MANAGEMENT OF ENDOCRINE DISEASE

Regenerative therapies in autoimmune Addison’s disease

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Abstract

The treatment for autoimmune Addison’s disease (AAD) has remained virtually unchanged in the last 60 years. Most patients have symptoms that are relatively well controlled with exogenous steroid replacement, but there may be persistent symptoms, recurrent adrenal crisis and poor quality of life, despite good compliance with optimal current treatments. Treatment with conventional exogenous steroid therapy is also associated with premature mortality, increased cardiovascular risk and complications related to excessive steroid replacement. Hence, novel therapeutic approaches have emerged in the last decade attempting to improve the long-term outcome and quality of life of patients with AAD. This review discusses the recent developments in treatment innovations for AAD, including the novel exogenous steroid formulations with the intention of mimicking the physiological biorhythm of cortisol secretion. Our group has also carried out a few studies attempting to restore endogenous glucocorticoid production via immunomodulatory and regenerative medicine approaches. The recent advances in the understanding of adrenocortical stem cell biology, and adrenal plasticity will also be discussed to help comprehend the science behind the therapeutic approaches adopted.

Current therapeutic approaches in AAD

Autoimmune Addison’s disease (AAD) is an inevitably fatal disease in the absence of treatment, and affected patients have to take lifelong steroid replacement for survival. The synthesis of 11-deoxycortisone in 1937 and eventually cortisone and fludrocortisone has transformed AAD from a lethal condition into a chronic but manageable disease. However, the treatment for primary adrenal failure has been virtually unchanged since then, and patients with AAD have a lifelong dependency on daily steroid replacement with the ever-present risk of adrenal crisis. The common glucocorticoid replacement regimen is hydrocortisone (15–20 mg) or cortisone acetate (25–37.5 mg) taken in divided doses, twice or three times daily. The use of synthetic glucocorticoids such as prednisolone, which is deemed to give a more stable glucocorticoid effect throughout the day, has also been advocated. However, their variable pharmacodynamic pattern and potential long-term undesirable metabolic effects have rendered synthetic glucocorticoids less favoured as the first-line treatment (1, 2, 3). The synthetic mineralocorticoid, 9α-fludrocortisone, is usually taken once daily at a dose of 50–200 µg, as guided by blood pressure, electrolytes and plasma renin level. For most AAD patients, daily steroid replacement is adequate to control symptoms but does not restore them to full health. One of the main drawbacks of conventional hydrocortisone replacement therapy is its failure to mimic the physiological circadian rhythm of cortisol secretion. Endogenous cortisol production peaks in the early morning, within 1 h prior to awakening and falls progressively during the day to reach a nadir in the evening (4). Twice-daily hydrocortisone therapy results in a supra-physiological rise of cortisol concentration upon steroid ingestion followed by a rapid fall in serum levels in 4–6 h, leading to a sub-physiological serum concentration at pre-dose (5). Nevertheless, tissue cortisol concentrations do not mirror serum concentrations,
and there is no tight correlation between symptoms and serum cortisol levels (6, 7).

Novel drug formulations have been developed in the past decade attempting to mimic the physiological secretary pattern of endogenous steroids. These include the modified release formulations of hydrocortisone tablets (Plenadren; Chronocort) and continuous subcutaneous hydrocortisone infusion. Plenadren (formerly DuoCort) is a dual-release formulation with combined immediate and extended-release design allowing once-daily dosing (8, 9). Although this new formulation has been shown to be effective, demonstrating improvement in quality of life and biometabolic parameters, the trials to date have either been carried out with small sample sizes or lack a randomised control group (9, 10, 11). In particular, a direct comparison to patients taking prednisolone, which might be considered as the current standard if once-daily steroid replacement is the goal, is lacking. Similarly, Chronocort, a multi-particulate hydrocortisone formulation with twice-daily dosing has been shown to be effective in reducing androgen excess in congenital adrenal hyperplasia by restoring the end of night cortisol peak, but no study has yet been carried out in patients with Addison’s disease (12, 13, 14). On the other hand, continuous subcutaneous hydrocortisone infusion allows fine-tuning of the delivery of glucocorticoid to mimic circadian biorhythm but is still lacking of long-term efficacy and safety evidence (15, 16, 17, 18). Although each of these treatment approaches hold promise in selected AAD patient groups, particularly those with concomitant diabetes mellitus or metabolic syndrome, robust longer-term data demonstrating improved patient outcomes are desirable before these treatments are more widely adopted.

It is important to bear in mind that endogenous steroid production involves a 24-h cyclical pulsatile ultradian rhythm, with the amplitude of peaks decreasing during course of the day (19, 20). There is considerable evidence indicating that pulsatile circulating glucocorticoid levels lead to transcriptional pulsing at a genetic level via transient glucocorticoid receptor (GR) activation (21, 22). This pulsatile pattern is essential in maintaining GR-dependent transcription regulation that responds to circulating hormone levels (21, 23), in which non-pulsatile glucocorticoid level had been shown resulting in aberrant mRNA and protein levels (21). On the other hand, differences in circulating cortisol-binding globulin concentration, tissue cortisone to cortisol recycling and cortisol disposal in various physiological conditions also influence end-organ cortisol action. Hence, the lack of the physiological adaptation and secretary pattern of the conventional oral glucocorticoid replacement regimes might contribute to the metabolic abnormalities and increased cardiovascular risk among AAD patients (24, 25). In a parallel way, disruption to adrenocorticotrophic hormone (ACTH) and glucocorticoid physiological rhythm has been implicated in depression and insomnia (26, 27). This article reviews recent developments that attempt to restore endogenous glucocorticoid production using innovative treatment approaches and the future potential of regenerative therapy in AAD.

Background and natural history of AAD

AAD is a chronic condition caused by selective immune destruction of the steroid-producing cells in the adrenal cortex. It is a relatively rare endocrine condition with a prevalence of 110–140 cases per million and an annual incidence of 4.7–6.2 per million people in white populations (28, 29, 30). However, in common with several other autoimmune disorders, the incidence of AAD has been rising, with about 8500 people in the UK suffering from AAD and around 200 newly diagnosed people each year (31, 32). The common age of onset is between 30 and 50 years of age, and it is more prevalent in females (female-to-male ratio of 1.5–3.5:1) (28, 29, 31, 33, 34). It is commonly associated with autoimmune thyroid disease (~70%), Type 1 diabetes (~20%) and other autoimmune conditions, constituting the Type 2 polyendocrinopathy syndrome (APS2), which is inherited as a complex polygenic trait. Although patients survive with lifelong steroid replacement, the long-term outcome is compromised by many potential complications associated with chronic steroid replacement, particularly vertebral fracture and Type 2 diabetes. Although the life expectancy of AAD patients has been considered normal, analyses from large population-based studies have shown increased mortality with risk of premature death among young AAD patients before the age of 40, commonly due to acute adrenal crisis, infection or sudden death (25, 30, 35). In addition to the complications discussed, patients have to prepare for the ever-present risk of an unexpected adrenal crisis, which is inevitably fatal without prompt medical intervention. The average age of diagnosis for AAD is 39 years, and this results in many years of intensive health care consumption and reduced workforce capacity, with significant impact on the health economy.

Patients affected with AAD display a wide spectrum of clinical presentation and severity. AAD is commonly associated with a prolonged course of subclinical adrenal hypofunction. For instance, a patient with 9-year history
of pigmentation was found to have normal serum cortisol level with no symptoms of adrenal failure, despite having raised ACTH and 21-hydroxylase antibody levels (36). Conversely, a small number of patients progress more rapidly and present with adrenal crisis at disease onset (34). Interestingly, approximately 40% of established autoimmune Addison’s disease patients are reported to have never been hospitalised with an adrenal crisis (37, 38). A few risk factors have been identified predicting the development of overt adrenal insufficiency among asymptomatic patients with positive adrenal antibodies. These include higher 21-hydroxylase autoantibody index (39), younger age (35), HLA haplotypes, particularly DRB1*0404-DQ8 and DRB1*0301-DQ2 (40, 41), male gender and biochemical evidence of adrenal dysfunction (raised ACTH or renin level) upon discovery of adrenal antibodies (42). It is also relatively common to find cases of delayed diagnosis in AAD. A cross-sectional study found that 20% of the patients suffered for more than 5 years before being diagnosed (43). AAD has generally been thought to be a chronic irreversible condition in which patients will be dependent on lifelong exogenous steroid replacement after diagnosis. However, at least three individuals have been reported undergoing spontaneous recovery of adrenal function, either partially or fully, following years of established Addison’s disease (44, 45, 46). We also witnessed a patient with AAD (unpublished) who reported the ability to regularly miss steroid doses with little consequence. He showed spontaneous improvement in adrenal function after gradual down-titration of exogenous steroid replacement dose. He had established AAD for 10 years with no other associated autoimmune diseases. He has now discontinued exogenous steroid replacement for 2 years without adrenal crisis, despite going through inter-current episodes of diarrhoea and flu-like symptoms. His adrenal antibody is still weakly positive and both ACTH and renin levels remain elevated.

These observations have challenged our traditional concept of the natural history of AAD. Detailed studies of the plasticity of adrenal cortex have shed some light on a potential explanation for the natural history of AAD observed as well as opening up a new horizon for developing regenerative therapy in AAD.

As endocrinologists, we generally perceive the natural history of autoimmune diseases as being that of progressive end-organ destruction with the common models of Type 1 diabetes and Hashimoto thyroiditis in mind. Nevertheless, many autoimmune diseases do have a relapsing-remitting course, as seen in rheumatoid arthritis, multiple sclerosis and also in Graves’ disease, where tissue destruction is not complete and recovery of function and/or clinical remission are frequently observed.

Adrenal plasticity

The adrenal cortex is one of the most plastic tissues in the human body. For more than 60 years, it has been known that bilateral subcapsular enucleation of the adrenals in rodent models leads to the regeneration of adrenocortical mass within 4–6 weeks (47, 48). Steroidogenic function can be detected as early as day-8 after enucleation, whereas full steroidogenic capacity continues developing for up to 6 weeks (47, 49). A similar picture was also found when the adrenal cortical tissue with capsule attached was autotransplanted to a flank muscle pocket in a rodent model undergoing bilateral adrenalectomy (50). Interestingly, either hypophysectomy or administration of exogenous glucocorticoids to the rodent model after adrenal enucleation inhibited the regrowth of adrenal glands and suppressed steroidogenesis (48). This suggested that adrenal plasticity is primarily regulated by the adrenocorticotropic hormone (ACTH). Indeed, compelling clinical experience shows that both adrenal size and steroidogenic function are strongly regulated by daily pulsatile ACTH secretion. During chronic exogenous glucocorticoid therapy, ACTH secretion is suppressed leading to adrenal atrophy, and functional adrenal failure ensues if exogenous steroids are rapidly withdrawn. Conversely, adrenal gland hyperplasia is seen during Cushing’s disease after excessive pituitary ACTH secretion. These clinical observations suggest that ACTH is the main regulator of the highly plastic adrenal cell mass and adrenal steroidogenesis.

Adrenal biology and adrenocortical stem/progenitor cells (ACSCs)

Adrenal plasticity suggests the presence of adrenocortical progenitor or stem cells (ACSCs) that continuously repopulate the adrenal cortex upon stimulation by their trophic peptide hormone (ACTH₁₋₃₉). The widely accepted model generated from rodent studies suggests that ACSCs are located in the capsule or subcapsular region of the adrenal cortex (51, 52, 53, 54). After organogenesis, the adrenal cortex differentiates into 3 distinct zones: zona glomerulosa, zona fasciculata and zona reticularis. Pulse-chase studies using bromodeoxyuridine (BrdU) and thymidine-H³ showed centripetal migration of clonal cells in a radial pattern from the capsular region over time (55, 56). A transgenic mouse model utilising beta-galactosidase...
reporters under the control of a steroidogenic promoter also revealed variegated expression of reporter genes in cord-like radial stripe patterns (57). These lines of evidence suggest radial clonality of adrenocytes, with ACSCs undergoing a continuous, zone-specific differentiation programme, starting from the periphery of the gland and eventually undergo apoptosis at the corticomedullary junction (58, 59, 60). However, the major proviso is that these data were generated in rodent models, where adrenocortical organisation differs from that of human, great apes and Old World primates (i.e. rhesus macaques and baboons) by lack of zona reticularis (61, 62).

In recent years, genetic lineage tracing studies of the various adrenal morphogenic signalling pathways and transcription factors have provided insight into the potential sources and influencing factors for ACSCs (63). During the 4th week of human gestation, the adrenogonadal primordium (AGP) is formed and expresses the key transcription factor steroidogenic factor 1 (SF1). SF1 is an orphan nuclear receptor paramount in transcriptional regulation of various genes encoding the steroidogenic enzymes (i.e. cytochrome P450 side-chain cleavage enzyme and steroid 21-hydroxylase), and genes involved in the regulation of steroid biosynthetic pathway (i.e. melanocortin 2 receptor (MC2R)) (64, 65, 66). It plays a pivotal role in adrenocortical proliferation and differentiation. Adrenal agenesis was demonstrated in homozygous Sf1 knocked-out mice that died shortly after birth from adrenal insufficiency, whereas mice heterozygous for Sf1 were viable with small adrenal glands (67, 68). Similarly, no homozygous loss-of-function mutation in SF1 has been reported in humans to date, likely due to the fact that complete loss of SF1 is not compatible with life (69).

By 8th week of the embryogenesis, the bipotential AGP separates into the gonadal primordia and adrenal primordial (fetal adrenal cortex) (63). Once separated from the AGP, SF1 expression is maintained via a fetal adrenal-specific SF1 enhancer (FAdE) (70). The initiation of the development of the adult definitive cortex then takes place during week 8–9 of gestation, which becomes evident between the capsular cells and fetal adrenal cells (71, 72). The fetal adrenal cortex regresses rapidly after birth with two layers of differentiated zones in the adult adrenal cortex – zona glomerulosa and zona fasciculata – that can be distinguished at birth. However, the zona reticularis only becomes evident morphologically 3 years after birth and is complete at puberty (73).

Although the adrenal capsule/subcapsular region has been ascertained to be the stem cell niche for adult progenitor/stem cells, the phenotypic characteristics of ACSCs have yet to be fully defined. In rodent models, lineage-tracing studies using an inducible Cre transgene under the control of FAdE showed that all SF1-positive adult adrenocortical cells were derived from the fetal adrenal zone (74). However, the nature of the definitive ‘SF1 enhancer’ that maintains SF1 transcription in adult adrenal gland in humans remains elusive, and the molecular and cellular mechanism that coordinates the transition from fetal to the definitive adult adrenal cortex remains uncharacterised. The second potential marker for ACSCs was unravelled after the discovery of the Sonic Hedgehog (SHH) signalling pathway. SHH is a secreted signalling protein that acts upon target cells by binding to a twelve-pass transmembrane-binding protein, the Patched1 (Ptc1) membrane receptor (75). In the absence of the SHH protein, Ptc1 inhibits the accumulation of the seven-pass transmembrane protein, the Smoothed (Smo). When the SHH ligand binds to the cell surface receptor Ptc1, it relieves the Ptc1-mediated repression of the Smo protein, leading to downstream activation and expression of the GLI family transcription factors (Fig. 1) (76, 77). This signalling pathway is vital in the regulation of embryonic development and adult stem cell maintenance (78, 79, 80, 81). SHH co-localises with SF1 and is only found at the subcapsular region of the adrenal cortex (82). Interestingly, further lineage tracing studies revealed that SHH-expressing cells gave rise to the differentiated adrenocortical cells (82). Additionally, lineage tracing showed that the descendants of the downstream activator of the SHH pathway, GLI1, which is only found in the adrenal capsule, also gave rise to differentiated adrenocortical cells (82). Consistent with these findings, conditional deletion of Shh in steroidogenic adrenocortical cells (under the Sf1 promoter) demonstrated marked adrenal hypoplasia, with the adrenal gland being 5–10 times smaller than the wild type but with a normal-sized medulla (83, 84). Despite the clear size reduction with a thin capsule, the adrenal gland managed to maintain proper zonal organisation and steroidogenic cell differentiation (84). These observations suggest that the SHH pathway does not influence the initiation of adrenal differentiation but is essential for optimal growth of the adrenal capsule to maintain the pluripotency of adrenocortical stem cells. Nevertheless, it remains undetermined if this proliferative ability of capsular cells is due to the direct effect of SHH as a capsular cell mitogen or a secondary signal that acts on the capsule mesenchyme. Interestingly, the Shh mutant mice suffered from adrenal insufficiency when they aged,
Despite having normal glucocorticoid levels when they were young (83). Hence, it remains a possibility that humans with impaired SHH signalling might suffer from adrenal insufficiency, particularly when they grow older or when elevated steroid production is required during stress (85). In essence, ACSCs could potentially originate from two groups of cells: either those originating from the fetal adrenal cortex or GLI1-/SHH-positive cells from the capsule/subcapsular region, both of which ultimately give rise to SF1-positive, differentiated adrenocortical cells. Wood and Hammer proposed a two-lineage unifying model suggesting the potential sources of adrenocortical stem/progenitor cells (86), but the exact mechanistic and molecular basis for the definitive relationship between the two lineages remains unclear.

The regulation of adrenocortical stem/progenitor population involves a dynamic process comprising both activation and inhibition pathways, combining the exquisite orchestration of paracrine and endocrine signals to achieve an integrated homeostatic mechanism for organ maintenance. Apart from ACTH acting as the main regulator of adrenal cell mass and steroidogenesis, a few important transcription factors have been identified contributing to the maintenance of ACSCs and adrenal steroidogenesis. Firstly, SF1 has been shown to serve as both a transcriptional activator and repressor of ACTH-dependent target genes, which is well depicted in its action on pre-B-cell transcription factor 1 (PBX1) and DAX1 transcription factor. PBX1 is a direct target of SF1 and acts as the downstream activator of SF1-dependant steroidogenesis (87). DAX1 is an orphan transcription factor specifically enriched in the subcapsular region of the adrenal cortex (88, 89). It is another common target gene for SF1, and the gene product was found to serve as a repressor of SF1-dependant transactivation, leading to the inhibition of steroidogenesis in the subcapsular region of the adrenal cortex (90). This contributes to the maintenance of ACSCs in the undifferentiated state with proliferative capacity (91, 92). DAX1-deficient patients usually exhibit adrenal hypoplasia resulting in adrenal insufficiency (93, 94). The widely accepted model suggests a synergistic glucocorticoid receptor (GR)/SF1-dependant induction of DAX1 expression, which in turn leads to the repression of SF1-mediated steroidogenesis (Fig. 2) (95, 96). Binding of ACTH to MC2R stimulates adenylyl cyclase and induces cAMP production, which then unblocks both GR and SF1 from DAX1 promoters. This will then shut off DAX1 transcription and initiate adrenal steroidogenesis (95, 96). Hence, as DAX1 is only present in the subcapsular adrenal cells, steroidogenesis is inhibited in these undifferentiated progenitor cells and the ‘stemness’ of ACSCs is maintained. Interestingly, young DAX1-knockout mice initially exhibited a hyperfunction adrenal state consistent with the loss of DAX1-dependant

**Figure 1**
Depicts the gene expression and signalling pathways that regulate adrenal progenitor cells and adrenal steroidogenesis. DAXI inhibits the activation of SF1-mediated steroidogenesis and maintains the cells in an undifferentiated state. ACTH induces the clearance of glucocorticoid receptor (GR) and SF1 from the DAX1 promoter, resulting in transcriptional silencing of the DAX1 gene and initiation of SF1-mediated steroidogenesis. SF1/DAX1 also participates in tissue fate decisions through dynamic regulation of GATA4/inhibin and GATA6 transcription factors in adrenals and gonads. SHH is secreted from the subcapsular cell to activate SHH signalling in the capsule by binding to the PTCH1 transmembrane receptor, leading to downstream activation and expression of the GLI1 family transcription factors.
inhibition of steroidogenesis (97) but was followed by the attenuation of steroidogenic capacity when they aged, with profound loss of the subcapsular region, and concomitant adrenal dysplasia was observed (97, 98). This model is in keeping with a number of patients with DAX1 mutations demonstrating enhancement of steroidogenic function state prior to the development of adrenal failure in later childhood (99, 100).

Overall, there is compelling evidence to support the presence of ACSCs and we will discuss our experience attempting to restore adrenal steroidogenesis by manipulating adrenal plasticity using various therapeutic approaches.

Regenerative medicine in autoimmune Addison’s disease

AAD is a disease with insidious onset and plasma ACTH concentrations rise in the preceding months to years before the diagnosis of AAD. The adrenal cortex is known to contain high concentrations of steroids (in the micromolar range), and this local steroidogenesis might ensure a glucocorticoid-induced immune privilege, in which adrenal antigen presentation within the adrenal cortex will be suppressed by the high ambient cortisol concentration (101).

In addition, local steroidogenesis exerts potent anti-inflammatory action by suppressing pro-inflammatory cytokines and chemokines secreted by steroid-producing adrenocytes. These include IL-1, IL-6, IL-8, IL-18 and TNF-α, IFN-γ and CXC Chemokine Ligand 10 (CXCL10), which have a key role in the immune-endocrine crosstalk to regulate steroidogenesis (102, 103). Hence, any reduced local glucocorticoid production could result in enhanced pro-inflammatory state and loss of immune tolerance to adrenal antigens. Antigen presentation will be enhanced followed by inflammatory infiltration in the adrenal cortex, leading to adrenal cell destruction. This will then promote further adrenal antigen presentation, inflammatory cell recruitment and a spiral of adrenal cell destruction, failing steroidogenesis with lower intra-adrenal glucocorticoid concentrations. Compensatory increases in the ACTH concentration by the pituitary will help to regenerate and repopulate adrenocortical cells in response to the immune destruction. However, when adrenocortical cell renewal is out-balanced by adrenocortical destruction, adrenal insufficiency will ensue and correspond to the overt clinical phase of AAD.

Our group has undertaken a few studies attempting to modify the factors contributing to the pathophysiology of AAD, aiming to restore adrenal steroidogenesis. We first undertook a clinical study using B lymphocyte-depleting therapy with anti-CD20 antibodies (104) to modulate the immune response in 6 newly diagnosed AAD patients. We observed a rapid and significant suppression of the adrenal function after the introduction of exogenous steroid therapy, within a month of being diagnosed with AAD. On average, there was a reduction in serum cortisol levels of more than 50% in 3 weeks after diagnosis and starting glucocorticoid replacement (104). The removal of trophic influence of ACTH after exogenous steroid therapy, within a month of being diagnosed with AAD. On average, there was a reduction in serum cortisol levels of more than 50% in 3 weeks after diagnosis and starting glucocorticoid replacement (104). The removal of trophic influence of ACTH after exogenous steroid could partly lead to the rapid decrease in adrenal steroid biosynthesis. In this study, one out of six patients demonstrated a progressive rise in serum cortisol and aldosterone secretion. She was able to discontinue steroid replacement 15 months after her initial B cell depletion treatment and subsequently had an extended 17-month ‘remission’ of her adrenal failure. This study suggests that the natural history of AAD is modifiable and that Addison’s disease is potentially remediable by disease-modifying drugs targeted at the immune response. This is in line with
Figure 3
Represents a highly simplified schematic overview of potential immunomodulatory and regenerative therapeutic approaches in autoimmune Addison's disease (AAD).

Potential novel therapeutic approaches in AAD
1. High-dose ACTH1-24 therapy in AAD (108)
2. Immunomodulatory therapy- B-cell depletion therapy in AAD (104)
3. Cellular reprogramming/ manipulation of adrenocortical progenitor/stem cells – cell line/ animal studies only; no clinical study available (112-120)
Type 1 diabetes mellitus and Graves’ ophthalmopathy, in which immunomodulatory drugs administered early on in natural history have also shown some success (105, 106, 107). The low but detectable serum cortisol at diagnosis demonstrates the persistence of adrenal steroidogenesis in some patients, which has the potential to be rescued by either immunomodulatory or regenerative approaches.

AAD exhibits a potential for regenerative medicine therapy as a disease model in several ways (Fig. 3). Firstly, the adrenal cortex harbours adrenocortical progenitor/stem cells that continuously repopulate the adrenal cortex under ACTH influence. Secondly, the intrinsic plasticity of the adrenal cortex is potentially preserved in AAD as ACSCs may be ‘invisible’ to the immune response owing to the lack of steroidogenic enzyme expression. In a separate study, we performed a 20-week trial of high-dose ACTH$_{1-24}$ (1 mg tetracosactide) therapy in 13 patients with established autoimmune Addison’s disease (108). Two out of the 13 patients had significant residual adrenal function at baseline (serum cortisol $>100$ nmol/L; $<200$ nmol/L) and showed significant improvement in steroidogenic function during ACTH therapy (Fig. 2). Both of these patients were able to withdraw oral glucocorticoid and mineralocorticoid replacement during the study, but one of the patients had a progressive decline in steroidogenic function once ACTH treatment was discontinued, requiring reinstatement of glucocorticoid replacement after a 28-week steroid-free period. The other patient remains well without steroid replacement after ACTH therapy was stopped for 5 years. Nevertheless, despite good steroidogenic function with a peak cortisol level of $>400$ nmol/L on synacthen stimulation testing, she still does not have normal adrenal function to date, with persistently elevated plasma ACTH and renin levels on follow-up. The local glucocorticoid concentrations generated after ACTH therapy might be high enough to dampen the immune response against the adrenal cortex in this patient, and hence, maintained a good level of adrenal steroidogenesis, despite subnormal adrenal function with elevated plasma ACTH and renin levels.

This study highlights that steroidogenic function in patients with established AAD is potentially salvageable and that residual adrenal function is therefore important as a future therapeutic target in AAD. This may also explain why patients with spontaneous recovery of established Addison’s disease have occasionally been reported, (44, 45, 46) likely owing to endogenous ACTH stimulation of residual adrenal function once the patients had reduced or stopped their steroid medication (against medical advice). Interestingly, residual adrenal function did not correlate with the duration of disease and useful residual adrenal steroidogenic function was noted in patients diagnosed with AAD for 4 years or more, rather than within 2 years of diagnosis. One previous study of autoimmune Addison's disease patients showed increases in serum cortisol in 10 of 27 patients during tetracosactide testing, despite prior dexamethasone administration (0.5 mg twice) (109). Along with our own data, this suggests that low-level residual steroidogenic function may be present in up to 30% of autoimmune Addison’s disease patients.

Residual adrenal function in patients with established AAD is comparable with residual islet β-cell function, which is well described in about 20% of patients with autoimmune Type 1 diabetes. This represents a parallel state of partial destruction of the target organ in an autoimmune disease process. Residual insulin secretion in Type 1 diabetes patients has been correlated with improved glycaemic control and fewer microvascular complications (110, 111). Interestingly, approximately 40% of autoimmune Addison’s disease patients are reported to have never been hospitalised with an adrenal crisis, and this could be owing to low-level residual adrenal function. Residual islet β-cell function is marked biochemically by insulin C-peptide level, but we have yet to identify a good biochemical marker to quantify or assess residual adrenal function. This is clinically important as we need to understand more about the heterogeneity of the natural history of AAD. The adrenal function of those with ‘useful’ residual adrenal function might also be remediabile by therapeutic approaches other than exogenous steroid replacement. Hence, it is important to identify good biochemical markers of residual adrenal function to enable this patient group to be distinguished in the future.

**Future therapeutic approaches**

Our current approach is aimed at manipulating endogenous adrenocortical stem cells to enhance adrenal steroidogenesis. There have been some studies looking at the possibility of cellular reprogramming for cell-based therapy in AAD. However, there are significantly fewer studies in AAD as compared to the evaluation of stem cell-derived islet beta cell replacement therapy in Type 1 diabetes. In recent years, several studies attempted to produce cells resembling adrenocortical cells from human and murine cell sources. The cells of origin comprised embryonic or bone marrow-derived stem cells (including induced ‘iPS’ from fibroblasts), as well as mesenchymal cells derived from adipose tissue or umbilical cord.

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(112, 113, 114, 115, 116, 117, 118, 119, 120). Although these cell sources demonstrated their capability to be reprogrammed to steroidogenic phenotypes by the overexpression of SF1 and cAMP treatment, none fully resembled adrenocortical cells with a complete endogenous steroid profile. This is likely due to the fact that cellular reprogramming to adrenocortical-like cells involves a complex interplay between various transcription factors and signalling pathways (i.e. DAX1, SHH etc.), which will never be achieved by SF1 overexpression solely. Cell-based therapy in AAD is certainly still at an early pre-clinical stage, with a lack of evidence from an in vivo adrenal insufficiency model. There are also safety concerns with regards to forced expression of SF1 exogenously using a vector or adenovirus. Regenerative medicine therapies employing stem cell biology have potential promise for definitive treatment of AAD in the future, but more efforts are required to unravel the key factors in adrenal cell reprogramming to render cell-based therapy a reality in AAD.

Declaration of interest
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