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Review

Current concepts in glucocorticoid resistance

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ABSTRACT

Glucocorticoids (GCs) are the most potent anti-inflammatory agents known. A major factor limiting their clinical use is the wide variation in responsiveness to therapy. The high doses of GC required for less responsive patients means a high risk of developing very serious side effects. Variation in sensitivity between individuals can be due to a number of factors. Congenital, generalized GC resistance is very rare, and is due to mutations in the glucocorticoid receptor (GR) gene, the receptor that mediates the cellular effects of GC. A more common problem is acquired GC resistance. This localized, disease-associated GC resistance is a serious therapeutic concern and limits therapeutic response in patients with chronic inflammatory disease. It is now believed that localized resistance can be attributed to changes in the cellular microenvironment, as a consequence of chronic inflammation. Multiple factors have been identified, including alterations in both GR-dependent and -independent signaling downstream of cytokine action, oxidative stress, hypoxia and serum derived factors. The underlying mechanisms are now being elucidated, and are discussed here. Attempts to augment tissue GC sensitivity are predicted to permit safe and effective use of low-dose GC therapy in inflammatory disease.

Contents

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1. Glucocorticoids as anti-inflammatory agents

Despite huge advances in our understanding and more than 50,000 published manuscripts in the last 10 years, a comprehensive understanding of glucocorticoid (GC) action in inflammation remains elusive [1]. As the most robust anti-inflammatory agents known, natural and synthetic GCs are widely prescribed to treat a range of inflammatory and immune diseases in the clinic. Although in recent years, several novel therapies have been introduced, GC remain the first-line treatment for long-term control of asthma, Crohn's disease and ulcerative colitis [2,3]. However, in addition to an increased susceptibility to side-effects [3], patients also present a significant variation in response.

2. Glucocorticoid receptor structure

GCs exert their effects through the glucocorticoid receptor (GR, or NR3C1), a member of the nuclear hormone receptor superfamily [4]. The GR comprises three major functional domains, an N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and C-terminal ligand-binding domain (LBD). The DBD and the LBD are linked by a hinge region. During evolution the ancestral corticosteroid receptor has diverged into the GR, and mineralocorticoid receptor (MR, or NR3C2). These share 94% amino acid identity in the DBD, and 56% in the LBD [5,6]. Recent evidence suggests an important pro-inflammatory role for MR in macrophage cells, which contrast strongly with the anti-inflammatory role of the GR, despite such close structural homology [8].

Recent studies revealed that alternative translation start sites in the GR NTD give rise to greater diversity of protein species. These now appear to play a role in regulating cellular sensitivity to GCs [5]. In particular, the GR α-D proteins have less transcriptional activity compared with other GR α translated protein isoforms [9]. In the U2-OS osteosarcoma cell line, expression of the relatively inactive GR α-D3 may contribute to glucocorticoid-induced apoptosis resistance [10]. Phosphorylation is a key factor in modulating the activity and the stability of GR, with the main sites of phosphorylation located in the NTD [11]. The NTD of the human GR spans residues 1–417 and contains the transcriptional activation function-1 (AF1) domain [12]. AF-1 recruits diverse proteins to the GR to regulate target gene expression, including TATA-binding protein, and MED14 [13,14].

The DBD is located in the central amino acid sequence of the GR. Residues 418–487 of the human GR form this domain, which bind to its DNA targets, termed GC response elements (GREs). This specific binding capability is achieved by its two highly conserved zinc finger motifs [15].

The LBD adopts a complex globular tertiary structure, including eleven α helices and four short β sheet that folded as a central pocket for ligands [16]. The LBD gates ligand access, and also recruits chaperones and coactivators [17]. There is a transcriptional activation function-2 (AF2) residue towards its C-terminal end. The AF2 consists of residues 526–556 and has significant ligand-dependent function, acting to recruit co-activator complexes with the motif LXXLL [18–26].

3. Glucocorticoid receptor function

In the absence of ligand, GR α primarily resides in the cytoplasm as part of a multisubunit complex, including Hsp90, Hsp70, Hsp40, immunophilins, CyP40, and P23 [26]. Hsp90 is the fundamental protein in this complex and combines with the LBD of GR α [12] to stabilize the optimal and high affinity structure of the ligand binding pocket within the receptor [26]. In response to GCs, the GR α complex rapidly undergoes a conformational change and subsequently dissociates from the heat shock proteins. After replacing immunophilin FBK51 with FBK52, ligand-bound GR is able to produce rapid non-genomic actions through interactions with signaling pathways via cytosolic kinases [27]. Subsequently, the ligand bound GR α translocates into the nucleus, driven by the dynein motor protein [28].

However, this simple dogma has been recently challenged, based on the observations that intracellular GR localization under ligand-free conditions is frequently seen to be heterogeneous, with both nuclear and cytoplasmic expression. Nuclear localization in the absence of added ligand requires the first GR nuclear localization signal (NLS1), and is progressive during cell cycle progression through G1. During mitosis GR is excluded from condensed chromosomes, and in early G1 following cytokinesis the GR is strictly excluded from the nucleus. Therefore in addition to the very rapid (minutes) kinetics of nuclear translocation seen in response to ligand binding there is an additional slow (hours) partial nuclear translocation driven by cell cycle [29]. This discovery is important as immunohistochemical analysis of tissue has been used to infer GR activation based on detection of nuclear protein, but this is an unreliable surrogate.

Activated GR binds to consensus elements in the host cell genome to activate or repress gene transcription. These sites are cell-type specific, and are determined, in part, by chromatin structure [30,31]. Multiple mechanisms have been inferred to explain anti-inflammatory GR action. These include transcription of anti-inflammatory mediators and transcriptional inhibition of proinflammatory cytokines. The latter occurs through inhibition of the activity of proinflammatory transcription factors via a tethering mechanism [12,32]. Important factors include activator protein 1 (AP-1) and NF-κB [33,34].

4. Regulation of access of GC to cells: P-glycoprotein activation

As one of the ATP-binding cassette (ABC) transporters, the drug efflux pump P-glycoprotein 170 is responsible for transporting structurally and functionally unrelated drugs out of cells [3]. This protein is encoded by the multidrug resistance gene MDR1 (ABCB1) [35]. Recent studies on blood lymphocytes reported the high expression level of MDR1 in GC resistant inflammatory diseases [36,37]. Meanwhile, it has been shown that certain single nucleotide polymorphisms within MDR1 are associated with GC resistance [38]. However, to date this is only reported in GC resistant inflammatory bowel disease and rheumatoid arthritis. Therefore future research in other diseases, e.g. GC resistant pulmonary inflammation is needed [39].

5. Genetic and post-translational variation in GR structure and function

5.1. Familial GC resistance syndrome

Inactivating mutations of the GR gene cause familial GC resistance [40–52] (Table 1). This syndrome is characterized by hypercortisolism without features of Cushing's syndrome and was firstly explained as a GR mediated disorder in 1976 [53,54]. High adrenocorticotrophin levels stimulate an over-secretion of non-corticosteroid adrenal steroids, such as aldosterone and androgen. Therefore clinical manifestations of this syndrome are hypertension, hypokalemia and/or symptoms of androgen excess which occur as menstrual abnormalities and hirsutism in females [39]. Familial GC resistance is very rare, in all cases due to mutations in the GR α gene, most of which affect the function of either LBD or DBD [55].