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ACCELERATED COMMUNICATION

Dexamethasone Up-Regulates the Inhibitory Adaptor Protein Dok-1 and Suppresses Downstream Activation of the Mitogen-Activated Protein Kinase Pathway in Antigen-Stimulated RBL-2H3 Mast Cells

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ABSTRACT

The glucocorticoid dexamethasone suppresses antigen-induced degranulation, cytokine production, and intermediate signaling events in RBL-2H3 mast cells, although the exact mechanisms are uncertain. By microarray analysis, we discovered that expression of the inhibitory adaptor protein, *d*ownstream *o*f tyrosine *k*inase (Dok)-1, was up-regulated 4-fold in dexamethasone-treated RBL-2H3 cells. The up-regulation was apparent with as little as 1 to 10 nM dexamethasone. Treatment with dexamethasone also enhanced tyrosine phosphorylation of Dok-1, augmented recruitment of Ras GTPase-activating protein (RasGAP) by Dok-1, and inhibited activation of the mitogen-activated protein (MAP) kinase pathway in antigen-stimulated cells. The same effects were obtained by transient overexpression of Dok-1 but not by overexpression of Dok-1 that was mutated in RasGAP-binding domain. The negative regulatory role of Dok-1 was further validated by the expression of small interfering RNA directed against Dok-1, which enhanced activation of MAP kinase and subsequent release of arachidonic acid and tumor necrosis factor-α. These findings identify Dok-1 as mediator of the antiallergic actions of dexamethasone and as a negative regulator of the MAP kinase pathway and downstream release of inflammatory mediators.

Mast cells initiate allergic responses to antigen by rapid secretion of granules that contain preformed inflammatory mediators and by producing a variety of arachidonic acid-derived inflammatory lipids and cytokines. Glucocorticoids are among the most effective agents for the treatment of mast cell-related allergic diseases. The glucocorticoids act by different mechanisms via the glucocorticoid receptor to either positively (transactivation) or negatively (transrepression) regulate gene transcription (Adcock, 2001; Hayashi et al., 2004). The anti-inflammatory actions of glucocorticoids are attributed specifically to suppression of transcriptional activity of factors that regulate cytokine gene transcription in a variety of inflammatory cells. However, such actions would not account for the ability of glucocorticoids to suppress degranulation and production of inflammatory lipids, and there is accumulating evidence that these drugs interrupt intermediate signaling events that lead to mast cell activation.

Dexamethasone and other glucocorticoids inhibit at least two key signaling cascades in antigen-stimulated RBL-2H3 cells, a rat mast cell line. One is the activation of phosphatidylinositol-3-kinase and downstream signaling events that lead to degranulation (Andrade et al., 2004). Inhibition of this pathway has been attributed to the suppression of phosphorylation of an adaptor protein, Grb2-associated binder 2, and its association with phosphatidylinositol-3-kinase. Another is suppression of the MAP kinase (Erk) pathway, the phosphorylation of phospholipase A_2 by Erk, and release of

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ABBREVIATIONS: MAP, mitogen-activated protein; Dok, downstream of tyrosine kinase; Erk, extracellular-regulated protein kinase; Mek, mitogen-activated protein kinase kinase; MKP-1, mitogen-activated protein kinase phosphatase-1; RasGAP, Ras GTPase-activating protein; siRNA, small interfering RNA; TNFα, tumor necrosis factor α; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

arachidonic acid (Rider et al., 1996; Cissel and Beaven, 2000). The inhibitory actions of dexamethasone are mediated via the glucocorticoid receptor, are of slow onset, and are apparent with as little as 5 to 10 nM dexamethasone.

The precise mechanisms by which dexamethasone suppresses signaling events are unclear. Dexamethasone does not inhibit early antigen-mediated signaling events but rather acts downstream, which, in the case of the Erk pathway, seems to be at the level of Raf-1. Dexamethasone causes disassembly of the Raf-1/heat shock protein-90 complex and blocks recruitment of Raf-1 by Ras (Cissel and Beaven, 2000). Dexamethasone also up-regulates expression of the MAP kinase phosphatase, MKP-1, in RBL-2H3 cells and may thus negatively regulate Erk (Kassel et al., 2001). MKP-1 is a dual-specificity protein phosphatase that dephosphorylates and inactivates Erk and p38 MAP kinase. Dexamethasone has since been shown to attenuate activation of both kinases and up-regulate MKP-1 in several types of cells (Lasa et al., 2002; Engelbrecht et al., 2003; Jeong et al., 2003; Bazuine et al., 2004). However, the up-regulation of MKP-1 is apparent within 30 min and begins to subside by 8 h (Kassel et al., 2001), a time course that does not fit with the relatively slow attenuation of the Erk response to antigen in dexamethasone-treated cells. The attenuation is apparent only after 4 h and reaches a maximum by 12 to 14 h (T. Hiragun, D. S. Lissel, and M. A. Beaven, unpublished data). In addition, increased MKP-1 activity would not account for the inhibitory effect of dexamethasone on the activation of Raf-1 or the downstream MAP kinase kinase, Mek.

As reported here, examination of the effects of dexamethasone on expression of gene transcripts by microarray analysis revealed that treatment of RBL-2H3 cells with dexamethasone resulted in a substantial increase in transcripts for *d*ownstream *o*f tyrosine *k*inase (Dok)-1, which is a known negative regulator of Ras activation (Veillette et al., 2002). Dok-1 is the prototypic member of a family of five known Dok adaptor proteins (Carpino et al., 1997). Among these, Dok-1, Dok-2, and Dok-3 are abundant in hematopoietic cells, including RBL-2H3 cells (Abramson et al., 2003). Dok family adaptors possess an amino-terminal pleckstrin homology domain, a central phosphotyrosine binding domain, and several tyrosine residues in a carboxyl-terminal, which are phosphorylated on ligation of immunoreceptors (Veillette et al., 2002). The tyrosine-phosphorylated Dok proteins can then recruit negative regulators of signaling pathway such as Src homology 2 domain-containing 5′-inositol phosphatase-1, C-terminal Src kinase, and RasGAP. Phosphorylation of tyrosines 295 and 361 specifically is critical for association of Dok-1 with RasGAP (Shah and Shokat, 2002). Dok-1 and Dok-2 are tyrosine-phosphorylated when mast cells are stimulated by antigen via the IgE receptor FcεRI (Abramson et al., 2003). Dok-1, in particular, participates in the inhibitory effect of FcγRIIB on FcεRI-mediated signaling events on coaggregation of these receptors (Ott et al., 2002; Kepley et al., 2004). Here, we provide evidence that basal levels of Dok-1 can negatively regulate antigen-induced activation of the Ras/Erk pathway and that the induction of Dok-1 by dexamethasone could account for the inhibitory action of dexamethasone on this pathway and on downstream production of arachidonic acid and TNFα.

Materials and Methods

Reagents and Antibodies. Dexamethasone, dinitrophenyl-human serum albumin, and anti-DNP IgE were from Sigma-Aldrich (St. Louis, MO). The polyclonal antibodies against phosphorylated forms of and total Erk1/2 (Thr202/Tyr204) and Mek1/2 (Ser217/221) were from Cell Signaling Technology (Beverly, MA). The polyclonal antibodies against Dok-1 (M-276), Dok-3 (S-20), glucocorticoid receptor (H-300), and monoclonal antibody against RasGAP (B4F8) were from Santa Cruz Biotechnology (Santa Cruz, CA). The polyclonal antibody against Dok-2, monoclonal antibodies against phospho-Raf-1 (Ser338), and phosphotyrosine (4G10) were from Upstate Biotechnology (Lake Placid, NY). The monoclonal antibodies against Raf-1 and heat shock protein-90 were from BD Biosciences PharMingen (San Diego, CA). The tumor necrosis factor α (TNFα) enzyme-linked immunosorbent assay kit was from Biosource International (Camarillo, CA).

Cell Culture. RBL-2H3 cells were maintained in minimal essential medium supplemented with 15% fetal calf serum (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, and antibiotic-antimycotic (Invitrogen, Carlsbad, CA). Cultures were incubated overnight (18 h) at 37°C with 50 ng/ml anti-DNP-IgE in the presence or absence of dexamethasone. In some experiments, the time of exposure to dexamethasone was varied where indicated. The cultures were then washed twice with glucose saline/PIPES buffer (Choi et al., 2002) and stimulated in the same buffer for 15 min unless otherwise indicated. The cultures were then placed on ice for subsequent assays.

Immunoprecipitation and Western Blot Analysis. Cells were washed twice with ice-cold phosphate-buffered saline and then lysed in lysis buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, 25 μg/ml aprotinin, and 2 μg/ml pepstatin) and incubated for 30 min on ice. Cell lysates were incubated with the specified antibodies and then with protein G-Sepharose 4 Fast Flow beads (Amersham Biosciences Inc., Piscataway, NJ). The beads were washed four times with the lysis buffer and boiled with 2× SDS sample buffer for 5 min. For immunoblotting, proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membrane (Immobilon-P; Millipore Corporation, Billerica, MA). The membrane blots were probed with the indicated primary antibodies, and the immunoreactive proteins were visualized by use of horseradish peroxidase-conjugated secondary antibodies and chemiluminescence.

Microarray Analysis. Total RNA was extracted from nontreated RBL-2H3 cells and from cells treated with 100 nM dexamethasone for 1, 4, or 12 h by using RNeasy Mini kit (QIAGEN, Valencia, CA). Total RNA (8 μg) was converted to single-stranded cDNA and then to double-stranded cDNA by using SuperScript choice system (Invitrogen) and T7-oligo(dT) promoter primer kit (Affymetrix, Santa Clara, CA). Double-stranded cDNA was converted to biotin-labeled antisense cRNA and then fragmented by the use of a RNA transcript labeling kit (Enzo Life Sciences, Farmingdale, NY). The fragmented cRNA (15 μg) was hybridized to Rat Expression Set 230A and B GeneChip arrays (Affymetrix) for 16 h. The GeneChip was scanned and the data analyzed by the GeneSpring 6.2 program (Silicon Genetics, Redwood City, CA).

Dok-1 Expression Vectors. Single-stranded cDNA was generated with SuperScript II (Invitrogen) from 5 μg total RNA which was extracted from mouse bone marrow-derived mast cells with RNeasy Mini Kit (QIAGEN). Mouse Dok-1 was amplified from cDNA by polymerase chain reaction with pfuTurbo DNA Polymerase (Stratagene, La Jolla, CA) using the following primers: sense primer, 5′-GACTAGTGGAATCGCCTGGGCCATGAAC-3′; and antisense primer, 5′-ATAAGAATGCGGCCGCGCAACTGTCTCAGGTGGAACC-3′. The polymerase chain reaction fragment was digested with SpeI and NotI and then ligated into the same sites of pEF6/V5-His vector (Invitrogen). Dok-1

mutant (Y295/361F), which is unable to bind to RasGAP (Shah and Shokat, 2002), was generated by mutagenesis using the following primers: Y295F sense primer, 5′-GCCCTCCAGCCCTATTTGCG-GAGCCTTTAG-3′; Y295F antisense primer, 5′-CTAAAGGCTCCG-CAAATAGGGCTGGAGGGC-3′; Y361F sense primer, 5′-CCAAA-GAGGACCCCATCTTTGATGAACCTGAAGGCC-3′; and Y361F antisense primer, 5′-GGCCTTCAGGTTCATCAAAGATGGGGTC-CTCTTTGG-3′, by QuikChange Site-Directed Mutagenesis Kit (Stratagene).

Dok-1 siRNA Expression Vector. The siRNA vector targeted against rat Dok-1 was generated with the Silencer Express Kit pSEC neo (Ambion, Austin TX) and the following oligonucleotides (the target sequence was 187–207 of rat Dok-1, XM_232106): sense oligonucleotide, 5′-GAACTACACAAATTCAGCCAGGCGTATCATCCGGTGT-TTCGTCCTTTCCACAAG-3′; and antisense oligonucleotide, 5′-CGGCGAAGCTTTTTCCAAAAAAGATGATACGCCTGGCTGAACTA-CACA-AATTCA-3′, according to the manufacturer's instruction. A negative control siRNA was generated by using the oligonucleotides that were provided in the Silencer Express Kit.

Transient Transfection. All plasmids were purified by EndoFree Plasmid Maxi Kit (QIAGEN) before transfection. RBL-2H3 cells were suspended in Dulbecco's modified Eagle's medium containing 25 mM HEPES (Invitrogen) at a concentration of 4×10^7 cells/ml. Cells (100 μl) were mixed with 15 μg of plasmid and transfected by electroporation (Gene Pulser; Bio-Rad, Hercules, CA) at 250 V/250 μF. The percentage of transfected cells was approximately 50%. The transfected cells were used within 24 h for each experiment.

Measurement of Release of Arachidonic Acid and TNFα. Release of arachidonic acid was determined as described previously (Collado-Escobar et al., 1990). In brief, RBL-2H3 cells were incubated with IgE and 0.2 μCi/ml [^{14}C]arachidonic acid overnight, washed, and then stimulated with antigen for 15 min in the glucose saline/PIPES buffer. Medium and cell lysate were assayed for ^{14}C using a scintillation counter (Beckman Coulter, Fullerton, CA), and

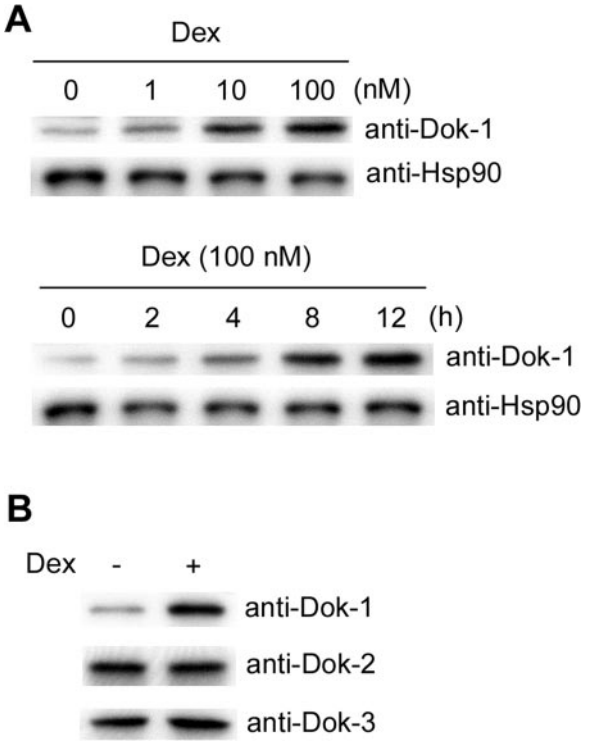

Fig. 1. Dexamethasone increases the expression of Dok-1 in RBL-2H3 mast cells. A, RBL-2H3 cells were treated with various concentrations of dexamethasone for 18 h (top) or with 100 nM dexamethasone for indicated time periods (bottom). Immunoblots were prepared from whole-cell lysates for detection of Dok-1 and, as a control, heat shock protein-90 (Hsp90) by use of specific antibodies (anti-). B, RBL-2H3 cells were treated with 100 nM dexamethasone for 18 h. Immunoblots were prepared from whole-cell lysates for detection of Dok-1, Dok-2, and Dok-3 with use of the specific antibodies. A representative immunoblot of three experiments is shown for both.

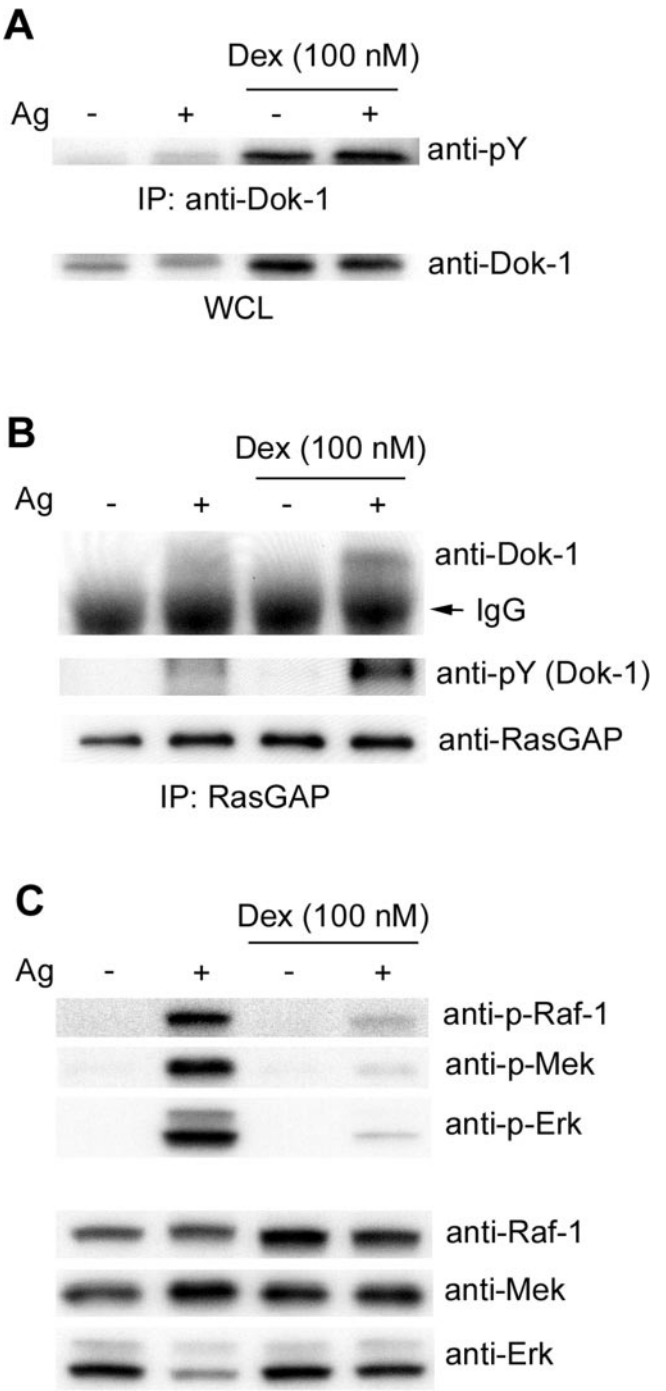

Fig. 2. Dexamethasone enhances the tyrosine phosphorylation of Dok-1 and the interaction of Dok-1 with RasGAP. RBL-2H3 cells were treated with 100 nM dexamethasone and IgE for 18 h, and then stimulated or not with antigen (Ag) for 15 min. A, whole-cell lysates were immunoprecipitated (IP) with anti-Dok-1 antibody and tyrosine phosphorylation of Dok-1 was detected by use of anti-phosphotyrosine (pY) antibody. B, whole-cell lysates were immunoprecipitated with anti-RasGAP, and coimmunoprecipitated Dok-1 was detected with the use of anti-Dok-1 or anti-pY antibody. C, immunoblots were prepared from whole-cell lysates by use of antibodies that recognized phosphorylated (p-) Raf-1 (Ser338), Mek1/2 (Ser217/221), or Erk1/2 (Thr202/Tyr204), or the proteins themselves. A representative immunoblot of three experiments is shown for each.

values for [^{14}C]arachidonic acid that was released into the medium were expressed as a percentage of total intracellular ^{14}C. Release of TNFα was determined with the use of an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions. For this determination, cells were stimulated with antigen in complete growth medium in 12-well plates (10^6 cells/well/ml) for 90 min.

Results and Discussion

Dexamethasone Increases the Expression of Dok-1. The microarray analysis of dexamethasone-treated RBL-2H3 cells revealed no remarkable change in expression of mRNA for proteins that are commonly associated with the transduction of signals in antigen-stimulated mast cells (Rivera, 2002). However, among the transcripts that showed the highest increases was that of Dok-1 (probe ID, 1384292). Dok-1 mRNA increased 1.8-, 4.3-, and 2.9-fold by 1, 4, and 12 h, respectively, after the addition of dexamethasone. Examination of the expression of Dok-1 protein by immunoblotting indicated that expression of Dok-1 increased in a time- and dose-dependent manner (Fig. 1A). The increase was apparent with as little as 1 nM dexamethasone. The action of dexamethasone was of slow onset but was evident at 4 h and more so by 8 and 12 h. The potency and onset of action of dexamethasone were closely reminiscent of the previously observed inhibitory effects of dexamethasone on activation of the Erk pathway (Cissel and Beaven, 2000). It is interesting to note that there was no change in expression of the two other Dok proteins that exist in RBL-2H3 cells, namely Dok-2 and Dok-3 (Abramson et al., 2003) (Fig. 1B).

Dexamethasone Increases the Association of Tyrosine-Phosphorylated Dok-1 with RasGAP. The increased expression of Dok-1 was associated with an increase in the amount of tyrosine-phosphorylated Dok-1 in resting cells (Fig. 2A). Antigen stimulation further enhanced the extent of phosphorylation (Fig. 2A). Antigen also induced a shift in migration of Dok-1, which is consistent with the multiple sites phosphorylated on this molecule (Shah and Shokat, 2002; Shinohara et al., 2004). We next examined the interaction of Dok-1 with RasGAP because phosphorylation of Dok-1 is required for this interaction. Immunoprecipitation of RasGAP revealed that significantly more Dok-1 and tyrosine-phosphorylated Dok-1 was associated with RasGAP after antigen stimulation in dexamethasone-treated cells than in nontreated cells (Fig. 2B). However, the limitation in this and subsequent experiments was that none of the commercially available antibodies provided satisfactory immunoblots of Dok-1 when coimmunoprecipitated with RasGAP. As reported previously (Cissel and Beaven, 2000), dexamethasone suppressed downstream activation of the MAP kinase pathway, as indicated by reduced activating phosphorylations of Raf-1, Mek, and Erk1/2 (Fig. 2C).

Overexpression of Dok-1 Increases Its Association with RasGAP and Suppresses MAP Kinase Pathway. Experiments were undertaken to determine whether the upregulation of Dok-1 by dexamethasone could be mimicked by transient overexpression of wild-type Dok-1 but not by expression of a mutated murine Dok-1 (Y295/361F) that is unable to bind to RasGAP (Shah and Shokat, 2002). As in dexamethasone-treated cells, the overexpression of Dok-1 substantially increased the amount of tyrosine-phosphorylated Dok-1 that was associated with RasGAP in antigen-

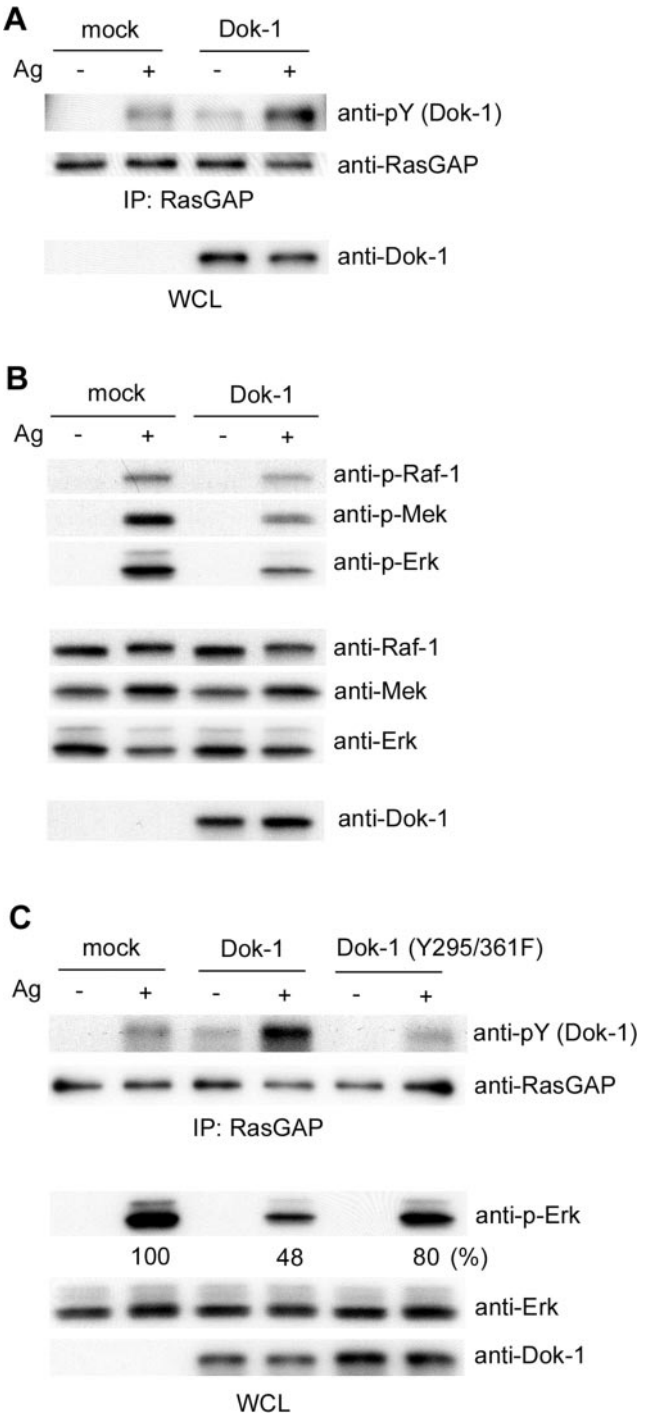

Fig. 3. Transient overexpression of Dok-1 suppresses MAP kinase pathway via the interaction of Dok-1 with RasGAP. RBL-2H3 cells were transfected with vector alone (mock), wild-type Dok-1 (Dok-1), or mutant Dok-1(Y295/361F), and then incubated with IgE overnight. Cells were stimulated or not with antigen (Ag) for 15 min and then lysed. A, whole-cell lysates were immunoprecipitated (IP) with anti-RasGAP antibody, and immunoblots were prepared for detection of coimmunoprecipitated tyrosine-phosphorylated Dok-1 and RasGAP with anti-phosphotyrosine (pY) and anti-RasGAP antibodies. B, immunoblots were prepared from whole-cell lysates (WCL) by the use of antibodies that recognized phosphorylated (p-) Raf-1 (Ser338), Mek1/2 (Ser217/221), or Erk1/2 (Thr202/Tyr204), or the proteins themselves. C, immunoblots were prepared for detection of tyrosine phosphorylated Dok-1, RasGAP, and phosphorylated Erk1/2 (Thr202/Tyr204) in RasGAP immunoprecipitates and whole-cell lysates as described for the previous panels. Densitometric data for phosphorylated Erk2 are indicated numerically (relative mean values of three experiments). A representative immunoblot of three experiments is shown for each.

stimulated cells (Fig. 3A). The overexpression also resulted in significant suppression of the activating phosphorylations of Raf-1, Mek, and Erk1/2 (Fig. 3B), although possibly not to the same extent as that observed with dexamethasone (compare with Fig. 2C). This difference might be related to the efficiency of transfection of Dok-1 (~50%) or to the induction of other inhibitory factors by dexamethasone in addition to Dok-1.

In contrast to wild-type Dok-1, expression of the Dok-1 mutant did not enhance the amount of tyrosine-phosphorylated Dok-1 that was associated with RasGAP immunoprecipitates after antigen stimulation (Fig. 3C, upper). The similarity with mock-transfected cells indeed suggested that the Dok-1 associated with RasGAP was endogenous Dok-1 rather than mutated Dok-1 (compare the second and final lanes in Fig. 3C, top). In other experiments, direct immunoprecipitation with anti-Dok-1 antibody revealed that, like wild-type Dok-1 (Fig. 2A), mutated Dok-1 was tyrosine-phosphorylated, presumably at sites other than tyrosines 295 and 361, and this phosphorylation was increased by antigen stimulation (data not shown). In addition to the apparent failure of phosphorylated mutant Dok-1 to associate with RasGAP, the Dok-1 mutant was significantly less active than wild-type Dok-1 in suppressing the phosphorylation of Erk1/2, even though expression of the Dok-1 mutant was higher than wild-type Dok-1 (Fig. 3D). Therefore, the suppression of Erk activation by Dok-1 was apparently mediated through its association with RasGAP.

The Effect of siRNA Targeted against Dok-1. To verify that Dok-1 negatively regulates the MAP kinase pathway, cells were made to transiently express siRNAs against Dok-1. Such cells exhibited much-reduced expression of Dok-1 (Fig. 4A) as well as enhanced antigen-induced phosphorylation of Erk1/2 (Fig. 4, B and C) compared with cells that were transfected with a negative control. Release of [^{14}C]arachidonic acid from [^{14}C]arachidonate-labeled RBL-2H3 cells (Fig. 4D) and production of TNFα (Fig. 4E) were similarly enhanced (by 30–40%). The generation of arachidonic acid and TNFα in antigen-stimulated mast cells is dependent on Erk (Zhang et al., 1997) and was used to monitor events downstream of Erk1/2.

Concluding Comments. Our results support the notion that Dok proteins are negative regulators of immunoreceptor-mediated signaling in B cells and mast cells, in which

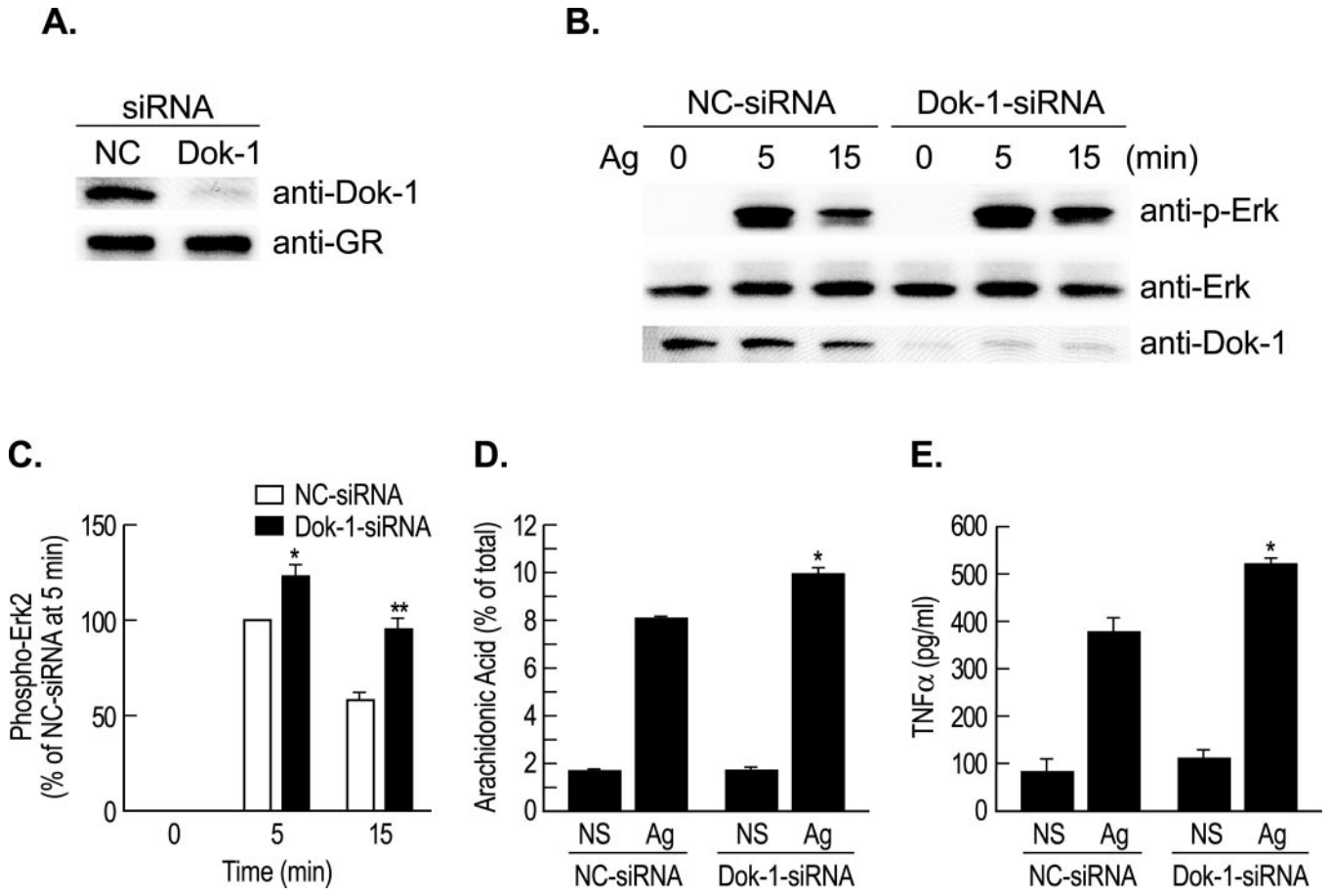

Fig. 4. siRNA targeted against Dok-1 enhances the phosphorylation of Erk and subsequent release of arachidonic acid. RBL-2H3 cells were transfected with negative control siRNA (NC-siRNA) or siRNA targeted against Dok-1 (Dok-1-siRNA) and then incubated with IgE overnight. A, immunoblots were prepared from whole-cell lysates for detection of Dok-1 or glucocorticoid receptor (GR) as a control with the use of anti-Dok-1 or anti-GR antibody. B, transfected cells were stimulated with antigen (Ag) for the indicated time. Immunoblots were prepared from whole-cell lysates with the use of antibodies which recognized phosphorylated (p-) Erk1/2 (Thr202/Tyr204), Erk, and Dok-1. C, densitometric data for phosphorylated Erk2 (as in B) are shown. Values given are the mean ± S.E.M. of six experiments and are expressed as relative values. D, transfected cells were labeled with [^{14}C]arachidonic acid before stimulation or not (NS) with antigen for 15 min. Release of [^{14}C]arachidonic acid into the medium (mean ± S.E.M. of three experiments) is expressed as a percentage of intracellular ^{14}C. E, release of TNFα was also measured in transfected cells 90 min after the addition of antigen (mean ± S.E.M. of three experiments). Asterisks indicate significant increase in response to antigen in Dok-1-siRNA–transfected cells by paired t test (⋆, $p < 0.05$, and ⋆⋆$p < 0.01$).

they are activated by inhibitory and activating receptors such as FcγIIB and FcεRI (Tamir et al., 2000; Abramson and Pecht, 2002; Ott et al., 2002; Abramson et al., 2003). Our data indicate that suppression of expression of Dok-1 with an anti-Dok-1 siRNA modestly augments antigen-induced phosphorylation of Erk and downstream production of arachidonic acid and TNFα in RBL-2H3 cells. Overexpression of Dok-1, in contrast, results in increased association of Dok-1 with RasGAP and decreased activation of the Raf-1/Mek/Erk pathway (this article) and production of TNFα (Abramson et al., 2003) in antigen-stimulated cells. The notable finding is that dexamethasone markedly increases expression of Dok-1 and suppresses activation of the Raf-1/Mek/Erk pathway (Cissel and Beaven, 2000), as well as the generation of arachidonic acid (Rider et al., 1996) and TNFα (Andrade et al., 2004). In particular, the effects of dexamethasone on Dok-1 expression are of slow onset and are apparent with as little as 1 to 10 nM dexamethasone, as are the inhibitory effects of dexamethasone on the Raf-1/Erk pathway and release of arachidonic acid (Rider et al., 1996; Cissel and Beaven, 2000). In total, our studies suggest that the expression and inhibitory effects of Dok-1 on antigen/FcεRI-mediated signaling events are relatively small but that they become significant once Dok-1 is up-regulated by dexamethasone. Therefore, in addition to the well-documented inhibitory effects of dexamethasone on cytokine gene transcription, dexamethasone may act also by induction of inhibitory signaling factors, of which MKP-1 and Dok-1 are two such examples. If so, then glucocorticoids chosen for selectivity toward suppression of cytokine gene activation to minimize side effects (sometimes referred to as dissociated glucocorticoids) (Schacke and Rehwinkel, 2004) may lack an important component of anti-inflammatory activity.

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