Annexin A1 and glucocorticoids as effectors of the resolution of inflammation

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Abstract | Glucocorticoids are widely used for the management of inflammatory diseases. Their clinical application stems from our understanding of the inhibitory effect of the corticosteroid hormone cortisol on several components of the immune system. Endogenous and exogenous glucocorticoids mediate their multiple anti-inflammatory effects through many effector molecules. In this Opinion article, we focus on the role of one such effector molecule, annexin A1, and summarize the recent studies that provide insight into its molecular and pharmacological functions in immune responses. In addition, we propose a model in which glucocorticoids regulate the expression and function of annexin A1 in opposing ways in innate and adaptive immune cells to mediate the resolution of inflammation.

Inflammation is a primordial response that functions to protect the host against invasion by pathogens or exposure to xenobiotics. However, overly aggressive or prolonged inflammatory responses can be detrimental to the host. Therefore, higher organisms have evolved mechanisms to ensure that the inflammatory response is limited in time and space. Many endogenous anti-inflammatory and pro-resolving mediators function to counteract the properties of pro-inflammatory factors and to ensure a prompt resolution of inflammation. The concept that inflammation is resolved in a time- and space-specific manner is emerging, such that different outcomes can be produced according to the stage or site at which a given pathway or mediator becomes operative. The action of anti-inflammatory and pro-resolving mediators and pathways on several components of the inflammatory response is crucial for restoring tissue structure and homeostasis. Elucidation of their effects on immune cells could lead to the identification of the molecular target (or targets) of a given mediator, thereby prompting innovative drug discovery for the treatment of chronic inflammatory conditions.

Glucocorticoids are the first class of endogenous anti-inflammatory mediators that have been successfully used for therapeutic purposes; budesonide and beclomethasone are widely used for the treatment of asthma, prednisolone is used for rheumatoid arthritis and other autoimmune diseases, and mometasone and hydrocortisone are used for eczema and psoriasis. In healthy individuals, the circadian release of glucocorticoids (such as cortisol and corticosterone) from the adrenal glands facilitates the control of several homeostatic processes that are crucial for health. During inflammation, increased levels of cortisol dampen local and systemic inflammatory events, thereby favouring proper resolution of the inflammatory response. These fundamental pathophysiological functions of glucocorticoids are achieved through many molecular mechanisms, which can be broadly divided into genomic mechanisms (involving transactivation or transpression of gene transcription) and non-genomic mechanisms (that are rapid and independent of de novo protein synthesis).

In this Opinion article, we focus on one downstream mediator of glucocorticoids, the 37 kDa protein annexin A1 (also known as lipocortin 1; encoded by ANXA1). We review the recent evidence indicating that glucocorticoids regulate the synthesis and function of annexin A1, possibly through a combination of both genomic and non-genomic processes, depending on the cell type and the time of induction. In addition, we describe the emerging data showing that glucocorticoids can differentially affect the annexin A1 pathway in cells of the innate and adaptive immune system, in which this pathway can have opposing effects. We propose that the annexin A1 pathway is an important mediator of the anti-inflammatory effects of glucocorticoids.

Effects in innate immunity

Annexin A1 is a member of a superfamily of annexin proteins that bind acidic phospholipids with high affinity in the presence of Ca²⁺. There are 13 mammalian annexin proteins, each of which has specific biological functions. Similarly to other annexin proteins, annexin A1 is expressed in resting cells and binds to acidic phospholipids in the presence of Ca²⁺; original studies of annexin A1 therefore focused on its role in granule fusion and exocytosis, in which it was shown to promote the fusion of vesicle membranes with plasma membranes in reconstituted systems.

The actions of annexin A1. In resting conditions, human and mouse neutrophils, monocytes and macrophages constitutively contain high levels of annexin A1 in their cytoplasm. Following cell activation (for example, by neutrophil adherence to endothelial-cell monolayers), annexin A1 is promptly mobilized to the cell surface and secreted. The molecular mechanisms that are responsible for this rapid secretion are cell specific. In macrophages, the
Box 1 | The molecular mechanisms by which glucocorticoids affect gene expression

**Genomic effects**

Glucocorticoids have numerous effects on gene expression, such that it has been proposed that ~1% of the genome might be modulated by these drugs\(^1\). The glucocorticoid receptor is retained in an inactive state by several chaperone proteins, including heat-shock protein 70 (HSP70) and HSP90, and following ligand binding, the glucocorticoid–receptor complex translocates to the nucleus, where it modulates gene expression through transrepression or transactivation.

Transrepression occurs when the glucocorticoid–receptor complex interacts with transcription factors: it can sequester them in the cytoplasm, promote their degradation or inhibit them through other mechanisms. Through transrepression, the glucocorticoid–receptor complex prevents the transcription of the target genes, including pro-inflammatory cytokines, growth factors, adhesion molecules, nitric oxide, prostanoids and other autacoids\(^3\). In addition, glucocorticoids transrepress the transcription of nuclear factor-κB (NF-κB)-induced pro-inflammatory genes by inducing the activation of histone deacetylase 2, which reduces the access of NF-κB to the promoter regions of its target genes\(^7\).

Transactivation involves the direct binding of the glucocorticoid–receptor complex to specific nucleotide sequences (termed glucocorticoid-response elements) to promote the transcription of genes, such as interleukin-10, β-adrenergic receptor, interleukin-1-receptor antagonist and dual-specificity protein phosphatase 1 (DUSP1), many of which have anti-inflammatory functions\(^4\). The anti-inflammatory effects of glucocorticoid-mediated transactivation can also be reinforced indirectly; for example, DUSP1 downregulates mitogen-activated-protein-kinase signalling and thereby inhibits cytokine synthesis. Finally, it is possible that annexin A1 (encoded by ANXA1) functions as a mediator of some of these glucocorticoid effects, as suggested by decreased levels of DUSP1 expression in Anxa1\(^–/–\) lung fibroblasts\(^2\) (see the main text for more details).

**Non-genomic effects**

Glucocorticoids can also have rapid effects, which occur within a few minutes, by modulating the degree of activation and responsiveness of target cells. These actions do not require de novo protein synthesis and are referred to as non-genomic effects\(^1\). Initially reported in monocytes, the non-genomic actions of glucocorticoids have been recently described in T cells\(^2\) and platelets\(^5\). However, it is unclear how non-genomic effects contribute to the overall therapeutic efficacy of glucocorticoids in controlling vascular inflammatory pathologies.

ATP-binding cassette (ABC) transporter system has been shown to be responsible for the secretion of annexin A1 (REF 12), whereas in pituitary cells the phosphorylation of annexin A1 at the amino-terminal serine 27 residue is required\(^13\). In human neutrophils, a high proportion (>60%) of cytoplasmic annexin A1 is stored in gelatinase granules\(^3,14\) and can be rapidly mobilized following exposure of the cells to weak activating signals, such as low concentrations of chemoattractants or adhesion to endothelial-cell monolayers\(^15,16\). Cell activation generally leads to the relocation of annexin A1 to the outside leaflet of the plasma membrane, to which it is tethered in a calcium-dependent manner. In addition, extracellular concentrations of Ca\(^2+\) of ≥1 mM lead to a conformational change in the protein, such that the N-terminal region that is otherwise buried becomes exposed and the active form of annexin A1 is generated\(^14\) (FIG. 1).

Several studies using experimental models of inflammation have shown that administration of annexin A1, or of the bioactive peptides that comprise its N-terminal region, to mice results in potent inhibition of neutrophil trafficking\(^17,18\). In one study\(^19\), annexin A1 administration to mice during an inflammatory response had an important anti-inflammatory effect, as revealed by analysis of the mesenteric microcirculation. This effect was associated with the detachment of adherent neutrophils from the vascular wall, which resulted in a reduction in the number of cells that migrated into the subendothelial-matrix tissue\(^19\) (FIG. 2). These in vivo effects are supported by in vitro analyses showing that annexin A1 or annexin A1-derived peptides inhibit neutrophil adhesion to and transmigration through endothelial-cell monolayers\(^20,21\). Quantification of the interaction of neutrophils with human umbilical-vein endothelial-cell monolayers under flow has recently confirmed these observations, showing that low concentrations of annexin A1 (10 nM) significantly inhibit neutrophil adhesion\(^22\). The observed inhibitory activities of recombinant annexin A1 or annexin A1-derived peptides are consistent with the phenotype of Anxa1\(^–/–\) neutrophils, which show increased transmigration in the cremaster microcirculation of Anxa1\(^–/–\) mice\(^23\). This phenotype is probably neutrophil-intrinsic because Anxa1\(^–/–\) neutrophils show higher chemotaxis and CD11b upregulation in response to chemoattractants than wild-type neutrophils\(^23\). These data are in agreement with an earlier study in which glucocorticoid treatment increased the levels of annexin A1 in circulating leukocytes and inhibition of annexin A1 prolonged leukocyte migration time in the post-capillary venules of the hamster cheek pouch\(^24\).

Further investigation of Anxa1\(^–/–\) mice has shown that these mice develop more-severe joint disease (characterized by increased destruction of the cartilage and a thicker pannus at day seven) than wild-type mice in a model of monoarticular antigen-induced arthritis\(^25,26\). Increased levels of mRNA encoding interleukin-1 (IL-1) and IL-6 were also detected in the joints of these mice\(^26\), which is consistent with the known requirement for IL-6 in this model of arthritis\(^27\). In addition, Anxa1\(^–/–\) lung fibroblasts showed higher IL-1-induced IL-6 production, an effect that was shown to be an indirect consequence of decreased levels of dual-specificity protein phosphatase 1 (DUSP1; also known as MKP1) in these cells\(^28\). The potential involvement of DUSP1 in the mechanism of action of annexin A1 is reminiscent of what has been observed following glucocorticoid treatment, in which the induction of DUSP1 expression leads to the inhibition of mitogen-activated protein kinase signalling and thereby to a reduction in cytokine signalling and cell activation (BOX 1). Overactivation of intracellular signalling pathways that induce the release of effector molecules might also contribute to the observed lethality from endotoxic shock in Anxa1\(^–/–\) mice following the administration of an otherwise sublethal dose of lipopolysaccharide (LPS)\(^29\). Compared with wild-type mice, LPS-induced endotoxic shock in Anxa1\(^–/–\) mice was associated with a delayed and more prolonged increase in the levels of tumour-necrosis factor, IL-1 and IL-6 in the blood, as well as with increased production of these cytokines by peritoneal macrophages.

In summary, exogenous and endogenous annexin A1 counter-regulate the activities of innate immune cells, in particular extravasation and the generation of pro-inflammatory mediators, and this ensures that a sufficient level of activation is reached but not exceeded.

**ALXR: the annexin A1 receptor.** Annexin A1 signals through a seven-membrane-spanning G-protein-coupled receptor (GPCR)\(^30\) known as formyl peptide receptor 2 (FPB2; also known as ALXR in humans), which is also the receptor for the anti-inflammatory molecule lipoxin A\(_4\). (BOX 2). Here, we use the term ALXR, to...
Figure 1 | Mobilization of annexin A1 in activated cells and its potential mode of action. Following cell activation, such as through adhesion to endothelial-cell monolayers, intracellular annexin A1 is mobilized to the plasma membrane. Depending on the cell type, annexin A1 is then externalized and/or secreted through one of three mechanisms: through the activation of the ATP-binding cassette (ABC) transporterABCA1 (a); through phosphorylation of its amino-terminal serine 27 residue followed by membrane localization to specific lipid domains before it moves to the outer leaflet of the plasma membrane and is secreted (b); or through fusion of annexin A1-containing granules with the plasma membrane, followed by release of annexin A1 (c). In the presence of ≥1 mM Ca\(^{2+}\), extracellular annexin A1 undergoes a conformational change that leads to exposure of the N-terminal region and binding to its receptor ALXR (also known as FPR2). Annexin A1 can function in an autocrine, paracrine and juxtacrine (involving cell–cell contact) manner to activate ALXR signalling. The juxtacrine interaction between annexin A1 that is tethered on the surface of the producing cell and ALXR on the target cell might be the most plausible mechanism of action in inflammatory conditions, as ‘activated’ annexin A1 could bind to acidic phospholipids at plasma Ca\(^{2+}\) concentration of ≥1 mM and could therefore be presented to target cells that express ALXR. The annexin A1–ALXR pathway can be manipulated by glucocorticoids, which induce the expression of the gene encoding annexin A1, ANXA1, as well as ALXR, by innate immune cells, thereby increasing the effects of this anti-inflammatory circuit.

In addition to sharing a receptor, a common function for annexin A1 and lipoxin A\(_4\) has been proposed recently to explain why germ-free mice show inflammatory hyporesponsiveness\(^{39}\). Compared with wild-type mice, germ-free mice showed increased levels of endogenous annexin A1 and lipoxin A\(_4\), and this was associated with increased levels of the anti-inflammatory cytokine IL-10 in the gut; the increased IL-10 levels were responsible for the reduced inflammation observed in these mice. A role for endogenous IL-10 in the anti-inflammatory effects of annexin A1 is also consistent with the observation that exposure of macrophages to annexin A1-derived bioactive peptides in vitro induces IL-10 secretion\(^{40}\). Moreover, the finding that glucocorticoids also mediate some of their anti-inflammatory effects through the induction of expression of IL-10 (REF. 41) and DUSP1 (as discussed above) supports the idea that annexin A1 and glucocorticoids have overlapping activities in the regulation of inflammatory responses. However, the contribution made by IL-10 and DUSP1 to the range of biological actions that are elicited by annexin A1 and glucocorticoids remains to be determined.

Glucocorticoids induce annexin A1 and ALXR expression. Administration of glucocorticoids to healthy human volunteers leads to an increase in the levels of annexin A1 expression by circulating monocytes and neutrophils\(^{42}\). The levels of annexin A1 are also regulated by glucocorticoids during disease. More specifically, leukocytes from patients with Cushing’s disease (which is associated with high levels of cortisol) have higher intracellular levels of annexin A1, and leukocytes from patients with Addison’s disease (which is associated with low levels of cortisol) have lower intracellular levels of annexin A1 than healthy controls\(^{43}\). However, the mechanism by which glucocorticoids regulate annexin A1 has not been defined. The ANXA1 promoter does not seem to contain a canonical glucocorticoid-response element, but it does contain a partial consensus-binding site that mediates responsiveness to IL-6 (REF. 45), which suggests that glucocorticoids indirectly regulate the expression of annexin A1. However, more work is needed to determine the mechanisms that are involved in the cell-specific induction of annexin A1 expression by glucocorticoids, as it remains possible that glucocorticoid-response elements might be present in other regions of the ANXA1 promoter that are yet to be explored.
Despite the probable involvement of many transcription factors and the differential effects of glucocorticoids in distinct cell types, a link between glucocorticoid administration and annexin A1 expression has been confirmed in Anxa1−/− mice bearing a LacZ reporter construct under the control of the Anxa1 promoter44. Treatment of these mice with dexamethasone increased the activity of the Anxa1 promoter in circulating neutrophils and monocytes, leading to a significant increase in LacZ expression as early as 2 hours after dexamethasone injection45. The timing of the increase in promoter activity in these cells following dexamethasone injection correlates with that of annexin A1 expression in cells from wild-type mice. This further supports the idea that annexin A1 expression is regulated by glucocorticoids (as shown in the human studies), although the molecular mechanism underlying this process remains unclear.

Glucocorticoids have also recently been shown to induce the expression of ALXR. Incubation of human monocytes with dexamethasone or other synthetic glucocorticoids induced the de novo synthesis of ALXR, leading to an increase in mRNA levels after 4–6 hours and protein levels after 12–24 hours46. A microarray study of human monocytes that had been treated with the glucocorticoid fluticasone for 16 hours showed that FPR1 mRNA (which encodes a receptor that is structurally related to ALXR) was upregulated, but ALXR mRNA was not47. However, it is worth noting that fluticasone-mediated induction of ANXA1 mRNA was also not detected under the conditions used in this study. Another study showed that dexamethasone treatment led to the upregulation of ALXR expression by human neutrophils, as well as by skin tissues in a mouse model of dermatitis48. We think these data support the hypothesis that the upregulation of specific anti-inflammatory GPCRs (such as ALXR) is involved in a mechanism that mediates the anti-inflammatory effects of glucocorticoids. In addition, we propose that the link between glucocorticoids and the expression of annexin A1 and ALXR involves a genomic mechanism, and therefore requires de novo protein synthesis, although this needs to be further substantiated experimentally.

Finally, the finding that glucocorticoids can also cause a rapid non-genomic mobilization and secretion of annexin A1 by target cells49 has led to the hypothesis that, during an inflammatory response, glucocorticoids first rapidly increase the availability of annexin A1 at the cell surface (where its receptor is located) through non-genomic mechanisms. They then (within 2–4 hours) upregulate the expression of the genes that encode annexin A1 and ALXR through genomic mechanisms. In the context of resolution of inflammation, recent work indicates that the interaction between annexin A1 and ALXR has a role in controlling leukocyte apoptosis and leukocyte clearance by macrophages (Box 3).

In conclusion, we believe that glucocorticoids positively regulate the annexin A1–ALXR pathway, thereby assuring an appropriate level of activation of innate immune cells while limiting the duration of the pro-inflammatory response.

Effects in adaptive immunity The widespread use of glucocorticoids as a treatment for inflammatory diseases is largely due to their ability to simultaneously block both innate and adaptive immune
Box 2 | Pharmacophysiology of ALXR

The receptor for lipoxin A₄ (ALXR; also known as FPR2), which is also thought to be the receptor for annexin A₁, is a seven-membrane-spanning G-protein-coupled receptor (GPCR) that is structurally related to formyl peptide receptor 1 (FPR1). ALXR can recognize various different types of ligand, including several short-lived lipids, such as lipoxin A₄ and its stable analogues, as well as proteins and peptides, such as serum amyloid protein A (SAA) and those derived from viral glycoproteins. Although it was originally cloned from monomyelocytic cells, ALXR is expressed by a wide range of cells, including epithelial cells, endothelial cells, T cells and cells of the central nervous system (CNS). In the CNS, ALXR is expressed by microglial cells, in which it mediates the activating and pro-inflammatory properties of amyloid-β peptide.

The long list of ALXR agonists underlies the versatility of this GPCR and its ability to bind a wide range of molecules (such as lipids, proteins and peptides). It is probable that different receptor regions, and perhaps conformations, might be required for, or are induced after, binding to each distinct agonist. Consistent with this hypothesis, a comparison of lipoxin A₄ and SAA-mediated responses in human neutrophils showed that SAA activated the pro-inflammatory transcription factor nuclear factor-κB, whereas lipoxin A₄ inhibited the effect of SAA. In addition, many ALXR agonists, but not lipoxin A₄, can induce a rapid and transient increase in the concentration of intracellular calcium.

Both annexin A₁ and lipoxin A₄ induce rapid phosphorylation of extracellular-signal-regulated kinase. More recent studies have used genomic and proteomic approaches to determine the downstream targets of annexin A₁-induced signalling. For example, CC-chemokine receptor 10 (CCR10) has been shown to be one such target in an epithelial cell line and in cells from the mouse trachea and ileum. In addition, annexin A₁ has been shown to upregulate IL1RA (interleukin-1 receptor antagonist) and downregulate CCR2 among the genes it regulates in human monocytes. Small chemical ligands that function as selective agonists for ALXR but not FPR1 have recently been developed. Importantly, these small molecules showed anti-inflammatory properties in mice, which supports the hypothesis that ALXR is an important anti-inflammatory GPCR during acute inflammation.

Box 3 | Annexin A₁ and ALXR in leukocyte apoptosis

Endogenous glucocorticoids control the rate of clearance of apoptotic neutrophils by macrophages, as shown by the delay in apoptotic-cell clearance in the absence of 11β-hydroxysteroid dehydrogenase type 1, an enzyme that is required to convert endogenous glucocorticoids into active corticosterone. Such a mechanism is consistent with the well-known effects of exogenous glucocorticoids in inhibiting neutrophil apoptosis, while inducing eosinophil and lymphocyte apoptosis, and promoting the non-inflammatory removal of apoptotic cells by macrophages.

Three main effects of annexin A₁ in leukocyte apoptosis have been described. First, exogenous administration of annexin A₁ promotes human neutrophil apoptosis owing to transient calcium fluxes and dephosphorylation of the pro-apoptotic protein BCL-2-antagonist of cell death (BAD). Interestingly, glucocorticoids inhibit neutrophil apoptosis, revealing a surprising converse effect to that of annexin A₁ (discussed in REF 6). Second, endogenous annexin A₁ is released from apoptotic neutrophils and acts on macrophages to promote the phagocytosis and removal of the apoptotic cells. Third, glucocorticoid-treated macrophages secrete annexin A₁, which then acts in a autocrine or paracrine manner to increase the engulfment of apoptotic neutrophils. The molecular mechanisms that are responsible for these effects of annexin A₁ have not been fully elucidated and may involve the activation of ALXR (also known as FPR2) and/or binding to phosphatidylinerine, a phospholipid that is externalized on the outer leaflet of the plasma membrane of apoptotic cells that can interact with a specific receptor on the phagocyte, thereby favouring the engulfment of apoptotic cells.

Although these observations need to be confirmed in vivo, they suggest that annexin A₁ might alter the fate of the extravasated neutrophils and promote their removal by macrophages, thereby contributing to the resolution of inflammation.

responses. With regard to the inhibition of adaptive immune responses, glucocorticoids have marked effects on T-cell activation and differentiation pathways. In naive T cells, glucocorticoids negatively modulate early T-cell receptor (TCR)-initiated signalling events and inhibit the activation of key downstream transcription factors, such as activator protein 1 (AP1), nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT). In vitro studies using isolated CD4⁺ T cells and in vivo studies in mice have shown that glucocorticoids promote T helper 2 (Th2)-type responses directly and indirectly, an effect that would explain their therapeutic efficacy in Th1-cell-driven autoimmune diseases. Glucocorticoids are also widely used for the treatment of Th2-cell-driven diseases, such as asthma, in which they act by suppressing the production of cytokines that propagate inflammation in these diseases, such as IL-5 and IL-13, while increasing the synthesis of anti-inflammatory cytokines, such as IL-10 (REFS 56,57). Recent work has identified new effects of annexin A₁ on T cells; surprisingly, however, these effects are the opposite to those exerted by glucocorticoids.

Annexin A₁ and T-cell activation.

Until recently, little was known about how annexin A₁ influences the adaptive immune response, possibly because T cells express lower levels of annexin A₁ (REF 42) than myeloid cells.

Indeed, naive T cells contain ~100-fold less ANXA1 mRNA and protein than neutrophils and macrophages; however, annexin A₁ expression is upregulated in Th1 cells following activation and differentiation (FDA, unpublished observations). The first evidence for a role of annexin A₁ in T-cell function came from the studies of its effects, at nanomolar concentrations, on T cells that had been stimulated with CD3- and CD28-specific antibodies. Exposure to annexin A₁ had no effect on naive T cells, as they express negligible levels of ALXR. However, following stimulation with CD3- and CD28-specific antibodies, annexin A₁ led to the upregulation of ALXR expression on the surface of the T cells in a time- and concentration-dependent manner. Interestingly, this occurred in parallel with the mobilization and release of endogenous annexin A₁ (REF 58). Upregulation of the annexin A₁−ALXR pathway in T cells following treatment with annexin A₁ led to increased and prolonged phosphorylation of AKT and ERK after the cells were stimulated with CD3- and CD28-specific antibodies. This is in agreement with the observation that T cells from Anxa1⁻/⁻ mice show a lower (suboptimal) signalling response following stimulation than wild-type mice. The increase in ERK activation by annexin A₁ also occurs in neutrophils and other cells in the innate immune system. However, the consequences of this increase differ between cell types—ERK and AKT activation in T cells leads to cell proliferation, whereas ERK activation in neutrophils leads to a loss of cell adhesion and can have an anti-inflammatory effect. The divergent outcomes of the ERK signalling pathways suggest that many checkpoints exist to control cell behaviour and responses, and highlight the importance of cell type and of the microenvironment in determining the biological function of a molecule.
Other downstream signalling responses can also be modulated (both positively and negatively) by exogenous annexin A1 or by its deletion in T cells. For example, stimulation of T cells with annexin A1 together with CD3- and CD28-specific antibodies increases the activation of several key transcriptional factors that are downstream of the TCR64: AP1, NF-κB and NFAT. Accordingly, reduced activation of these transcription factors was detected in Anxa1−/− T cells following stimulation59. So, in contrast to glucocorticoids, these studies indicate that annexin A1 transduces a stimulatory signal to promote T-cell activation, probably through ALXR (FIG. 3).

**Annexin A1 and effector Tc1-cell differentiation.** Many factors, including the micro-environment where T cells encounter their antigen and the affinity of the antigen for the TCR, have an important role in the early phase of TCR signalling and therefore in determining the activation of specific gene programmes that lead to the differentiation of naive T cells into effector Tc1 cells49. Understanding how subtle changes in the strength of TCR signalling modulate T-cell differentiation is of particular importance for understanding and treating many autoimmune diseases, in which T cells become responsive to signals that would otherwise be ignored. Over the past 20 years, the number of molecules that have been shown to be involved in the regulation of early events in TCR signalling has increased enormously, and in many cases a link has been found between the dysfunction of these molecules and the development of autoimmune diseases62. Recent work suggests that annexin A1 might belong to the group of molecules that fine-tune TCR signalling (FIG. 3).

Activation of naive T cells in non-polarizing conditions (TCR stimulation and IL-2) or Tc1-cell-polarizing conditions (TCR stimulation and IL-2, IL-12 and IL-4-specific blocking antibodies) in the presence of exogenous annexin A1 promotes the differentiation of IL-2- and interferon-γ (IFNγ)–producing Tc1 cells and suppresses the production of the Tc1-type cytokines IL-4 and IL-13 (REF. 58). Consistent with these findings, in vitro differentiation of T cells from Anxa1−/− mice revealed a reduction in the production of Tc1-type cytokines and increased skewing to a Tc2-cell phenotype63. In agreement with these in vitro data, systemic treatment of mice with annexin A1 immediately after immunization with collagen exacerbated the severity of collagen-induced arthritis at disease onset59. However, these observations are in contrast to those from studies of Anxa1−/− mice with antigen-induced arthritis46, which showed increased disease severity in the absence of annexin A1. These apparently contradictory results could stem from the fact that a single time point (7 days after challenge) was assessed in the Anxa1−/− mice with antigen-induced arthritis and that this disease is monoarticular (it only affects the injected joint). In addition, the comparison of two such different effects — the pharmacological effects of annexin A1 administration in collagen-induced arthritis and the effect of the absence of annexin A1 expression in antigen-induced arthritis — could further confound any conclusions drawn. It is therefore difficult at this stage to determine whether either model might be useful for understanding the role of annexin A1 in human disease. Of interest in this regard, and in agreement with the studies of collagen-induced arthritis, annexin A1 has recently been shown to have an activating effect on synovial fibroblasts from patients with rheumatoid arthritis, in which it complements the actions of tumour-necrosis factor and contributes to the release of metalloproteinase1 (REF. 63). More studies in these and other models are needed to clarify the role of the annexin A1–ALXR pathway in arthritis and in distinct phases of the experimental arthritic response.

Further studies of human cells provide clues that annexin A1–mediated stimulation of T cells might be relevant to human disease. First, ANXA1 mRNA and protein levels were shown to be higher in circulating T cells from patients with rheumatoid arthritis than those

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**Figure 3 | The annexin A1–ALXR pathway and strength of T-cell receptor signalling.** Engagement of the T-cell receptor (TCR) triggers several signalling pathways, including the mitogen-activated protein kinase (MAPK) pathway. Activation of this pathway can lead to the expression of genes such as interleukin-2 (IL-2), which is responsible for T-cell activation and proliferation. Activation of T cells also results in the release of annexin A1 and the expression of its receptor ALXR. This pathway seems to fine-tune the strength of TCR signalling and exert homeostatic control on T-cell activation under physiological conditions (a). Higher expression of annexin A1 during pathological conditions could increase the strength of TCR signalling through the MAPK signalling pathway, thereby causing a state of hyperactivation of T cells (b). By contrast, low levels of annexin A1 expression, which occurs during pathological immunodeficiency, could be associated with a hyporesponsive state of T cells owing to the decreased strength of TCR signalling (c).
from healthy individuals. Second, synovial T cells expressed higher levels of annexin A1 than tonsil T cells from the same patient. Therefore, it is tempting to speculate that the dysregulated expression of annexin A1 in T cells might contribute to the hypersensitivity of these cells to antigen stimulation and to the development of an overexuberant immune response in disease states.

**Glucocorticoids and annexin A1 in T cells.**

The modulation of the annexin A1–ALXR pathway in innate immune cells by glucocorticoids (as described above) prompted us to investigate the effects of glucocorticoids on annexin A1 expression in T cells. Incubation of mouse and human T cells with dexamethasone induced a time-dependent decrease in both ANXA1 mRNA and annexin A1 expression levels. These results contrast with those that were obtained from studies of innate immune cells, but may explain how the immunosuppressive effects of glucocorticoids can be achieved given the stimulatory effects of annexin A1 on T cells. It might therefore be possible that glucocorticoids inhibit T-cell activation and proliferation by reducing the expression of annexin A1. Two pieces of evidence support this hypothesis: first, T cells from Anxa1−/− mice proliferate less in response to CD3 and CD28 stimulation, and second, incubation of dexamethasone-pretreated human T cells with recombinant annexin A1 for 12 hours almost completely reversed the suppressive effects of dexamethasone pretreatment and restored their ability to proliferate following stimulation with CD3- and CD28-specific antibodies.

The clinical relevance of these findings is supported by a recent study in humans. Treatment of five patients with rheumatoid arthritis with the synthetic glucocorticoid methylprednisolone acetate was shown to reduce the expression of annexin A1 in circulating T cells by ≥ 50% (REF. 64). Together, these results suggest that inhibition of annexin A1 expression might be one of the mechanisms responsible for the immunosuppressive effects of glucocorticoids on T cells, and that glucocorticoids exert different effects on annexin A1 expression in innate and adaptive immune cells.

The modulation of annexin A1 expression in T cells by glucocorticoids might also explain a well-known but poorly understood aspect of glucocorticoid pharmacology, the cell-specific time latency of their beneficial effects. The inhibitory effects of glucocorticoids on macrophages and neutrophils requires short incubation times (less than 2 hours), whereas much longer incubation times (more than 6–12 hours) are needed for the efficient inhibition of T-cell activation. We propose that this time-dependent difference reflects the differential effects of glucocorticoids on annexin A1 expression in these cells. Future investigation will be needed to determine the molecular mechanisms that are responsible for the differential modulation of annexin A1 expression by glucocorticoids in the innate and adaptive immune system. In this regard, a thorough and detailed analysis of the transcriptional regulation of ANXA1 in innate and adaptive immune cells might provide some clues, such as the involvement of cell-specific transcription factors. Indeed, we have found three consensus GATA-binding protein 3 (GATA3)-binding sites in the Anxa1 promoter (FDA, unpublished observations). This transcription factor is preferentially expressed by T cells but not monocytes and is a well-known regulator of T,2-cell differentiation. Interestingly, glucocorticoids have been reported to inhibit GATA3 transcriptional activity. We speculate that inhibition of GATA3 activity by glucocorticoids occurs upstream of their suppressive action on annexin A1 expression in T cells.

**Conclusions**

On the basis of recent studies of the adaptive immune response in Anxa1−/− mice and previous studies of the innate immune system, we propose a new hypothesis to explain the link between annexin A1 and glucocorticoids. The model suggests that part of the anti-inflammatory effect of glucocorticoids on the innate immune system is mediated through the release of annexin A1 and the subsequent activation of ALXR, in an autocrine or paracrine manner, in neutrophils and macrophages. By contrast, the immunosuppressive effect of glucocorticoids on the adaptive immune system is mediated through the inhibition of annexin A1 expression by T cells (FIG. 4). We speculate that daily treatment with glucocorticoids or intermittent therapy with high doses of these drugs would induce a constant release of annexin A1 by innate immune cells, and this would have a pro-resolving effect on the inflammatory response. In parallel, glucocorticoids would reduce the co-stimulatory effect of annexin A1 on T-cell activation, thereby lowering the strength of the TCR signal (FIG. 3) and maintaining these cells in an unresponsive state.

In this model, annexin A1 has important opposing properties during innate and adaptive immune responses, as it inhibits innate immune cells and promotes T-cell activation, which is a property that is shared with
other immune regulators, such as galectin 1 (REF 68) and T-cell immunoglobulin domain and mucin domain 3 (TIM3)18. A complete understanding of the effects of glucocorticoids and annexin A1 on immune responses, and the identification of ALIX as its main receptor, should aid the development of selective agonists for the resolution of inflammatory responses and antagonists for the control of adaptive immune responses.

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doi:10.1038/nri2470


Perspectives


Acknowledgements

Work in our laboratory is funded by the Arthritis Research Campaign UK, the Wellcome Trust, the British Heart Foundation and the Medical Research Council UK. We apologize to the many colleagues whose work could not be cited here owing to space limitations.

Competing financial interests

The authors declare competing financial interests: see web version for details.

DATABASES


ACT | ANXA1 | DUSP1 | ERK1 | ERK2 | FPR1 | FPR2 | FPRL1 | GATA3 | IL-1 | IL-2

FURTHER INFORMATION

Mauro Perretti’s homepage: http://www.wfri.qmul.ac.uk/staff/mauro.html

Fulvio D’Acquisto’s homepage: http://www.wfri.qmul.ac.uk/staff/fulvio.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF