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ENDOTHELIAL CELLS OF THE BLOOD-BRAIN BARRIER: A TARGET FOR GLUCOCORTICOIDS AND ESTROGENS ?

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1. ABSTRACT

Adhesion molecules are involved in the leukocyte recruitment of leukocytes at the blood-brain barrier. For this reason, it is important to understand how the regulation of their gene expression controls lymphocyte adhesion to endothelial cells in microvessels. Indeed, due to their specificity and diversity, adhesion molecules involved in extravasation play an essential role in the recruitment of activated leukocytes and activation of inflammation. Multiple sclerosis results from a chronic inflammation of the CNS which is mediated by infiltration of inflammatory cells from the immune system. Administration of glucocorticoids is a routine method to control multiple sclerosis since naturally derived or synthetic glucocorticoids are potent immunosuppressive and anti-inflammatory agents. Glucocorticoids also have beneficial effects in stabilizing the blood-brain barrier, as steroid hormones regulate the expression of adhesion molecule genes in endothelial cells.

Other hormones such as estrogens modulate many endothelial cell biological activities, among them adhesion to leukocytes. They regulate expression of adhesion molecules genes on endothelial cells and are useful for the treatment of experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis.

The effects of glucocorticoids and estrogens on the expression of adhesion molecules on endothelial cells, including microvascular endothelial cells of the blood-brain barrier, are reviewed in this paper, as well as the involvement of these hormones in the therapy of experimental autoimmune encephalomyelitis and multiple sclerosis.

2. INTRODUCTION

The blood-brain barrier (BBB) is an important and highly specialized structure that controls passage of molecules and cells from blood to the extracellular fluid environment of the brain. Indeed, until recently, CNS was regarded as an immunologically privileged site, where the BBB prevented the entry of circulating lymphocytes into the cerebral parenchyma. However, when inflammation of the CNS occurs, mononuclear cells do gain access to the brain. To study the molecular mechanisms involved in lymphocyte recruitment across the BBB, experimental autoimmune encephalomyelitis (EAE) is used as animal model for human demyelinating diseases such as multiple sclerosis. In EAE, entry of the activated $CD4^+$ T cells into the CNS can be studied to determine the traffic signals involved in T cell recruitment across the BBB (1). This entry causes edema, inflammation and demyelination of the white matter in the CNS.

Glucocorticoids (GCs) play ignificant roles in various physiological processes, such as development of organs, modulation of the immune function, adapting to stress and control of energy homeostasis and cognitive functions (2). GCs exert their effects at the BBB level by regulating the expression of adhesion molecules and

Table 1. Adhesion molecules involved in inflammation : leukocyte adhesion molecules and their ligands on endothelial cells. CD and integrin nomenclatures are in brackets

Leukocyte molecules	L-selectin (CD62L)	PSGL-1 (CD162)	E-selectin ligand-1 & CLA bearing sialyl-Lewis$_x$	VLA-4 (CD49d/CD18) (alpha$_4$ beta$_1$)	LFA-1 (CD11a/CD18) (alpha$_L$ beta$_2$)	Mac-1 CR3 (CD11b/CD18) (alphaM beta$_2$)	PECAM-1 (CD31)	(CD 99)
Endothelial cell counter-ligand	Sialyl-Lewis$_x$ on appropriate ligand	P-selectin (CD62P)	E-selectin (CD62P)	VCAM-1 (CD106)	ICAM-1 (CD54) ICAM-2 (CD102)	ICAM-1 (CD54)	PECAM-1 (CD31)	(CD 99)
Actions	Tethering (T) Rolling (R)	T,R	T,R	T, R Tight adhesion (TA)	TA	TA	Diapedesis (D)	D
Leukocytes involved	Neutrophils (N) Monocytes (M) Lymphocytes B&T (B&T) Natural killer cells (NK)	N, M, B&T, NK	N, M, B&T, NK	N, M, B&T, NK	M, B&T, NK	N,M, NK	N, M, NK, subsets of T	N, M, B&T, NK

inhibiting T cell recruitment. Due to their immunosuppressive and anti-inflammatory activities, GCs are used as standard therapy for many disorders, in particular acute exacerbations of multiple sclerosis (MS).

Estrogens, another group of steroid hormones, have significant roles in reproductive physiology, development of the pituitary gland and brain and in inflammatory reactions (3). Estrogens also elicit several biological responses in endothelial cells, including cerebrovascular endothelial cells.

This review summarizes data on the effects of GCs and estrogens on endothelial cells (EC), with particular emphasis on cerebral endothelial cells, the main component of the blood-brain barrier (BBB) and discusses the usefulness of these hormones in treatment of EAE and MS.

3. GLUCOCORTICOIDS AND THE BLOOD BRAIN BARRIER

3.1. Glucocorticoids and inflammation

The basic principles of inflammation were established by Elie Metchnikoff at the end of the 19th century (4). In depth understanding of the inflammatory response has allowed identification of the molecular mechanisms underlying the activation and the recruitment of the lymphocytes from the circulation and their trafficking across endothelial barriers. Adhesion molecules and inflammatory mediators involved in inflammation have been identified, demonstrating the importance of adhesion molecules on the control of acute inflammation, a phenomenon characterized by immediate infiltration of polymorphonuclear neutrophils (PMN), followed by monocytes and eventually lymphocytes (5). It is actually known that during inflammation specific combinations of signaling and adhesion molecules regulate leukocyte recruitment into extravascular tissue (6,7).

At the present time, a general paradigm of leukocyte-endothelial cell interactions in the inflammatory response is accepted (8).

GCs have been proposed to exert their anti-inflammatory effects principally by redirecting lymphocyte traffic and inhibiting the expression of cytokines and adhesion molecules (9). GCs also have a direct effect on the lipid mediators of inflammation by inhibiting the expression of enzymes involved in their biosynthesis, as in the case of cytokine-induced cyclo-oxygenase-2 (10). In addition, GCs inhibit the expression of the inducible nitric oxide synthase, required for nitric oxide synthesis, in vascular endothelial cells (11).

3.2. Regulation of the expression of adhesion molecules by glucocorticoids

After giving a short description of the leukocyte-endothelial cell interactions (for more details, see reference 7), we will focus on the adhesion molecules of endothelial cells regulated by GCs. The molecules involved in this description are summarized in Table 1.

Leukocytes first attach to the endothelium lining the post-capillary venules before they migrate towards the inflamed tissues. This initial interaction (tethering) is followed by loose "rolling" contacts, mediated by the selectin family of adhesion molecules. Rolling allows the leukocytes to loosely contact the endothelium, and initiates the activation of the next step : firm adhesion. At the molecular level, this step involves the integrin family of adhesion molecules. The integrins are found on the activated leukocytes and bind to their counter-receptors on the endothelium. These counter-receptors are members of the immunoglobulin gene superfamily, and are also adhesion molecules (ICAMs : intercellular adhesion molecules). After the leukocytes are firmly adhered to the endothelium, they migrate on the apical surface of the endothelium. These interactions are mediated by beta2 integrins on the leukocyte and ICAMs on the endothelium, and finally transmigration (or diapedesis) occurs. It is worth noting that all of the preceding steps in leukocyte emigration are potentially reversible, but this is not more the case for diapedesis. In addition, whereas tethering, rolling, adhesion and crawling on the apical surface involve heterophilic interactions between one class of molecules on the leukocyte and a second set on the endothelial cell, the two major interactions presently known in diapedesis are homophilic: in other words, interacting molecules are the same on the leukocyte and the endothelium at the tight junctions. The molecules involved in these homophilic interactions are PECAM-1/CD 31 and CD 99 and they act

Table 2. Adhesion molecules expressed in endothelial cells: HBECs: human brain endothelial cells ; HUVECs: human umbilical vein endothelial cells ; ECV304: human vascular endothelial cell line, and involved in leukocyte recruitment at the BBB. Effects of GCs

Name of cells or cell lines	Name of the genes	Description	Inducers of gene expression	GCs used and effects on gene expression	References
HBECs HUVECs	E-selectin ELAM-1	Endothelial leukocyte adhesion molecule 1	TNFalpha Interleukin-1beta LPS(Lipopolysaccharide)	DEX Down-regulation	14,15,16
HUVECs	P-selectin		Interleukin-4	DEX No effect	20
HUVECs EAhy926	ICAM-1 (CD54)	Intercellular adhesion molecule 1	LPS(Lipopolysaccharide)	DEX Down-regulation	21,22,23
ECV304	ICAM-1 (CD54)	Intercellular adhesion molecule 1	Thyroid hormone T3(3,5,3'triiodothyronine)	DEX Down-regulation	24
HBECs HUVECs	ICAM-1 (CD54)	Intercellular adhesion molecule 1	TNFalpha	DEX MP (high doses) Down-regulation	25,26
HBECs	VCAM-1	Vascular cell adhesion molecule 1	TNFalpha	MP (high doses)	25,26

at sequential steps as the leukocytes cross the endothelial barrier.

After transmigration of leukocytes at the junctions of endothelial cells, they pass across the basal lamina and the extracellular matrix to arrive at the inflammatory site. Little is known at the molecular level about these processes. PECAM-1 is involved in the passage of leukocytes across the basal lamina (12), and beta1 and beta2 integrins are known to promote migration through the extracellular matrix (13).

Selectins, and some members of the immunoglobulin superfamily, which are indeed involved in leukocyte recruitment are expressed in endothelial cells (Table 2) and the effects of GCs on the expression of these molecules in these cells have been studied by several laboratories.

Dexamethasone (DEX) is a synthetic glucocorticoid and potent anti-inflammatory agent currently used to analyze the effects of GCs on various genes. Expression of E-selectin by cerebral microvascular endothelial cells is up-regulated by cytokines and lipopolysaccharide (14). Up-regulation of E-selectin expression in aortic endothelial cells and HUVECs by inflammatory mediators such as TNF-alpha or lipopolysaccharide is markedly reduced in the presence of DEX (15, 16). L-selectin level is increased, whereas E-selectin level is reduced 24 hours after an intravenous administration of methylprednisolone (IVMP), and this modification of E-selectin expression on monocytes may limit monocyte recruitment to areas of tissue destruction in MS (17).

Unlike E-selectin which is synthesized in endothelial cells in response to TNF-alpha or related agonists, P-selectin is constitutively expressed by endothelial cells. Inflammatory cytokines augment synthesis of P-selectin in human tissues with chronic or allergic inflammation (18,19). In human endothelial cells, the interleukin-4 induced expression of P-selectin is decreased by the proteasome inhibitor ALLN (N-acetyl-leucinyl-leucinyl-norleucinal-H), the antioxidant PDTC (pyrrolidine dithiocarbamate), or sodium salicylate, but not DEX (20). ICAM-1 expression (up-regulated by lipopolysaccharide) is inhibited by DEX in HUVECs and in the human vascular endothelial cell line EAhy926, a hybridoma of HUVECs and the human epithelial cell line A549 (21, 22, 23). The same effect of DEX was observed in the ECV304 human endothelial cell line, where ICAM-1 expression stimulated by the thyroid hormone (T3), interleukin-1beta or lipopolysaccharide was inhibited significantly (24).

The effects of another glucocorticoid, MP, on cerebral endothelium have been studied at low (65 µg/ml) and high (650 µg/ml) concentrations and have been compared to the effects of DEX. These effects were studied both in basal conditions and after stimulation of human brain endothelial cells (HBECs) or HUVECs with pro-inflammatory cytokines, IFN-gamma or TNF-alpha. Only high dose MP down-regulated TNF-alpha-induced expression of VCAM-1. Neither IFN-gamma-induced HLA-DR expression nor TNF-alpha-induced ICAM-1 expression was influenced by DEX or MP treatment (in either cell lineage) (25). A more recent study by the same authors confirmed that high doses of MP reduced TNF-alpha-induced ICAM-1 and VCAM-1 expression on HBECs (26).

3.3. Regulation of leukocyte-endothelium interactions by glucocorticoids

Leukocyte-endothelium cell interactions are indeed mediated by various adhesion molecules and can potentially lead to transendothelial migration of activated leukocytes. Disruption of leukocyte trans-endothelial migration across an altered BBB is a prominent feature of many neuro-inflammatory diseases. Thus, understanding leukocyte-endothelium interactions is an essential step for developing of potential therapies for controlling inflammation. Therapeutic strategies have been developed in animal models (27) to overcome tissue damage induced by excessive leukocyte infiltration. The many models studying leukocyte migration across the BBB have recently been reviewed (28).

The role of GCs in T cell recruitment across the BBB has been studied by several groups. Engelhardt *et al.* used an *in vitro* transendothelial system to delineate the exact role of ICAM-1 in T cell interactions with endothelium. Established endothelial cell lines from ICAM-1-deficient mice were used to compare T cell interactions with ICAM-1-deficient brain endothelium mice

to those with wild-type endothelium *in vitro*. This group demonstrated that ICAM-1 and ICAM-2 are essential for transendothelial migration of T cells (29). Recent studies have led to the hypothesis that GCs induce down-regulation of expression of adhesion molecules which in turn reduces adhesion of leukocytes to the endothelial layer (25,30). This is further supported by experiments in humans where glucocorticoid (GC) treatment decreases peripheral blood mononuclear (PBMN) cell infiltration at sites of inflammation with a concomitant peripheral leukocytosis (30). Pitzalis *et al.* (31) recently analyzed the effects of GCs on leukocyte-endothelial interactions and reported that the mechanisms by which GCs modulate cell adhesion are complex and multifactorial. They conclude that GCs can act on target cells at multiple levels and regulate cell adhesion molecules (CAM) expression differently depending on the cell type and the stimuli considered. In addition, GCs may regulate CAM expression in different ways in response to various cytokines, and different CAM may be more or less sensitive to the effects of GCs in response to the same stimulus.

3.4. Mechanisms of glucocorticoid action

The mechanisms of GC action have been extensively studied (9,32) and are either genomic and non genomic. Genomic mechanisms are mediated via the glucocorticoid receptor (GR), a cytoplasmic receptor. The GC-GR complex can directly induce gene transactivation and influence translational and post-translational processes. Binding of specific DNA sequences called GRE (glucocorticoid response elements) by the GC-GR complex allows activation of gene expression of the relevant genes. The GC-GR complex can also stabilize the DNA structure and prevent the binding of transcription factors. In addition, it is also well-documented that considerable cross-talk occurs between GR and other transcription factors such as AP-1 (activator protein-1) and NF-kappaB (nuclear factor-kappaB) which can modify their respective biological activities (33). Cross-talk between GR and other transcription factors allows these mediators to affect a common set of genes, including those coding for adhesion molecules. For example, NF-kappaB activation has been shown to precede the transcriptional activation of ICAM-1 (34). GCs counteract the induction of CAM gene expression by cytokines and other molecules, as discussed above. In contrast to transactivation, in which the GR binds to DNA as a homodimer to induce gene expression, during transrepression (where the GR modulates negatively gene expression), the receptor interacts directly or indirectly with transcription factors such as AP-1 or NF-kappaB. GCs mediated repression of these transcription factors is thought to form the molecular basis of anti-inflammatory action of glucocorticoids.

Non-genomic pathways also allow GCs to act at multiple levels on target cells (35). Non-genomic effects of GCs are either specific or non specific. Specific non-genomic effects occur within a few minutes and are mediated by the steroid-specific membrane receptors. Non specific non-genomic effects occur within seconds (only at high GCs dosages) and seem to result from direct interactions with biological membranes. For example, it has been shown in thymocytes that MP inhibits calcium and sodium cycling across the plasma membrane, but has little effect on protein synthesis. These non-genomic effects of GCs lead to cellular apoptosis and have possible implications for human neuroimmunological diseases (36,37).

3.5. Glucocorticoids and multiple sclerosis

The effect of GCs on cerebral endothelial cells is not restricted to the regulation of adhesion molecule expression. Other molecules such as endothelin receptors are also under GC control. In HBECs, DEX down-regulates high affinity endothelin receptors by 40 %. Thus, GCs may counteract some endothelin-induced events in cerebral endothelium, like adhesion molecule expression or permeability changes, which are implicated in the development of cerebrovascular and/or inflammatory brain disorders (38). In immortalized rat brain endothelial cells (RBE4), histamine H1 and H2 receptors are expressed, and mRNA levels of both receptors were down-regulated by GCs treatment. This mechanism may be involved in GCs-mediated effects on cerebrovascular permeability and brain edema (39).

Increased expression of matrix metalloproteinases by the CNS vascular endothelial cells may contribute to BBB disruption. A recent study showed that MMP-9, but not MMP-2, was up-regulated by TNF-alpha and interleukin-1beta in cerebral endothelium (rat brain endothelial cell lines**)** at 24 hours. DEX partially inhibited this effect. The mechanism by which steroids affect BBB permeability is not well understood, but the up-regulation of MMP-9 may be one possible mechanism of action (40). Inhibition of BBB disruption in EAE has been demonstrated experimentally. Therapeutically administered DEX (0.1-1mg/kg body weight) dose-dependently reduced albumin movement across the BBB, and DEX at a dose of 1 mg/kg completely suppressed abnormal BBB permeability in all tissues (41). Metalloproteinases which are known to disrupt the BBB are elevated in the cerebrospinal fluid of MS patients and are also inhibited by GCs (42).

Statins induce apoptotic death of several cell types. Fluvastatin, at concentrations from 1 to 2 microM, blocks growth and induces apoptosis of the endothelial cell line EAhy926. DEX (1 microM) blocked not only fluvastatin-induced apoptosis, but also apoptosis induced by serum deprivation, TNF-alpha, oxidation, DNA damage and mitochondrial disruption. Thus, GCs probably play a role in the prevention of vascular injury (43).

Modulation of receptor activity and prevention of BBB disruption are important effects of GCs. Indeed, GCs are the most potent immunosuppressive and anti-inflammatory drugs currently used. The therapeutic dose is quite variable and depends on the disease, but ranges from very low to extremely high. In the case of neuro-inflammatory diseases, the standard approach is currently glucocorticosteroid pulse therapy (37). Corticosteroid pulse therapy is a strong inducer of leukocyte apoptosis. Induction of apoptosis might contribute to down-regulation

of T-cell activity and thereby terminate inflammation in the CNS. After *in vivo* corticosteroid treatment, apoptosis of non-stimulated peripheral blood leukocytes was significantly augmented in all 3 MS groups of patients (44). In addition, it was shown that MP augments T-cell apoptosis *in situ* in a dose-dependent manner in adaptive transfer EAE (45).

Another research group found that megadose IV MP over 5 days (1 g daily during the first 3 days and 0.5 g daily during the remaining 2 days) decreased brain inflammation by reducing the expression of adhesion molecules on mononuclear cells from blood and cerebrospinal fluid of MS patients. After treatment, the mean proportions of VLA-4, LFA-1 and ICAM-1 on blood lymphocytes and monocytes of 23 MS patients decreased (46). The effect of MP therapy on patients with different subtypes of MS (n= 71) versus 29 healthy subjects were evaluated in the study. The group was divided into three subtypes: relapsing-remitting (RRMS [n=26]), secondary progressive (SPMS [n=20]) and primary progressive (PPMS [n=25]). Treatment of MS patients in exacerbation with IV MP caused a significant reduction in the serum levels of soluble VCAM-1 and E-selectin (47). In addition, treatment of MS patients with a single dose of MS (1g) show a decrease of transendothelial migration of peripheral blood mononuclear cells significantly after 3 hours following intravenous treatment. However, this reduced level of transmigration increased again after 24 hours, which indicates that a single infusion is not enough for obtaining a persistent reduction of transmigration (48). Three days of MP administration at a dose of 1 g/day resulted in a significant reduction of cytokine production (n=18 MS patients) and inhibited lymphocyte endothelial adhesiveness (49).

The combination of GCs and type I interferons decrease BBB permeability *in vitro* (49). However, they did not prevent the increase in BBB permeability induced by the pro-inflammatory stimulus, lipopolysaccharide. It appears that the beneficial clinical effect of GCs and interferon therapy is not mediated by a direct action on BBB permeability, but by a more general sensitivity to GCs, because pretreatment with type I interferon potentiates the effects of GCs by two orders of magnitude (50). The side effects of the classical GC therapy are well documented (51). Currently, high dose GC therapy is used for treatment of MS relapses, and one mode of action could be inhibition of cytokine-induced expression of CAMs, mediating leukocyte/BBB interactions and finally chronic leukocyte recruitment across the BBB. Recently, the design of new steroidal drugs has been proposed as a possible beneficial therapy for MS patients. As the immunosuppressive and anti-inflammatory actions of GCs are known to be largely independent of glucococorticoid receptor DNA binding (35,52), this DNA binding-independent cross-talk function could be used to design these new steroidal drugs. These drugs would specifically transrepress NF-kappa B mediated induction of CAM, without the severe side effects of classical GC therapy (53). In the future, GR^{dim} mice could be used in pharmaceutical drug research, because this animal model permits to distinguish between the two modes of action of gene regulation: transactivation by direct binding of the receptor to its specific response elements, and transrepression via cross-talk with other transcription factors (54).

4. ESTROGENS AND THE BLOOD-BRAIN BARRIER

4.1. Endothelial cells as target of estrogens

At the level of the BBB, modulation of the glucose transporter 1 expression by 17beta-estradiol (E2) has been reported. E2 treatment caused dose- and time-dependent increases in glucose transporter 1 protein expression by microvessels (55). In addition, estrogens have cytoprotective effects. For example, E2 and a low concentration of tamoxifen promoted cytoprotection of cultured rat cerebral endothelial cells treated with 3-nitropropionic acid, a mycotoxin inducing brain damage (56). Estrogens act via estrogen receptors (ER), which are members of the nuclear hormone receptor family.

Low levels of ER are found in endothelial cells (HUVECs and human coronary artery cells) and estradiol treatment up-regulates these estrogen receptors (57). Recently, three other genes were found to be up-regulated by E2 in female human aortic endothelial cells, using differential display analysis. Significant increases in mRNA expression were observed for aldose reductase (3.4-fold), caspase homologue-alpha protein (4.2-fold), and plasminogen activator inhibitor-1 intron e (2.3-fold). Up-regulation of all three genes, occurred with a similar time course and was temporally clustered at a 24 hours hormone treatment. These genes may be potentially important for vascular function in human endothelial cells (58). These few examples demonstrate that endothelial cells are indeed a target for estrogen action.

4.2. Regulation of the expression of adhesion molecules by estrogens

Estrogens increase the expression of endothelial adhesion molecules (E-selectin, ICAM-1 and VCAM-1) and leukocyte binding to TNF-alpha-stimulated HUVECs. E-selectin expression is enhanced by estradiol at physiological doses during the first hours of exposure to TNF-alpha. VCAM-1 expression is also enhanced by estradiol at 24 hours (59). However, at pharmacological doses and longer period of exposure (48 hours) estrogens decrease the expression of cytokine-induced adhesion molecules gene in cultured endothelial cells. E2 strongly (60-80 %) inhibited E-selectin, ICAM-1 and VCAM-1 induction in HUVECs activated by interleukin 1 (60). These two examples illustrate the dual effect of estrogens, which depends on the time and dose of exposure.

The E-selectin promoter is down-regulated by E2 through either ER alpha or ER beta, requiring the NF-kappa B site at position - 94 to - 85 within the promoter (61). E2, but not the alpha enantiomer, was able to inhibit both basal and interleukin-1beta stimulated expression of ICAM-1, as well as NF-kappa B activation in immortalized rat brain endothelial cells (62).

E2 also decreased VCAM-1 expression by inhibiting transcription factors: NF-kappa B, AP-1 and

Table 3. Adhesion molecules expressed in endothelial cells: HBECs: human brain endothelial cells ; HUVECs: human umbilical vein endothelial cells ; HSVECs: human saphenous vein endothelial cells, and involved in leukocyte recruitment at the BBB. Effects of estrogens (ES)

Name of cells or cell lines	Name of the genes	Description	Inducers of gene expression	ES used and effects on gene expression	References
HBECs HUVECs	E-selectin ELAM-1	Endothelial leukocyte adhesion molecule 1	TNF alpha Interleukin-1beta	E2 (17beta-estradiol) Down-regulation	59,60
HUVECs	ICAM-1 (CD54)	Intercellular adhesion molecule 1	Interleukin-1beta	E2 Down-regulation	59,60,62
HUVECs	VCAM-1 (CD 106)	Vascular cell adhesion molecule 1	TNF alpha (24 hours exposure)	E2 Up-regulation	59
HUVECs	VCAM-1 (CD 106)	Vascular cell adhesion molecule 1	Interleukin-1beta	E2 Down-regulation	60,64
HSVECs	VCAM-1 (CD 106)	Vascular cell adhesion molecule 1	LPS(Lipopolysaccharide)	E2 Down-regulation	63

GATA. This was found in lipopolysaccharide-induced VCAM-1 expression in human vascular endothelial cells. This is of particular interest, because endothelial VCAM-1 is an important mediator of mononuclear cell adhesion (63). Induction of VCAM-1 by interleukin-1beta is also regulated by E2 in HUVECs. Pre-incubation with E2 (250 or 500 pg/ml) suppressed the induction of VCAM-1 mRNA expression by this interleukin (64). All of these data are summarized on table 3.

4.3. Mechanisms of estrogen action

Estrogens and the mechanisms underlying their action have been extensively reviewed (65,66). Their effects are mediated by direct and indirect genomic, as well as non-genomic pathways. The direct genomic mechanism involves both nuclear forms of ER-alpha or ER-beta.

The estrogen-ligand complex, once formed, acts as a transcription factor by binding to an ERE (estrogen response element) or to fos-jun heterodimers which bind to an AP-1 response element (67). The indirect genomic action occurs when activation of a form of ER, possibly associated with cell membranes, stimulates second messengers such as adenylyl cyclase, protein kinases A, B, C and MAP kinase. Many cell substrates are then phosphorylated, among them transcriptional regulators, for example CREB, and then able to act at the DNA regulatory region CRE (cAMP responsive element). This can be followed by the regulation of genes without ERE.

Non-genomic effects can occur at low or high estrogen concentrations, either at nanomolar or lower levels of estradiol concentrations, or at micromolar concentrations. It was shown that these non-genomic effects clearly involve another receptor system than the intracellular ER (actually membrane ER). These non-genomic effects have been described for neurons at the lower estrogen concentrations, and neuroprotective effects have been reported for a number of cell culture models, but not on endothelial cells, at high estrogen concentrations (66).

4.4. Estrogens and experimental autoimmune encephalomyelitis

Estrogens are known to induce a potent suppression of EAE, the animal model of MS. This was obtained by a long term treatment with high levels of 17beta-estradiol (68). More recently, estrogens have been also used in a low-dose therapy. Diestrus (< 100 microg/ml in serum) levels of E2 reduced the clinical manifestations of active EAE in both male and female mice (69). E2 treatment was shown to drastically decrease the recruitment of inflammatory cells into the CNS at the onset of the disease. In addition, systemic inhibition of TNF-alpha expression was observed (70,71). The effects of E2 on gene expression in EAE were evaluated using DNA microarrays. Interestingly, E2 treatment affected only about 10 % of the 12000 genes tested, but only 18 cytokine, chemokine/receptor, adhesion molecule or activation genes were up- or down-regulated more than 2.4-fold by this treatment (72). Thus, this study clearly showed that the estrogen effect is restricted to specific genes. The down-regulated genes in mice splenocytes included TNF-alpha (decreased 10.4-fold), an important proinflammatory cytokine in EAE and MS, Prgp (another TNF superfamily member), RANTES (known to be increased in EAE and MS) and NCAM (increased in cerebrospinal fluid from patients with active MS). Up-regulated genes included CTLA-4 (known to inhibit T cell activation), two interferon gamma-induced genes, TGF-beta3, IL18, chemokines, VCAM-1 and disintegrin metalloprotease (thought to regulate TNF-alpha production). These set of known and previously unexpected E2-sensitive genes may be of interest for developing novel strategies to treat EAE and possibly MS.

5. CONCLUSION AND PERSPECTIVES

The activated cerebrovascular endothelium plays a crucial role in recruitment of activated leukocytes during chronic inflammatory diseases like MS. This activated endothelium is an attractive target for pharmacological intervention and inhibition of endothelial cell activation and consequent leukocyte recruitment may improve therapy of such diseases.

Glucocorticoid hormones are among the steroids who have become an established treatment of acute relapses in MS. GC-dependent suppression of adhesion molecules expression is now an accepted mechanism of action of these anti-inflammatory drugs. This is especially important in the case of ICAM-1 and ICAM-2, which have been identified as key regulators of the transendothelial migration of autoaggressive T cells (29). The fact that GCs have a wide range of action allows for their efficacy in various pathologies. Indeed, GCs are among the most potent immunosuppressive and anti-inflammatory drugs.

However, long-term therapies are usually accompanied by severe side effects: atrophy of the skin, osteoporosis, myopathy and psychosis (2).

Development of new GR ligands showing highly potent anti-inflammatory and immunosuppressive properties, but reduced side-effects, is an interesting approach to improve the GC therapy in the future (73). Because they act at the level of adhesion molecules gene expression involved in leukocyte recruitment, these new synthetic GCs (called dissociated GCs) could be valuable tools for a more convenient therapy of EAE and MS. The possibility to dissociate the two main activities of GCs, i.e. transactivation and transrepression, provides a novel concept of drug discovery.

Because selective delivery of dexamethasone into activated endothelial cells using an E-selectin-directed immunoconjugate was recently obtained (74), analogous strategies could be used to delivery of dissociated GCs to inhibit endothelial cell activation in the future.

In addition, one should keep in mind that regulation of endothelial cell functions is now a novel field with potential therapeutic impact.

Recently, the pregnancy hormone estriol was used for treatment of MS, because this hormone is well known to decrease relapses, probably by mediating a shift in immune responses from T helper 1 to T helper 2. A significant decrease in lesion numbers was observed when patients were treated with oral estriol (8 mg/day). This treatment could have relevance to other autoimmune diseases that also improve during pregnancy (75).

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Abbreviations: BBB: blood-brain barrier, CAM: cell adhesion molecule, CNS: central nervous system, DEX: dexamethasone, EAE: experimental autoimmune encephalomyelitis, E2: 17beta-estradiol, ER: estrogen receptors, GC: glucococorticoid, GCs: glucocorticoid hormones, GR: glucocorticoid receptor, HBECs: human brain endothelial cells, HUVECs: human umbilical vein endothelial cells, ICAM-1, -2, -3: intercellular adhesion molecule-1, -2, -3, LFA-1: leukocyte function associated molecule-1, MP: methylprednisolone, MS: multiple sclerosis, PECAM-1: platelet endothelial cell adhesion molecule-1, PMN: polymorphonuclear neutrophils, TNF: tumor necrosis factor, VLA-4: very late antigen -4.

Key Words: Blood-Brain Barrier, Endothelial Cells, Adhesion Molecules, Glucocorticoid Hormones, Estrogens, Experimental Autoimmune Encephalomyelitis, Multiple Sclerosis, Review

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