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Inhibitory Effect of Açaí (*Euterpe oleracea* Mart.) Pulp on IgE-Mediated Mast Cell Activation

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ABSTRACT: The palm fruit açaí is known to have potential health benefits due to its antioxidant scavenging capacities. Pretreatment of IgE-sensitized mouse primary cultured mast cells with açaí pulp resulted in the dramatic suppression of antigen-induced degranulation in a dose-dependent manner. Similarly, açaí suppressed IgE-mediated degranulation and transcription of the cytokine genes from a cultured mast cell line of rat basophilic leukemia (RBL)-2H3 cells. Açaí could selectively inhibit FcεRI signaling pathways. Furthermore, the FcεRI-mediated complementary signaling pathway was also suppressed by açaí. These results demonstrate that açaí is a potent inhibitor of IgE-mediated mast cell activation.

KEYWORDS: mast cells, signal transduction, protein tyrosine kinase, açaí, *Euterpe oleracea* Mart.

INTRODUCTION

The activation of mast cells plays a central role in allergic responses. The aggregation of high-affinity IgE receptor (FcεRI) on mast cells induces a number of biochemical events leading to degranulation and production of multiple cytokines and leukotrienes.[1] Antigen-induced engagement of FcεRI triggers the activation of Lyn protein−tyrosine kinase (PTK), resulting in rapid tyrosine phosphorylation of FcεRIβ and γ subunits within the immunoreceptor tyrosine-based activating motif (ITAM).[2] Lyn is a member of the Src family nonreceptor type PTK. Lyn constitutively associates with FcεRIβ and localizes in the plasma membrane by its myristoylation and palmitoylation signals.[3] Kinase activity of Lyn is regulated by the balance of tyrosine phosphorylation: dephosphorylation of C-terminal regulatory Tyr^{527} by CD45 and autophosphorylation of Tyr^{416} in the activation loop.[4] Phosphorylation of ITAM of FcεRI recruits and activates Syk protein−tyrosine kinase, an essential kinase for the activation of mast cells.[5−7] Activated Syk phosphorylates LAT to activate phospholipase C-γ (PLC-γ) and mitogen-activated protein kinases (MAPKs) such as c-Jun N-terminal kinase (JNK) and extracellular signal regulated kinase (ERK).[7−9] There is a complementary signaling pathway besides this Lyn−Syk−LAT pathway in mast cells. Another member of the Src family PTK, Fyn, phosphorylates Grb2-associated binder-2 (Gab2), which controls the activation of phosphatidylinositol 3-kinase (PI3K). The product of PI3-kinase, phosphatidylinositol 3,4,5-triphosphates (PIP_3), targets the pleckstrin homology domain of signaling molecules including PLC-γ and Akt to the plasma membrane. This complementary signaling pathway is required for the regulated degranulation and transcription of the cytokine genes in response to antigen stimulation in mast cells.[10]

Dietary intake of natural fruits may contribute to the prevention of many chronic disorders. Açaí (*Euterpe oleracea* Mart.), a palm fruit native to the Amazon estuary, contains a variety of phytochemicals and has been used as a major food source or as a folk medicine by native people from ancient times.[11] Recent papers showed that açaí contains phenolic compounds, and much attention has been paid to its potential health benefits, due to its antioxidant scavenging activities.[11−14] Polyphenols from apple extracts have an antiallergic effect on patients with atopic dermatitis and on allergic model mice.[15] Analysis of the biochemical properties revealed that açaí could induce the apoptosis of HL-60 leukemia cells and antiproliferative activities in HT-20 human colon adenocarcinoma cells.[16,17] However, studies on the biological relevance of açaí have yet to be investigated in detail.[17] We thus attempted to examine the antiallergic activities of açaí whole fruit pulp by investigating the role of açaí on IgE-mediated mast cell activation.

MATERIALS AND METHODS

Materials. Mouse monoclonal anti-dinitrophenyl IgE (anti-DNP IgE) and protein A−agarose beads were purchased from Sigma (St. Louis, MO). Anti-phosphotyrosine (pTyr) mAb (4G10), anti-JNK, and anti-Gab2 antibodies were obtained from Upstate Biotechnology (Lake Placid, NY). Anti-FcεRIβ monoclonal antibodies (mAb) were kindly provided by Dr. Juan Rivera[18] and Dr. Reuben P. Siraganian (National Institutes of Health, Bethesda, MD). Anti-Lyn and anti-PLC-γ2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-JNK (Thr^{183}/Tyr^{185}), anti-phospho-p44/42 ERK (Thr^{202}/Tyr^{204}), anti-p44/42 ERK, anti-phospho-Akt (Ser^{473}), and anti-Akt antibodies came from Cell Signaling Technology (Beverly, MA). Anti-mouse FcεRIα was obtained from eBioscience (San Diego, CA) and rabbit anti-mouse IgG/IgE antibody from Jackson Immuno Research (West Grove, PA). Açaí pulp was directly homogenized and separated by centrifugation for 10 min at 15000*g*. The resulting supernatants were passed through a 0.45 μm filter (Millipore, Bedford, MA). The precleared, filtered açaí pulp was directly added to the cell culture medium at either 1/30 or 1/60 dilution. Sterilized water was utilized as a vehicle control.

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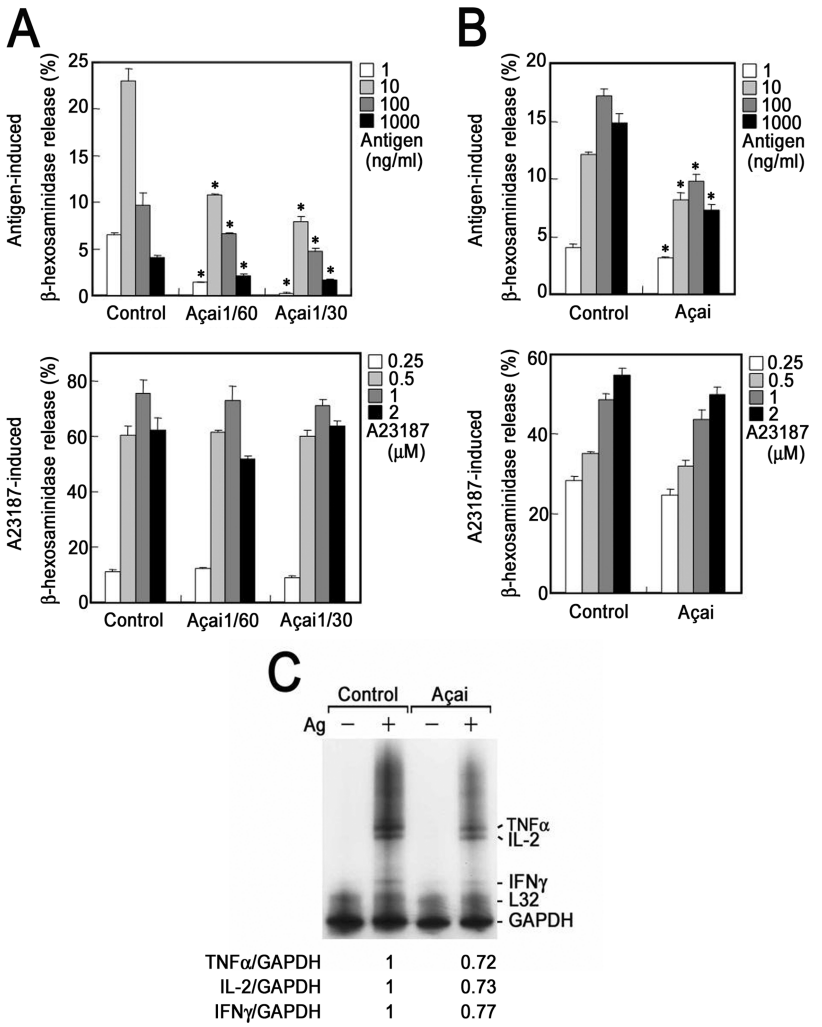

Figure 1. Açaí inhibits IgE-mediated mast cell activation (A, B) Analysis of β-hexosaminidase release. BMMC (A) and RBL-2H3 cells (B) were sensitized with anti-DNP IgE overnight and then preincubated either with or without (control) açaí (1/30 or 1/60 dilution) for 4 h prior to stimulation. Cells were stimulated with either antigen DNP-BSA or A23187 at the indicated concentrations for 1 h. Release of β-hexosaminidase was presented as a percentage of the total activity. The results are the mean value ± SD from three independent experiments. Statistical significance was determined by a two-sided Student's *t* test. ∗, $P < 0.01$, compared with the control of each antigen concentration. Statistical analysis was performed using Excel 2000 (Microsoft) with the add-in software Statcel2. (C) RNase protection assay. RBL-2H3 cells were sensitized with anti-DNP IgE overnight, pretreated with or without (control) açaí (1/30) for 4 h, and then stimulated with (+) or without (−) 30 ng/mL of antigen DNP-BSA (Ag) for 1 h. Total RNA was extracted and hybridized with the ^{32}P-labeled RNA probes of rat cytokines. After RNase treatment, the protected double-stranded RNA was separated by the urea gel and analyzed by the autoradiography. The results were representative of two experiments. The numbers at the bottom of the figures are the results of densitometric analysis of TNFα, IL-2, and INFγ normalized by GAPDH.

Cell Culture. Bone marrow cells from C57BL6/J mice were cultured in a complete medium for 4 weeks in RPMI-1640 (Sigma) and supplemented with 15% (v/v) heat-inactivated fetal calf serum (FCS), 100 U/mL of penicillin, 1× nonessential amino acids (Invitrogen, Carlsbad, CA), 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, and 50% of murine myelomonocytic leukemia WEHI-3 cells prepared in a medium as a source of mast cell growth factors. After 4 weeks, cells were differentiated into mouse bone marrow-derived mast cells (BMMC) with the surface expression of FcεRI and used for experiments.[19] Rat basophilic leukemia RBL-2H3 cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM, Sigma) with 100 U/mL of penicillin and 10% (v/v) FCS.[20]

Analysis of β-Hexosaminidase Release. The release of β-hexosaminidase was measured as described previously.[20−24] In brief, cells were cultured overnight with anti-DNP IgE (1/5000) and pretreated with or without açaí pulp for 4 h prior to stimulation. Then, cells were washed once with Tyrode−Hepes buffer (10 mM Hepes, pH 7.4, 127 mM NaCl, 4 mM KCl, 0.5 mM KH_2PO_4, 1 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM $LiCl_2$, 5.6 mM glucose, and 0.1% BSA) and stimulated with different concentrations of the antigen DNP-BSA (2,4-dinitrophenylated bovine serum albumin, LSL, Tokyo, Japan) or Ca^{2+} ionophore A23187 (Sigma) in the same buffer. After 1 h of incubation at 37 °C, the medium was recovered for analysis of β-hexosaminidase activity. Total β-hexosaminidase activity was measured using cell lysates obtained by the addition of 1% NP-40 in the same buffer. The cultured supernatants and cell lysates were incubated with 1.3 mg/mL of *p*-nitrophenyl-2-acetoamido-2-deoxy-β-D-glucopyranoside (Nacalai, Osaka, Japan) in 0.1 M sodium citrate (pH 4.5) for 30 min at 37 °C. The reaction was terminated by the addition of 0.2 M glycine (pH 10.7), and release of the product 4-*p*-nitrophenol was monitored by absorbance at 405 nm using a Microplate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA).

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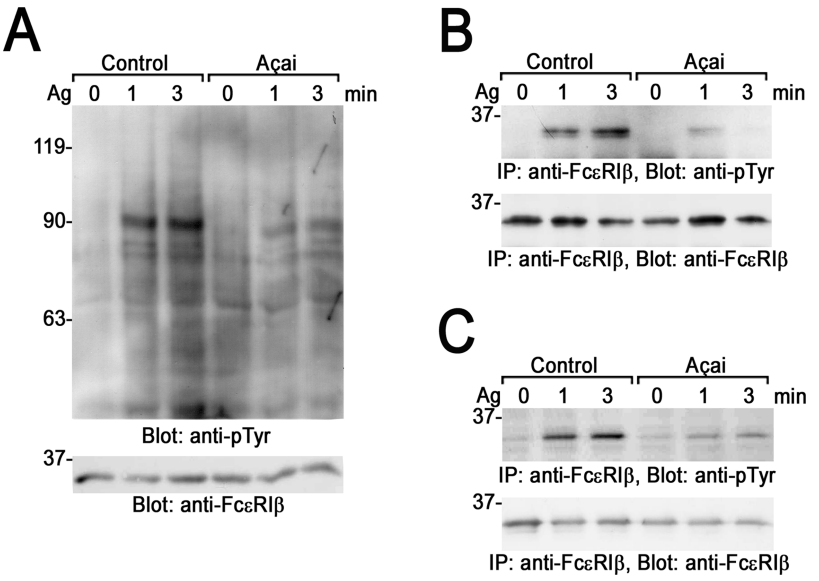

Figure 2. Açaí inhibits IgE-mediated protein−tyrosine phosphorylation of FcεRIβ. (A) Analysis of protein−tyrosine phosphorylation. BMMC were sensitized with anti-DNP IgE overnight, preincubated either with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Total cell lysates were separated by SDS-PAGE and analyzed by the immunoblotting with anti-pTyr (upper panel) or anti-FcεRIβ antibody (lower panel), respectively. (B, C) Analysis of protein−tyrosine phosphorylation of FcεRIβ. BMMC (B) or RBL-2H3 cells (C) were sensitized with anti-DNP IgE overnight, preincubated with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Cells were solubilized in Triton lysis buffer, and cell lysates were immunoprecipitated with anti-FcεRIβ mAb. Anti-FcεRIβ immunoprecipitates were separated by SDS-PAGE and analyzed by immunoblotting with anti-pTyr (upper panels) or anti-FcεRIβ antibody (lower panels), respectively. Molecular sizing markers are indicated at the left in kilodaltons. The results are representative of three experiments.

Analysis of Multiple Cytokine mRNA Transcription by RNase Protection Assay. Multiple cytokine mRNA transcription was analyzed as described previously.[21−24] RBL-2H3 cells cultured overnight with anti-DNP (1/5000) were preincubated either with or without (control) açaí pulp (1/30) for 4 h. Cells were then stimulated with 30 ng/mL antigen DNP-BSA for 1 h. Total RNA was purified by RNeasy Mini Kit (Qiagen, Hilden, Germany). ^{32}P-Labeled RNA probes were synthesized using Rat Cytokine Multi-Probe Template Sets and a Riboquant In Vitro Transcription Kit (BD Biosciences, San Jose, CA). The synthesized probes were hybridized with total RNA by using a Multiprobe RNase Protection Assay Kit (BD Biosciences). After the RNase treatment, the protected double-stranded RNAs were separated by the urea gel and visualized with autoradiography.

Cell Activation, Immunoprecipitation, and Immunoblotting. BMMC or RBL-2H3 cells cultured overnight with anti-DNP IgE were pretreated either with or without (control) açaí pulp (1/30) for 4 h prior to stimulation. For cell activation, cells were washed once with Tyrode−Hepes buffer and then stimulated with 30 ng/mL antigen DNP-BSA in the same buffer for the indicated times.

For the preparation of total cell lysates, cells were rinsed twice with PBS and lysed by the direct addition of a 2× SDS sample buffer. For the preparation of detergent-soluble lysates, cells were solubilized in Triton lysis buffer (1% Triton X-100, 50 mM Tris, pH7.4, 150 mM NaCl, 10 mM EDTA, 100 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1 μg/mL of aprotinin). After centrifugation, the supernatants were mixed with the SDS sample buffer. For immunoprecipitation, detergent-soluble lysates were reacted with the indicated antibody prebound to protein A−agarose for 90 min at 4 °C. The beads were washed four times with Triton lysis buffer, and precipitated proteins were eluted by heat treatment at 100 °C for 5 min with the 2× SDS sample buffer.

Total cell lysates, detergent-soluble lysates, and immunoprecipitated proteins were separated by SDS−polyacrylamide gel electrophoresis (SDS-PAGE) and electronically transferred onto a polyvinylidene difluoride membrane (Millipore). The membrane was preincubated with 5% milk in TBST (25 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween 20) and then probed with primary antibody in TBST. After washing with TBST, the membrane was reacted with a horseradish peroxidase conjugated secondary antibody and washed extensively in TBST. In all blots, proteins were visualized by the chemiluminescence reagent (Western Lightning, PerkinElmer Life Sciences, Boston, MA).[25,26]

In Vitro Protein Kinase Assay. RBL-2H3 cells cultured overnight in anti-DNP IgE were pretreated either with or without (control) açaí pulp (1/30) for 4 h prior to stimulation. Cells were washed once with Tyrode−Hepes buffer and then stimulated with 30 ng/mL antigen DNP-BSA in the same buffer for the indicated times. Cells were solubilized in Triton lysis buffer, and cell lysates were immunoprecipitated with an anti-Lyn antibody. Anti-Lyn immunoprecipitates were washed twice with the kinase buffer without ATP and then incubated with 40 μL of the kinase buffer (40 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 4 μM ATP, 4 μCi [γ-^{32}P] ATP) and 2.5 μg of acid-treated enolase (Sigma) for 30 min at room temperature. Reactions were terminated by heat treatment at 100 °C for 5 min with the 2× SDS sample buffer. Proteins were separated by SDS-PAGE, and gels were incubated with 1 N KOH at 56 °C for 1 h to remove the phosphoserine and most of the phosphothreonine. After the gel had been dried, the radiolabeled proteins were visualized using autography.[22,23]

RESULTS

Açaí Inhibits IgE-Mediated Activation of Mast Cells. Aggregation of high-affinity IgE receptor (FcεRI) by multivalent antigens induces degranulation from mast cells. In performing this process, we attempted to analyze the effect of the tropical fruit açaí on IgE-mediated mast cell degranulation by measuring

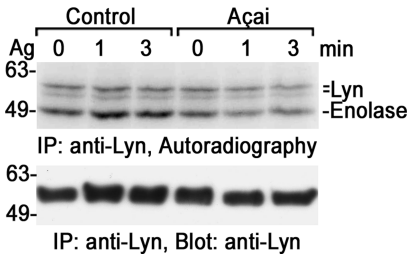

Figure 3. Açaí inhibits IgE-mediated activation of Lyn protein−tyrosine kinase. RBL-2H3 cells were sensitized with anti-DNP IgE, preincubated either with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Cells were solubilized in Triton lysis buffer, and cell lysates were immunoprecipitated with an anti-Lyn antibody. Anti-Lyn immunoprecipitates were subjected to the in vitro protein kinase assay using enolase as the exogenous substrate. Radioactive proteins were separated by SDS-PAGE and analyzed using autoradiography. Anti-Lyn immunoprecipitates were analyzed by immunoblotting with an anti-Lyn antibody (lower panel). Molecular sizing markers are indicated at the left in kilodaltons. The results are representative of three experiments.

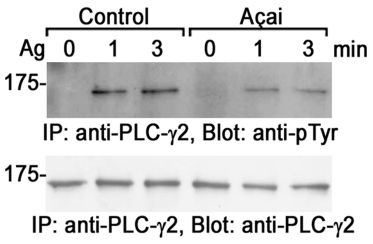

Figure 4. Açaí decreases IgE-mediated protein−tyrosine phosphorylation of PLC-γ2. RBL-2H3 cells were sensitized with anti-DNP IgE overnight, preincubated with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Cells were solubilized in Triton lysis buffer, and cell lysates were immunoprecipitated with anti-PLC-γ2 antibody. Anti-PLC-γ2 immunoprecipitates were separated by SDS-PAGE and analyzed by the immunoblotting with anti-pTyr (upper panel) or anti-PLC-γ2 antibody (lower panel), respectively. The results are representative of three experiments.

the release of β-hexosaminidase. IgE-sensitized mouse primary cultured mast cells (BMMC) were pretreated with açaí pulp (açaí) and then stimulated with the antigen DNP-BSA at different concentrations. Pretreatment of cells with açaí inhibited IgE-mediated degranulation in a dose-dependent manner (Figure 1A, top panel). Antigen DNP-BSA at 10 ng/mL induces the highest degree of degranulation in both control and açaí-pretreated cells. In contrast, açaí could not suppress Ca^{2+} ionophore A23187-induced release (Figure 1A, bottom panel). The suppression of IgE-mediated degranulation was not observed when açaí was extracted with ethanol (data not shown). Similar results were obtained when açaí was added to the cultured mast cell line RBL (rat basophilic leukemia)-2H3 cells (Figure 1B). An inhibitory effect was also observed when the pretreatment period was 10 min; however, pretreatment of cells with açaí for only 1 min could not inhibit IgE-mediated degranulation, suggesting that the effect of açaí is not due to the inhibition of the in vitro enzymatic reaction of β-hexosaminidase in the assay system (data not shown).

Besides degranulation, aggregation of FcεRI induces transcription of multiple cytokines genes. Sensitized RBL-2H3 cells were pretreated either with or without açaí and then stimulated with the antigen (Figure 1C). The effect of açaí on cytokine gene transcription was quantitatively measured by RNase protection assay. Antigen-induced transcription of cytokine genes such as tumor necrosis factor α (TNFα), interleukin-2 (IL-2), and interferon γ (INFγ) was inhibited by the pretreatment of cells with açaí, whereas that of housekeeping genes (L32 and GAPDH) was not affected (Figure 1C). Collectively, these results demonstrate that açaí has a function to suppress IgE-mediated mast cell activation.

Açaí Inhibits IgE-Mediated Activation of Lyn Protein−Tyrosine Kinase. Next, we examined the effect of açaí on IgE-mediated cellular signaling pathways leading to the degranulation and production of cytokines. First, we examined IgE-mediated tyrosine phosphorylation of cellular protein (Figure 2A). Pretreatment of BMMC with açaí did not affect the overall protein−tyrosine phosphorylation in resting cells (Figure 2A, lane 1 vs 4). However, antigen-induced increase in tyrosine phosphorylation of cellular proteins was inhibited by the pretreatment of BMMC with açaí (Figure 2A, lanes 2 and 3 vs 5 and 6). The membrane was stripped and reprobed with an anti-FcεRIβ mAb to confirm the loading of equal amounts of protein (Figure 2A, bottom panel).

Aggregation of FcεRI causes rapid tyrosine phosphorylation of FcεRIβ and γ subunits by Lyn, which propagates the receptor-activating signals to downstream signaling pathways. Thus, we tested the effect of açaí on protein−tyrosine phosphorylation of FcεRIβ, a substrate of Lyn. Pretreating BMMC with açaí dramatically suppressed the antigen-induced tyrosine phosphorylation of FcεRIβ (Figure 2B). A similar result was obtained by using RBL-2H3 cells (Figure 2C). Therefore, these results suggest that pretreatment of mast cells with açaí could inhibit the antigen-induced tyrosine phosphorylation of FcεRIβ, the substrate of Lyn protein−tyrosine kinase.

The intrinsic kinase activity of Lyn was examined with in vitro kinase assay using enolase as the in vitro substrate. Consistent with phosphorylation of FcεRIβ, pretreatment of cells with açaí suppressed FcεRI-mediated increase in the phosphorylation of enolase (Figure 3). In addition, in vitro reaction of anti-Lyn immunoprecipitates with açaí resulted in the suppression of the kinase activity of Lyn (data now shown). Altogether, açaí inhibits the cellular signaling pathways at the initial event, which is responsible for the antigen-induced degranulation and production of multiple cytokines.

Açaí Inhibits IgE-Mediated Activation of Cellular Signaling Pathway. Aggregation of FcεRI induces the activation of cellular signaling pathways leading to the activation of mast cells. Therefore, we attempted to analyze in detail the effect of açaí on antigen-induced activation of signaling pathways. Sequential activation of nonreceptor type protein−tyrosine kinases after FcεRI aggregation results in the activation of PLC-γ. PLC-γ is an effector molecule responsible for FcεRI-mediated intracellular Ca^{2+} mobilization, which induces the degranulation as well as the transcription of cytokine genes. Pretreatment of cells with açaí inhibited FcεRI-mediated increase in tyrosine phosphorylation of PLC-γ2 (Figure 4). Similar results were obtained when BMMC was tested (data not shown).

Then we tested the effect of açaí on IgE-mediated activation of downstream protein kinases, JNK and ERK, both of which regulate the production of cytokines (Figure 5). FcεRI-mediated activation of JNK was abrogated by the pretreatment with açaí;

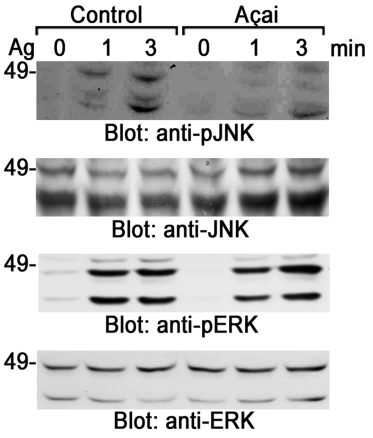

Figure 5. Effect of açaí on downstream protein kinases. RBL-2H3 cells were sensitized with anti-DNP IgE overnight, preincubated either with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Detergent-soluble lysates (for JNK) and total cell lysates (for ERK) were separated by SDS-PAGE and analyzed by immunoblotting with anti-phospho-JNK (Thr^{183}/Tyr^{185}), anti-JNK, anti-phospho-p44/42 ERK (Thr^{202}/Tyr^{204}), and anti-p44/42 ERK antibodies, respectively. The results are representative of three experiments.

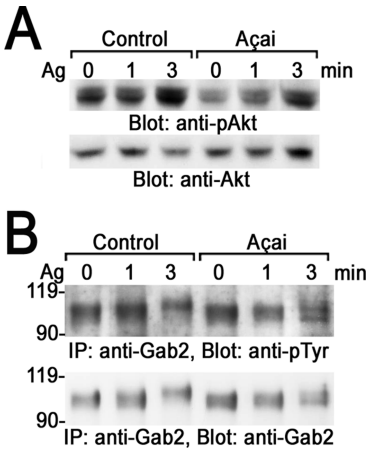

Figure 6. Effect of açaí on complementary signaling pathways. RBL-2H3 cells were sensitized with anti-DNP IgE overnight, preincubated either with or without (control) açaí (1/30) for 4 h, and then stimulated with 30 ng/mL antigen DNP-BSA (Ag) for the indicated times. Cells were directly lysed in 2× SDS sample buffer (for Akt) or solubilized in a Triton lysis buffer, and cell lysates were immunoprecipitated with an anti-Gab2 antibody. Total cell lysates (A) and anti-Gab2 immunoprecipitates (B) were separated by SDS-PAGE and analyzed by immunoblotting with anti-phospho-Akt (Ser^{473}) and anti-Akt antibodies (A) or anti-pTyr and anti-Gab2 (B), respectively. The results are representative of three experiments.

however, activation of ERK was apparently not inhibited. This suggests that açaí selectively inhibits the downstream signaling pathways of JNK, but not ERK. It is still possible that crude açaí pulp contains a minor fraction which mimics the FcεRI-mediated activation of ERK. Similar results were obtained when BMMC was tested (data not shown).

Aggregation of FcεRI induces the activation of conventional (Lyn−Syk−LAT) and complementary signaling pathways (Fyn−Gab2−PI3K), both of which contribute to the activation

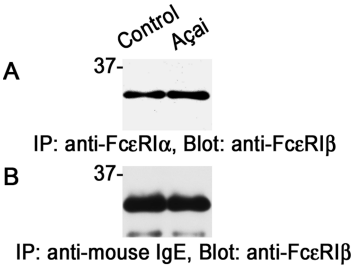

Figure 7. Effect of açaí on the expression of FcεRI and binding of IgE to FcεRI. (A) RBL-2H3 cells were pretreated either with or without (control) açaí pulp (1/30) for 4 h. Anti-FcεRIα antibody was added to the cell culture medium to react with FcεRI on cell surfaces. Cells were rinsed twice with PBS and solubilized in a Triton lysis buffer. Anti-FcεRIα immunoprecipitates were separated by SDS-PAGE and analyzed by immunoblotting with an anti-FcεRIβ antibody. (B) RBL-2H3 cells cultured overnight with anti-DNP IgE were pretreated either with or without (control) açaí pulp (1/30) for 4 h. Cells were rinsed twice with PBS and solubilized in the Triton lysis buffer. Anti-mouse IgE immunoprecipitates were separated by SDS-PAGE and analyzed by immunoblotting with the anti-FcεRIβ antibody. Molecular sizing markers are indicated at the left in kilodaltons. The results are representative of three experiments.

of mast cells. Therefore, finally we tested the effect of açaí on Gab2, which plays a critical role in the activation of PI3K and its downstream molecule Akt.[27] Pretreatment of cells with açaí moderately inhibited FcεRI-mediated phosphorylation of Akt and tyrosine phosphorylation of Gab2 (Figure 6). Similar results were obtained when BMMC was tested (data not shown). These results demonstrate that açaí inhibits FcεRI-mediated activation of cellular signaling molecules such as PLC-γ and JNK. Phosphorylation of complementary signaling molecules such as Gab2 and Akt are moderately inhibited by açaí. Although the activation of ERK is the downstream of LAT, açaí could not suppress the IgE-mediated activation of ERK.

Açaí Does Not Affect the Expression of FcεRI and Binding of IgE to FcεRI. To demonstrate whether açaí affects the expression of FcεRI on cell surfaces, RBL-2H3 cells were pretreated either with or without (control) açaí pulp (1/30), and an anti-FcεRIα antibody was added to the cell culture medium to react with FcεRI on cell surfaces. Anti-FcεRIα immunoprecipitates, which contain FcεRI expressed on cell surfaces, were collected and analyzed by immunoblotting with an anti-FcεRIβ antibody. As shown, açaí does not affect the expression of FcεRI on cell surfaces (Figure 7A). To demonstrate whether açaí affects the binding of IgE to FcεRI, RBL-2H3 cells cultured overnight with anti-DNP IgE were pretreated either with or without (control) açaí pulp (1/30). The IgE-binding form of FcεRI on cell surfaces was immunoprecipitated with an anti-mouse IgE antibody. Anti-IgE immunoprecipitates were analyzed by immunoblotting with the anti-FcεRIβ antibody. This experiment demonstrated that açaí does not affect the binding of IgE to FcεRI (Figure 7B).

DISCUSSION

Genetic studies have shown that Lyn has both positive and negative regulatory functions on IgE-mediated degranulation.[28,29] FcεRI-mediated activation of mast cells is thought to be Lyn-dependent; however, Lyn is also responsible for phosphorylating the immunoreceptor tyrosine-based inhibitory motif

(ITIM)-bearing receptors that inhibit mast cell activation.[30−32] In vivo relevance of ITIM-bearing receptors on mast cell function was further demonstrated by the analysis of mice lacking those receptors.[33,34] A recent paper also showed that Src family PTK Hck is a candidate molecule for the selective regulation of the Lyn in mast cells.[35] The present results demonstrate that açaí inhibits IgE-mediated activation of Lyn, tyrosine phosphorylation of FcεRIβ, degranulation, and the transcription of cytokine genes in mast cells (Figures 1−3). This would suggest that açaí affects the positive role of Lyn for phosphorylating ITAM of FcεRIβ, but presumably does not affect the negative regulatory role for phosphorylating ITIM-bearing inhibitory receptors. One of the possible explanations is that açaí affects the conformation and/or intracellular localization of Lyn, resulting in the selective inhibition of the positive roles of Lyn in mast cells.

Dramatic suppression of the antigen-induced tyrosine phosphorylation of FcεRIβ by açaí causes apparent inhibition of the downstream signaling molecules. IgE-mediated tyrosine phosphorylation of PLC-γ2 and phosphorylation of JNK were suppressed by pretreatment with açaí (Figures 4 and 5). IgE-mediated activation of the complementary pathway including Gab2 and Akt was also suppressed by açaí (Figure 6). Recent studies indicated that Gab2 locates downstream of both Fyn and Syk, and Syk more dominantly associates with Gab2.[27,36,37] The results in this study suggested that inhibition of Lyn by açaí leads to the suppression of Syk and Gab2/Akt and, therefore, IgE-mediated degranulation and cytokine production. Dramatic inhibition of Lyn may account for the suppression of IgE-mediated activation of these cellular signaling pathways; the signaling properties of Lyn-deficient mast cells remain controversial because Lyn seems to have both positive and negative regulatory roles on FcεRI-mediated mast cell signaling pathways. On the other hand, inducible phosphorylation of ERK was not inhibited, although ERK is located downstream of LAT-mediated Ras activation (Figure 5).[38] These results suggest that there is a selective inhibitory mechanism of the downstream signaling molecules by açaí. It is possible that some minor components of açaí stimulate the marginal activation of Ras or ERK in mast cells, because we utilized a soluble crude fraction of açaí.

The present study demonstrates the inhibitory role of açaí on IgE-mediated mast cell activation. Several tropical fruits that have antioxidant scavenging activities were examined for whether or not they could suppress IgE-mediated mast cell activation. Our preliminary experiments demonstrated that many tropical fruits had antioxidant scavenging activities (data not shown). So far, in what we have tested, only açaí could suppress IgE-mediated mast cell activation, suggesting that this function of açaí is not simply due to common antioxidant scavenging capacities among these tropical fruits. Furthermore, the fat-soluble fraction of açaí could not suppress the IgE-mediated mast cell activation (T.H., N.H-T., and K.S., unpublished observation). Thus, by exploring the soluble fraction of açaí, this study will pave athe way to the development of a new class of antiallergic drugs.

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ABBREVIATIONS USED

FcεRI, high-affinity IgE receptor; PTK, protein−tyrosine kinase; PLC-γ, phospholipase C-γ; JNK, c-Jun N-terminal kinase; ERK, extracellular signal regulated kinase; Gab2, Grb2-associated binder-2; BMMC, bone marrow-derived mast cells; RBL, rat basophilic leukemia; DNP, dinitrophenyl; pTyr, phosphotyrosine.

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