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journal homepage: www.elsevier.com/locate/mceMolecular mechanisms of glucocorticoid action in mast cells[☆]Emmanuel Oppong^{*}, Nesrin Flink, Andrew C.B. Cato

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ABSTRACT

Glucocorticoids are compounds that have successfully been used over the years in the treatment of inflammatory disorders. They are known to exhibit their effects through the glucocorticoid receptor (GR) that acts to downregulate the action of proinflammatory transcription factors such as AP-1 and NF- κ B. The GR also exerts anti-inflammatory effects through activation of distinct genes. In addition to their anti-inflammatory actions, glucocorticoids are also potent antiallergic compounds that are widely used in conditions such as asthma and anaphylaxis. Nevertheless the mechanism of action of this hormone in these disorders is not known. In this article, we have reviewed reports on the effects of glucocorticoids in mast cells, one of the important immune cells in allergy. Building on the knowledge of the molecular action of glucocorticoids and the GR in the treatment of inflammation in other cell types, we have made suggestions as to the likely mechanisms of action of glucocorticoids in mast cells. We have further identified some important questions and research directions that need to be addressed in future studies to improve the treatment of allergic disorders.

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1. Introduction

Glucocorticoids are steroid hormones demonstrated some 60 years ago to possess potent anti-inflammatory properties (Hench et al., 1950a,b). It was shown that the naturally occurring glucocorticoid, cortisone, alleviated the suffering of patients with rheumatoid arthritis. Since then different synthetic glucocorticoids such as dexamethasone, prednisolone or budesonide have been effectively used in the treatment of inflammatory disorders. One of the routinely prescribed uses of glucocorticoids is in the treat-

ment of allergy, a hypersensitive disorder of the immune system that is triggered within minutes as a result of adverse reactions to normally harmless substances such as food, insect bites or environmental pollutants.

At the cellular level, allergens are first captured by antigen presenting cells (APC) that in turn activate T-helper 2 cells (Th2 cells). These cells stimulate B cells to produce allergen-specific immunoglobulin E (IgE) that binds to the high affinity IgE receptor (Fc ϵ RI) expressed on the surface of mast cells (Dimov and Casale, 2010). Aggregation of the IgE bound Fc ϵ RI leads to activation of mast cells resulting in degranulation, a process whereby preformed mediators such as histamine are released through fusion of granule containing vesicles with the plasma cell membrane. Besides this, lipid mediators such as prostaglandins and leukotriene as well as newly synthesised chemokine and cytokines are released (Gilfillan and Rivera, 2009; Schwartz, 1994; Turner and Kinet, 1999). Collectively, the expression and release of these mediators from activated mast cells lead to symptoms like sneezing, reddening/itching of the skin and eyes that all together constitute the allergic response that can be treated by glucocorticoids (Barnes, 1998).

Glucocorticoids act at the molecular level by binding to an intracellular glucocorticoid receptor (GR), a ligand inducible transcription factor that exists in an inactive complex in the cytoplasm of target cells (Grad and Picard, 2007; Olefsky, 2001). Upon hormone binding, the receptor becomes activated and moves into the nucleus to regulate target gene expression. Two main mechanisms by which glucocorticoids exert their anti-allergic effects have been described: a genomic mechanism, by which the GR re-

Abbreviations: APC, antigen presenting cell; IgE, immunoglobulin E; GR, glucocorticoid receptor; LAT, linker of activation of T-cells; SLP-76, SH2 domain containing leukocyte protein of 76 kDa; ITAM, immunoreceptor tyrosine based activation motifs; SYK, spleen tyrosine kinase; MAPK, mitogen-activated protein kinase; NTAL, non-T-cell activation linker; PI3K, phosphatidylinositol 3-kinase; Gab2, Grb2-associated binder 2; BMMC, bone marrow derived mast cells; FITC, fluorescein isothiocyanate; PLC, phospholipase C; IP3, inositol 1,4,5-trisphosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-triphosphate; DOK 1, Doking protein 1; SLAP, Src-like adaptor protein; GRE, glucocorticoid response elements; PTP, protein tyrosine phosphatases; DUSP, dual specificity phosphatase; Erk, extracellular regulated kinases; JNK, c-Jun N-terminal kinases; PEP, proline-, glutamic acid-, serine- and threonine-rich (PEST)-domain enriched tyrosine phosphatase; IL, Interleukin; TNF α , tumour necrosis factor; SHIP1, SH2 domain containing polyinositol-5phosphatase 1.

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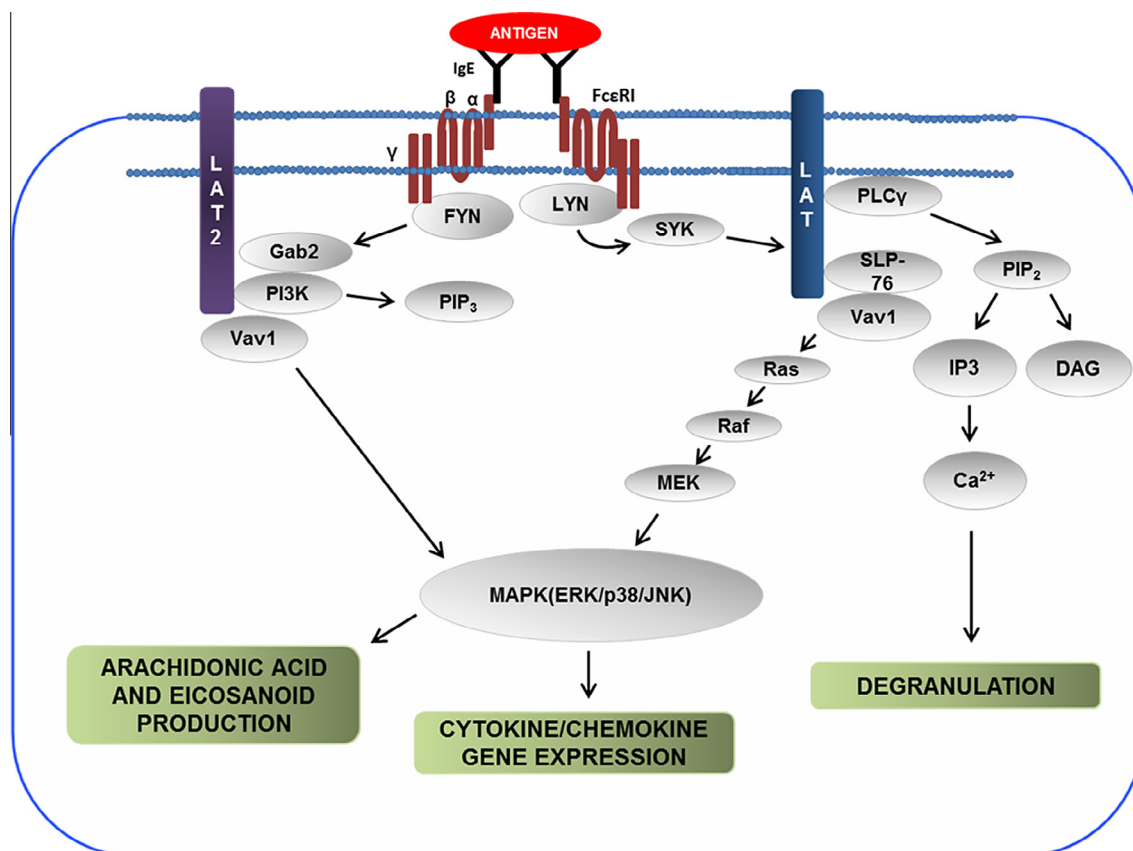


Fig. 1. Schematic representation of mast cell signalling pathway. Aggregation of the FcεRI leads activation of the Lyn pathway through Syk to downstream targets. Alternatively the Fyn pathway is also used by mast cells for signal propagation. Both pathways lead to three main downstream events: arachidonic and eicosanoid generation, cytokine gene expression and degranulation.

quires at least 30 min after ligand binding to exert its effects; and a second mechanism, termed rapid action that takes place within seconds to minutes after hormone exposure (reviewed in Cato et al., 2002; Haller et al., 2008; Stahn and Buttgerit, 2008). This rapid action may involve plasma membrane and cytoplasmic activities of the GR, since the responses are far too fast to come from genomic effects. In this review, we will discuss both the genomic and rapid mechanisms of glucocorticoids and describe how they exert their anti-allergic effects by impacting on activated mast cells.

Mast cells are immune cells found in numerous parts of the body. They originate from precursors of the haematopoietic lineage and circulate in blood and the lymphatic system (Kitamura et al., 1978; Nakano et al., 1985). Mast cells differentiate and mature under the influence of stem cell factor in resident tissues such as the epithelium of the intestines, airways or gastrointestinal tract (Galli et al., 2005; Metcalfe et al., 1997). They express on their surface a high affinity IgE receptor (FcεRI) that consists of an α-subunit containing an extracellular IgE binding domain, a transmembrane β-subunit and two γ-subunits (Fig. 1). The β- and γ-subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs) for mast cell signal amplification and propagation respectively (Kraft and Kinet, 2007; Rivera and Gilfillan, 2006). Although the main receptor involved in allergic responses is the FcεRI, mast cells also express other receptors on their surface. An example is the Kit receptor which is able to cross-talk with the FcεRI pathway to enhance downstream signalling events (Tkaczuk et al., 2004).

Mast cell signalling pathways have been reviewed extensively (Gilfillan and Rivera 2009; Alvarez-Errico et al., 2009) and will therefore be described only briefly in this article. Following

allergen-mediated crosslinking of FcεRI, several signal transduction pathways involving mainly tyrosine phosphorylation are activated (Gilfillan and Rivera, 2009). The proximal signalling events involve Lyn, a Src-like kinase that already weakly associates with the FcεRI in resting mast cells (Eiseman and Bolen, 1992; Vonakis et al., 1997; Wilson et al., 2000). Upon FcεRI aggregation, more Lyn molecules are recruited to FcεRIβ subunit where they associate and phosphorylate the ITAMs of the β subunit at Tyr²¹⁹ (On et al., 2004; Yamashita et al., 1994). Lyn also phosphorylates the ITAMs of the FcεRIγ subunits and this generates binding sites for the recruitment of spleen tyrosine kinase (Syk) (Scharenberg et al., 1995). The recruited Syk gets phosphorylated by Lyn and this leads to the phosphorylation of other signalling molecules like the linker of activation of T-cells (LAT), SH2 domain containing leukocyte protein of 76 kDa (SLP-76) and Vav1. The signal is further propagated downstream to substrates such as the mitogen activated protein (MAP) kinases (Sanderson et al., 2010; Scharenberg et al., 1995) (Fig. 1). Mast cells can also use an alternative signalling pathway involving Fyn that phosphorylates Grb2-associated binder 2 (Gab2). Gab2 in turn activates the p85α subunit of phosphatidylinositol 3-kinase (PI3K) leading to the production of phosphatidylinositol (3,4,5) triphosphate (PIP₃) (Fig. 1) which also controls mast cell responses (reviewed in Metcalfe et al., 2009).

2. Levels of modulation of mast cell activity by glucocorticoids

2.1. Glucocorticoid-mediated regulation of mast cell signalling

Initial studies on how glucocorticoids inhibit the allergic action of mast cells concentrated on the action of the hormone on the

process of degranulation. Although allergen-mediated degranulation was inhibited by glucocorticoids, degranulation triggered by the use of calcium ionophore was not. This indicated that the hormone was functioning at an upstream level during mast cell activation (Benhamou et al., 1986; Robin et al., 1985). In bone marrow derived mast cells (BMMC), flow cytometry analysis of cells stained with fluorescein-isothiocyanate (FITC) labelled IgE antibody indicated that the surface expression of the FcεRIα was suppressed after glucocorticoid treatment (Benhamou et al., 1986; Robin et al., 1985; Yamaguchi et al., 2001). Intriguingly, analysis of the mRNA levels of the α, β and γ subunits did not show any change in expression following glucocorticoid treatment (Yamaguchi et al., 2001). This suggests a post-transcriptional effect of the hormone possibly at the level of FcεRI stability.

Subsequent studies on the effects of glucocorticoids were focussed on the early signalling events following mast cell activation. Although glucocorticoids negatively regulate Erk1/2 activity following activation of RBL-2H3 mast cells, Rider et al. (1996) showed that this effect was not accompanied by a change in expression of the early signalling molecules Lyn or Syk nor did it affect the phosphorylation of Syk or FcεRIβ. Therefore, they argued that the attenuation of mast cell activation by the hormone must occur downstream of these signalling molecules (Cissel and Beaven, 2000; Rider et al., 1996). Indeed, the same group later showed that glucocorticoids inhibit Raf-1 activity by disrupting the interaction of Raf-1 and HSP-90: a complex that is important for the association of Ras with Raf-1 for further propagation of the signalling cascade to Erk1/2 (Cissel and Beaven, 2000; Rider et al., 1996). However, Hiragun et al. (2006) reported that glucocorticoids do have an effect on the early signalling molecules, albeit in an indirect manner. They showed that the phosphorylation of Syk, following mast cell activation is indirectly impaired by glucocorticoids through an increased expression of an inhibitory adapter protein, Src-like adaptor protein (SLAP). In RBL-2H3 mast cells, Syk was shown to associate with SLAP upon activation of the cells (Hiragun et al., 2006). This interaction was further enhanced when SLAP was transiently overexpressed in the cells. Additionally, siRNA mediated knockdown of SLAP reversed the inhibition of Syk phosphor-

ylation (Hiragun et al., 2006). Whether SLAP also affects other molecules involved in the mast cell signalling pathway remains to be determined.

The effect of glucocorticoids on mast cell signalling is not only restricted to the Lyn–Syk–LAT pathway but it also extends to the Fyn–Gab2–PI3K pathway. Activation of this latter pathway is characterised by the production of phosphatidylinositol (3,4,5)-triphosphate (PIP₃) (Fig. 1) and it is suppressed by glucocorticoids (Andrade et al., 2004). This effect of the hormone also correlates with a decrease in PI3K activity, reduction of calcium release, and an impaired association of p85α with Gab2 (Andrade et al., 2004). Other effects of glucocorticoids in mast cells are reduced phosphorylation of p38, PLCγ1 and JNK1/2 (Maier et al., 2007; Obiri et al., 2012).

In addition to the effects of glucocorticoid action on FcεRI signalling pathway, glucocorticoids also inhibit cross-linked Kit receptor induced mast cell responses such as migration, p38 MAP kinase phosphorylation, TNFα and IL-6 production (Jeong et al., 2003). Thus, glucocorticoids exert their effect on mast cell activation at different points in the signalling cascade and a summary of these stages is presented in Table 1.

2.2. Glucocorticoid regulation of mast cell action through activation of phosphatases

In addition to the different mechanisms for attenuating mast cell action described above, the activation of protein tyrosine phosphatases (PTPs) by glucocorticoids provides another mode of downregulating mast cell action by dephosphorylation. Phosphatases play critical roles in the regulation of cell signalling by controlling the rate and duration of signal transduction (Tonks, 2006). They function mainly by dephosphorylating tyrosine, serine or threonine residues of protein kinases to inhibit inflammatory signal transduction and are classified into four main groups (Faucian and Saper, 1996). The first family of PTPs referred to as class I, is made up of the classical PTPs and the dual specificity phosphatases (DUSPs). The former dephosphorylates phosphotyrosine residues of its target substrate while the latter regulates phosphoserine, phosphothreonine, and phosphotyrosine residues, mainly in the family of mitogen activated kinases (MAPK) (Groom et al., 1996; Keyse, 1998; Lang et al., 2006; Saxena and Mustelin, 2000). The second class of phosphatases is made of the 18 kDa low molecular weight phosphatases (LMW-PTPs). This class of phosphatases targets receptor protein tyrosine kinases (RPTKs) that influence cellular processes such as growth and migration (Raugei et al., 2002). The third class of PTPs contains the cell cycle kinase phosphatases CDC25A, CDC25B, and CDC25C that dephosphorylate cyclin-dependent kinases while the fourth group is formed by asparagine-based phosphatases that target phosphotyrosine and phosphoserine residues (For review of PTPs see Alonso et al., 2004; Bayón and Alonso, 2010).

The class I PTPs are the only phosphatases so far reported to respond to glucocorticoid treatment. In the group of classical PTPs, the PEST-domain-enriched tyrosine phosphatase (PEP) has been shown to be positively regulated by glucocorticoids (Maier et al., 2007; Obiri et al., 2012). PEP, a non-receptor phosphatase expressed in hematopoietic cells is described as a negative regulator of T-cell receptor signalling (TCR) (Alonso et al., 2004; Cloutier and Veillette, 1999; Cohen et al., 1999). For example, in mouse T-cell hybridoma BI-141, stable expression of PEP inhibited production of lymphokines, a downstream response of T-cell receptor activation (Cloutier and Veillette, 1999). Additionally, the activation of isolated effector T-cells from PEP^{−/−} mice exhibited enhanced phosphorylation of T-cell receptor upstream molecules, LCK at Tyr³⁹⁴ and ZAP 70 at Tyr³¹⁹ as compared to PEP^{+/+} cells

Table 1
Glucocorticoid-mediated inhibition of components of the mast cell activation pathway.

Mast cell target	Glucocorticoid action	References
<i>Surface receptors</i>		
FcεRI	Reduction of surface expression of FcεRIα. The mRNA of α, β, and γ subunits are however unaffected	Rider et al., (1996), Yamaguchi et al. (2001)
<i>Signalling molecules</i>		
Lyn	Expression remains unchanged	Rider et al. (1996)
Syk	Phosphorylation is reduced by upregulation of SLAP	Hiragun et al. (2006)
PLCγ1	Phosphorylation is reduced	Obiri et al. (2012)
PI3K	Activity is downregulated	Andrade et al. (2004)
p38	Phosphorylation is reduced	Maier et al. (2007)
Erk1/2	Activity and phosphorylation of Erk1/2 is down regulated through multiple mechanisms	Cissel and Beaven (2000), Kassel et al. (2001), Rider et al. (1996)
JNK1/2	Phosphorylation is reduced	Obiri et al. (2012)
Ca ²⁺	Intracellular calcium mobilization is reduced	Obiri et al. (2012)
<i>Adaptor molecules</i>		
LAT	Expression not affected	Andrade et al. (2004)
Gab2	Decreased association of Gab2 with p85α	Andrade et al. (2004)
Dok1,SLAP	These inhibitory adaptors are upregulated	Hiragun et al. (2005, 2006)

(Hasegawa et al., 2004), indicating that PEP is a negative regulator of TCR.

On the contrary, the absence of PEP has been reported to inhibit mast cell action suggesting that PEP is a positive regulator of mast cell signalling (Obiri et al., 2012). In experiments carried out in both PEP^{+/+} and PEP^{-/-} mice, markers of mast cell activation such as serum histamine, the number of degranulated mast cells in the skin, and passive systemic anaphylaxis were significantly decreased in the knockout as compared to the wild-type mice (Obiri et al., 2012). The level at which PEP functions in mast cells is not known but in analogy to its function in T cells, it may possibly target and influence the activity of upstream signalling molecules such as Lyn, Fyn and the ITAMs that regulate FcεRI activation of mast cells. Analysis of the action of glucocorticoids in BMMC from PEP^{+/+} and PEP^{-/-} mice revealed that hormone-mediated inhibition of JNK1/2 and PLCγ1 phosphorylation following FcεRI cross-linking was abolished in the knockout mast cells (Obiri et al., 2012). Furthermore, glucocorticoid-mediated downregulation of calcium mobilization and serum histamine release observed in PEP^{+/+} BMMC was impaired in the PEP^{-/-} BMMC (Obiri et al., 2012). These studies suggest that in addition to its positive effect in regulating anaphylaxis, PEP also positively regulates glucocorticoid action. Future work is therefore needed to clarify how PEP contributes to the antiallergic action of glucocorticoids by positively regulating glucocorticoid action and at the same time positively regulating the anaphylactic response.

DUSPs are the other members of class 1 PTPs that are upregulated by glucocorticoids (Maier et al., 2007). DUSP family is subdivided into four groups on the basis of their structure (Farooq and Zhou, 2004). The type 1 DUSP, also referred to as atypical DUSP, consists of 19 members and is characterised by the presence of a protein tyrosine phosphatase domain (PTP), but by a lack of MAPK binding domain (MKB). Nevertheless, certain members of this class: DUSP3, DUSP14, DUSP18, DUSP19, DUSP21 and DUSP22 are able to target MAPK (reviewed in Bayón and Alonso, 2010) (Table 2). The Type II subgroup possesses both PTP and MKB and consists of DUSP1, DUSP2, DUSP4, DUSP5, DUSP6, DUSP7, and DUSP9. Type III subgroup has an N-terminal domain in addition to its PTP and MKB. DUSP10 is the only member of this group. The last subgroup is Type IV that has a PEST domain in addition to the MKB and PTP. Members of this group include DUSP8 and DUSP16 (Alonso et al., 2004; Hammer et al., 2006). To determine

whether glucocorticoids function in mast cells by modulating the expression of these DUSP genes, we have analysed the level of mRNA expression of these genes in the presence of the synthetic glucocorticoid, dexamethasone in BMMC. While the expression of DUSP1, DUSP2, DUSP4 and DUSP9 is upregulated, the expression of DUSP8, DUSP16, and DUSP18 is downregulated. However, the others DUSPs were not affected by the hormone (Table 2). This suggests a rather complex glucocorticoid-mediated regulation of mast cell action via phosphatases that needs to be clarified in the future.

Of all the DUSPs reported to be transcriptionally regulated by glucocorticoids, only DUSP1 and DUSP2 have been functionally characterised in mast cells. A knockout of DUSP2 was reported to protect mice from peripheral arthritis as measured by a decrease in ankle thickness (Jeffrey et al., 2006). Additionally, antigen-stimulated DUSP2^{-/-} BMMC showed a reduced expression of IL-6, TNFα, and cyclooxygenase-2 (Cox-2) as compared to DUSP2^{+/+} BMMC (Jeffrey et al., 2006). The effects of glucocorticoids in DUSP2 knockout BMMC have however not been analysed. DUSP1 has so far been extensively analysed in mast cells. DUSP1 was first identified as a glucocorticoid induced phosphatase that inhibited the phosphorylation of Erk1/2 and its downstream target Elk-1 in RBL-2H3 mast cells, in a genome wide affymetrix study (Kassel et al., 2001). The mechanism of Erk1/2 dephosphorylation in that study was shown to occur via a dual function of glucocorticoids. A short term (4 h) glucocorticoid treatment that resulted in an increase in DUSP1 expression and a long term glucocorticoid effect (16 h) that stabilised DUSP1 level by inhibiting its proteasomal degradation. In this way, increased level of DUSP1 is made available for the dephosphorylation of Erk1/2 (Kassel et al., 2001). In subsequent studies, the role of DUSP1 in the anti-allergic action of glucocorticoids was analysed using BMMC from DUSP1 knockout mice. This study showed no loss of glucocorticoid-mediated dephosphorylation of Erk1/2 in the knockout cells. In contrast, dephosphorylation of p38 MAPK was impaired but only at the early times (4–8 h) of glucocorticoid treatment (Maier et al., 2007). Additionally, loss of DUSP1 seems not to affect glucocorticoid-mediated inhibition of a selected number of cytokine genes although hormone-mediated inhibition of Cox-2 was partly dependent on this gene. Furthermore, DUSP1^{-/-} mice were found to exhibit increased susceptibility to anaphylaxis but were still responsive to glucocorticoids (Maier et al., 2007). To investigate an overall role

Table 2
Glucocorticoid regulation of dual specificity phosphatases in mast cells.

DUSPs	MAPK substrate	Glucocorticoid regulation	References
<i>Atypical</i>			
DUSP3	ERK,p38, JNK	–	Alonso et al. (2001), Takagaki et al. (2004) and Todd et al. (2002)
DUSP14	ERK, p38, JNK	–	Marti et al. (2001)
DUSP18	JNK	↓	Wu et al. (2006)
DUSP19	JNK	–	Nakamura et al. (2002) and Zama et al. (2002)
DUSP22	ERK,JNK,p38	–	Alonso et al. (2002), Aoyama et al. (2001) and Chen et al. (2002)
DUSP23	ERK,JNK,p38	–	Takagaki et al. (2004) and Wu et al. (2004)
<i>Classical</i>			
DUSP1	JNK,p38,ERK	↑	Franklin and Kraft (1997), Kassel et al. (2001) and Wu and Bennett (2005)
DUSP2	ERK,p38	↑	Chu et al. (1996) and Maier et al. (2007)
DUSP4	JNK,p38, ERK	↑	Caunt et al. (2008) and Maier et al. (2007)
DUSP5	ERK	–	Mandl et al. (2005)
DUSP6	ERK	–	Groom et al. (1996) and Muda et al. (1996)
DUSP7	ERK	–	Keyse (2008)
DUSP8 ^a	JNK, p38	↓	Keyse (2008)
DUSP9	ERK,p38	↑	Dickinson et al. (2011) and Maier et al. (2007)
DUSP10	JNK,p38	–	Tanoue et al. (1999)
DUSP16 ^a	JNK,p38	↓	Tanoue et al. (2001)

↑ Upregulation.

↓ Downregulation.

– No effect.

^a Unpublished observation by Stefanie Bormann.

of DUSP1 in anti-inflammatory action of glucocorticoids, Maier et al. (2007) made use of two additional animal models of inflammation: dinitrobenzene induced contact hypersensitivity and lipopolysaccharide (LPS) induced endotoxic shock models. They found that glucocorticoid administration protected both DUSP1 knockout and wild-type mice from inflammation (Maier et al., 2007). Thus, they concluded that the anti-inflammatory effect of glucocorticoids is independent of DUSP1. It was argued that other phosphatases such as DUSP9 and DUSP4 that are upregulated in the absence of DUSP1 by glucocorticoids may compensate for the loss of DUSP1 (Maier et al., 2007). The compensatory roles of these other DUSPs have so far not been determined.

2.3. Glucocorticoid regulation of mast cell action through adaptor proteins

Apart from the regulation of action of kinases and phosphatases, glucocorticoids also regulate mast cell action through altered expression of adaptor proteins as presented in Table 1. Adaptor proteins act as either positive or negative regulators of FcεRI mediated signalling (reviewed in Alvarez-Errico et al., 2009). They may be categorised either as membrane or cytoplasmic adaptors, based on the presence or absence of a putative transmembrane domain that influences their cellular location. Membrane adaptors form platforms for the assembly of signalling molecules by providing docking sites for phosphorylation events. Cytoplasmic adaptors on the other hand interact and recruit signalling proteins in both phosphotyrosine-dependent and independent manner to membrane adaptors for further signalling activities (Alvarez-Errico et al., 2009; Rivera, 2002).

The reported effects of glucocorticoid on adaptors are mostly on the cytoplasmic molecules. For example, in the inhibition of the Fyn pathway by glucocorticoids, treatment of RBL-2H3 mast cells with dexamethasone did not affect the interaction of LAT (membrane) with p85α, but rather it impaired the phosphorylation of Gab2 (cytoplasmic) and its association with p85α (Andrade et al., 2004). Another reported effect of glucocorticoids on cytoplasmic adaptors is the induction of inhibitory adaptors that terminate signalling cascades. Doking protein 1 (Dok 1) and Src-like adaptor protein (SLAP) are examples of such inhibitory adaptors that were found to be transcriptionally upregulated by glucocorticoids (Hiragun et al., 2005, 2006). Studies on Dok1 in RBL-2H3 mast cells showed that glucocorticoid treatment did not only increase the expression of this gene, but also enhanced phosphorylation of the gene product and its interaction with RasGTPase activating protein (Hiragun et al., 2005). Consequently, the signalling cascade Raf-1, MEK and Erk of the MAP kinase pathway was inhibited. Furthermore, siRNA mediated knock-down of Dok1 in RBL-2H3 mast cells reversed the down regulation of Erk1/2 phosphorylation (Hiragun et al., 2005). In the case of SLAP, glucocorticoid-mediated increase in expression of this gene resulted in a reduced phosphorylation of Syk, LAT and PLCγ2 in RBL-2H3 mast cells (Hiragun et al., 2006). The inhibitory effect could also be reversed by siRNA mediated knockdown of SLAP (Hiragun et al., 2006). A characteristic feature of adaptor proteins is the presence of several binding motifs that allow their interaction with numerous signalling molecules including phosphatases (reviewed in Alvarez-Errico et al., 2009). Thus, it is conceivable that Dok1 may cause downregulation of signal transduction through indirect dephosphorylation by interacting with phosphatases such as SH2 domain containing polyinositol-5 phosphatase 1 (SHIP1). Alternatively, association of Dok1/SHIP1 with the inhibitory FcγRIIB which is also expressed on the surface of mast cells could lead to inhibition of the signalling cascade (Abramson et al., 2003; Huber et al., 1998; Ott et al., 2002).

2.4. Glucocorticoid-mediated regulation of mast-cell chemotactic factor

A common feature associated with inflammatory conditions is the increased migration and accumulation of mast cells at the inflamed site. Mast cells migrate to these sites under the influence of stem cell factor (SCF), a chemotactic factor produced mainly by resident cells such as fibroblasts (Meininger et al., 1992; Nilsson et al., 1994). In addition to its chemotactic property, SCF, also termed Kit ligand, is a major growth factor important for the development, survival, adhesion, and activation of mast cells (reviewed in Da Silva et al., 2006). Glucocorticoids have been reported to decrease the number of mast cells in human inflamed tissue biopsies (Goldsmith et al., 1990; Lavker and Schechter, 1985), an effect that was hypothesized to occur through the ability of the hormone to downregulate SCF production (Finotto et al., 1997). However, Kassel et al., 1998 reported a different mechanism of regulation of SCF by glucocorticoids in lung fibroblasts. They showed an initial inhibitory phase which occurs within 3 h followed by a stimulatory phase that reaches a maximum level at twenty-fours (Kassel et al., 1998). Thus, further studies are required to better understand how glucocorticoid-mediated regulation of SCF production may impact on mast cell recruitment.

3. Mechanisms of glucocorticoid action

Only a handful of the effects of glucocorticoids described in mast cells have been mechanistically explained. On the whole the actions of glucocorticoids are mediated by the GR which is made up of distinct functional domains: a central DNA binding domain (DBD), a C-terminal ligand binding domain containing the activation domain AF2 and an N-terminal that harbours the activation domain AF1. The GR is mainly cytoplasmic in its unliganded form and reside in complex with molecular chaperones such as Hsp90. Upon hormone binding, it undergoes conformational changes that allow it to translocate into the nucleus to bind to DNA and to regulate the expression of specific genes (Grad and Picard, 2007). Before the GR binds to its sites, certain molecular events are thought to occur. For example, binding of AP-1, has been proposed to be necessary for maintaining an “open” chromatin conformation that facilitates GR binding (Biddie et al., 2011). The GR then utilizes multiple molecular functions such as recruitment of coregulators complexes through protein–protein interactions and transcriptional crosstalk with other cofactors to modulate gene expression (reviewed in Kassel and Herrlich, 2007).

Glucocorticoids negatively regulate gene transcription by inhibiting the activities of transcription factors (TFs) such as activator protein (AP-1) or nuclear factor (NF-κB). In this mode of action, liganded monomeric GR downregulates the expression of proinflammatory genes by being “tethered” to AP-1 or NF-κB already bound to DNA (Heck et al., 1994; Kassel et al., 2004; Luecke and Yamamoto, 2005). The GR can also cooperate with other coregulator proteins such as GRIP1 to exhibit its negative transcriptional effect. Chromatin immunoprecipitation analysis performed in activated bone marrow derived macrophages showed that glucocorticoid-mediated repression of cytokine genes such as IL1α, IL1β, CCL4, and TNFα correlated with the recruitment of GR and GRIP1 to NF-κB binding sites (Chinenov et al., 2012). However, this inhibitory effect of glucocorticoids was attenuated in conditional GRIP1 knockout macrophages. Thus, GRIP1 is a potential mediator of the anti-inflammatory action of the GR (Chinenov et al., 2012). Another means by which the GR negatively regulates the expression of target genes has recently been reported in the promoter of thymic stromal lymphopoietin (TSLP), a cytokine that triggers atopic dermatitis (Surjit et al., 2011). The repression of the expression this

gene appears to be through the binding of two monomeric GRs to a negative GRE (nGRE) of the target promoter (Hudson et al., 2013).

Most studies investigating transcriptional mechanisms of glucocorticoids in anti-inflammation have been carried out in cells other than mast cells. However, as activation of pro-inflammatory genes in mast cells require transcription factors such as AP1 and NF- κ B (reviewed in Tshori and Nechushtan, 2012), in the same way as in other cell types, the possibility exists that these factors could also be used in the anti-allergic action of glucocorticoids in mast cells. Alternatively, there could be distinct mechanisms used by the GR for the anti-allergic action of glucocorticoids.

3.1. Glucocorticoid regulation of DUSP1 gene expression

One of the proteins that have received great attention in the anti-allergic action of glucocorticoids in mast cells is DUSP1, although its contribution to the anti-inflammatory action of glucocorticoids is still controversial. It has been reported that dimerization-defective as well as transactivation-negative GR are unable to activate the DUSP1 promoter after glucocorticoid treatment (Kassel et al., 2001). However, analysis of the effect of glucocorticoids on the expression of DUSP1 in macrophages isolated from dimerization mutant GR mice (GR^{dim}), showed no impairment in the expression of this gene (Abraham et al., 2006). Endogenous DUSP1 expression and reporter constructs containing distinct glucocorticoid responsive regions in the promoter and upstream sequences of DUSP1 gene were activated by dimerization-defective GR molecules (Abraham et al., 2006; Tchen et al., 2010). These studies are inconsistent with the findings in mast cells where the regulation of DUSP1 expression by GR requires dimerization of the receptor (Kassel et al., 2001). The discrepancies could be due to the use of constructs with different-length promoter sequences. A further possibility exists that certain regions in the DUSP1 promoter may contain elements that require dimerization of the GR, while other regions may be regulated by receptor monomers. The inconsistencies may also be due to the different cell types used in the studies.

In another study, hormone-activated GR and the coactivator p300 were shown to be involved in glucocorticoid-induced hyperacetylation of histone H3 that potentiated DUSP1 gene transcription (Shipp et al., 2010). In a different study, an indirect mechanism involving interaction of the GR with DNA-bound CCAAT/enhancer-binding protein beta (C/EBP β) in the promoter of human DUSP1 has also been identified (Johansson-Haque et al., 2008). Furthermore, a posttranscriptional regulation of DUSP1 expression by glucocorticoids has also been presented in murine macrophage RAW 264.7. In this case, DUSP1 levels were reduced through the action of LPS-induced miRNA101 leading to a prolonged phosphorylation of JNK1/2 and p38 MAPK (Zhu et al., 2010). Pre-treatment of the cells with glucocorticoids inhibited the expression of miRNA-101 and this correlated with upregulation of DUSP1 and dephosphorylation of JNK and p38 (Zhu et al., 2010). Whether this regulatory pathway also occurs in mast cells has not been determined.

4. Rapid action of glucocorticoids in mast cells

The mechanisms of action of glucocorticoids in mast cells described so far cannot account for all the effects of the hormone observed in an organism upon glucocorticoid administration. Certain effects of the hormone cannot be explained by the genomic action of the GR. These actions of glucocorticoids occur within seconds to minutes and are insensitive to transcriptional and translational inhibitors (Croxtall et al., 2000).

Glucocorticoids were found to rapidly inhibit allergic reactions within 5 min in an *in vivo* model of allergic asthma (Zhou et al.,

2008). In that study, it was shown that the hormone rapidly decreased lung mast cell degranulation as demonstrated by toluidine blue staining of degranulated cells (Zhou et al., 2008). Utilizing a whole cell patch clamp technique, a decrease in exocytosis of mast cells was correlated with glucocorticoid-mediated decrease in membrane capacitance (Zhou et al., 2008). Additionally, rapid decreases in histamine and calcium release from mast cells by glucocorticoids have also been reported (Liu et al., 2007; Zhou et al., 2008). Mechanistic studies on the rapid action of glucocorticoids in mast cells are lacking. Nevertheless, studies from other cell types suggest that the rapid actions of the hormone are initiated at the plasma cell membrane. At this cellular location, the GR would interact and modulate the activities of membrane proteins to exert its rapid effects. For example, rapid phosphorylation of protein kinase B by glucocorticoids has been reported and this has been shown to require the interaction of the GR with the membrane protein caveolin (Matthews et al., 2008). Furthermore, a short term exposure of T-cells to glucocorticoids was shown to cause a rapid dissociation of the protein complex at the plasma membrane that consist of the GR, Hsp90 and Src resulting in impaired T-cell receptor signalling (Lowenberg et al., 2006). These studies suggest that the actions of the GR at the plasma membrane may be important for it to exert its rapid effects. However, studies on how the GR targets the proximal signalling molecules in mast cells are required to gain further understanding of the rapid action of glucocorticoids.

5. Conclusion and perspectives

Glucocorticoids play a vital role in the inhibition of allergic response. This inhibitory action of the hormone may occur through inhibition of signalling pathways in mast cells. Alternatively, glucocorticoids can also inhibit signaling cascades in mast cells through the expression of phosphatases that are upregulated by glucocorticoids. The protein tyrosine phosphatases that are known to modulate immune responses have emerged as one of the potential mediators of glucocorticoid action with DUSP1 being the best characterised to date. Although the precise mechanism of DUSP1 regulation by glucocorticoid is still not fully understood, the report that other phosphatases such as PEP, DUSP4 and DUSP9 are upregulated in the absence of DUSP1 indicates that systematic analyses of the action of these phosphatases are required. Additionally, the observation that certain DUSP (DUSP8 and DUSP16) are downregulated by glucocorticoids in mast cells introduces a level of complexity that needs to be resolved. Finally, since glucocorticoids have also been shown to act in a rapid manner, analysis of how this rapid effect contributes to the overall action of the hormone, will provide a thorough understanding of glucocorticoid action in mast cells.

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