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Mini review

Glucocorticoid resistance as a major drive in sepsis pathology

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ABSTRACT

Sepsis is an acute systemic inflammatory disease. Glucocorticoids (GCs), which function by binding to the GC receptor GR have very powerful anti-inflammatory activities, yet they are hardly useful in sepsis. We can thus consider sepsis as a GC resistant disease. We here review the literature which has investigated this GC resistance, and summarize the mechanisms of GC resistance that have been observed in other diseases and in experimental models. We also discuss the importance of GC resistance in sepsis, in terms of the contribution of this phenomenon to the pathogenesis of sepsis.

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1. Introduction

Since the first application of hydrocortisone in the suppression of rheumatoid arthritis in 1948 [1], multiple other compounds with glucocorticoid (GC) activity have been applied, in numerous formulations, for the treatment of inflammatory disorders, such as asthma, allergy, septic shock, multiple sclerosis and inflammatory bowel disease [2–5]. GCs belong to the most prescribed drugs in the world. The fraction of the population in the USA using GCs is estimated to be 1.2% [6]. GC act via binding to their intracellular receptor, the glucocorticoid receptor (GR). In human leukocytes, transcription of 20% of the protein coding genes is regulated by GR [7], suggesting the importance of this nuclear transcription factor.

Although synthetic GCs are powerful drugs to combat inflammatory disorders, side effects and reduced responsiveness limit their applicability. Glucocorticoid resistance (GCR) is seen in multiple diseases and has several explanations.

Abbreviation: 11β-HSD, 11β-hydroxy steroid dehydrogenase; AA, alopecia areata; ACTH, adrenocortico-throphic hormone; AF1, activation function 1; ALL, acute lymphoblastic leukemia; Alum, aluminum hydroxide; ARE, adenylate uridylate (AU)-rich elements; CBG, cortisol binding globulin; CBP, cAMP-responsive element-binding protein (CREB)-binding protein; CFA, complete Freund's adjuvant; CLP, cecal ligation & puncture; COPD, chronic obstructive pulmonary disease; CRH, corticotropin releasing hormone; DBD, DNA binding domain; G6P, glucose-6-phosphatase; GC, glucocorticoid; GCR, glucocorticoid resistance; GR, glucocorticoid receptor; GRE, glucocorticoid responsive elements; HAT, histone acetyl transferase; HPA, hypothalamic-pituitary-adrenal; HSP, heat-shock protein; IBD, inflammatory bowel disease; LBD, ligand-binding domain; LPS, lipopoly saccharides; MIF, migration inhibitory factor; NCoR1, nuclear hormone repressor 1; nGRE, negative GRE; NLS, nuclear localization signals; NTB, N-terminal transactivation domain; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; PCK, phosphoenol pyruvate carboxy kinase; PP5, protein phosphatase 5; SIRS, systemic inflammatory response syndrome; SR, steroid resistant; SWI/SNF, switching/sucrose non-fermenting; TrxR1, thioredoxin reductase 1.

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Sepsis is one such GCR disease and is estimated to hit at least 20 million people per year worldwide. Since the mortality is around 30%, sepsis forms one of the most urgent unmet medical needs of today [8–10]. Despite the number of infectious diseases is declining steadily, sepsis is still increasing in frequency, a.o. because of the aging of the population [11–13].

2. Glucocorticoids and their receptor

Glucocorticoids (cortisol in humans and corticosterone in rodents) are steroid hormones synthesized and released by the adrenal glands. GC secretion displays a circadian rhythm and is tightly regulated via the hypothalamic-pituitary-adrenal gland (HPA) axis [14] (see Fig. 1) and is influenced by several factors such as serotonin [15], GABA [16] and pro-inflammatory cytokines [17,18] such as TNF, IL-1 and IL-6. These factors trigger the hypothalamus to release corticotropin releasing hormone (CRH) in the hypothalamo-pituitary venous system. CRH enhances the production of adrenocorticothrophic hormone (ACTH) in the pituitary. ACTH applies a trophic action on the adrenal glands and stimulates the production and secretion of GCs. GC hormones are derived from the precursor cholesterol by a cascade of enzymatic reactions, involving cytochrome P450 [19]. Fine-tuning of blood GC levels appears, a.o. by a negative feedback loop, whereby increased GC levels inhibit the HPA axis at different points [20]. The activity and availability of GCs is also regulated after their secretion, via cortisol binding globulin (CBG) and albumin [21]. Less than 10% of cortisol is freely available to passively diffuse across cellular membranes, via their lipophilic nature. 11β-hydroxysteroid dehydrogenase (11β-HSD) regulates the glucocorticoid activity in cells by converting part of the bioactive cortisol back into the inactive precursor cortisone [22].

Once entered into cells, GCc have plenty of physiological functions, such as regulating metabolic homeostasis, inflammation, immune responses, development and reproduction [23–25]. GCs function by binding to their intracellular receptor, the GC receptor (GR), which is encoded by the *NR3C1* gene in humans and *Nrc3c1* in mice (see Fig. 1). GR is a ligand-inducible transcription factor belonging to the nuclear receptor superfamily [26]. The tissue specific expression profile of the GR is controlled at distinct levels via transcriptional, post-transcriptional and post-translational regulation. Alternative transcription start sites in exon 1 results in 13 variants of the human GR [27] and also alternative splicing near the 3′ UTR in exon 9 generates two isoforms (hGRα and hGRβ) [28]. The classical human GRα consists of 777 amino acids, whereas GRβ is only 742 amino acids long and is unable to bind ligand, resulting in a dominant negative protein [29,30]. Adenylate uridylate (AU)-rich elements (AREs) present in the 3′UTR of GR mRNA affects the stability of the mRNA and GR protein expression [31]. Several reports also showed that micro RNAs can further fine-tune the GR expression levels [32–35]. Also, alternative translational initiation, i.e. alternative methionine start codons, have been identified in GR mRNA molecules, resulting in eight hGRα subtype of proteins [36]. Finally, numerous post-translational modifications of the GR protein have been described to influence its biological activity [37,38].

GR is a modular protein comprised of three functional domains; an N-terminal transactivation domain (NTD), a central DNA binding domain (DBD) and a C-terminal ligand-binding domain (LBD) (see Fig. 1). The NTD contains the ligand-independent constitutive transcriptional activation function 1 (AF1), important for initiation of transcription. The DBD consists of two highly conserved zinc fingers and are essential for DNA binding and dimerization respectively. A hinge region is defined and located between the DBD and LBD and provides structural flexibility. A second (ligand-dependent) transcriptional domain, called AF2, is situated in the LBD [39,40]. This domain is important for ligand binding [41] and for interaction with transcriptional cofactor, through their LXXLL motifs [42].

In unstimulated cells, GR is mainly kept in the cytoplasm in an inactive state and bound to several chaperone proteins, such as heat-shock protein (hsp) 90, hsp70, immunophilins (eg, KFBP51, FKBP52, Cyp44 and PP5), and other factors, which prevent GR degradation and assists it in its maturation [43,44]. Upon ligand binding, GR undergoes conformational changes resulting in the dissociation of the interacting proteins and in nuclear translocation via two nuclear localization signals (NLS) [45]. In the nucleus, GR has been found to function as a monomeric as well as a homodimeric protein (see Fig. 1).

In cells of naive mice, monomeric GR resides at DNA half-site motifs. However after stimulation with exogenous GCs, GR dimers assemble near GC/GR-activated genes at the expense of monomeric binding [46]. GR exerts its classical transcriptional activity through binding of glucocorticoid responsive elements (GRE), typically found in promoters of target genes. These GREs contain an inverted hexameric imperfect palindrome sequence separated by 3 base pairs, in which each half site is bound by one receptor subunit of the homodimeric GR [47]. Such GR binding sequences are abundantly represented in the genome, however recent genome-wide ChIPseq analyses demonstrated that only a small fraction is really used by GR [48]. Why some sequences are used for gene regulation and others not, is still a topic of investigation, but this selectivity may allow flexibility for GR-DNA binding and gene regulation, e.g. in the context of tissue specific regulation by GRE genes. Recently, it was shown that chromatin accessibility predetermines the GR binding patterns [49–51]. As seen by ChIPseq, GR binding sites are often located far from promoters and transcription start sites, suggesting that responsive elements can loop towards promoter regions of target genes in order to regulate transcription [52].

Once GR is bound by ligand and bound on GRE, the receptor undergoes subtle structural changes, leading to the exposure of sequences as helix 11 and helix 12 of the LBD [53–55]. It is believed that these sequences, along with surface sequences of the AF1 and AF2 domains, form a platform that attracts nuclear transcriptional coactivators with chromatin-remodeling activities, that favors the formation of the transcription initiation complex. These includes the proteins p300 and the homologous cAMP-responsive element-binding protein (CREB)-binding protein (CBP), members of the p160 family (SRC-1, SRC-2 and SRC-3) and the mating-type switching/sucrose non-fermenting (SWI/SNF) complex [56–59], as well as many others. These cofactors have many functions, a.o. intrinsic histone acetyltransferase activity (HAT) allowing the transcription initiation complex of RNA-polymerase II to initiate and promote transcription. Gene induction plays an essential role in the anti-inflammatory functions of GCs, e.g. via the induction of genes coding for anti-inflammatory proteins, such as GILZ, MKP-1, IL-10 and IL1-RA [5].

Additionally, next to transcriptional activation, GR can also repress the expression of target genes via binding to negative GRE (nGRE) elements, of similar structure as GRE elements, or iGRE elements, which are very different in nature, and have first been described by Surjit *et al.* [60], or via a DNA-independent mechanism by protein–protein interaction with other nuclear transcription factors [61]. The latter involves mainly monomeric GR and may take place at promoters without GRE (tethering mechanism) [62] or at promoters that contain GRE and responsive elements of transcription factors (composite promoters). Many of the immunosuppressive functions of GR are carried out by induction of GRE genes, but also by direct interaction of GR with NF-κB, AP-1 and STAT [5,62].