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Dehydroepiandrosterone as a regulator of immune cell function☆

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ABSTRACT

Dehydroepiandrosterone (DHEA) is a C19 steroid of adrenal origin. Notably, its secretion declines with age, a phenomenon referred to as the "adrenopause". For many years, the physiological significance of DHEA remained elusive. However, many studies have now shown that DHEA has significant immune modulatory function, exhibiting both immune stimulatory and anti-glucocorticoid effects. Although several of these studies are limited by the fact that they were carried out in rodents, who are incapable of adrenal DHEA production, and therefore have very low circulating levels of this steroid, evidence from the study of immune cells is now accumulating to suggest a role for DHEA in regulating human immunity. This ability to regulate immune function has raised interest in the therapeutic potential of DHEA as a treatment for the immunological abnormalities that arise in subjects with low circulating levels of this hormone. This has included attempts at reversing the impaired immune response of older individuals to vaccination and restoring immune regulation in patients with chronic autoimmune disease. This review summarises the reported effects of DHEA on immune function and discusses the therapeutic potential of this steroid in geriatric medicine and particularly in age-related disease with an immune component.

1. Introduction

Dehydroepiandrosterone (DHEA) is a pregnenolone derived C19 steroid that is synthesised predominantly in the zona reticularis of the human adrenal gland by the steroidogenic enzyme CYP17A1, formerly P450c17 [1]. In response to stimulation by the endogenous hormone adrenocorticotrophin, DHEA is secreted from the adrenal cortex alongside its sulfated precursor DHEAS. Together, these steroids are the most abundant in human circulation, reaching peak concentrations of approximately 10 μM (DHEAS) and 10 nM (DHEA) during the third decade of life [2–4], with the dominance of DHEAS attributable to its slower metabolic clearance rate and longer half-life [5,6]. Once maximal levels of DHEAS are attained, serum concentrations undergo a steady age-related decline (Fig. 1), such that by 70 years of age, DHEA/DHEAS levels are 20–30% of those achieved in early adulthood [7–9]. This fall, termed the "adrenopause" occurs in both sexes and is associated with a reduction in the size of the zona reticularis [10]. Interestingly, current evidence suggests the "adrenopause" to be a phenomenon confined to higher primates [4,11,12], which may have specific implications for health in old age in these species. However, such exclusivity may be a reflection of the limited number of species in which age-associated alterations in DHEA/DHEAS levels have been investigated.

To date, no biological function has been identified for DHEAS independent of its role as a precursor to DHEA, whilst for many years the only role attributed to DHEA was that of an intermediate in sex steroid biosynthesis [[13], Fig. 2], where it acts a precursor to approximately 30–50% of circulating androgens in men and 100% of circulating estrogens in postmenopausal women [7,14]. However, a number of studies have now revealed that in addition to its role as a sex steroid precursor, DHEA is a potential regulator of immune function, eliciting immune stimulatory effects *in vivo*, often counteracting the immune-suppressive effects of glucocorticoids [15]. As a result and considering the significant decline in DHEA levels that occurs with age, it has been suggested that the fall in DHEA may in part explain the deterioration of immune function that accompanies human aging [16–18]. Moreover, it has been proposed that DHEA supplementation may be a novel and inexpensive approach by which to reverse or minimise immune senescence. Due to its immune modulatory effects, DHEA is also being proposed as a potential therapeutic option for the treatment of autoimmune and chronic inflammatory diseases, as these have been shown to be associated with low circulating DHEAS levels.

In this brief review, we will summarise those studies that have assigned an immune-regulatory role to DHEA and examine the evidence to support DHEA supplementation as a route to reversing the immune dysregulation that arises with DHEA deficiency.

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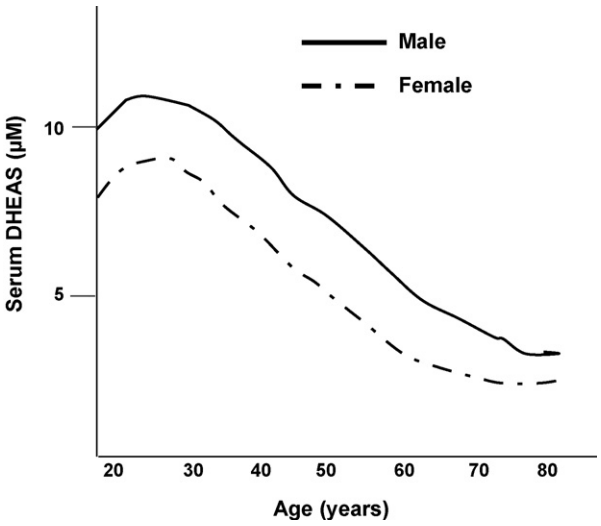

Fig. 1. Human adrenopause. The figure is a schematic representation of the decline in serum DHEAS levels with age in men and women, termed adrenopause, showing that maximum circulating levels are seen at age 20–30 years and thereafter levels gradually decrease.

2. DHEA and immune cell function

The first section of this review will discuss *in vitro* [19–33] and *in vivo* murine-based studies [34–42] that have shown DHEA to elicit both immune stimulatory and anti-glucocorticoid effects. Despite highlighting novel roles for this steroid, an important caveat of these studies is that many have employed DHEA at concentrations exceeding those found in human circulation. This is particularly relevant to rodent studies, where DHEA is administered in pharmacological doses in order to compensate for the inability of rodent adrenals to synthesise DHEA [43]. Thus, care should be taken when attempting to relate findings from rodent studies directly to humans.

2.1. Cytokine production

Most of the data that has attributed an immune modulatory role to DHEA comes from studies that investigated its effect upon cytokine secretion. As shown in Table 1, DHEA regulates cytokine production from both myeloid and lymphoid cells, with the majority of reports showing this steroid to be a potent enhancer of interleukin (IL)-2 secretion from T helper (T_H)-1 cells. Of note, Suzuki et al. demonstrated that human T cells directly exposed to physiological concentrations of DHEA (10^{-9} M) exhibited increased IL-2 secretion upon activation by various stimuli [19], findings that are in agreement with the earlier work of Daynes et al. [36], which reported lymphocytes from DHEA-treated mice to produce greater

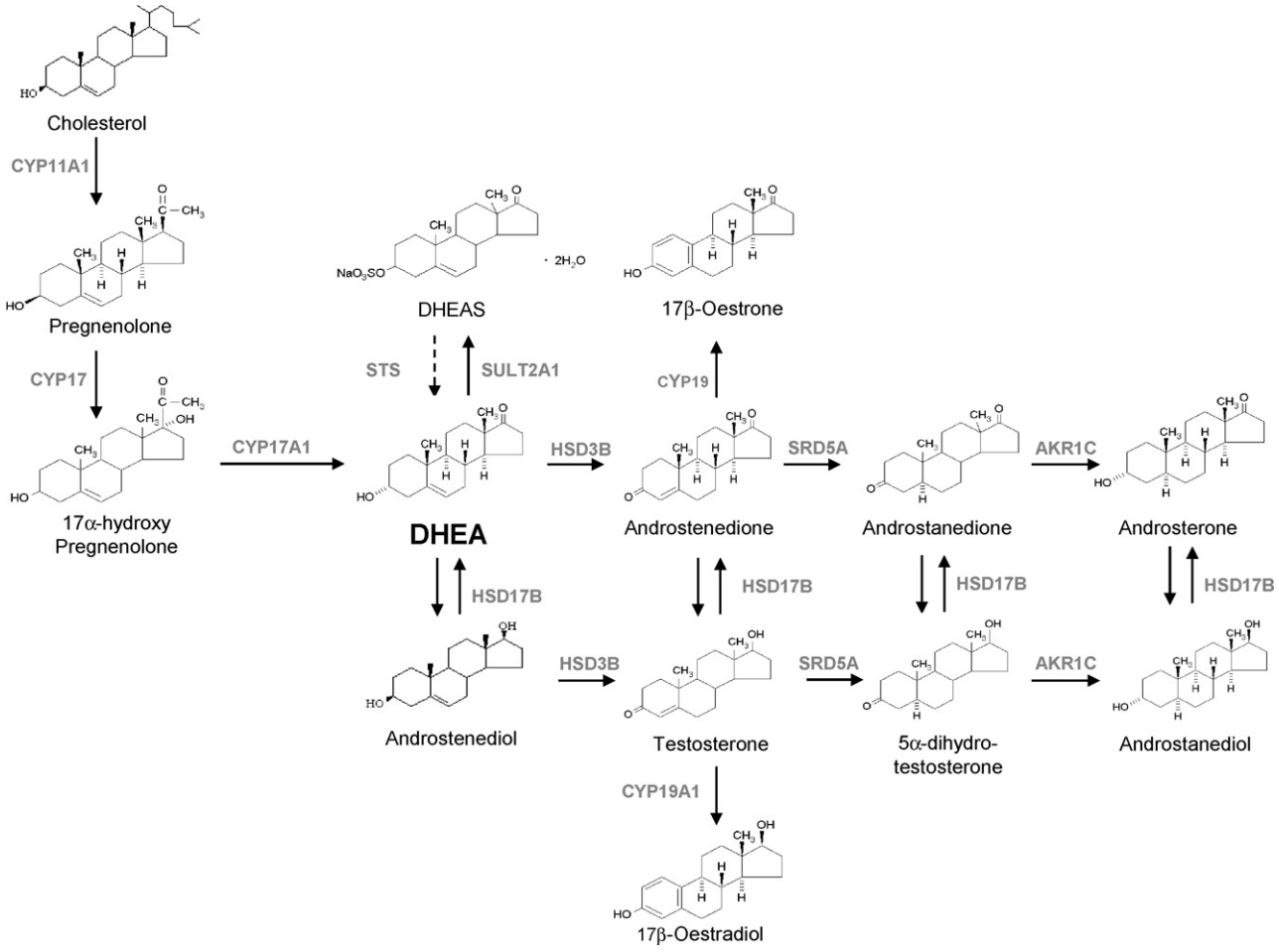

Fig. 2. Dehydroepiandrosterone (DHEA)—a crucial precursor in sex steroid biosynthesis. STS, steroid sulfatase; SULT2A1, sulfo-transferase; CYP11A1, Cytochrome P450scc; CYP17A1, Cytochrome P45017α; CYP19A1, P450 aromatase; HSD3B, 3β-hydroxysteroid dehydrogenase isoenzymes; SRD5A, 5α-reductase isoenzymes; AKR1C, 3α-hydroxysteroid dehydrogenase isoenzymes; HSD17B, 17β-hydroxysteroid dehydrogenase isoenzymes.

Table 1
Summary of studies investigating the effect of DHEA/DHEAS treatment on cytokine secretion from murine and human immune cells.

Reference	Model	Cell type	DHEA treatment	Cytokine	Effect
[18]	*In vitro*	Human PBMC	24 h at 10^{-6} to 10^{-9} M	IL-6	↓
[19,21]	*In vitro*	Human T lymphocytes	48 h at 10^{-8} to 10^{-11} M	IL-2	↑↑
[20]	*In vitro*	Murine lymphocytes		IL-2	↑
[25,26]	*In vitro*	Murine splenocytes	24–72 h at 10^{-5} to 5×10^{-9} M	IL-2, IL-3, IL-4, IFNγ	↓↓
[33]	*In vitro*	Murine splenocytes	48 h at 6 μM with ConA or LPS stimulus.	IL-1, IL-2, IFNγ	↓
				IL-10	↑
				TNFα, IL-4, IL-6	No effect
[24,41]	*In vitro*	Murine peritoneal cells/macrophages	DHEA (10^{-6} to 5×10^{-9} M) + LPS stimulus	TNFα, IL-1β	↓↓
[22,36,37,42]	*Ex vivo*	Murine splenocytes	s.c. or i.p. 1–3 days prior to cell isolation	IL-2, IL-3, IL-4, IFNγ	↑↑
				IL-10	↓
[17,23]	*Ex vivo*	Murine splenocytes	Oral for 3 or 12 weeks prior to cell isolation	IL-2, IFNγ	↑
				IL-4, IL-6, IL-10, TNFα	↓
[16]	*Ex vivo*	Mesenteric lymph node lymphocytes	s.c. 24 h prior to cell isolation.	IL-6	↓

Abbreviations: i.p., intra-peritoneal; s.c., subcutaneous; IL, interleukin; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma.

levels of IL-2 upon stimulation than control. Whilst promoting cytokine secretion from T_H-1 cells, DHEA negatively regulates the production of the T_H-2 cytokines IL-6 [16,18,23] and IL-10 [17]. Consequently, the fall in circulating DHEA levels that accompanies physiological aging (Fig. 1) has been proposed to contribute to the dysregulated cytokine balance (reduced T_H-1 cytokine production, increased T_H-2 cytokine production) seen in older individuals [16–18]. However, IL-6 levels are elevated in the serum of aged mice [16], whilst splenocytes from aged mice spontaneously secrete IL-10 at levels higher than those produced by splenocytes from younger mice [17] despite the fact that these animals do not undergo "adrenopause". Although these data suggest that the age-related alterations to T_H cell cytokine responses may not be the direct result of a decline in DHEA/DHEAS with age, interestingly DHEAS supplementation in mice reversed these age-related alterations in cytokine production [16,17]. Thus, these findings support a possible role for DHEA supplementation in reversing immune decline in old age in humans and also in the treatment of age-related conditions where a skewing of the immune response towards a T_H-2 cytokine profile could contribute to pathology, such as poor resolution of mycobacterial infections. Dysregulation of both IL-6 and IL-10 synthesis is also believed to underlie the pathology of a number of autoimmune diseases, including rheumatoid arthritis [44] and systemic lupus erythematosus (SLE) [45]. Consequently, DHEA/DHEAS supplementation may also be of therapeutic value for the management of these conditions, which have been shown to be associated with low DHEAS (see Section 4.2).

Other pro-inflammatory cytokines negatively regulated by DHEA include TNF-α [24,41] and IL-1β [24]. In two models of endotoxin shock, the protective effects resulting from exogenous administration of DHEA were attributed to its ability to significantly reduce lipopolysaccharide (LPS)-mediated increases in TNF-α secretion [24,41]. Exactly how DHEA elicits its anti-inflammatory effect with respect to cytokine production is still unclear, although evidence indicates it may be mediated in part through its ability to inhibit the activation of the nuclear transcription factor NF-κB pathway [46,47]. Du et al. [46] demonstrated the significant reduction in TNF-α secretion they observed in DHEA-treated splenocytes to be associated with a decrease in both the activation and nuclear translocation of NF-κB [46]. Similarly, Poynter and Daynes [47] reported DHEAS administration to reduce both NF-κB activity and spontaneous IL-6 production in the spleens of aging mice.

2.2. Lymphocyte proliferation

The published reports on the effect of DHEA on lymphocyte proliferation are contradictory. Whilst some studies have shown that pre-treatment of murine splenocytes with DHEA can increase both concanavalin (ConA) and LPS-induced proliferation [22,23], other studies employing mixed splenocyte cultures [24,25,28,29] or purified human lymphocytes [27] have demonstrated an anti-proliferative activity for DHEA. This conflicting evidence may be due to differences in technical procedure, in particular the concentrations of DHEA used and the variations in the length of time cells were exposed to the steroid. However, what is noticeable is that in those studies that employed a dose of DHEA close to the physiological concentration, proliferation was enhanced [22,27], whereas supraphysiological doses produced an inhibition of proliferation [28,29].

2.3. Immune cell cytotoxicity

In a recent prospective study, Tomlinson et al. [48] reported respiratory infections to be a major cause of excess mortality in patients with hypopituitarism, which included subjects suffering from secondary adrenal insufficiency (AI). With AI a condition characterised by near total DHEA deficiency [49], the susceptibility of these patients to infection indicates a link between DHEA and immunity. Respiratory infections are caused by both viral and bacterial pathogens, and there is evidence that DHEA/DHEAS can enhance immune responses to both classes of pathogens.

Anti-viral immunity is mediated primarily by cytotoxic T lymphocytes and natural killer (NK) cells, which induce cell death in infected cells. The efficiency of these cells is enhanced by the release of pro-inflammatory cytokines derived primarily from monocytes/macrophages. Human-based *in vitro* studies support a role for DHEA in regulating immune cell cytotoxicity. Suzuki et al. [19] observed that T lymphocytes exposed to physiological concentrations of DHEA (10^{-9} M) mediated more potent cytotoxicity following antigenic stimulation than control, with further investigation attributing this enhancement to a DHEA-mediated increase in IL-2 secretion [19].

In monocytes, DHEA treatment has been shown to modulate LPS-induced cytotoxicity [31,32]. In an elegant study, McLachlan et al. [31] demonstrated that monocytes stimulated with either LPS (0.2 ng/ml) or DHEA (10^{-7} to 10^{-11} M) alone showed no signs of activation, but in combination these agents induced the expression of a number of cytotoxic markers, including increased secretion of pro-inflammatory cytokines and enhanced cytotoxicity against tumour cell lines [31]. This ability of DHEA to lower the threshold for LPS-induced activation of monocytes suggests the presence of this hormone at sites of infection may allow for the more efficient killing of infectious agents [31].

DHEAS has also been shown to regulate immune cell cytotoxicity. In a series of *in vitro* assays, Solerte et al. [50] reported DHEAS treatment to increase NK cell cytotoxicity in a dose dependent manner by enhancing NK cell production of insulin-like growth factor I

(IGF-I) [50]. However, it is currently unknown whether NK cells like macrophages, express functionally active steroid sulfatase capable of metabolising DHEAS to DHEA [51], and therefore one cannot rule out the possibility that these effects were mediated through conversion of DHEAS to DHEA within the cell.

In relation to bacterial infections, our own studies have shown that neutrophil bactericidal function declines with age, paralleling the loss of DHEA/DHEAS in adrenopause [52]. Moreover, in a study of post-surgical infections in elderly patients with hip fracture, we found an association between a low DHEAS:cortisol ratio and a higher incidence of bacterial infections, with the largest proportion involving respiratory infections [53]. Importantly, preliminary *in vitro* studies showed that incubation of primed neutrophils with cortisol inhibited neutrophil bactericidal function (superoxide generation) and that this could be overcome by co-incubation with a physiological concentration of DHEAS [53]. Clinical trials involving DHEA replacement are now urgently required to determine if this *in vitro* effect can be repeated *in vivo* with obvious clinical benefit for older patients following surgery.

2.4. DHEA—an antagonist of glucocorticoid induced immune suppression

As our studies in neutrophils suggest [53], as well as enhancing immune function directly, DHEA/DHEAS has also been shown to have indirect benefits for immunity by counteracting the immune-suppressive effects of glucocorticoids.

2.4.1. Thymic involution

Thymic involution is a well described feature of immune aging, with the reduced ability to produce naive T cells resulting in an increased memory:naive T cell ratio and consequently an impaired response to new antigenic challenges, including vaccinations [54]. In a murine model of stress-induced thymic involution, Riley [55] were the first to propose an anti-glucocorticoid effect for DHEA. The group demonstrated corticosterone levels in mice subjected to "rotational stress" to be elevated when compared to control, and that this increase was associated with thymic involution. Corticosteroids induce thymic involution primarily by the induction of apoptosis in thymocytes developing within the thymus. Supplementation with DHEA prior to stress exposure reduced these involution effects [55]. In other murine studies, DHEA has also been shown to protect against dexamethasone (DEX) induced thymic involution [34,35]. Of note, May et al. reported that a single subcutaneous injection of DHEA (1.6 mg) 3 days prior to DEX treatment could reduce thymic involution by approximately 50% [34]. These authors showed that this effect was likely to be due to DHEA inducing enhanced survival of thymocytes, as thymocytes isolated from the DHEA-treated mice were more resistant to DEX-induced apoptosis [34]. However, it should be noted that this anti-apoptotic effect of DHEA was not observed when thymocytes were treated simultaneously with DHEA and DEX *in vitro* [34]. Therefore, this ability of DHEA to confer protection on thymocytes only *in vivo* suggests that its effects are indirect and may involve either generation of survival factors from other cells within the thymus (thymic epithelial cells), or require metabolism of DHEA to active downstream products (see Section 3.1).

2.4.2. Cytokine secretion

Glucocorticoid treatment of T lymphocytes has contrasting effects on cytokine secretion: suppressing IL-2 production whilst enhancing IL-4 production [56]. Exposure to DHEA reverses this trend in IL-2 secretion. In an informative study, Daynes et al. demonstrated that the suppressive effects on IL-2 production resulting from increased glucocorticoid levels *in vivo* could be overcome by DHEA administration [36]. Similarly, exposure of lymphocytes *in vitro* to DHEA prevented glucocorticoid-induced inhibition of IL-2 secretion from activated T cells [37].

2.4.3. DHEA, glucocorticoids and infection

In the first study of its kind, Loria et al. [38] demonstrated that a single subcutaneous injection of DHEA could increase survival rates in a murine model of lethal viral infection by 32%. Although the group did not identify the molecular basis for this increase in host resistance, they did propose that DHEA-mediated protection by counteracting the effects of increased glucocorticoid levels that accompany viral infection [38]. In support of this theory, Ben-Nathan et al. revealed through a model of stress-enhanced viral encephalitis [39], that DHEA administration prevented glucocorticoid-induced involution of lymphoid organs and subsequent immunosuppression, as evidenced by the lower virus levels in the blood and brain of DHEA-treated mice compared to control [39].

2.4.4. How does DHEA mediate its anti-glucocorticoid effects?

In spite of the protection conferred by DHEA against the immune-suppressive effects of glucocorticoids, its mechanism of action is still not fully understood. However, there are several possibilities. Whilst DHEA is unable to bind directly to the androgen receptor, it is a precursor to a range of androgens, including both testosterone and 5-α-dihydrotestosterone (Fig. 2). With evidence demonstrating that both the androgen and glucocorticoid receptor recognise and bind to the same hormone response element [57], it may be that by activating the androgen receptor, DHEA-derived androgens compete with the glucocorticoid receptor (GR) for binding to glucocorticoid-regulated target genes. That DHEA may compete directly with glucocorticoids for occupancy of the GR or interfere with GR trafficking has been ruled out by studies performed in hepatocytes [58] and transfected COS-7 cells respectively [59]. However, the lower glucocorticoid levels present within immune cells may allow DHEA to function as a competitive inhibitor of glucocorticoid and GR interaction. In addition to influencing the interaction of an activated GR with its target genes, studies in neuronal cells have shown exposure to estrogen (another downstream metabolite of DHEA) significantly reduces transcription of the GR gene [60], suggesting DHEA may regulate indirectly the number of intracellular glucocorticoid receptors.

At the tissue level, glucocorticoid exposure is regulated in part by two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD), namely 11β-HSD1 and 11β-HSD2. *In vivo* 11β-HSD1 functions predominantly as an oxo-reductase generating biologically active cortisol from inactive cortisone [61]. In contrast, 11β-HSD2 functions exclusively as a dehydrogenase, catalysing the conversion of cortisol to cortisone [62]. In an elegant study, Apostolova et al. [63] demonstrated that DHEA could significantly reduce both the mRNA levels and activity of 11β-HSD1 in adipose tissue, whilst Balazs et al. revealed DHEA treatment of renal cells markedly enhanced both the mRNA expression and activity of 11β-HSD2 [64]. With current evidence suggesting 11β-HSD1, and not 11β-HSD2 is expressed in a number of immune cells, including lymphocytes [65], dendritic cells [66] and macrophages [67], we speculate that DHEA may exert an anti-glucocorticoid action upon immune cells by modulating local production of active glucocorticoids. Whether such inhibition of 11β-HSD1 would be achieved through the direct actions of DHEA or require its metabolism to downstream products is currently unknown, although recent work favours the latter scenario. In two studies, Morfin's group demonstrated 7α-hydroxy-DHEA to be a substrate for 11β-HSD1 [68], and that the presence of this metabolite reduced 11β-HSD1-mediated production of active cortisol [69]. Taken together, these studies suggest a role for DHEA and/or its metabolites in regulating the activity

of glucocorticoids at a pre-receptor level and provide a future area of investigation for groups interested in the mechanisms by which DHEA counteracts the immune-suppressive actions of glucocorticoids.

3. How does DHEA mediate its immune-enhancing effects?

A current obstacle to defining the extent of the biological function of DHEA and DHEAS is the lack of a fully characterised and specific receptor for these major steroids [70]. In line with the classic theory of steroid action, intracellular binding sites for DHEA have been identified in both murine [20] and human [21] T lymphocytes, suggesting DHEA regulates immune function through a receptor dependent process. Indeed, T cell clones that lack expression of this DHEA binding site are unresponsive to DHEA [20]. However, in both the aforementioned studies [20,21], the addition of 5-α-dihydrotestosterone to the binding assay displaced bound DHEA from the receptor complex. Thus, it seems increasingly likely that DHEA mediates its actions via interaction with a range of receptors and signalling pathways rather than through interaction with a specific receptor [70].

In addition to signalling through ligand dependent transcription factors, steroids can also exert their physiological effects through interaction with plasma membrane associated receptors [71,72], and recent studies on endothelial cells have identified such a receptor for DHEA [73,74]. This receptor exhibits both high affinity (K_d 48.7 pM) and specificity for DHEA, and is coupled to G proteins [73,74]. Further investigations have revealed activation of this receptor to elicit rapid cellular signalling [74,75], and since many of the immune modulatory effects of DHEA are observed only hours after exposure to the steroid, this suggests that a membrane-bound receptor for DHEA may be present on the surface of immune cells, though this remains to be confirmed.

3.1. Evidence for the indirect action of DHEA on immune cell function

As DHEA is a precursor to both androgens and estrogens (Fig. 2), the immune-enhancing effects observed following DHEA treatment could be mediated by the actions of a downstream steroid. In support of this notion, a series of studies have shown downstream derivatives of DHEA to possess immune modulatory activities superior to those of their parent steroid. For example:

- In a mouse model of lethal Coxsackievirus B4 infection, subcutaneous injection of the DHEA metabolites androstenediol or androstenetriol conferred 100% protection against viral-induced mortality, whilst DHEA offered no protection [76,77].
- *In vitro* activation assays revealed that androstenetriol markedly enhanced both the proliferation and cytokine secretion of mitogen-stimulated lymphocytes above the levels observed following DHEA treatment. Furthermore, unlike DHEA, androstenetriol counteracted the suppressive effects of glucocorticoid treatment on Con A induced lymphocyte proliferation [25].
- The addition of the DHEA metabolite 7α-hydroxy-DHEA to a co-culture of tonsil derived B and T lymphocytes significantly increased the production of antigen specific antibodies by B cells. DHEA on the other hand had no effect [78].

Furthermore, steroidogenic enzymes involved in the downstream conversion of DHEA are expressed and functionally active in a range of human immune cells, including macrophages [79], lymphocytes [80] and peripheral blood mononuclear cells [81], suggesting DHEA metabolism within the immune cell is involved in mediating its immune-enhancing effects.

4. DHEA supplementation

The second part of this review aims to summarise recent studies performed in both rodents [22,82–91], and humans [92–105], that have investigated whether DHEA supplementation can reverse the immune dysregulation that occurs coincident with DHEA deficiency.

4.1. DHEA and aging

Physiological aging coincides with a fall in circulating DHEA/DHEAS levels (Fig. 1). After reaching peak concentrations in the third decade of life, serum levels of both hormones decrease at a rate of approximately 1–2% per annum, such that by 70 years of age, DHEA/DHEAS levels are 20–30% of those achieved in early adulthood [7,9]. Alongside this fall in DHEA/DHEAS levels is a decline in immune function termed immune senescence [106], symptoms of which include: a decline in IL-2 secretion from stimulated T cells and a shift towards a Th2 cytokine profile [106], decreased proliferation of T cells in response to antigenic challenge [107], reduced neutrophil bactericidal function [108] and a reduction in NK cell cytotoxicity [109]. Due to the known immune modulatory effects of DHEA, some researchers have suggested an endocrine link to immune senescence [16–18], and that DHEA supplementation may be a novel approach by which to reverse the immune senescent phenotype. Here we highlight those studies that have examined this theory in old (>65 years) and middle-aged humans.

4.1.1. DHEA as a vaccine adjuvant

Aging individuals elicit a poor immune response when challenged with foreign antigens, which in the case of vaccination prevents the development of long lasting protective immunity [110,111]. For example, less than half of adults over the age of 65 years produce a protective antibody response to the annual influenza vaccination. To increase vaccine efficacy in the elderly, researchers have attempted to reverse their impaired immune response by administering DHEA as a vaccine adjuvant.

In animal studies, this approach has proved successful, with DHEA administration augmenting the immune response of aged mice to a range of vaccines [82–84]. Araneo et al. [82] demonstrated that DHEA treatment could promote intense primary and secondary antibody responses against recombinant hepatitis B surface antigen. Similarly, Danenberg et al. reported a single subcutaneous injection of DHEA 4 h prior to immunization could significantly increase the humoral response to influenza vaccination [83], which notably led to increased host resistance when challenged with live influenza virus. When summarising, Danenberg et al. proposed this increased resistance to be the result of a DHEA-mediated decrease in the circulating levels of the T helper type 1 cytokine IFN-γ [83], suggesting DHEA can reverse the increase in IFN-γ levels that accompanies physiological aging.

Data from human studies however are less impressive. On the back of their successful animal model [83], Danenberg et al. studied the effect of DHEA treatment on the immune response of seventy one old subjects (aged 61–89 years) to influenza vaccination [92]. DHEA was administered orally to participants at a dose of 50 mg for four consecutive days (starting 2 days before vaccination) and the antibody response to the vaccine measured 28 days post vaccination. Surprisingly, the group reported a significant decrease in the levels of protective antibody titres attained by subjects who received DHEA compared to those assigned to placebo treatment [92], a finding the group replicated in a second prospective study 1 year later [93]. In another randomised double blind study, Evans et al. [94] investigated whether DHEAS supplementation could enhance the immune response of sixty six elderly

individuals (median age 70 years) to tetanus toxoid. No significant difference was reported in the levels of protective antibody between the placebo and DHEAS treated groups [94].

There are however a handful of studies that have displayed increased efficacy of influenza vaccination following DHEAS treatment [94–96], although only one gave a result of significance. In this study, a single subcutaneous injection of DHEAS (7.5 mg) administered simultaneously to influenza vaccination was found to significantly increase serum antibody titres in elderly volunteers (mean age: 78 years) [96]. Interestingly, this response was limited to a small number of the study cohort who had low pre-vaccination antibody titres and circulating DHEAS levels [96], suggesting that low serum DHEAS may act as a biomarker for poor vaccine responses and indicate a beneficial response to DHEA or DHEAS supplementation.

Besides the differences that exist in DHEA physiology between rodents and humans [43], are there any other reasons as to why DHEA supplementation fails to reverse the impaired immune response of a majority of old individuals to vaccination? One possibility may be that the immune cells of older subjects have reached a tipping point at which they are no longer responsive to the immune-enhancing effects of DHEA, a theory supported by the relative success of DHEA supplementation in the middle-aged.

4.1.2. DHEA supplementation in the middle-aged

In a prospective randomised double blind study, postmenopausal women (mean age 56.1 years) were treated daily with either DHEA (50 mg) or placebo for three weeks before *ex vivo* analysis was performed on a range of immunological traits [97]. Compared to the changes observed after placebo treatment, DHEA was found to result in a two-fold increase in mean NK cell cytotoxicity and significantly enhance NK cell number. However, DHEA treatment decreased the number of circulating $CD4^+$ T helper cells and suppressed Con A-induced lymphocyte proliferation [97]. In another study, Khorram et al. demonstrated that 20 weeks of DHEA treatment (daily dose of 50 mg) could significantly enhance immune function in nine age-advanced men (mean age 63 years) [98]. In agreement with the findings of Casson et al. [97], this group reported DHEA treatment to result in a 22–37% increase in NK cell number and a 45% increase in NK cytotoxicity [90]. Furthermore, DHEA treatment was found to increase the number of circulating monocytes and B cells. However, no change was observed in the number of total T cells ($CD3^+$) or T cell subsets ($CD4^+$, $CD8^+$), although proliferation of these cells in response to PHA stimulation markedly increased [98]. More recently, Kohut et al. [99] investigated the effect of an "androgenic dietary supplement" containing 150 mg DHEA on the immune function of middle-aged men (range 50–59 years), but unlike previous studies failed to report any beneficial effects after four weeks of treatment [99].

Although encouraging, these data alone should not justify the widespread use of DHEA in healthy aging subjects. Furthermore, importantly it remains to be determined whether the increase in immune cell activity observed following DHEA supplementation correlates with a more effective immune response.

4.2. DHEA in the management of autoimmune disease

Circulating concentrations of DHEAS are decreased in patients who suffer with chronic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease [112–114]. This association coupled to the reported immune modulatory effects of DHEA has led a number of groups to investigate whether DHEA supplementation could be of therapeutic value in the management of these conditions.

4.2.1. Systemic lupus erythematosus (SLE)

In a preliminary open label study, van Vollenhoven et al. [100] evaluated the effect of a daily oral dose of DHEA (200 mg) on ten female patients with mild to moderate SLE. After 6 months of treatment, a significant reduction was reported in both disease activity and corticosteroid requirements [100], findings that led numerous studies to further investigate the clinical benefits of DHEA supplementation in SLE [reviewed recently in [101]]. Of note, Chang et al. conducted a randomised double blind placebo controlled trial in 120 adult women with active SLE, and revealed DHEA treatment (200 mg/day for 6 months) could significantly reduce the number of SLE flares and improve patient assessment of disease activity [102]. In a similar study, Petri et al. reported a daily dose of DHEA (200 mg) could significantly reduce corticosteroid requirements in lupus patients without having an adverse effect on disease activity [103]. Exactly how DHEA mediates these effects is still unclear, but its ability to reduce immune cell apoptosis [115] and rectify the cytokine imbalance that underlies SLE pathology [116] may offer some explanation.

4.2.2. Rheumatoid arthritis (RA)

Exogenous administration of DHEA has been shown to confer protection against the development of rheumatoid arthritis in rodents. In a rodent model of rheumatoid arthritis (collagen-induced arthritis, CIA), Williams et al. [85] demonstrated that repeated administration of DHEA during arthritis induction delayed the onset and reduced the severity of CIA, a phenomenon that was later replicated in a murine model of antigen-induced arthritis [86]. In both these studies, DHEA supplementation significantly reduced the circulating levels of IgG autoantibodies [85,86], highlighting a potential mechanism of action.

In the only study to our knowledge that has investigated the effect of DHEA supplementation on the pathophysiology of human RA, eleven patients with established RA received daily DHEA treatment (200 mg) for 16 weeks. Despite increasing serum levels of DHEA, this therapy had no beneficial effect on RA pathology [104]. However, as low levels of DHEA precede the onset of RA [117], DHEA supplementation may prove to be more successful if administered during the early phase of RA.

As outlined earlier, DHEA negatively regulates the production of TNF-α and IL-1β [24], two pro-inflammatory cytokines renowned for their role in the pathogenesis of RA. In two studies employing fibroblast-like synoviocytes from patients with active RA, Dulos and co-workers found TNF-α and IL-1β significantly increased both the activity and expression of Cytochrome p450 enzyme 7b (Cyp7b) [118,119], a steroidogenic enzyme, which catalyses the conversion of DHEA to 7α-OH-DHEA [120]. With evidence suggesting 7α-OH-DHEA is capable of exerting anti-glucocorticoid effects [121], it is plausible that because of the low circulating levels of DHEAS in RA patients (which would lead to increased Cyp7b activity), elevated levels of 7α-OH-DHEA within the inflamed synovium would counteract the immune-suppressive actions mediated by endogenous glucocorticoids. Indeed, in murine CIA, the severity of arthritis was found to correlate with increased Cyp7b activity [122]. On this note, DHEA supplementation in early RA patients may reduce 7α-OH-DHEA levels within the synovium by lowering TNF-α and IL-1β levels, leading to a reduction in both Cyp7b activity and expression. Alternatively, direct inhibition of Cyp7b may offer an innovative treatment for RA [119].

4.2.3. Inflammatory bowel disease (IBD)

To date, only one study has evaluated the effect of DHEA supplementation in patients with active IBD [105]. In this phase II pilot trial, DHEA was administered to twenty patients (seven with Crohn's disease, thirteen with ulcerative colitis) at a dose of 200 mg/day for 56 days, and proved a safe and effective method

by which to reduce disease activity. However, the decision not to include a placebo group in the study means a randomised placebo controlled trial is needed to confirm these findings.

4.2.4. Possible side effects of DHEA supplementation

In the studies detailed above, DHEA was generally well-tolerated, although as with any treatment, adverse effects were observed. Of these, mild cases of acne and hirsutism were the most common [100,101,103,105], but very rarely did these conditions lead to patient withdrawal from a study. However, it should be noted that the long-term effects of DHEA treatment at supraphysiological concentrations still need to be investigated. Worryingly, studies in rodents have shown long-term exposure to pharmacological concentrations of DHEA results in hepatocellular carcinoma [123,124]. This association should be of particular concern when administering high doses of DHEA (200 mg/day) to subjects with a history of sex hormone-dependent malignancy.

4.3. DHEA and trauma

Major stress such as sepsis or polytraumatic injury is thought to lead to an intra-adrenal shift in steroid biosynthesis, with excessive production of glucocorticoids at the expense of DHEAS [125]. With glucocorticoids being potent suppressors of immune function, this shift may in part explain the prolonged and severe immune suppression that accompanies traumatic injury. Indeed, our own work has shown the increased cortisol:DHEAS ratio in elderly hip fracture patients to be associated with reduced neutrophil superoxide production when compared to control [53]. Whether administration of DHEA can restore immune function in hospitalised trauma patients has yet to be investigated, although a rationale for such studies is provided by the success of DHEA supplementation in a number of rodent models of trauma.

In three models of trauma-haemorrhage [22,87,88], Chaudry's group demonstrated that a single subcutaneous injection of DHEA post-trauma could restore several parameters of depressed immune function. This included reversing trauma-induced suppression of splenocyte proliferation [22,87], TNF-α, IL-1 and IL-6 production by macrophages [87,88] and IL-2, IL-3 and IFN-γ secretion from splenic T cells [22,88]. Moreover, in a model of hemorrhagic shock, DHEA supplementation normalised splenocyte apoptosis and lymphocyte migration [89], whilst DHEA-treated thermally injured mice displayed increased resistance to pathogenic challenge when compared to untreated controls [90]. This protection conferred by DHEA post-trauma has since been replicated in models of induced sepsis [87,91], where of note, Oberbeck et al. reported DHEA administration to improve the survival rate of septic mice by 34% [91]. With a high rate of infection documented amongst hospitalised trauma patients [126], DHEA supplementation may prove to be a novel and cost effective means by which to reduce both the morbidity and mortality arising from infection post-trauma.

Despite being able to restore immune function in a number of trauma models, the mechanism by which DHEA elicits this effect is still unclear. As described above, DHEA may mediate its beneficial effects through direct action on immune cells, but there is also evidence to suggest its effects may be mediated indirectly. In a model of trauma-haemorrhage, administration of estradiol was found to reverse immune suppression and assist in host recovery [127]. In a further study, DHEA administration prevented the trauma-induced increase in cortisol production [22], suggesting DHEA restores immune function by normalising the cortisol:DHEA ratio.

Thus, although evidence supporting the usefulness of DHEA supplementation in healthy elderly subjects remains at best equivocal, the case for supplementation in older adults in situations where a raised cortisol:DHEAS ratio occurs and is likely to suppress immunity (physical trauma such as hip fracture or surgery) is more compelling.

5. Summary and conclusions

The majority of data that has assigned an immune modulatory role to DHEA has originated from studies performed *in vivo* in rodents or *in vitro* in human and rodent cells. Their findings however cannot be translated directly to humans due to the differences in DHEA physiology between rodents and humans, and the pharmacological doses administered. Indeed, in human-based studies no consistent effects of DHEA on immune function have been reported, with the exception of its success in reducing the clinical symptoms in patients with SLE. Interestingly, these results were also achieved with supraphysiological doses of DHEA (200 mg/day), which raises the question of whether the beneficial effects on immune function are only seen at higher serum levels. This may in turn suggest that as far as the immune system is concerned, that DHEA plays a key role in counteracting raised cortisol levels seen during times of stress, or in chronic inflammatory conditions such as SLE. To reveal its true role clinical studies may have to try supplementing with doses higher than 50 mg/day, the dose used in most humans studies, in order to reset the cortisol:DHEA/DHEAS ratio and reduce immune suppression [128].

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