DJ-1 regulates mast cell activation and IgE-mediated allergic responses

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Background: DJ-1 is an antioxidant protein known to reduce levels of reactive oxygen species (ROS), but its presence or function in mast cells and allergic diseases is unknown.
Objectives: We sought to determine the role and mechanism of DJ-1 in allergic responses *in vitro* and *in vivo*.
Methods: ROS and DJ-1 levels in serum or culture medium were measured with ELISA kits. The role of DJ-1 was evaluated in mast cell cultures and passive cutaneous anaphylaxis in normal or DJ-1 knockout (KO) mice. The mechanism of DJ-1 action was examined by using immunoblotting, immunoprecipitation, RT-PCR, and other molecular biological approaches.
Results: Patients with atopic dermatitis had increased levels of ROS and diminished levels of DJ-1. DJ-1 KO mice exhibited enhanced passive cutaneous anaphylaxis and augmented ROS levels in sera and bone marrow–derived mast cells (BMMCs). Furthermore, antigen-induced degranulation and production of TNF-α and IL-4 were significantly amplified in DJ-1 KO and anti–DJ-1 small interfering RNA–transfected BMMCs compared with that seen in wild-type (WT) BMMCs. Studies with these cells and BMMCs transfected with small interfering RNAs against the phosphatases Src homology domain 2-containing protein tyrosine phosphatase (SHP) 1 and SHP-2 revealed that the DJ-1 KO phenotype could be attributed to suppression of SHP-1 activity and enhancement of SHP-2 activity, leading to strengthened signaling through linker for activation of T cells, phospholipase Cγ, and mitogen-activated protein kinases.
Conclusions: A deficiency or constitutive activation of DJ-1 can have implications in mast cell–driven allergic diseases, such as asthma and anaphylaxis. (J Allergy Clin Immunol 2013;131:1653-62.)

Key words: *DJ-1, reactive oxygen species, mast cells, allergy, FcεRI-mediated signals*

There is evidence that allergic disorders, such as asthma, rhinitis, and atopic dermatitis (AD), are associated with oxidative stress.[1,2] One regulator of intracellular and extracellular reactive oxygen species (ROS) levels is DJ-1, for which no role has been reported in mast cells and allergy. However, this protein plays a critical role in antioxidative stress activity by reducing ROS levels and transcriptional regulation. Loss of function of DJ-1 is associated with some types of neurodegenerative diseases and cancer.[3] The antioxidant activity of DJ-1 is achieved through several mechanisms, namely self-oxidation at cysteine residues 46, 53, and 106 (C46, C53, and C106, respectively) to eliminate ROS[4-6] and interaction with superoxide dismutase, glutathione peroxidase, and catalases to enhance their ability to remove ROS.[7]

Mast cells are key mediators of IgE-mediated allergic diseases, including asthma, rhinitis, and AD. Activation of mast cells through cross-linking of FcεRI-bound IgE with antigen results in release of 3 classes of mediators: granule-associated mediators, cytokines, and inflammatory lipids.[8,9] The initiating signaling event is the activation of Src family tyrosine kinases, including Lyn.[10,11] Lyn then phosphorylates the immunoreceptor tyrosine-based activation motifs of the β and γ subunits of adjacent FcεRI receptors. The subsequent recruitment and activation of spleen tyrosine kinase (Syk) by the phosphorylated immunoreceptor tyrosine-based activation motif results in activation of signaling molecules, such as linker for activation of T cells (LAT) and downstream signaling pathways that lead to release of various allergic mediators.[12,13] In addition, other Src family kinases, including Fyn, Hck, and Fgr, initiate signals required for optimal activation of mast cells.[14-16]

Other, less well understood components of mast cell activation are ROS, such as superoxide anion and hydrogen peroxide (H_2O_2). These diffusible molecules are produced by virtually all cells, including mast cells.[17] Earlier reports indicated that activated mast cells have increased intracellular ROS levels.[18,19] Also, ROS enhances histamine release from mast cells,[18,20] and H_2O_2 in particular can regulate tyrosine phosphorylation of phospholipase C (PLC) γ and LAT.[21] However, the mechanisms by which ROS influence mast cell signaling is largely unclear.

The above considerations led us to study the allergic reaction in DJ-1–deficient mice, as well as the reactivity of mast cells from these mice. For the first time, we report that DJ-1 modulates ROS levels in mast cells and, as a consequence, regulates the activity of

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Abbreviations used
AD: Atopic dermatitis
BMMC: Bone marrow–derived mast cell
DNP: 2,4-Dinitrophenyl
H_2O_2: Hydrogen peroxide
KO: Knockout
LAT: Linker for activation of T cells
MAP: Mitogen-activated protein
PCA: Passive cutaneous anaphylaxis
PI3K: Phosphoinositide 3-kinase
PLC: Phospholipase C
ROS: Reactive oxygen species
SHP: Src homology domain 2-containing protein tyrosine phosphatase
siRNA: Small interfering RNA
Syk: Spleen tyrosine kinase
TEMPO: 2,2,6,6-Tetramethylpiperidin-1-oxyl
WT: Wild-type

Fyn, Syk, and LAT, as well as responses to antigen, *in vitro* and *in vivo*.

METHODS

Reagents

Reagents were from the following sources: antibodies detecting activating phosphorylations of Syk (Y352 or Y525/526), LAT (Y191), Gab2 (Y452), PLCγ1 (Y783), p85 phosphoinositide 3-kinase (PI3K; Y458), and Src homology domain 2-containing protein tyrosine phosphatase (SHP) 2 (Y542) were from Upstate Biotechnology (Lake Placid, NY); antibodies detecting activated phosphorylated forms of Akt (T308), Erk1/2 (T202/Y204), p38 (T180/Y182), and c-Jun N-terminal kinase (T183/Y185) were from Cell Signaling Technology (Danvers, Mass); and antibodies against Lyn, Fyn, Fgr, SHP-1, SHP-2, and DJ-1 were from Santa Cruz Biotechnology (Santa Cruz, Calif). Unless otherwise stated, other reagents were from Sigma (St Louis, Mo).

Generation of passive cutaneous anaphylaxis in mice and sera from patients with AD

Passive cutaneous anaphylaxis (PCA) was induced by injection of antigen (2,4-dinitrophenyl [DNP]–BSA) into mice previously primed with DNP-specific IgE in one ear.[22] Evans blue dye in ear tissues was extracted in formamide for assay at 620 nm. Mast cells in the ear tissues were histologically examined, as described elswhere.[16] The animal study was approved by the Institutional Animal Care and Use Committee at Konkuk University. Patients with AD (n = 56) who visited the Seoul Allergy Clinic, Seoul, Korea, participated in this study. They fulfilled the criteria of Hanifin and Rajka.[23] The patients had been experiencing a wide range of AD severity with a SCORAD index score of 35.0 ± 19.8 (22 with mild, 17 with moderate, and 17 with severe disease) at the time of study (see Table E1 in this article's Online Repository at www.jacionline.org).[24] All medications were discontinued for at least 2 weeks before the study, although topical application of 1% hydrocortisone was allowed. Healthy control subjects (n = 15) had no history of specific diseases, including allergic disease. The study was approved by the Institutional Review Board of Eulji University, Daejeon, Korea.

Mice, cell culture, and stimulation of bone marrow–derived mast cells

DJ-1 knockout (KO) and wild-type (WT) C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, Me). Bone marrow–derived mast cells (BMMCs) were from mouse bone marrow and prepared by means of culture for 4 weeks in a 50% enriched medium (RPMI 1640 containing 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, antibiotics, and 10% FBS) containing 10 ng/mL IL-3.[25] For individual experiments, cells (2×10^5 cells/1.5-mL tube) were primed overnight with DNP-specific IgE (50 ng/mL) and then stimulated with 25 ng/mL antigen in Tyrode-BSA buffer (20 mmol/L HEPES [pH 7.4], 135 mmol/L NaCl, 5 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 5.6 mmol/L glucose, and 0.05% BSA) or complete growth medium, as indicated.

Measurement of ROS levels in sera and cells

ROS levels were measured with the OxiSelect *In vitro* ROS/RNS Assay Kit (Cell Biolabs, San Diego, Calif). BMMCs (2×10^5 cells/1.5 mL) were incubated with dichlorofluorescein diacetate (20 μmol/L) for 10 minutes at 37°C and washed before stimulation with antigen for 10 minutes in Tyrode-BSA buffer. Dichlorofluorescein fluorescence (excitation, 492 nm; emission, 535 nm) of 100 μL of lysed cells (0.5% Triton-X 100) was monitored in a GENios fluorescent plate reader (ReTiSoft, San Diego, Calif).[26] Cells were fixed in 4% formaldehyde for 10 minutes for measurement of intracellular ROS levels in dichlorofluorescein-loaded cells by using confocal microscopy. Confocal images were obtained in an Olympus FV-1000 confocal laser scanning microscope with an Apochromat 60× objective (Olympus, Center Valley, Pa).

Measurement of degranulation and TNF-α, IL-4, and DJ-1 levels

IgE-primed BMMCs were stimulated with antigen in Tyrode-BSA buffer for 10 minutes or as indicated. Degranulation was determined based on measurement of release of the granule marker β-hexosaminidase.[27] Otherwise, cells were stimulated with antigen for 8 hours in complete media for measurement of TNF-α and IL-4 levels with ELISA kits from Invitrogen–Biosource Cytokine & Signaling (Camarillo, Calif) or measurement of DJ-1 by using ELISA kits from R&D Systems (Minneapolis, Minn).

Immunoprecipitation and immunoblot analysis

IgE-primed BMMCs stimulated with antigen in Tyrode-BSA buffer for 7 minutes or as indicated were lysed with ice-cold lysis buffer (20 mmol/L HEPES [pH 7.5], 150 mmol/L NaCl, 1% Nonidet p-40, 10% glycerol, 60 mmol/L octyl-β-glucoside, 10 mmol/L NaF, 1 mmol/L Na_3VO_4, 1 mmol/L phenylmethylsulfonyl fluoride, 2.5 mmol/L nitrophenylphosphate, 0.7 μg/mL pepstatin, and a protease inhibitor cocktail tablet). Lysates were kept on ice for 30 minutes and then centrifuged at 13,000*g* for 10 minutes at 4°C. Equal aliquots of protein were subjected to immunoprecipitation and immunoblotting analysis.[16]

Transfection of DJ-1 DNA plasmid and small interfering RNAs against DJ-1, SHP-1, SHP-2 or Syk

BMMCs were transfected with DNA plasmids (10 μg/5×10^6 cells, unless stated otherwise) by using the Amaxa Nucleofector (Lonza Cologne AG, Cologne, Germany) and used within 48 hours of transfection. To knock down target proteins, BMMCs (5×10^6 cells) were transfected with 100 nmol/L siGENOME ON-TARGETplus SMARTpool against target proteins or ON-TARGETplus siCONTROLpool as controls (Dharmacon, Chicago, Ill) and used 48 hours after transfection.

Flow cytometric analysis

IgE-primed BMMCs from DJ-1–deficient or WT mice were incubated with fluorescein isothiocyanate–conjugated anti-IgE or anti-CD117 (c-Kit)–phycoerythrin (BD Biosciences, San Jose, Calif). Multicolor analysis was performed in a FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ).

Measurement of activities of SHP-1, SHP-2, and tyrosine kinases *in vitro*

SHP-1 and SHP-2 were immunoprecipitated from lysates of BMMCs stimulated or not with antigen in Tyrode-BSA buffer for 7 minutes.