRNA level of the proto-oncogenes c-fos, c-jun, and junB. PKC was found to be associated with the regulation of these cell processes in response to both stimuli.

MATERIALS AND METHODS

Cell culture. Ablon transformed murine liver-derived mast cells (ABFTL), mouse bone marrow-derived mast cells (MBMMCs), and rat basophilic leukemia cells (RBL) were maintained in RPMI-1640 supplemented with 2 mM L-glutamine, 2 mM nonessential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin (GIBCO, Paisley, Scotland), 50 μg/mL β-mercaptoethanol (Fisher Scientific, Medford, MA), and 10% fetal calf serum (FCS; Bio-lab, Jerusalem, Israel). MBMMCs were maintained, in addition to the RPMI medium, in 50% mouse spleen-derived conditioned medium.

IgE-Ag calcium ionophore and phorbol-12-myristate-13-acetate (PMA)-mediated activation. ABFTL, MBMMCs, and RBL cells were incubated for 2 days at 37°C in the presence or absence of 200 ng PMA/mL (Sigma, St Louis, MO). Then the cells were transferred to RPMI serum-free medium for overnight incubation with or without a supplement of 20 ng PMA/mL. IgE sensitization was performed by incubating replicates of 2.5 × 10⁷ cells with 2.5 μg of monoclonal IgE against dinitrophenol (DNP) (kindly provided by Dr F.-T. Liu, La Jolla, CA) for 45 minutes at 37°C in a final volume of 200 μL modified Tyrode’s solution containing 1.0 mM Ca²⁺, 0.3 mM Mg²⁺, and 1 mM gelatin (TG), in the presence or absence of 100 μM H-7 (Sigma) or 10 μM Stauroporine (Sigma). The cells were washed once with 1 mL TG by centrifugation at 400g for 5 minutes at room temperature and resuspended in 450 μL TG. Then the cells were incubated at 37°C for various time intervals after the addition of 50 μL TG containing defined concentrations of DNP coupled to bovine serum albumin (DNP-BSA) (kindly provided by Dr F.-T. Liu), in the presence or the absence of 100 μM H-7, 10 mM Stauroporine or 20 ng PMA/mL. Another set of experiments was centrifuged and resuspended in 450 μL TG, followed by the addition of 50 μL of PMA or 4α-phorbol (20 ng/mL final concentration). Another set of cells was exposed to calcium ionophore A23187 (Sigma) (0.2 μM/L final concentration) in the presence or absence of 100 μM H-7 and incubated for 30 minutes at 37°C. Following activation, samples of cells were taken for proliferation assay. Cell viability before and after each experiment was greater than 95% as determined by trypan blue exclusion. The remaining cells were lysed and the total RNA was isolated.

H-thymidine incorporation assay. Cell activation was performed as described previously. Following activation the cells were cultured in 200 μL of FCS-free medium in round-bottomed 96-well microtiter plates (Nunc, Roskilde, Denmark) at a density of 0.5 to 1 × 10⁵ cells/well at 37°C. The cells were then labeled with 1.0 μCi of 'H-thymidine (Nuclear Research Center, Negev, Israel) for 4 or 16 hours and transferred onto glass fiber filter paper where they were water-lysed and washed in an automated cell harvest unit. The incorporated radioactivity was measured in a liquid scintillation counter.

Materials and Methods

Cell-free PKC assay. PKC activity in the cytosolic and membrane fractions of the cells was determined as previously described. Reaction mixtures contained 10 mM MgCl₂, 1 mM CaCl₂, 100 μg of histone (type III-S) with or without 20 μg of phosphatidylserine, 5 μg of 1,2-D-oleyl-RAC-glycerol (DAG) (Sigma), and 10 μM L-32-ATP (Amersham, Amersham, UK) in a final volume of 250 μL of 200 mM Tris-HCl. Before addition to the reaction mixtures, phosphatidylserine and DAG were prepared by evaporation under N₂ and sonicated in 200 mM Tris HCl at pH 7.5. Enzyme reactions were started by the addition of 50 μL of cytosol or membrane fractions to the reaction mixtures and the samples incubated for 10 minutes at 37°C. Reactions were stopped by pipetting 100 μL from each sample onto a square (2.5 × 2.5 cm) filter paper 3mm Chr (Whatman Inc, Clifton, NJ), which was then immediately washed with agitation in 200 mL of ice-cold 10% trichloroacetic acid (TCA) for 10 minutes. This was followed by a 20-minute agitated wash in 10% TCA at room temperature and a fourth wash overnight. The papers were then soaked in 95% ethanol for 5 minutes followed by soaking in ether for an additional 5 minutes, and then allowed to air-dry before counting.

Isolation of cytosolic RNA. Cells were pelleted and lysed in lysis buffer containing heparin, spermidine, and NP-40 (Sigma), centrifuged immediately to remove the nuclei, and processed by phenol and chloroform:isooamyl alcohol extraction. The RNA was precipitated overnight at -20°C in ethanol and sodium chloride, then washed in 70% ethanol and the concentration determined in a spectrophotometer.

Northern and dot-blot analysis. For Northern and dot-blot analysis, samples of 2 to 10 μg of RNA were concentrated and denaturated in a mixture of formaldehyde (BDH, Pool, England) and formamide (Fluka Chemie, Buchs, Switzerland) for 10 minutes at 65°C. For dot-blot, the RNA was loaded on a nitro paper and for Northern blot the samples were separated overnight on an agarose-MOPS gel (Sigma). The gel was blotted onto a nitro filter for 48 hours. Then, in both methods, the nitro was baked for 2 hours at 80°C under vacuum, and then prehybridized at 42°C for 4 hours in a solution containing 5X SSC, 50% formamide, 0.5% sodium dodecyl sulfate (SDS) (IBI, New Haven, CT), 0.5 mg/mL.
the L30 gene was used as a control for loading of equivalent total RNA.44

The DNA fragments were labeled with 32P-dCTP (Amersham) using the random primed labeling technique45 up to a specific activity of 3 x 106 cpm/µg. Labeled probes were used at a final concentration of 2 x 105 cpm/mL hybridization mixture.

RESULTS

Inhibition of mast cell proliferation by IgE-Ag stimulation.

In order to study the effects of short-term immunologic stimulation on mast cell proliferation, a growth-factor independent liver-derived mast cell line (ABFTL)42 was sensitized with monoclonal IgE anti-DNP followed by exposure to various doses of DNP-BSA for 15 minutes. The cells were then incubated for up to 4 hours with 3H-thymidine. Stimulation by this trigger inhibited 3H-thymidine uptake by these cells (Fig 1A). The inhibition reached a plateau at 50% ± 18% (mean ± SE, n = 6) at a concentration of 10 ng DNP-BSA/106 cells. Prolonged incubation with the antigen, up to 16 hours, did not change the maximal level of inhibition (data not shown). To check whether the effect of IgE-DNP on ABFTL proliferation was in direct response to the immunologic trigger and not an indirect result of the transformation of these cells by the Abelson virus, 3H-thymidine uptake was measured in IgE-DNP-stimulated MBMMC and RBL cells. Inhibition of 3H-thymidine incorporation was observed both in IgE-DNP-

DNP-BSA (ng/10^6 cells)

![Inhibition of mast cell and RBL proliferation by short-term IgE-DNP stimulation. ABFTL (A), MBMMC (B), or RBL (C) were grown for 24 hours in FCS-free medium. After sensitization with IgE anti-DNP, the cells were exposed to different concentrations of DNP-BSA for 15 minutes. The cells were cultured in microplates at a concentration of 0.5 x 10^6 cells/well followed by the addition of 1.0 µCi of 3H-thymidine. Stimulation by this trigger inhibited 3H-thymidine uptake by these cells (Fig 1A). The inhibition reached a plateau at 50% ± 18% (mean ± SE, n = 6) at a concentration of 10 ng DNP-BSA/10^6 cells. Prolonged incubation with the antigen, up to 16 hours, did not change the maximal level of inhibition (data not shown). To check whether the effect of IgE-DNP on ABFTL proliferation was in direct response to the immunologic trigger and not an indirect result of the transformation of these cells by the Abelson virus, 3H-thymidine uptake was measured in IgE-DNP-stimulated MBMMC and RBL cells. Inhibition of 3H-thymidine incorporation was observed both in IgE-DNP-

![Antigen dose-dependent elevation of the expression of c-fos, c-jun, junB, and junD proto-oncogenes. ABFTL were grown for 24 hours in FCS-free medium. After sensitization with IgE anti-DNP, the cells were stimulated for 30 minutes to defined concentrations of DNP-BSA. After stimulation, cytoplasmic RNA was extracted as described in Materials and Methods and then loaded on an agarose-MOPS gel (10 µg/lane), electrophoresed, and blotted onto a Nytran paper (Schleicher & Schuell, Dassel, Germany). The hybridization of the Nytran with the 32P-labeled probe of c-fos, junD, and L30 was performed as described in Materials and Methods. After 36 hours of autoradiography, the Nytran was stripped and rehybridized to a radioactive-labeled junB probe, autoradographed for 18 hours, then stripped once again and hybridized to a radioactive-labeled c-jun probe, followed by autoradiography for 24 hours. The results are represented as arbitrary units of the densitometry of each band of the Northern blot. One representative experiment out of three. (○-○), c-fos; (■-■), junB; (●-●), c-jun; (△-△), junD; (○-○), L30.](image-url)
Fig 3. Kinetics of c-fos, c-jun, junB, and junD expression in IgE-Ag–stimulated ABFTL. IgE-sensitized ABFTL were exposed for various periods of time to 100 ng DNP-BSA/million cells. After stimulation, cytoplasmic RNA was extracted as described in Materials and Methods and then loaded on an agarose-MOPS gel (10 µg/lane), electrophoresed, and blotted onto Nytran paper. The hybridization of the Nytran with the 32P-labeled probes of c-fos, junD, and L30 was performed as described in Materials and Methods. Then the Nytran was stripped, and rehybridized to a radioactive junB probe, autoradiographed for 16 hours, stripped once again, and hybridized to a labeled c-jun probe, followed by autoradiography for 24 hours. The kinetics of c-fos (A) and of c-jun, junB and junD (B) are represented in section (C) as arbitrary units of the densitometry of each band of the Northern blots. In (C), the level of each proto-oncogene expression in each time point was calculated by dividing the intensity of the respective mRNA band by that of the control. The result was then divided by the value of the quotient between the intensity of the L30 band of the same time point and that of the lowest L30 band. One representative experiment out of three. (••••), c-fos; (□□□□), junB; (▲▲▲▲), c-jun; (○○○○), junD.

stimulated MBMMC and RBL up to a maximal level of 36% ± 5% (mean ± SE, n = 3) and 50% ± 12% (mean ± SE, n = 3), respectively, at a concentration of 10 ng DNP-BSA/10^6 cells (Fig 1B). To exclude any possibility of error in measuring proliferation as a result of change in thymidine uptake by the cells, changes in thymidine uptake by the cells, changes in thymidine pool sizes, or changes in DNA repair synthesis, we used the colorimetric MTT (tetrazolium) assay. By using this assay similar data were obtained relating to the inhibitory effect of IgE-DNP on the proliferation of all the cell types mentioned previously (data not shown).

IgE-Ag and A23187 induction of AP-1 proto-oncogene mRNA expression. It has become apparent that the process of cell proliferation involves gene expression, including expression of nuclear proto-oncogenes such as c-fos, c-jun, junB, and junD. To study whether such proto-oncogenes are expressed after IgE-Ag or A23187 stimulation of mast cells, we measured their mRNA level in activated and control cells. Figure 2 shows that stimulation of IgE-sensitized ABFTL by defined concentrations of DNP-BSA for 30 minutes resulted in elevation of the mRNAs for c-fos, c-jun, and junB, in a similar dose-dependent manner. Maximal levels of approximately 20-, 18-, and 30-fold above control for c-fos, c-jun, and junB, respectively, were observed at a concentration of 10 ng DNP-BSA/million cells, while the mRNA level of junD only slightly increased by this stimulus. The kinetics of the elevation of the mRNA level of c-jun and junB were similar, starting 5 minutes poststimulation, reaching maximal levels 25 minutes later,
and then gradually decreasing (Fig 3). The increase in c-fos mRNA was more transient than that of c-jun and junB, reaching a maximal level 5 minutes poststimulation, and then immediately decreasing (Fig 3). All mRNAs remained at a higher level than that of the control, also at 60 minutes after the trigger. The mRNA level of each of the proto-oncogenes mentioned was very low in unstimulated cells and remained unchanged at all time points checked. No significant changes in the mRNA level of the gene encoding the ribosomal protein L30, which (used here as a control), were detected in response to the immunologic stimulus. Accumulation of c-fos mRNA, 20-fold above control, was measured in the IgE-sensitized MBMMC, 30 minutes after exposure to DNP-BSA (Fig 4). RBL also responded to this trigger with the accumulation of c-fos mRNA, but at different kinetics, reaching a 23-fold maximal level above control 45 minutes after stimulation (Fig 4). The level of L30 mRNA was equal in control and stimulated cells. Thus, the similarity between the effects of short-term IgE-Ag on normal and transformed mast cells enabled us to continue our study using ABFTL, which due to the large number of cells that could be achieved, were much more convenient for work.

As mentioned in the introduction, calcium ionophore A23187 is a potent activator of mast cells that bypasses the membrane-associated biochemical events involved in the linking of the Fc,RI receptors. Therefore, to investigate whether the mRNA expression of the AP-1 nuclear proto-oncogenes could be induced by a mechanism other than that involved by excitation of Fc,RI receptors, their expression was investigated in calcium ionophore A23187-activated ABFTL. Stimulation of ABFTL by calcium ionophore A23187 was followed by an increase in the level of junB up to 20-fold above the basal level, while c-jun and junD mRNA levels were not affected by this stimulant (Fig 5). A23187-induced accumulation of junB mRNA was less sustained than in the presence of IgE-DNP, starting 30 minutes poststimulation, lasting about 15 minutes more, and then decreasing to the basal level. No significant changes in the level of the rRNA L30 were detected in A23187-stimulated cells, as compared with unstimulated ABFTL, at any time point analyzed.

The effects of PKC downregulation on proliferation of IgE-DNP or calcium ionophore A23187-activated ABFTL. The involvement of PKC in growth processes has been established in many reports. Therefore, we tried to elucidate a possible role for this enzyme in the mechanism of proliferation of ABFTL using several methods. Incubation of the cells for 30 minutes with defined concentrations of PMA, an activator of PKC, induced, as expected, a dose-dependent elevation of the enzyme's activity in the cell membrane, with maximal activity of 7.3 pmol/μg protein per minute at a concentration of 10 ng PMA/mL (Fig 6A). Inhibition of the enzyme activity was achieved by chronic treatment of the cells with 20 ng PMA/mL, which induced a time-dependent inhibition of total PKC activity (Fig 6B). Total inactivation of the enzyme occurred 72 hours after exposure to PMA.

Activation of mast cells by short-term PMA treatment inhibited the level of 3H-thymidine incorporation into the cells by 54% ± 1% (mean ± SE, n = 3) (Fig 7A, lane 1) while the inactive analogue of PMA, 4-α-phorbol, had no effect, compared with the level found in control cells (Fig 7A, lane 2). IgE-sensitized mast cells were triggered by DNP-BSA in the absence or presence of 100 μmol/L H-7 or 10 nmol/L Staurosporine, PKC inhibitors. The presence of H-7 (lane 4) and Staurosporine (lane 5) significantly reduced the level of 3H-thymidine uptake inhibition caused by the immunologic stimulus. Prolonged pretreatment of the cells with PMA (for 72 hours) blocked completely the inhibitory effect of IgE-DNP stimulation on the mast cell proliferation (Fig 7A, lane 6). The long-term PMA treat-
H-thymidine uptake (Fig 7B, lane 3). Inhibition of PKC or significant reduction of calcium ionophore A23187-activated ABFTL. Stimulation of thymidine uptake in unstimulated cells (Fig 7B, lane 1). H-7 total PKC activity found in the cells that were not exposed to PMA (lane 2). When PKC was depleted from the cells by long-term treatment of the cells with PMA followed by stimulation. Each experiment was followed by a 'H-thymidine uptake measurement, as described in Materials and Method. The results are calculated as the total PKC activity at a certain time point after exposure to PMA, divided by the activity in the membrane combined with that in the remains of the cell) was then measured by the cell free PKC assay, as described in Materials and Methods. The results are calculated as the total PKC activity found in the cells that were not exposed to PMA (percent of total). One representative experiment out of three.

Fig 7. The involvement of PKC in the proliferation of mast cells. In one set of experiments cells were triggered by PMA alone for only 30 minutes. In the second one, cells were exposed to H-7 or Staurosporine before activation. In a third set, cells were chronically treated with PMA followed by stimulation. Each experiment was followed by a 'H-thymidine uptake measurement, as described in Materials and Method. The type of activation represented by each bar is as follows: (A) 1, PMA (30 minutes); 2, 4a-phorbol; 3, IgE-DNP; 4, IgE-DNP + H-7; 5, IgE-DNP + Staurosporine; 6, PMA (72 hours) + IgE-DNP; 7, H-7; 8, Staurosporine; 9, PMA (72 hours). (B) 1, Calcium ionophore A23187; 2, calcium ionophore A23187 + H-7; 3, PMA (72 hours) + calcium ionophore A23187; 4, H-7; 5, PMA (72 hours). The results represent the mean ± SE of three separate experiments.
Fig 9. The role of PKC in IgE-DNP-induced c-fos expression. (A) Cells were either exposed or not to H-7 after IgE-DNP or calcium ionophore A23187 stimulation. (B) Cells were or were not exposed to 20 ng PMA/mL for 72 hours and then activated by IgE-DNP or by calcium ionophore A23187. The types of stimulation showed in each lane are as follows: (A) 1, control; 2, IgE-DNP; 3, IgE-DNP + H-7; 4, calcium ionophore A23187; 5, calcium ionophore A23187 + H-7; 6, H-7. (B) 1, control; 2, IgE-DNP; 3, PMA (72 hours) + IgE-DNP; 4, calcium ionophore A23187; 5, PMA (72 hours) + calcium ionophore A23187; 6, PMA (72 hours).

treatment of the cells by PMA did not affect the mRNA level of this proto-oncogene in unstimulated cells (Fig 9, A and B, lane 6). The mRNA of L30 did not significantly change in response to the treatments mentioned here.

Downregulation of PKC by either chronic PMA treatment of the cells or by H-7 inhibited the ability of IgE-DNP to induce the expression of c-jun and junB mRNA (Fig 10, lanes 1 through 4), whereas downregulation of PKC in unstimulated cells did not alter the mRNA levels of these two proto-oncogenes (Fig 10, lanes 1, 5, and 6). The mRNA of L30 did not significantly change in response to such treatments.

Fig 8. The effect of short-term PMA treatment on the expression of c-fos, c-jun, junB, and junD. ABFTL were exposed for 30 minutes to PMA at a concentration of 20 ng/mL, then the cytoplasmic RNA was extracted and treated as described in the legend to Fig 1. (A) c-fos expression. (B) c-jun, junB, and junD expression. One representative experiment out of three.
The results regarding the role played by PKC in the expression of the mRNA of c-fos, c-jun, and junB in IgE-DNP-stimulated cells were further confirmed by using two additional approaches. Using another PKC inhibitor, Staurosporine, resulted in an over-expression of c-fos (Fig 11) and a decrease in the level of the mRNAs of c-jun and junB, as compared with the level of their expression in the absence of Staurosporine. Combined activation of PKC by exposure of the IgE-sensitized cells to DNP-BSA and PMA together for 30 minutes slightly lowered the ability of IgE-DNP to induce the expression of c-fos mRNA, whereas it did not alter its ability to induce the expression of the mRNAs of the Jun proto-oncogenes.

Figure 12 shows that prolonged exposure of the cells to PMA or their treatment with H-7 blocked the ability of calcium ionophore A23187 to induce elevation of junB mRNA (Fig 12, lanes 1 through 4), whereas such treatments did not affect the mRNA of this proto-oncogene in unstimulated cells (Fig 12, lanes 5 and 6). The level of the mRNA of c-jun and junD (data not shown) and of L30 was not affected by the various treatments mentioned here.

A summary of the results is presented in Table 1.

**DISCUSSION**

The effect two mast cell exocytotic stimulants, IgE-Ag and calcium ionophore A23187, on mast cell proliferation and proto-oncogene expression was first investigated. The artificial stimulation by calcium ionophore A23187, in addition to the immunologic one, was studied to understand whether the proliferation and proto-oncogene expression in mast cells could be induced by different mechanisms, other than cross-bridging of the Fc,RI receptors. To study the direct effect of such stimuli on proliferation and to isolate the cell system from the influence of growth factors, we used the autonomous mast cell line ABFTL as the main cell target, whereas the IL-3-dependent primary culture of mast cells (MBMMC) was used only as a control. Short-term IgE-DNP stimulation of ABFTL, MBMMC, and RBL caused inhibition of the proliferation rate of these cells (Fig 1), suggesting that such a response to the immunologic stimulus is a general phenomena in mast cells.

Short-term activation of ABFTL by PMA, a PKC activator, was sufficient to inhibit proliferation of these cells (Fig 7). In addition, it was found that PKC activation, induced by IgE-Ag or by calcium ionophore A23187, was associated with the inhibitory effect of these stimuli on ABFTL growth (Fig 7). The involvement of PKC was evaluated first by using the prolonged treatment of the cells by PMA and H-7 or by using 4a-phorbol and Staurosporine to confirm the specificity of the involvement of this enzyme. Thus, it seems that PKC plays a negative role in the short-term proliferation of mast cells. Such an inhibitory role was found for PKC in the growth process of IL-3- and IL-4-dependent mast cells. Therefore, it may be possible that mast cell proliferation is determined by agents that regulate PKC activity.

IgE-DNP stimulation of ABFTL was followed by a transient expression of the mRNA of c-fos and a sustained...
expression of the mRNAs of c-jun and junB (Fig 3). It may be suggested that such differences in the time of appearance of the mRNAs of these proto-oncogenes might lead to a distinction in the pattern of appearance of the corresponding proteins. Thus, different combinations of Fos and Jun or Jun-Jun heterodimers could be formed at different times after the immunologic trigger, giving rise to different AP-1 subtypes. This, in turn, may affect the initiation of transcription of genes, which are responsive to the variations in the AP-1 species.

Stimulation of the cells by calcium ionophore A23187 only induced the expression of junB (Fig 5) and c-fos mRNAs. However, under these conditions the expression increased in a more delayed and transient pattern than that found when the cells were stimulated by IgE-Ag (kinetics of the expression of the mRNA of c-fos after calcium ionophore A23187 stimulation is not shown). Such differences in the proto-oncogene response to stimulation by the two mentioned triggers could be explained by differences in the biochemical pathways involved by cross-bridging of the Fc RI receptors as compared with those involved by activation with calcium ionophore A23187.

Elucidation of whether PKC is involved also in the regulation of the expression of the mRNA of the above-mentioned proto-oncogenes was next investigated. By using several methods of regulating PKC activity it was observed that this enzyme's activity was associated with upregulation of the level of the mRNAs of c-jun and junB proto-oncogenes in response to activation of the cells by either IgE-Ag or calcium ionophore A23187 (Figs 8, 10, and 11). On the other hand, the role played by PKC in the regulation of the expression of c-fos mRNA was found to be much more complex. PKC activity was associated with elevation of the level of the c-fos mRNA in response to activation by either short-term PMA treatment of the cells or calcium ionophore A23187 activation; however, inhibition of this enzyme activity during immunologic stimulation resulted in an enhancement of c-fos mRNA's expression (Fig 9). Hence, PKC exerts both positive and negative effects on the expression of the mRNA of c-fos in mast cells, depending on the type of stimulus involved. In explaining this phenomenon, it should be mentioned that in RBL cells, only a small proportion (<5%) of the total PKC is translocated to the membrane after stimulation with antigen,9 whereas PKA causes almost complete translocation of this enzyme.30,51 Moreover, the effects of PMA on RBL cells are exerted primarily through the PKC type III isoenzyme,51 while type II PKC exhibits marked changes in intracellular distribution during antigen stimulation.52 Therefore, it could be assumed that stimulation of mast cells via Fc RI receptors might result in activation of different types of PKC than those activated by short-term PMA trigger. Such differential activation of PKC might in turn result in diverse effects on the level of c-fos proto-oncogene mRNA expression. It has been shown that the dependence of PKC on calcium ions and phospholipids for its activation varies markedly, depending on the type of substrate available.50,53 Therefore, another possible explanation to the complex role played by PKC in the expression of the c-fos mRNA is that IgE-Ag, but not short-term PMA treatment or calcium ionophore A23187 activation, stimulates specific activator(s) or inhibitor(s) of c-fos's mRNA expression, which are regulated by PKC. Our results suggest that PKC regulates the balance between c-fos mRNA and those of c-jun and junB. As
mentioned previously, the relationship between Fos and Jun within the AP-1 complex probably regulates gene transcription and cell proliferation. Therefore, we suggest that the differences between the kinetics of the accumulation of the mRNAs of c-jun and junB, and that of c-fos, in response to the immunologic stimulus of mast cells, which are probably mediated by PKC, may regulate the transcription of various AP-1-responsive genes. However, the results do not exclude the possibility that the effects of PMA and the PKC inhibitors, H-7 and Staurosporine, on the various cellular responses studied in this work, were the result of other side effects of these agents other than inhibition of PKC activity.

It is interesting to discuss whether the jun proto-oncogenes play any role in the proliferation process of mast cells. The immunologic stimulation elevated the expression of the mRNAs of all the proto-oncogenes mentioned (Figs 2 and 3), yet partially inhibited mast cell proliferation (Fig 1). However, it failed to inhibit proliferation when PKC was depleted from the cells (Fig 7), while under these conditions the mRNA of c-fos was over-expressed (Fig 9) and the expression of mRNAs of the jun proto-oncogenes was downregulated (Fig 10). This finding may indicate that the over-expression of the c-fos mRNA may be responsible for the reduction of the inhibitory proliferative effect of IgE-Ag stimulation on ABFTL, while inhibition itself could be related to the expression of the mRNAs of the jun proto-oncogenes. Recently, it was reported that changes in the relationship between Fos and Jun content within the AP-1 complex are responsible for positive and negative effects of glucocorticoids on the transcription of the proliferin gene. We suggest that the dissimilarities between the pattern of the kinetics of the expression of the mRNAs of c-jun and junB and that of c-fos in response to the immunologic stimulus of ABFTL, which are probably mediated by PKC, may have a role in the regulation of the transcription of various AP-1-responsive genes. This finding may have a crucial role in the inhibitory effect of this stimulus on ABFTL proliferation because some of these genes could be related to cell growth. The positive correlation between the expression of the mRNA of c-fos gene and its protein has been shown in several systems including secretory cells. However, it still remains to be investigated whether such correlation exists in the mast cell system. Further investigations should concentrate on elucidation of the role played by AP-1 in the activation of mast cells and the involvement of PKC in this regard.

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