

Dual Specificity Phosphatase 1 Knockout Mice Show Enhanced Susceptibility to Anaphylaxis but Are Sensitive to Glucocorticoids

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Dual specificity phosphatase DUSP1 (otherwise known as mitogen-activated phosphatase 1 or MKP-1) dephosphorylates MAPKs, particularly p38, and negatively regulates innate immunity. Recent studies have shown that the DUSP1 gene is transcriptionally up-regulated by glucocorticoids (GCs) and that the antiinflammatory action of GCs is impaired in DUSP1^{−/−} mice. Here we show that GC-mediated dephosphorylation of ERK-1 and ERK-2 activated by IgE receptor cross-linking is unimpaired in bone marrow-derived mast cells (BMMCs) of DUSP1^{−/−} mice. Dephosphorylation of phospho-p38 MAPK is impaired but only at early times of GC treatment. Proinflammatory cytokine and chemokine gene expression (CCL2, IL-6, TNF α) is still down-regulated by GCs in BMMCs

from DUSP1^{−/−} mice, suggesting a compensatory mechanism for the GC action in these mice. In both DUSP1^{+/+} and DUSP1^{−/−} BMMCs, GC up-regulated the expression of several phosphatase genes (DUSP2, DUSP4, DUSP9, and PEST domain-enriched tyrosine phosphatase). DUSP1^{−/−} mice show enhanced mast cell degranulation and are highly susceptible to anaphylaxis, but these effects are still down-regulated by GCs. GCs also repressed other inflammatory responses such as dinitrofluorobenzene-induced contact hypersensitivity and lipopolysaccharide-induced mortality in DUSP1^{−/−} mice. Thus GC-mediated antiinflammatory action is largely independent of DUSP1. (*Molecular Endocrinology* 21: 2663–2671, 2007)

MAPKs PLAY IMPORTANT ROLES in all aspects of the immune response from innate to the adaptive immune system and from initiation of immune response to activation-induced cell death (1).

The mammalian MAPKs comprise three major groups that are classified on the basis of their sequence similarity, differential activation of agonists, and substrate specificity. These are the ERKs, the JNKs and p38 MAPK (2). These MAPKs are activated by phosphorylation of tyrosine and threonine residues within their active domains (3) and are inactivated by dephosphorylation of either residue. Dephosphorylation can be achieved by tyrosine-specific phosphatases, serine-threonine phosphatases, or dual-specificity (Thr/Tyr) phosphatases (DUSPs) (4–6). All three

classes of protein phosphatases can regulate MAPKs *in vivo* but the largest group dedicated to this function is the DUSPs (7, 8).

At least 10 mammalian DUSPs have been identified, and these constitute three subfamilies based on sequence similarity, substrate specificity, and subcellular localization (8, 9). Although information on the biochemical and structural basis of DUSP catalysis and substrate specificity has increased over the past years, the physiological roles of these enzymes are only just beginning to be unraveled.

DUSP1 (otherwise known as MAPK phosphatase-1) is an archetypal member of the dual-specificity phosphatases that dephosphorylates MAPKs. Recently DUSP1 has been shown to be a negative regulator in the innate immune response to lipopolysaccharides (LPS) (10–13), and DUSP1 deficiency leads to sustained activation of p38 MAPK and JNK in LPS-treated macrophages in response to Toll-like receptor signals.

DUSP1 gene expression has been shown to be enhanced by glucocorticoids (GCs) (14, 15), and because this group of hormones is widely used in the treatment of inflammatory diseases, it was suggested that the therapeutic action of these drugs could be mediated by DUSP1 (7). In more recent studies, it was demonstrated that the suppression of expression of a

First Published Online July 17, 2007

Abbreviations: BMMCs, Bone marrow-derived mast cells; DNFB, 2,4-dinitrofluorobenzene; DNP-HSA, dinitrophenyl-human serum albumin; DUSP, dual specificity phosphatase; GC, glucocorticoid; LPS, lipopolysaccharide; PEP, PEST [region rich in proline (P), glutamine (E), serine (S) and threonine (T)] domain-enriched tyrosine phosphatase.

Molecular Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

subset of proinflammatory genes in response to GCs is reduced in macrophages from DUSP1^{−/−} mice (16). Furthermore, *in vivo*, the antiinflammatory effect of dexamethasone on zymosan-induced inflammation was impaired in DUSP1^{−/−} mice (16). These studies therefore identify DUSP1 as an important target in the antiinflammatory action of GCs.

Here we have used bone marrow-derived mouse mast cells (BMMCs) to analyze the antiallergic action of GCs. We show that the expression of most proinflammatory genes induced by cross-linking the IgE receptor (FcεRI) is still down-regulated by GCs in BMMCs from DUSP1^{+/+} and DUSP1^{−/−} mice. The DUSP1^{−/−} mice showed enhanced mast cell degranulation and are highly susceptible to anaphylaxis. However, these responses are still inhibited by GCs in the DUSP1^{−/−} mice.

RESULTS AND DISCUSSION

Different Kinetics of GC-Mediated Dephosphorylation of p38 MAPK and ERK-1 and ERK-2

GC-mediated inhibition of phosphorylation of the MAPKs p38 MAPK but not the ERK-1 and ERK-2 has recently been shown to occur through DUSP1 using bone marrow-derived macrophages from DUSP1^{+/+} and DUSP1^{−/−} mice (16). We investigated whether the same regulation occurs in BMMCs from DUSP1^{+/+} and DUSP1^{−/−} mice in which we activated the MAPK signaling pathways by FcεRI cross-linking for 5 or 10 min. These cells were pretreated with the synthetic GC dexamethasone (DEX) for 1, 4, 8, and 12 h. The phosphorylation of the MAPKs p38 and ERK-1 and ERK-2 was analyzed using an immunoblot assay with phospho-specific antibodies to these kinases. As control, the levels of the nonphosphorylated kinases were determined as estimation of the amount of protein loaded on the gel.

FcεRI cross-linking for 5 and 10 min led to phosphorylation of the p38 MAPK and was inhibited by GC at 4, 8, and 12 h in the DUSP1^{+/+} BMMCs (Fig. 1). In the DUSP1^{−/−} BMMCs, GC-mediated inhibition of p38 phosphorylation was delayed till 12 h of hormone treatment. Thus, compared with the DUSP1^{+/+} BMMCs, the GC-mediated inhibition of p38 phosphorylation was temporarily impaired at 4–8 h of hormone treatment (Fig. 1). A slightly different regulation was observed in studies of ERK-1 and ERK-2 phosphorylation. First, phosphorylation of this MAPK was significantly more pronounced at 5 min of IgE receptor cross-linking than after 10 min and second, the GC-mediated dephosphorylation of ERK-1 and ERK-2 was observed starting at 8 h of dexamethasone treatment and persisted till 12 h in both DUSP1^{+/+} and DUSP1^{−/−} BMMCs. These results are not specific to the C57BL/6 strain of mice used in this study. Similar results were obtained using BMMCs from other

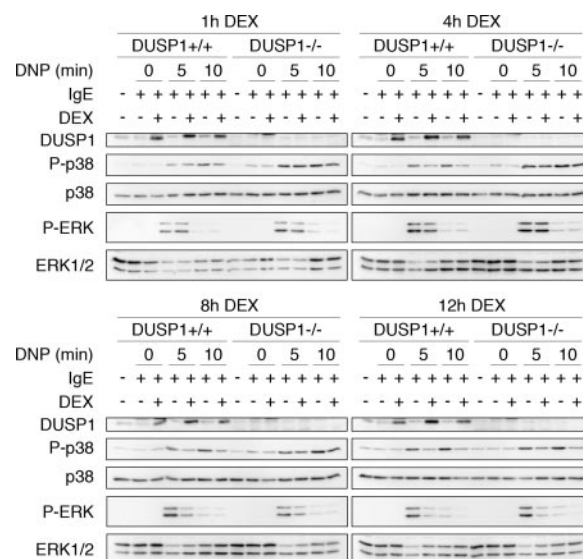


Fig. 1. GC-Mediated Inhibition of Phosphorylation of p38 MAPK Is Partly Impaired in DUSP1^{−/−} BMMCs

DUSP1^{+/+} or DUSP1^{−/−} BMMCs (2×10^6) were cultured in the presence of 100 nM dexamethasone or vehicle for the indicated period of time. Anti-DNP IgE (1 μ g/ml) was added to the medium 2 h before harvesting. Subsequently, cells were stimulated for 5 or 10 min with DNP-HSA (DNP, 500 ng/ml). Lysates were analyzed by immunoblotting using antibodies against DUSP1 and total and phosphorylated p38 MAPK as well as ERK1 and ERK2. DEX, Dexamethasone.

DUSP1^{+/+} and DUSP1^{−/−} strains such as DBA/1 and C3H/He (results not shown).

GC Up-Regulates the Expression of Other Phosphatase Genes in the Absence of DUSP1

The persistent GC-mediated dephosphorylation of p38 MAPK and ERK in DUSP1^{−/−} BMMCs could be due to an induced expression of other phosphatase genes. We found out in real-time PCR studies that, in addition to DUSP1, the expression of DUSP2, DUSP4, DUSP9, and the PEST domain-enriched tyrosine phosphatase (PEP) is induced by GC in DUSP1^{+/+} BMMCs (Fig. 2). These DUSPs dephosphorylate MAPKs (4–6), and PEP is reported to regulate MAPK signaling pathway in T cells (17). As opposed to DUSP1, the expression of the other phosphatases is up-regulated by GC in the DUSP1^{−/−} BMMCs. However the kinetics of GC-mediated up-regulation of expression differs for the different genes. For example, the basal and GC regulation of expression of DUSP2 is less pronounced in the DUSP1^{−/−} BMMCs compared with the wild-type cells. With DUSP9, the increased expression by GC is observed in the first 4 h. Thereafter this effect is abolished or even reversed at later time points in both cell types. On the contrary the GC-mediated expression of DUSP4 and PEP was evident at all the time points analyzed in the DUSP1^{+/+} and DUSP1^{−/−} BMMCs (Fig. 2).

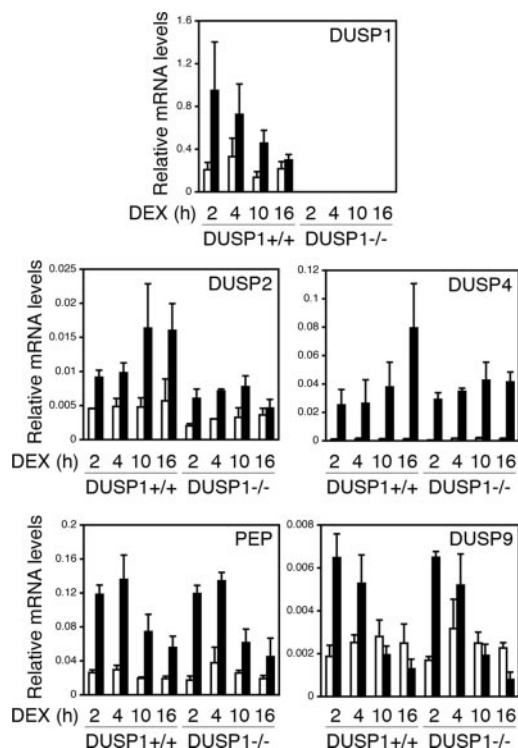


Fig. 2. Several Phosphatase Genes Are Transcriptionally Up-Regulated by Dexamethasone in DUSP1^{+/+} and DUSP1^{-/-} BMMCs

DUSP1^{+/+} or DUSP1^{-/-} BMMCs (3×10^6) were pre-treated with 100 nM dexamethasone (solid bars) or vehicle (open bars) for the indicated times. Total RNA was isolated, and mRNA levels were measured by quantitative real-time PCR using primers for DUSP1, DUSP2, DUSP4, DUSP9, and PEP and normalized against the transcript of the ribosomal subunit 36B4 gene. The bar chart shows the mean $\pm$ SD of three independent experiments. DEX, Dexamethasone.

Thus it is likely that these MAPK phosphatases, which are transcriptionally up-regulated by GC, contribute to the GC-mediated dephosphorylation of p38, ERK-1, and ERK-2 in the absence of DUSP1.

GC Effect on Cytokine Expression and Degranulation in DUSP1 Knockout BMMCs

In mast cells, Fc ϵ RI cross-linking increases MAPK signaling, which leads to the expression of several cytokine and chemokine genes and rapid degranulation (18, 19). These effects are all down-regulated by GC (20, 21). We therefore examined in DUSP1^{+/+} and DUSP1^{-/-} BMMCs the effect of dexamethasone on the expression of a number of cytokine and chemokine genes as well as on degranulation after IgE receptor cross-linking.

For studies on the effect of GC on antigen-induced cytokine gene expression, slightly different conditions were used for the activation of the cells

as previously shown in Fig. 1. BMMCs were pre-treated with dexamethasone for 4 h followed by cross-linking the IgE receptor for 30 min rather than the 5 or 10 min used in Fig. 1. These conditions produced the same results as shown in Fig. 1, *i.e.* phosphorylation of p38 MAPK was inhibited by dexamethasone in BMMCs from DUSP1^{+/+} but not in DUSP1^{-/-} mice (Fig. 3A). IgE cross-linking for 30 min did not show any effect on ERK-1 and ERK-2 phosphorylation because this MAPK is only activated for 5–10 min and, as such, dexamethasone had no effect on its activity at 30 min. Under these conditions of activation and GC treatment, Fc ϵ RI cross-linking led to an increased expression of all the proinflammatory cytokine and chemokine genes we examined, and there was no change in the degree of enhancement of expression of these genes in the DUSP1^{-/-} compared with the DUSP1^{+/+} BMMC with the exception of IL-4 (Fig. 3B). The level of increased expression of this cytokine was more than 2-fold down-regulated in the DUSP1^{-/-} compared with the DUSP1^{+/+} BMMCs. The reason for this is an increased basal expression of IL-4 in the DUSP1^{-/-} cells that strongly reduced the fold-induction after Fc ϵ RI cross-linking (results not shown). Nevertheless, the induced expression of IL-4 after Fc ϵ RI cross-linking was inhibited by dexamethasone (Fig. 3B). The only gene identified that showed an impaired GC repression in the DUSP1^{-/-} BMMCs was COX-2. In this case, although dexamethasone significantly inhibited the level of expression of this gene in the DUSP1^{+/+} BMMCs, it did not have any significant effect on expression in the DUSP1^{-/-} cells.

To further demonstrate that the inhibition of cytokine gene expression in the BMMCs is independent of DUSP1, we treated those cells with the protein synthesis inhibitor cycloheximide, which down-regulated DUSP1 expression and possibly other GC-induced genes (Fig. 3C). Cycloheximide treatment affected the expression of some of the cytokine genes, and this made interpretation of the results difficult. Nevertheless, on the whole, down-regulation of IgE/DNP-mediated expression of IL-1 α , IL-1 β , CCL2, and IL-6 by GC was less pronounced in the presence of cycloheximide than in its absence in both DUSP1^{+/+} and DUSP1^{-/-} BMMCs (Fig. 3D). This suggests that for the repression, a secondary action of GC involving the increased expression of genes other than DUSP1 is required.

In studies on degranulation, at least a 2-fold increase was observed in the DUSP1^{-/-} BMMCs over the wild-type cells, indicating that DUSP1 deficiency enhances degranulation in BMMCs (Fig. 4). Nonetheless, we observed a small but significant decrease in the degranulation after 24 h of dexamethasone treatment with about 40% decrease in DUSP1^{+/+} compared with 10% in DUSP1^{-/-} BMMCs (Fig. 4).

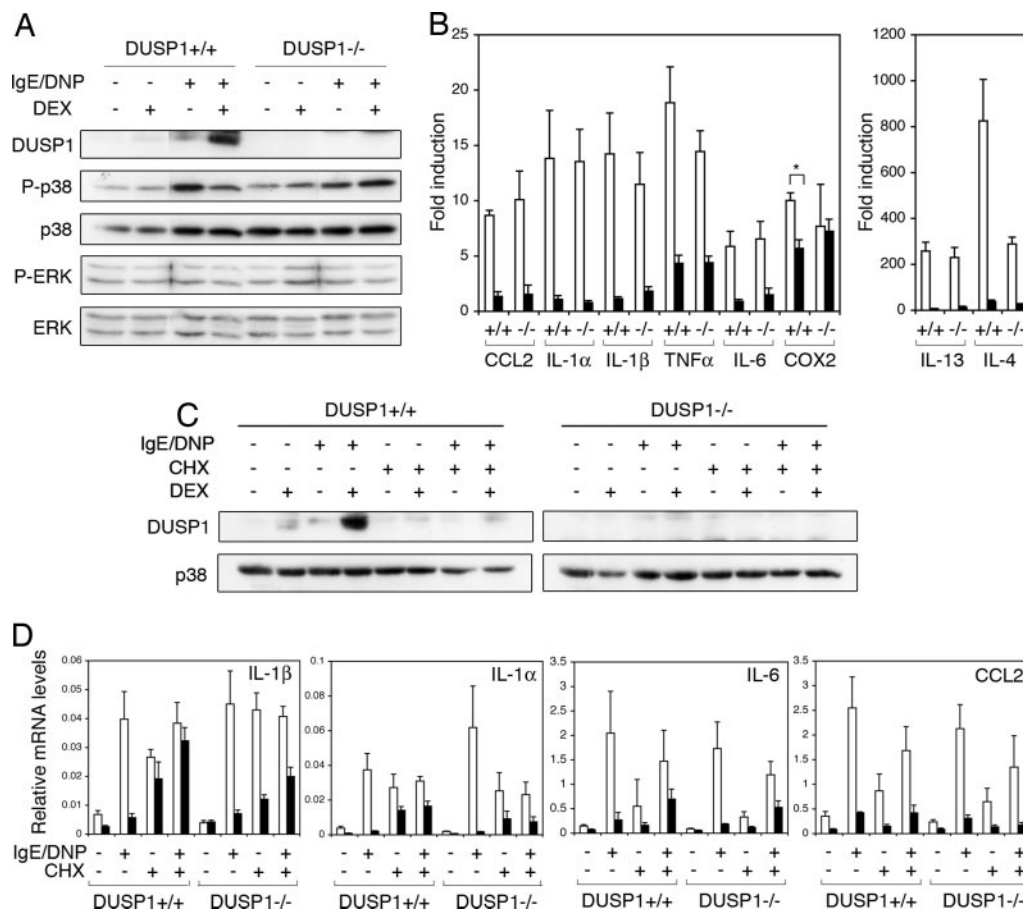


Fig. 3. Effect of Dexamethasone on Antigen-Induced Proinflammatory Gene Expression in DUSP1^{+/+} and DUSP1^{-/-} BMMCs. A, DUSP1^{+/+} or DUSP1^{-/-} BMMCs (2×10^6) were pretreated with 100 nM dexamethasone or vehicle for 2 h and then incubated with 1 μ g/ml anti-DNP IgE for 2 h followed by stimulation with DNP-HSA (500 ng/ml) for 30 min. Lysates were analyzed by immunoblotting using antibodies against DUSP1, total and phosphorylated p38 MAPK, and ERK1/ERK2. B, DUSP1^{+/+} or DUSP1^{-/-} BMMCs (3×10^6) were treated as in panel A except that they were stimulated with DNP-HSA (500 ng/ml) for 30 min in Iscove's modified Dulbecco's medium, and total RNA was isolated. mRNA levels of the indicated genes were measured by quantitative real-time PCR using primers for these genes and normalized against the transcript of the ribosomal subunit 36B4 gene. The fold induction was calculated as the relative mRNA level activated by DNP-HSA in the presence and absence of dexamethasone. The results are expressed relative to the mRNA levels of vehicle-treated, nonstimulated cells. The bar chart shows the mean $\pm$ SD of three independent experiments. C and D, Cells were treated as in panel A in the presence or absence of 5 μ g/ml cycloheximide (CHX), which was added 15 min before the dexamethasone treatment. DUSP1 and p38 MAPK protein levels were determined by immunoblotting. Transcript levels of IL-1 α , IL-1 β , CCL2, and IL-6 were measured by quantitative real-time PCR and normalized against the level of transcript of the ribosomal subunit 36B4 gene. The bar chart shows the mean $\pm$ SD of three independent experiments. Solid bars represent dexamethasone-, and open bars represent vehicle-treated cells. *, $P < 0.05$ (Student's *t* test). DEX, Dexamethasone.

DUSP1 Knockout Mice Show Increased Susceptibility to Anaphylaxis

Because allergen-induced degranulation of mast cells is a component of allergy and an extreme form of allergic reaction is anaphylaxis, we investigated whether the DUSP1^{-/-} mice are sensitive to passive systemic anaphylaxis.

DUSP1^{+/+} and DUSP1^{-/-} mice were sensitized with anti-DNP IgE, and passive systemic anaphylaxis was induced by iv injection of dinitrophenyl-human serum albumin (DNP-HSA). The mice were treated

with dexamethasone 3 h before the induction of anaphylaxis.

The DUSP1^{-/-} mice were found to be more sensitive to anaphylaxis measured by a decrease in body temperature compared with their wild-type counterparts (Fig. 5A). The decrease in rectal temperature was slightly more evident and persisted over a longer period of time in the DUSP1^{-/-} than in the DUSP1^{+/+} mice. Pretreatment with dexamethasone weakly but significantly reduced anaphylaxis to a level comparable to that seen in dexamethasone-treated DUSP1^{+/+} mice (Fig. 5A). As control, mice that were

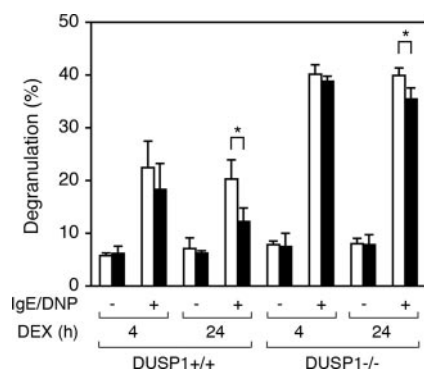


Fig. 4. Dexamethasone Inhibits Degranulation in DUSP1^{+/+} and DUSP1^{-/-} BMMCs

DUSP1^{+/+} or DUSP1^{-/-} BMMCs (2.5×10^5) were cultured in the presence (solid bars) or absence (open bars) of 100 nM dexamethasone for the indicated times. Anti-DNP IgE (1 μ g/ml) was added to the medium 2 h before harvesting. Subsequently, cells were stimulated for 30 min with DNP-HSA (DNP, 200 ng/ml). Degranulation was determined by measuring the enzymatic activity of β -hexosaminidase. The bar chart shows the mean percentage $\pm$ SD of three independent experiments. *, $P < 0.05$ (Student's t test). DEX, Dexamethasone.

wonly sensitized with IgE and in which the receptor was not cross-linked showed neither a change in body temperature nor an effect of dexamethasone (Fig. 5B).

Antiinflammatory Action of GC Is Independent of DUSP1

Several other inflammatory models showed that the DUSP1 mice are still sensitive to the antiinflammatory action of GCs. For example dinitrofluorobenzene (DNFB)-induced contact hypersensitivity, an effect that develops through an anti-DNP IgE antibodies production and an increase in mast cells recruitment (22), is inhibited by systemic GC treatment in both DUSP1^{+/+} and DUSP1^{-/-} mice (Fig. 6A). Topical treatment with GC was also performed by coapplication of dexamethasone and DNFB on the ears of the mice for 24 h after sensitization. The weights of punched biopsies after this treatment showed that GC decreased ear edema to the same extent in the DUSP1^{+/+} and DUSP1^{-/-} mice (Fig. 6B). Histological sections showed that in contrast to the solvent-treated ear specimen, cellular infiltrates and edema were seen in the DNFB-painted ears of both DUSP1^{+/+} and DUSP1^{-/-} mice (Fig. 6C). These infiltrates were mainly granulocytes and mononuclear cells. In the DNFB- and dexamethasone-treated ears, the edema was significantly reduced in both the DUSP1^{+/+} and DUSP1^{-/-} mice.

We also examined whether GC can protect DUSP1^{-/-} against endotoxic shock because these mice have been reported to be highly sensitive to LPS-induced mortality (10, 11, 13). In this study we confirmed that C57BL/6 DUSP1^{-/-} mice were highly

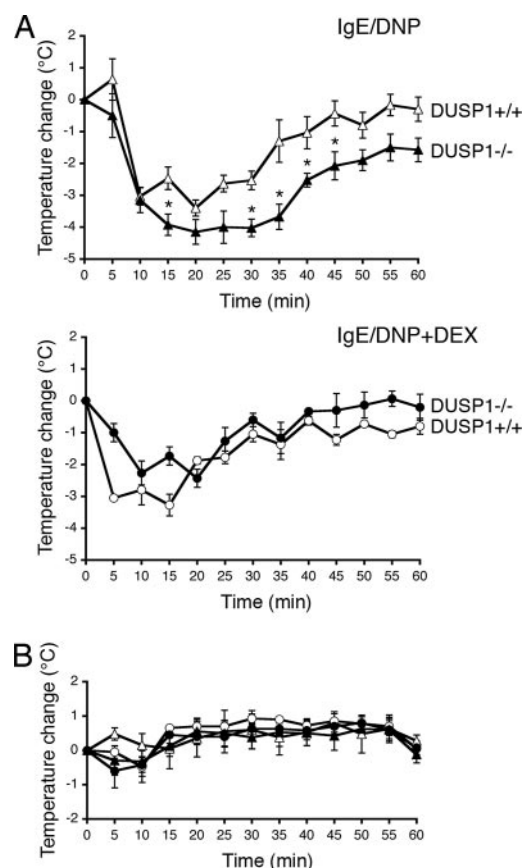


Fig. 5. Effect of Dexamethasone on Passive Systemic Anaphylaxis in DUSP1^{+/+} and DUSP1^{-/-} Mice

C3H/He, DUSP1^{+/+} mice (open symbols), and DUSP1^{-/-} mice (solid symbols) were injected ip with 1 mg/kg anti-DNP IgE. After 24 h, the mice received PBS (triangles) as vehicle or 1 mg/kg dexamethasone (circles) ip and 3 h later anaphylaxis was induced by iv injection of 200 μ l DNP-HSA (1 mg/ml) (A) or PBS (B). Changes in rectal temperature were periodically measured from time of antigen injection. Data are mean values $\pm$ SEM ($n = 4$). *, $P < 0.05$; comparison of PBS-treated DUSP1^{+/+} and DUSP1^{-/-} mice (Student's t test). DEX, Dexamethasone.

sensitive, exhibiting 50% mortality at 80 h after LPS (5 mg/kg body weight) challenge. The DUSP1^{+/+} mice in this genetic background did not show any obvious effect of the LPS treatment throughout the experimental procedure (results not shown). Dexamethasone (10 mg/kg body weight) given by oral gavage 24 h before and immediately before the LPS challenge, produced 100% protection against the LPS-induced mortality (Fig. 7A). Reduction of the dexamethasone concentration to 1 mg/kg body weight, and given ip 30 min before the LPS challenge, produced 100% protection over the first 40 h of LPS treatment. With time (~ 70 h post hormone treatment), no significant protection was observed, possibly due to a diminution of the action of dexamethasone (Fig. 7B). These results confirm recent studies that report that the effect of GCs on survival during sepsis is dependent on the concentra-

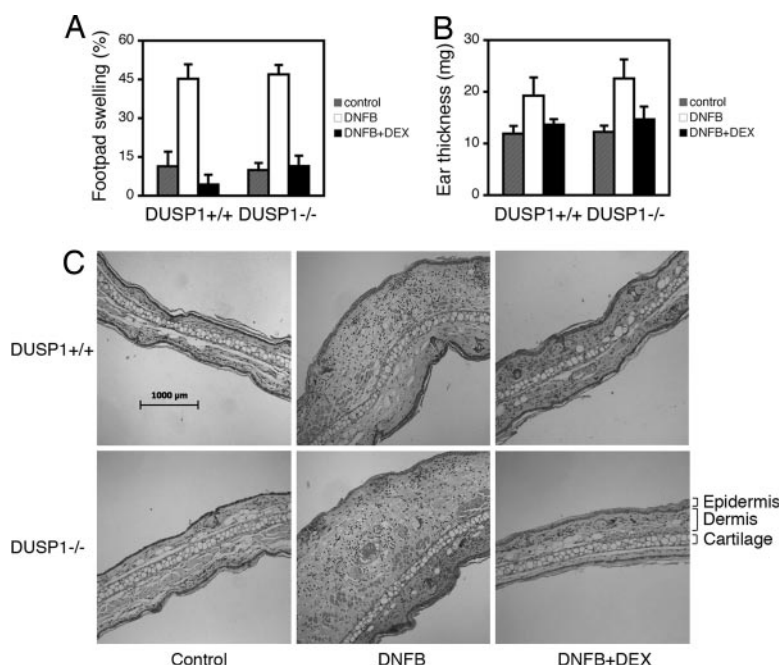


Fig. 6. Effect of Dexamethasone on a DNFB-Induced Contact Hypersensitivity Reaction in DUSP1^{+/+} and DUSP1^{-/-} Mice. A, 129/Sv × C56BL/6, DUSP1^{+/+}, and DUSP1^{-/-} mice were sensitized with 10 μ l 1% DNFB or vehicle as control on the preshaved abdomen. All mice were challenged 5 d later by application of 10 μ l 0.5% DNFB on the left footpad for 24 h, and the thickness of both footpads was measured. During the sensitization and elucidation phase, some mice received dexamethasone-phosphate via the wet diet and drinking water (20 mg/liter). The results are expressed as percentage swelling $\pm$ SEM ($n = 5$) relative to the nontreated control footpad. B and C, 129/Sv × C56BL/6, DUSP1^{+/+}, and DUSP1^{-/-} mice were sensitized with 25 μ l 0.5% DNFB on the preshaved abdomen for 2 consecutive days. The dorsal surface of both ears was treated 5 d later with 20 μ l 0.3% DNFB, DNFB plus 0.15 mg/ml dexamethasone, or vehicle alone for 24 h. Mice were killed and punched ear biopsies were weighed. Data are mean values $\pm$ SD ($n = 5$ –9). The biopsies were additionally processed for histological sections and stained with hematoxylin and eosin. DEX, Dexamethasone.

tion of the hormone (23, 24). Note that the latter experiment was carried out with DUSP1^{-/-} mice in C3H/He genetic background, which appeared to be more robust to the action of LPS. Only 30% mortality was observed for the DUSP1^{-/-} mice in this background compared with 50% in the C57BL/6 background 80 h after the injection of LPS (5 mg/kg body weight; results not shown). Thus a concentration of 15 mg/kg body weight of LPS had to be used for the DUSP1^{-/-} mice in the C3H/He genetic background. The wild-type DUSP1 mice in this genetic background did not succumb to the LPS treatment over the period of the experiment.

Taken together, our results show in different mouse models of inflammation that the GC-mediated up-regulation of DUSP1 is not enough for the antiinflammatory action of GCs. In particular, we have shown that in mast cells, the antiallergic effects of GCs are largely independent of DUSP1. This finding is in contrast with recent study in bone marrow-derived macrophages suggesting that the antiinflammatory action of GC is, in part, mediated by DUSP1. Our studies, however, agree with the observation in the same report that the GC-mediated dephosphorylation of p38 MAPK is partially impaired in DUSP1^{-/-} cells up to 8 h of dexa-

methasone treatment (16). The reason for the differences in the two studies is not known.

The most important finding in our study is that DUSP1-deficient mice are more susceptible to passive anaphylaxis than their wild-type counterparts. Thus anaphylaxis can be added to the list of inflammatory reactions that has been associated with the deficiency of DUSP1. The other defects are susceptibility to the lethal effects of endotoxin (10, 11, 13) and marked sensitivity to collagen-induced arthritis (12). These together imply that the DUSP1 gene is a negative regulator of a number of immune processes. Our finding that GCs still inhibit passive anaphylaxis in DUSP1-deficient mice and our results showing that other phosphatase genes are still up-regulated by GC in the DUSP1^{-/-} BMMCs would argue against a simple correlation between up-regulation of DUSP1 and inhibition of inflammation by GCs.

Taken together our studies demonstrate that GC-induced DUSP1 expression inhibits only a small subset of the signaling pathways that contribute to the inflammatory response. The different cell type and substrate specificities of the DUSP family of proteins and their regulation by proinflammatory and antiinflammatory cues would require careful analysis to de-

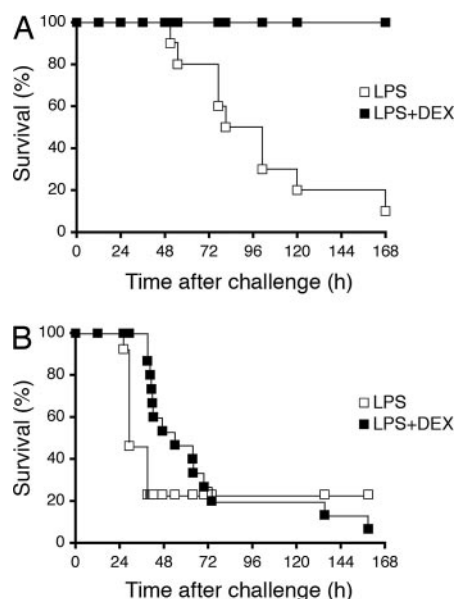


Fig. 7. Effect of Dexamethasone on LPS-Induced Septic Shock in DUSP1^{-/-} Mice

A, C57BL/6, DUSP1^{+/+}, and DUSP1^{-/-} mice received 10 mg/kg dexamethasone or vehicle orally for 2 consecutive days. On the second day the mice were additionally injected ip with 5 mg/kg LPS (*n* = 19). B, C3H/He, DUSP1^{+/+}, and DUSP1^{-/-} mice were injected ip with 1 mg/kg dexamethasone or PBS as vehicle; 30 min later, all mice received 15 mg/kg LPS ip (*n* = 12–14). Survival of the animals was monitored for 7 d. Only survival rates of DUSP1^{-/-} mice are presented. DEX, Dexamethasone.

termine the precise role of the individual family members in the immune system.

MATERIALS AND METHODS

Reagents

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Antibodies against p38 MAPK, ERK, and DUSP1 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against phosphorylated p38 MAPK and ERK were obtained from Cell Signaling (Beverly, MA). Horseradish peroxidase-coupled secondary antibodies were purchased from DAKO Corp. (Carpinteria, CA). Enhanced chemiluminescence reagents were obtained from Amersham Biosciences (Charlottesville, VA). Cell culture media and supplements were purchased from Invitrogen, Inc. (Carlsbad, CA).

Animals

DUSP1 ^{+/−} blastocysts generated by the laboratory of Bravo and co-workers (25) at Bristol-Myers Squibb Pharmaceutical Research Institute were supplied by The Jackson Laboratory (Bar Harbor, ME) and subsequently bred in house on a mixed 129/Sv × C57BL/6 background. Mice were also backcrossed for nine generations onto C57BL/6, C3H/He, and DBA/1. All experiments were performed according to European and German statutory regulations.

The genotype of the mice was determined by PCR analysis of genomic DNA from tissue biopsies as described previously (13).

Passive Systemic Anaphylaxis

Female mice of about 16 wk of age were sensitized by ip injection with anti-DNP IgE (1 mg/kg body weight) for 24 h. Dexamethasone (water soluble, 1 mg/kg) or vehicle alone, PBS, was administered by ip injection 3 h before the start of anaphylaxis. Anaphylaxis was initiated by iv injection of 200 μ l DNP-HSA (1 mg/ml PBS) or PBS, and rectal body temperature was measured with a MicroLife VT 1831 Vet-Temp (Tiershop, Trier, Germany) every 5 min over a period of 60 min.

LPS-Induced Septic Shock

Female and male mice of 12 to 14 wk of age received 10 mg/kg dexamethasone or vehicle, peanut oil/ethanol (10:1) via oral application on 2 consecutive days. On d 2, the mice were additionally injected ip with 5 mg/kg LPS (*Escherichia coli*) dissolved in PBS. In an alternative experiment, mice of 6 wk of age were injected ip with 1 mg/kg water-soluble dexamethasone or PBS as vehicle; 30 min later, the mice received 15 mg/kg LPS (*E. coli*) ip. Survival rate of the animals was monitored for 7 d.

DNFB-Induced Contact Hypersensitivity Reaction

For analyzing the systemic effect of dexamethasone on contact hypersensitivity reaction, female mice of about 12 wk of age were sensitized with 10 μ l 1% DNFB or vehicle alone (acetone-olive oil, 4:1) on the pre-shaved abdomen. All mice were challenged 5 d later by application of 10 μ l 0.5% DNFB on the left footpad for 24 h, and the thickness of the left and right footpads were measured with a spring-loaded caliper from Mitutoyo GmbH (Neuss, Germany). During the sensitization and challenge phase, dexamethasone-treated mice received dexamethasone-phosphate via the drinking water (20 mg/liter). This water was also used for preparation of the soft diet given to the mice. For topical application of dexamethasone, mice of about 14 wk of age were sensitized with 25 μ l 0.5% DNFB in acetone-olive oil (4:1) on the pre-shaved abdomen for 2 consecutive days. The dorsal surface of both ears were treated 5 d later with 20 μ l 0.3% DNFB, DNFB plus 0.15 mg/ml dexamethasone, or vehicle alone for 24 h. Mice were killed by cervical dislocation. Punched ear biopsies (8-mm diameter) were weighed as an indicator of edema formation. Thereafter they were fixed overnight with 4% neutral buffered formalin and embedded in paraffin wax. Sections (6 μ m) were taken with a rotary microtome (Leica RM 2155) and stained with hematoxylin and eosin. Sections were photographed using an inverted microscope (Zeiss Axioskop) fitted with a camera (AxioCam HRC; Carl Zeiss, Oberkochen, Germany) and AxioVision software for capture image.

Generation and Stimulation of BMMCs

BMMCs from sex- and age-matched 129/Sv × C57BL/6 or C57BL/6 DUSP1^{+/+} and DUSP1^{-/-} mice were generated as described previously (26). After 4–5 wk of differentiation in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate, 200 ng/ml stem cell factor, and 5 ng/ml IL-3 (PreproTech, Rocky Hill, NJ), BMMCs were treated with 100 nM dexamethasone or vehicle (0.01% ethanol) for different time periods. Two hours before the end of the incubation with dexamethasone, cells were treated with 1 μ g/ml anti-DNP IgE added to the medium. The cells were then washed and, unless other-

wise stated, they were resuspended in PBS containing 500 ng/ml DNP-HSA for the cross-linking of the IgE receptor.

Immunoblotting and Real-Time PCR Analysis

For detection of proteins, cells were lysed in sample buffer [80 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate, 2% β -mercaptoethanol, 10% glycerol, 0.1% bromophenol blue] and analyzed by immunoblotting. Total RNA was isolated using the NucleoSpin RNA II kit (Macherey Nagel, Düren, Germany). mRNAs were transcribed into cDNA and measured by real-time PCR using the ABI Prism Sequence Detection System 7000 (Applied Biosystems, Foster City, CA). The primers were purchased from Metabion (Martinsried, Germany) for the PCR analysis are as follows: 36B4: 5'-GGACCCGAGAAGACCTCCTT-3' and 5'-GCACATCACTCAGAATTTCAATGG-3'; DUSP1: 5'-GGATATGAAGCGTTTTTCGGCT-3' and 5'-GGATTCTGCACTGT-CAGGCA-3'; DUSP2: 5'-GATTGAGAGCCACCGGGTAC-3' and 5'-AGCTTCCCCCAGGAGTCAGT-3'; DUSP4: 5'-CCTGCTTAAGGTGGCTATGAGA-3' and 5'-GGTGTCTGGGAGGTACAGGG-3'; DUSP9: 5'-CCCCATCTCTGACCATTTGGA-3' and 5'-TGCGACAAGGCCTCATCA-3'; PEP: 5'-CAGCTTTTCCCCACCGT-TAAA-3' and 5'-AGACTCGGGGTGCTCGTTTCA-3'; CCL2: 5'-GTCCCTGTCTGCTTCTGGG-3' and 5'-GGCGTTAACTGCATC-TGGCT-3'; IL-6: 5'-TTGGGACTGATGCTGGTGAC-3' and 5'-TGGGAGTGGTATCCTCTGTGAAGT-3'; TNF α : 5'-AGTTCTATG-GCCCAGACCCTC-3' and 5'-GTTTGCTACGACGTGGGCTAC-3'; COX-2: 5'-GTCTGGTGCCTGGTCTGATGA-3' and 5'-GCTCCT-GCTTGATGATGTCGC-3'; IL1- α : 5'-GGAAGAGACCATCCAAC-CCA-3' and 5'-TGCTGACGAGCTTCATCAGT-3'; IL1- β : 5'-TT-TCCCGTGGACCTTCCAG-3' and 5'-TGAGTCACAGAGGATGG-GCTC-3'; IL-4: 5'-CTCACAGCAACGAAGAACACCA-3' and 5'-TTCAAGCATGGAGTTTCCCA-3'; IL-13: 5'-AGACCAGACTC-CCCTGTGCA-3' and 5'-GAATCCAGGGCTACACAGAACC-3'.

Degranulation Assay

Degranulation was measured by determining β -hexosaminidase release. BMMCs were treated with anti-DNP IgE as described above. Cells (2.5×10^5) were then incubated for 15 min in 200 μ l Tyrodes buffer (20 mM HEPES, pH 7.4; 135 mM NaCl; 5 mM KCl; 1 mM MgCl₂; 1.8 mM CaCl₂; 5.6 mM glucose; 0.05% BSA) supplemented with 200 ng/ml DNP-HSA. Cells were harvested, and enzymatic activity of β -hexosaminidase in cells and supernatant was measured as described previously (26).

Acknowledgments

We thank Sabine Müller, Cornelia Henkel, and Jutta Stober for their excellent technical assistance.

Received February 1, 2007. Accepted July 13, 2007.

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Disclosure Statement: J.V.M., S.B., J.T., U.H., M.K., M.S., A.M. have nothing to declare. A.C.B.C. consults for Bayer Schering Pharma AG.

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