

Review

The adrenal gland microenvironment in health, disease and during regeneration

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ABSTRACT

The adrenal gland is a key component of the stress system in the human body. Multiple direct and paracrine interactions between different cell types and their progenitors take place within the adrenal gland microenvironment. These unique interactions are supported by high vascularization and the adrenal cortex extracellular matrix. Alterations in the adrenal gland microenvironment are known to influence the progression of several pathological conditions, such as obesity and sepsis, and to be influenced by these disorders. For example, it has been suggested that activation of immune-adrenal crosstalk during sepsis induces elevated adrenal glucocorticoid levels, whereas crosstalk between adrenocortical cells and sonic hedgehog responsive stem cells was found to contribute to the increased size of the adrenal cortex during obesity. By contrast to sepsis, where activation of adrenal glucocorticoid production has protective effects, chronic exposure to high levels of glucocorticoids induces adverse effects, typically manifested in patients with Cushing syndrome, such as increased body weight, dyslipidemia, glucose intolerance, and hypertension. Therefore, a better understanding of factors involved in the regulation of the adrenal gland microenvironment is crucial. This review highlights bi-directional interactions occurring between the adrenal gland microenvironment and systemic responses during obesity and sepsis. Furthermore, it presents and discusses recent advancements and challenges in attempts to restore or regenerate adrenal gland function, including the use of oxygenated immune-isolating devices.

Key words: Adrenal insufficiency, ACTH, Cell transplantation, Hypothalamic-pituitary-adrenal axis, Immune-adrenal crosstalk, Obesity, Sepsis

INTRODUCTION

The adrenal gland is a key component of the body's

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stress system which is composed of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal medullary system (SAM).¹ It integrates two embryonically distinct endocrine systems within one organ capsule, the steroid hormone-producing cortex and the catecholamine-producing medulla. Adrenal cortex function is mostly regulated by the adrenocorticotrophic hormone (ACTH), produced

in the pituitary gland, whereas the adrenal medulla releases high levels of catecholamines in response to the activated sympathetic nervous system (SNS).^{2,3} During stress, these hormones induce various protective changes which ultimately contribute to restoration of body homeostasis. In particular, both glucocorticoids (GCs) and catecholamines (CAs) are known to acutely increase plasma glucose levels, to promote elevated cardiac output, to maintain high blood pressure, and to suppress inflammation.^{2,4,5} Activation of another key adrenal hormone, aldosterone, is required for maintenance of homeostasis. In particular, as part of the renin-angiotensin system, aldosterone is involved in regulation of plasma sodium (Na⁺) and extracellular potassium (K⁺) levels, thereby controlling arterial blood pressure.⁶

Glucocorticoid hormones, acting on all nucleated cells in the body, play an important role not only in regulation of homeostasis but also in the pathogenesis of different diseases. It is therefore of utmost importance to gain a deeper insight into the mechanisms regulating adrenal hormone production. Both adrenal gland hyperactivation and insufficiency can be life-threatening. In particular, patients with Cushing syndrome, induced by chronic hypersecretion of glucocorticoids, or those suffering from Conn syndrome, due to hyperaldosteronism, have an increased risk of developing metabolic syndrome,^{7,8} depression,^{9,10} osteoporosis¹¹ and/or cardiovascular diseases.^{12,13} Over the past few years, adrenal gland insufficiency has become a growing phenomenon. Patients with congenital adrenal hyperplasia or Addison Disease develop adrenal gland insufficiency which condition requires daily hormone supplementation. Furthermore, it predisposes affected patients to increased risk of mortality due to sepsis and to developing adrenal crisis, a condition requiring emergency treatment.¹⁴

The adrenal gland has astonishing regenerative capacity and ability to adapt to various physiological or pathological conditions.^{15,16} It should be noted that an extensive vascular network supports a paracrine interaction between the different cell types within the adrenal gland and their progenitors, together forming a unique microenvironment.^{17,18} These intraadrenal interactions are known to regulate adrenal gland function during physiological and pathophysiological conditions.¹⁹

Since complex cellular and paracrine interactions occurring within the adrenal gland have been described in detail in numerous review articles,¹⁸⁻²⁰ this review will highlight the connection between alterations in adrenal microenvironment function and the development of various diseases. As depicted schematically in Figure 1, this review article will also describe the impact of sepsis and obesity on adrenal gland adaptation, function, and damage. Finally, it will also provide a brief overview of some novel cellular and therapeutic approaches designed to restore adrenal gland function, which will furnish a deeper understanding of the adrenal microenvironment.

THE ADRENAL GLAND MICROENVIRONMENT

The adrenal gland combines two endocrine cellular systems of diverse embryogenic progeny within a common organ capsule, namely, the cortex and the medulla. Taking place within these two environmental niches is an interplay between various cells, including adrenocortical and chromaffin cells, their progenitors, neuronal cells, immune cells, endothelial cells, and glia cells.^{18,21-23} All these cells are known to influence each other's function, either directly or in a paracrine way, through secretion of various biologically active products, including steroid hormones, catecholamines, cytokines, neurotransmitters, and neuropeptides. The latter interactions are facilitated by the high vascularization of the adrenal organ and co-expression of various receptors for these substances in most of the cells present in the adrenal gland microenvironment.¹⁸

During non-stress conditions, adrenal glucocorticoids are secreted in circadian and ultradian cycles, reaching the highest concentration in plasma at the onset of the daily activity phase.²⁴ Rhythmicity of daily GC pulses are tightly controlled by the coordinated action of a central master circadian clock, in the suprachiasmatic nucleus of the hypothalamus (SCN), and peripheral clock machinery, located in the adrenal gland, including canonical clock protein BMAL1.²⁵⁻²⁷ While SCN controls the pulsatile secretion of two main regulators of adrenal steroidogenesis, namely, the corticotrophin-releasing hormone (CRH) and ACTH, production and secretion of adrenomedullary catecholamines is regulated by the sympathetic nervous system and splenic nerves.^{28,29}

Ample evidence supports the presence of bidirectional interactions between the adrenal cortex and the medulla.¹⁸ GCs were found to enhance synthesis of CAs both in vitro³⁰ and in vivo.³¹ This stimulatory effect was attributed to increased expression of phenylethanolamine N-methyltransferase (PNMT), which gene encodes for an enzyme that catalyzes the synthesis of epinephrine from norepinephrine. A key involvement of the cortex in the regulation of PNMT gene expression, and thus basal and stress-induced epinephrine production, was demonstrated in studies using mice deficient in either steroidogenic factor-1 (SF-1), glucocorticoid receptor (GR) or the corticotrophin-releasing hormone type 1 receptor (CRHR1).³¹⁻³³ Accordingly, patients with congenital adrenal hyperplasia (CAH) and Addison's disease showed reduced catecholamine biosynthesis.³⁴⁻³⁶

Similarly, intact function of the adrenal medulla was found to be involved in regulation of adrenocortical hormone synthesis. It has been demonstrated that co-culture of bovine adrenocortical cells with chromaffin cells resulted in a 10-fold increase in basal glucocorticoid secretion³⁷ through a mechanism involving increased expression of the steroidogenic acute regulatory protein (StAR).^{18,37,38} In line with these findings, deletion of the tyrosine hydroxylase (TH) gene in experimental animals revealed marked alterations in the ultrastructure of adrenocortical cells and reduced corticosterone levels.³⁹

The adrenal cortex is characterized by a high density of endothelial cells which lie in close proximity to steroid-producing cells.⁴⁰ Consequently, both adrenal hormones and endothelial cell-derived products were shown to influence the function of the adrenal vas-

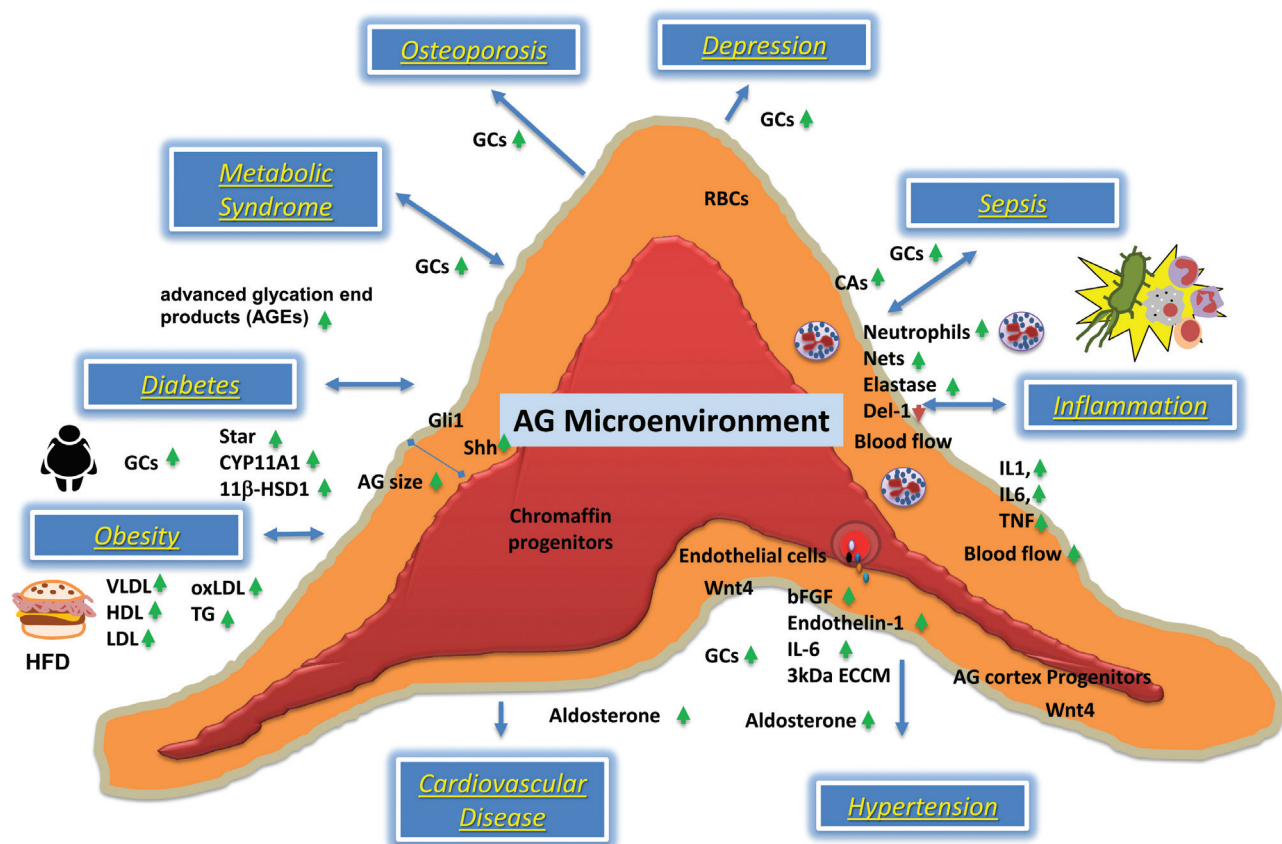


Figure 1. Bidirectional interactions between the adrenal gland microenvironment and systemic diseases. Chronic hypersecretion of glucocorticoids (GCs) and catecholamines (CAs) exert various systemic actions which are implicated in pathogenesis of many diseases. In turn, many disorders, such as e.g. obesity, have been shown to increase adrenal gland hormone production and expression of key steroidogenic enzymes including steroidogenic acute regulatory protein (StAR). The green arrow denotes an increased secretion or stimulatory action, whereas the red arrow denotes downregulation.

culature and steroid-producing cells, respectively.¹⁸ For example, an increased production of aldosterone and cortisol was observed when human adrenocortical cells were incubated with endothelial cell conditioned media (ECCM).⁴¹ Since ECCM is a cocktail of cell-derived factors, including endothelin-1, angiotensin-2 (AII), IL-6, and other as yet unidentified proteins, the exact mechanism of ECCM action remains unknown. Some studies reported that the stimulatory effect of ECCM on basal and ACTH-induced glucocorticoid production may involve endothelin-1 and nitric oxide (NO)-mediated activation of cyclic adenosine monophosphate (cAMP), but not protein kinase A (PKA)-dependent pathways.⁴¹ Other studies suggested involvement of a to date unknown protein with a molecular mass of around 3 kDa, isolated from ECCM, with the ability to increase aldosterone levels through the protein kinase C (PKC) pathway.⁴² Recently, a stimulatory action of basic fibroblast growth factor (bFGF) on β -catenin activity in adrenocortical cells was implicated in ECCM action.⁴³ The adrenal gland microenvironment is rich in both glucocorticoids and catecholamines, hormones which influence the function of adrenal vascular endothelial cells. Indeed, high levels of adrenal steroids may promote and sustain production of developmental endothelial locus-1 (Del-1), a protein that is selectively expressed in immune privileged organs and was demonstrated to act as a gatekeeper of adrenal gland inflammation.⁴⁴

It has become evident that the immune and endocrine systems interact with each other at various levels and that this interaction is crucially involved in the regulation of adrenal gland function during normal and stress conditions.³ This immune-endocrine crosstalk involves direct cellular interactions occurring between adrenal and immune cells, as well as bidirectional action of steroid hormones, catecholamines, interleukins, and various vasoactive or proteolytic enzymes.¹⁸ In addition, activation of toll-like receptor signaling in hematopoietic and liver cells was found to be involved in adrenal activation during endotoxemia.^{18,45} During non-stress conditions, various immune cells reside in both the adrenal cortex and the medulla, including tissue macrophages, dendritic cells, mast cells, and lymphocytes.⁴⁶⁻⁴⁹ These cells stay in close cell-cell contact with adrenocortical, chromaffin, and endothelial cells,^{47,49}

enabling them to exert bi-directional effects on other cell types. Immune cells play an important role in the regulation of adrenal homeostasis by sensing pathogens, removing apoptotic cells, and promoting tissue remodeling through e.g. secretion of growth factors.^{20,50} Furthermore, immune cells can regulate adrenal steroidogenesis through secretion of various cytokines, e.g. interleukins (IL)-1 and IL-6,^{51,52} and directly by cell-cell contact. For example, co-incubation of T cells with primary cultures of human adrenocortical cells resulted in increased secretion of dehydroepiandrosterone (DHEA) and cortisol.⁴⁹

A large number of studies demonstrate the immunomodulatory effects of adrenal hormones. For instance, a microarray analysis performed on dexamethasone-treated peripheral blood mononuclear cells (PBMC) obtained from healthy donors revealed that glucocorticoids might exert both inflammatory and anti-inflammatory actions. In resting PBMC cells, dexamethasone induced expression of various inflammatory genes, such as chemokines, cytokines, complement family members, and toll-like receptors. However, dexamethasone inhibited expression of these genes when cells were pre-treated with bacterial LPS.⁵ These effects are also evident *in vivo*. For example, chronic deficiency of adrenal hormones was recently shown to be associated with impaired natural killer cell function, which was linked to increased mortality risk.⁵³ On the other hand, an excess of glucocorticoids, which is typically manifested in patients with sepsis, contributes to immune paralysis, e.g. by promoting massive apoptosis of T cells in the thymus.⁵⁴ Due to the high expression of adrenergic receptors (ARs) by a variety of immune cells, catecholamines are also known to exert numerous immunomodulatory effects. In particular, activation of β 2-adrenergic receptors by catecholamines was found to promote IL-10 secretion while simultaneously decreasing production of pro-inflammatory TNF alpha in LPS-treated macrophages.⁵⁵ Additionally, a differential effect of epinephrine stimulation on cytokine production in circulating neutrophils and monocytes was reported. In fact, epinephrine was found to induce dose-dependently IL-8 production in unstimulated cells, but at the same time it suppressed IL-1 β , IL-8, and monocyte chemoattractant protein (MCP)-1 secretion in cells stimulated with LPS.⁵⁶

ROLE OF THE ADRENAL GLAND MICROENVIRONMENT IN VARIOUS DISEASES

In Systemic Inflammation and Sepsis

Sepsis is one of the extreme examples of severe, sustained physical stress and is characterized by abnormal host response to infection, resulting in systemic inflammation and dysregulation of metabolism that frequently culminates in life-threatening multiple organ dysfunction.⁵⁷ A fast adrenal response is critical to survive this adverse condition.^{58,59} Elevation of both catecholamines and glucocorticoids is crucial to prevent circulation collapse, to mobilize energy supplies, and to control initial overt inflammation. Although of vital importance for survival, regulation of adrenal gland function particularly in the later phase of sepsis, is still not fully understood. In many patients with sepsis, low ACTH levels are diagnosed despite normal or elevated cortisol levels in plasma. Multiple factors have been proposed as being involved in this process.⁶⁰ These include reduced metabolism of glucocorticoids,⁶¹ intraadrenal cytokines,⁶² increased blood flow,⁶³ and adrenal size.⁶⁴

During sepsis, the adrenal glands are heavily infiltrated by circulating immune cells.^{65,66} Once inside, many of these immune cells will secrete high levels of proinflammatory mediators, e.g. IL-1 β or IL-6, these cytokines stimulating adrenal hormone production.⁶⁷ For example, injection of IL-1 β in hypophysectomized rats was reported to enhance corticosterone production,⁶⁸ e.g. through activation of IL-1R expressed by adrenocortical or immune cells or by activation of the intraadrenal ACTH system.⁶² Furthermore, neutralization of TNF- α and IL-6 cytokines by blocking antibodies or IL-1 β or in IL-6 deficiency was found to be associated with significantly lower glucocorticoid levels after LPS administration.^{69,70} In addition, major involvement was observed of intact TLR-expression and -signaling during LPS-induced adrenal hormone production.⁷¹⁻⁷³ In sepsis, bacteria and their cell wall components are known to simultaneously activate toll-like receptors, which are expressed by most cells within the adrenal microenvironment, including myeloid, endothelial, adrenocortical, and medullary cells.⁷⁴⁻⁷⁶ Recently, mice deficient in a key TLR adaptor molecule, myeloid differentiation gene 88 (MyD88), in hematopoietic or adrenocortical cells

were used to dissect TLR-dependent responses in the adrenal microenvironment. In this study, a key role of hematopoietic and liver TLR signaling in LPS-induced HPA activation was demonstrated.⁷³ However, intact MyD88 expression in endothelial cells of the blood brain barrier was also found to contribute to glucocorticoid stimulation after LPS administration.⁷⁷

Increased size of the adrenal glands during sepsis was found to correlate positively with patients' survival rate.⁶⁴ One of the factors potentially involved in this finding could be increased adrenal steroidogenesis resulting from hyperplasia of adrenocortical cells and enhanced blood flow.⁶³ In fact, injection of either angiotensin II or ACTH simultaneously increases both adrenal steroidogenesis and adrenal blood flow in experimental animals. In the latter case, activation of the splanchnic nerve, vasoactive intestinal peptide (VIP),^{78,79} cytochromes 450,⁸⁰ and vasoactive substances released from granules of mast cells⁸¹ were found to be involved.

Chronic exposure of adrenal cells to IL-1 β and TNF α cytokines, neutrophil-derived reactive oxygen species (ROS), and proteolytic enzymes found in neutrophil-extracellular traps (NET) may lead to their damage.⁸² This in turn can contribute to adrenal exhaustion and is characterized by decreased adrenal steroidogenesis and blunted corticosterone response to ACTH stimulation.^{83,84} In fact, mortality of mice subjected to CLP-induced polymicrobial sepsis was correlated with increased occurrence of hemorrhages, apoptosis of cortical and chromaffin cells, and reduced response to exogenous ACTH injection.⁶⁶ A postmortem study found a positive correlation between the length of stay in intensive care units (ICUs) and exhaustion of the adrenal gland. In particular, the adrenal glands of patients with the longest stay in ICUs contained the lowest cholesterol content and reduced expression of key steroidogenic enzymes as compared to short-term stay patients.⁸⁵

In addition to suppressed function of adrenocortical cells in severe sepsis, an increasing number of studies suggest that the function of chromaffin cells is also compromised. For example, increased apoptosis of chromaffin cells mediated by C5a anaphylatoxin was noted in mice with polymicrobial sepsis.⁸⁶ Furthermore, it has been reported that in vivo administration of LPS

resulted in reduced excitability and neuropeptide Y release from adrenal chromaffin cells via TLR4- and nuclear factor- κ B-dependent pathways.⁷¹

In Obesity and the Metabolic Syndrome

Obesity, defined as a body mass index (BMI, the weight in kilograms divided by the square of the height in meters) above 30 kg/m², has become one of the most important public health problems not only in Western countries but also worldwide, it is being estimated to affect more than 107 million children and 600 million adults round the globe with steadily increasing prevalence.⁸⁷ Currently it is a major risk factor for the development of diabetes, cardiovascular diseases,⁸⁸ non-alcoholic steatohepatitis, liver failure, and some cancers.⁸⁹

Obesity is associated with alterations of adrenal gland function. Obese people as well as experimental animals, e.g. ob/ob mice or Zucker (fa/fa) rats, tend to have elevated levels of glucocorticoids and aldosterone in plasma.⁹⁰ Experimental studies have reported that the elevation of adrenal hormone levels found in these mice was associated with hyperactivity of the HPA axis, enlargement of the adrenal gland cortex, loss of leptin signaling, and increased conversion of 11-dehydrocorticosterone to corticosterone in peripheral tissues.⁹¹⁻⁹³ Loss of leptin signaling was also recently verified in another mouse model of obesity and type 2 diabetes, namely db/db mice, along with an additional increase in plasma 11-deoxycorticosterone and progesterone, which are precursors of aldosterone and corticosterone. Interestingly, elevated ratios of aldosterone and corticosterone to progesterone in these mice indicated that the increase in progesterone levels could be only partially responsible for the elevation in steroid production.⁹⁴ Leptin was shown to directly inhibit the secretion of adrenal hormones in primary cultures of bovine adrenocortical cells,^{95,96} which suggests that loss of leptin signaling may contribute to increased hormone levels. By contrast, suppressed plasma leptin levels were found in patients with primary aldosteronism, which suppression was resolved shortly after adrenalectomy.⁹⁷ One of the mechanisms potentially involved in increased aldosterone production in obese individuals and thereby contributing to loss of leptin signaling is the mineralocorticoid stimulatory action of adipocyte secretory

products. Indeed, adipocyte-conditioned medium obtained from primary cultures of human adipocytes was found to stimulate aldosterone secretion and to upregulate expression of steroidogenic enzymes in human adrenocortical cells.⁹⁸

In contrast to adipocyte-conditioned medium, plasma from obese people contains high levels of well-defined factors, such as triglycerides, fatty acids, and low- (LDL), very low- (VLDL) or high- (HDL) density lipoproteins. These in turn are known to stimulate adrenal steroidogenesis *in vitro* and *in vivo*.⁹⁹⁻¹⁰¹ Specifically, during stress conditions LDL and HDL lipoprotein-mediated delivery of cholesterol from the liver is required to sustain adrenal hormone production.¹⁰⁰ In fact, mice deficient in HDL-cholesterol receptor scavenger receptor B1 (SRB1) have decreased steroidogenesis and an increased mortality rate during endotoxemia.¹⁰² Interestingly, some cholesterol-free lipoproteins such as VLDL were found to increase aldosterone production and expression of genes encoding for steroidogenic acute regulatory protein (StaR) and aldosterone synthase (CYP11B2) in adrenocortical cells, independently of this steroid precursor.^{103,104} A recent study demonstrated an involvement of the PLC/IP3/PKC signaling pathway in VLDL-induced aldosterone production.^{104,105}

Patients with liver cirrhosis who have impaired lipoprotein-dependent delivery of cholesterol are at risk of developing adrenal insufficiency.¹⁰⁶ In line with these observations, individuals deficient in the LDL receptor or those carrying inactivating mutations in genes encoding for SRB1 demonstrate a much lower cortisol response to the ACTH test.^{107,108} Interestingly, a recent study suggests that maternal lipids could potentially reprogram offspring's HPA axis reactivity, as elevated levels of TG, HDL, and total cholesterol through pregnancy was associated with increased cortisol reactivity in children.¹⁰⁹

Adrenal gland function and structure undergoes dramatic changes during obesity. In a recent study, a high-fat diet (HFD) obesity model was used to evaluate these changes. Mice fed a HFD had increased body and adrenal weight, hyperplasia of the adrenal cortex, and increased secretion of corticosterone, aldosterone, and their precursors without affecting ACTH levels.¹¹⁰ Furthermore, elevated expression

of the main steroidogenic enzymes, including StAR, cholesterol side-chain cleavage enzyme (Cyp11A1), 11-beta-hydroxylase (Cyp11B1), and steroid 21-hydroxylase (Cyp21OH) was additionally observed. By tracking the fate of a glioma-associated oncogene (Gli)-1 positive progenitor cell population, cells known to differentiate into adrenocortical cells in response to increased sonic hedgehog (Shh) levels,¹¹¹⁻¹¹³ a rapid decrease in the number of these cells in the capsule was seen to occur after HFD. This suggests that both stem cell mobility and Shh activation may be involved in the increase of adrenal cortex size.¹¹⁰

The sympathoadrenal system (SAS) is composed of the adrenal medulla and the sympathetic nervous system which secrete epinephrine and norepinephrine, respectively. Activation of SAS is strongly implicated in the regulation of intermediary metabolism and the pathophysiology of obesity.^{114,115} Despite the existing conflicting data regarding adrenomedullary function during obesity,^{116,117} the results of a recent study demonstrated impaired adrenal secretion and storage of epinephrine under both fasting and feeding conditions.¹¹⁸

As evidenced in patients with Cushing syndrome, increased adrenal hormone production contributes to the obesity phenotype and its comorbidities.¹¹⁹ As for the mechanisms, elevated cortisol levels are known to promote differentiation of preadipocytes to adipocytes and to promote visceral obesity. Furthermore, GCs inhibit glucose uptake by peripheral tissues and stimulate gluconeogenesis in the liver,¹²⁰ thereby contributing to insulin resistance and the development of cardiovascular diseases.¹²¹ When GCs were continuously administered for four consecutive weeks into adult mice, they promoted an increase in body weight, an elevation in plasma leptin, insulin, and triglyceride levels, and reduced mobility.¹²² Interestingly, when glucocorticoids were administered in adolescent mice, blunted growth rate, induction of glucose clearance, and decreased bone density was observed.¹²³ These experimental models clearly demonstrated the ability of prolonged corticosterone to recapitulate symptoms typically found in adult Cushing syndrome in men.

As summarized in Table 1, both sepsis and obesity influence the adrenal adaptation process leading to increased adrenal size and enhanced production of

adrenal hormones. However, many septic patients, unlike obese individuals, will ultimately develop adrenal insufficiency and/or irreversible damage, requiring a life-long hormonal supplementation.

THERAPEUTIC APPROACHES TO RESTORE ADRENAL GLAND FUNCTION

Adrenal insufficiency (AI) is a rare but life-threatening disorder that requires constant hormone supplementation. Among many causes of AI, infections with certain bacteria and viruses play a key role. More specifically, suppressed function of the adrenal glands is diagnosed in patients with meningococcal meningitis, tuberculosis or adenoviral-induced adrenalitis.¹²⁴ Impaired adrenal gland function may also develop due to genetic factors, e.g. in patients with congenital adrenal hyperplasia (CAH). In these patients, suppressed cortisol and aldosterone production with androgen excess may develop as a result of several autosomal recessive diseases caused by mutations in key enzymes involved in adrenal hormone production.¹²⁵ It has been estimated that 21-hydroxylase deficiency (21OH) accounts for more than 95% of cases of patients with CAH.¹²⁶ Other important causes of adrenal dysfunction are sepsis-induced adrenal hemorrhages, medication-related causes,⁵⁸ and autoimmune-mediated adrenal destruction.^{124,127} In addition, the modern lifestyle, certain drugs or posttraumatic syndrome can increase the risk for AI.¹²⁸

Lifelong supplementation with hydrocortisone as a form of glucocorticoid replacement and fludrocortisone, used for mineralocorticoid replacement in the case of primary adrenal insufficiency, is essential in patients with AI.¹⁴ However, therapeutic hormone supplementation is challenging, particularly as regards restoration of the circadian secretion pattern of adrenal glucocorticoids. It therefore poses the risk of either underdosing, leading to adrenal crisis,^{14,128} or overdosing, which can eventually contribute to development of osteoporosis, impaired glucose tolerance or obesity.

Considering these drawbacks related to adrenal hormone supplementation, several alternative therapeutic approaches have been proposed and tested.¹²⁹ These include auto- and allotransplantations of the adrenal glands,¹³⁰ adenoviral-associated virus (AAV)-mediated gene targeting,^{131,132} and either allo- or xenotransplan-

Table 1. Adrenal gland (mal-)adaptation and its systemic impact during sepsis, obesity and regeneration. Adrenal gland function and structure is influenced by obesity and sepsis. Advances and challenges in regeneration or restoration of adrenal gland function.

SEPSIS	OBESITY	REGENERATION
Adrenal Gland (Mal-) Adaptation	Adrenal Gland (Mal-) Adaptation	Advances
Increased production of CAs and GCs	Adrenal hyperplasia	Increased survival of transplants with decellularized ECM
Increased adrenal gland size and blood flow	Increased expression of steroidogenic enzymes. Enhanced secretion of GCs and aldosterone	Transplantation of mixed cultures of adrenal cells, including capsular progenitors, improves aldosterone secretion
Chronic exposure to cytokines and neutrophil-derived anti-microbial agents (NETs, ROS, Enzymes)	Decreased storage and secretion of epinephrine. Decreased adrenal medullary responsiveness	Reprogramming of own patients stem cells by Sfl overexpression
Adrenal exhaustion: decreased cholesterol content, expression of key steroidogenic enzymes and GC response to ACTH	HPA-axis dysregulation	Use of devices with semipermeable membranes for immune protection
Systemic Response to Adrenal Hormones	Systemic Response to Adrenal Hormones	Challenges
Protection against cardiovascular collapse, overt inflammation and mobilization of glucose	Visceral obesity, glucose intolerance, hypertension, dyslipidaemia, cardiovascular diseases	Low aldosterone and too high testosterone levels
Induction of immune paralysis and increase in risk of secondary infections	Elevation in TG, cholesterol, VLDL and LDL	Lack of circadian secretion of GCs
	Differentiation of pre-adipocytes to adipocytes	Too low GCs levels to provide protein during extensive stress
	Alteration of SAS activity	Immune rejection
	GCs-output from Adipocytes (11 β -HSD1)	

tation of adrenocortical cells,¹³³⁻¹³⁹ or various stem cells reprogrammed into steroidogenic-like cells.¹⁴⁰⁻¹⁴² Apart from allotransplantation of a whole human adrenal with its intact microenvironment, all the other experiments were performed in animals, preferentially using rodent models.¹²⁹ Usually in these experiments adrenocortical cells were either transplanted directly under the kidney capsule or into the adrenal glands as single cell suspensions, or were incorporated inside various 3D scaffolds or devices, such as polycarbonate cylinders,¹⁴³ collagen sponges,¹³⁹ alginate slabs or oxygenated immunoisolating devices.¹⁴⁴

In many of these approaches, the transplanted adrenocortical cells managed to survive *in vivo* and to restore adrenal function in animals that underwent bilateral adrenalectomy.^{135,139,143} By contrast to adrenocortical cell transplantations, the majority of the studies using reprogrammed stem cells were performed only *in vitro*, hence the protective abilities of these adrenocortical-like cells are not known. Another

important aspect is whether the amount of steroids produced by these transplanted cells can sufficiently protect the animals during stress conditions. Interestingly, in one of the very few studies that addressed this important issue, despite restoration of glucocorticoid levels to levels very similar to those found in control mice with intact adrenals, addition of another stress factor in the form of laparotomy resulted in 100% mortality. Modulation of the experimental protocol with removal of the second adrenal gland 1 week after cell transplantation demonstrated a 42% survival rate within the first 14-days after exposure.¹³⁹

Better understanding of the adrenal gland micro-environment should greatly improve the functional efficiency of adrenal transplants. As in many of these approaches only a single clonal population of adrenocortical cells from the glucocorticoid-producing zone was used, few reports demonstrated successful restoration of aldosterone production, as e.g. in one study using primary cultures of bovine cells,¹⁴³ or in

another with transplantation of mixed populations of zona fasciculata and glomerulosa cells.¹⁴⁵ In both of these studies, an improved outcome could have been achieved by adding adrenal stem cell populations, which normally reside in the capsular and subcapsular region of the gland.^{17,146}

The adrenal gland progenitor pool is a heterogeneous population of cells mostly located in the adrenal capsule and subcapsular region.¹⁴⁶ Despite recent advances in adrenal stem cell biology, many aspects related to maintenance, self-renewal capacity, and the differentiation potential of these cells remain unknown. However, several pathways were found to play a key role in the regulation of these process, such as the mammalian wingless-type MMTV integration site (WNT) signaling pathway, hedgehog signaling, fibroblast growth factors, bone morphologic proteins, delta-like protein 1 or respondin family member 3, and proteins present within the adrenal extracellular matrix (ECM). The individual role which each of these regulatory factors plays in adrenal biology was recently summarized.²¹ Different populations of chromaffin stem cell/progenitor cells have been identified in adult adrenal tissues of cow,¹⁴⁷ human,²³ and mouse.²² When subjected to chronic stress, these multipotent stem cells were shown to give rise to new chromaffin cells, pointing to their active participation in adrenal stress adaptation.¹⁴⁸

Better understanding of the regulation of the function of adrenal stem cells and interaction with the adrenal microenvironment would improve our knowledge of adrenal regeneration and its adaptation to stress. For example, transplantation of adrenocortical cells *in vitro* into decellularized adrenal glands with intact ECM was shown to increase endocrine function and proliferation of transplanted cells.¹⁴⁹ Adrenal ECM has a unique composition of fibronectin, laminin, and collagen IV components that have been found to improve the cultivation of adrenal cells and, in the case of collagen, also responsiveness to ACTH.^{150,151} Although using decellularized adrenal glands may improve the functionality of the transplant and increase the number of transplanted cells, similarly to other xenotransplantations and auto-transplantations of reprogrammed stem cells there is a major challenge, which is immune reaction of the host.¹²⁹

In order to overcome this problem, an effort to create an “artificial adrenal gland” was recently attempted. In this study, a mixed population of primary bovine adrenal cells, embedded in alginate, was placed into an artificial device providing an oxygen-rich environment and simultaneous immune protection ensured by a semipermeable membrane. Upon transplantation into adrenalectomized rats, successful restoration of glucocorticoid production (cortisol) and long-term survival of the animals were observed.¹⁴⁴ Interestingly, this device was recently used in a patient with type 1 diabetes where it successfully restored the insulin production of beta pancreatic cells.¹⁵² Unique properties of the adrenal microenvironment, in particular its vast vascularization and, as a consequence, high oxygenation together with its anti-inflammatory and immunoprotective properties, make the adrenal gland suitable for transplantation. In fact, recently an intraadrenal transplantation of rat islets was performed in diabetic mice, this approach resulting in rapid normalization of glucose levels in these animals.¹⁵³

SUMMARY

Multiple direct and indirect paracrine interactions between different cell types take place simultaneously within the adrenal gland, forming a unique and flexible microenvironment. These interactions are known to regulate the function and adaptation of the adrenal gland during various pathological conditions, including obesity and sepsis. Deeper insight into the mechanisms involved in the regulation of the adrenal microenvironment should help to improve currently used therapeutic strategies for patients with adrenal insufficiency.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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