EXPERT REVIEWS

Experimental autoimmune oophoritis and α-melanocyte-stimulating hormone

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This article focuses on primary ovarian insufficiency (POI) and the experimental models used in recent years to explain the probable mechanisms of autoimmune oophoritis and idiopathic POI. The relationship between the immune system and the neuroendocrine system is also an important focus of this article. Activation of the immune system is necessary for maintaining homeostasis and this requires multiple interactions and regulation between the immune system and the neuroendocrine system. Neuropeptides, neuroendocrine mediators, are expressed and released primarily, but not exclusively, by the nervous system and have profound effects on the immune system. As an example of one of these peptides we describe the α-melanocyte-stimulating hormone and its anti-inflammatory properties.

The most important function of the immune system is to discriminate between self and nonself. The 'self' needs to be protected, whereas 'nonself' must be actively controlled in order not to be harmful. When the 'self' is recognized as 'nonself', in some pathological processes, activation of the immune system leads to autoimmune diseases [1]. Tregs are considered the primary mediators of peripheral tolerance [2]. Tregs are necessary for maintaining immunological unresponsiveness to self-antigens and for suppressing an excessive immune response that is deleterious for the host. The immune homeostasis is lost in autoimmune diseases. The human ovary can be the target of an autoimmune attack in various circumstances, including several organ-specific or systemic autoimmune diseases [3]. Autoimmunity is involved in several ovarian etiologies, such as primary ovarian insufficiency (POI), idiopathic infertility, polycystic ovary disease and endometriosis. There are a lot of potential autoimmune targets that indicate a variety of pathogenic mechanisms in ovarian disease, but their clinical and diagnostic relevance are still in controversy [3]. Autoimmune oophoritis (AO) is one of the main causes of POI and it is demonstrated by the presence of circulating steroid cell antibodies (StCAs) directed against steroidogenic enzymes [4], at ovary level it is characterized by intense lymphocytic infiltration of developing follicles and the specific targeting of ovarian autoantibodies to the theca cells with preservation of the granulosa cells. On the other hand, many antibodies directed to other targets are proposed as possible contributors to infertility in idiopathic POI [3,5–7]. To understand the pathophysiology of autoimmune POI, and other probable causes of idiopathic POI a number of different experimental models have been developed [8–11].

In the last few years, the importance of bidirectional communication between immune and neuroendocrine systems has been established [12,13]. Our laboratory has studied the relationship between these systems based on the induction of experimental AO and the well-known neuropeptide α-melanocyte-stimulating hormone (α-MSH). α-MSH is mainly found in the pituitary gland, although at low concentrations it is also found in the CNS and other tissues, such as skin, placenta, testes and ovaries. It is an important modulator of host reactions, including fever and inflammation, and is also involved in functions such as reproduction, sexual behavior [14]. Furthermore, it has been shown to play a role as an immunomodulator in experimental AO (EAO) [11]. This review focuses on autoimmune POI, the experimental models

used in recent years to explain probable mechanisms of AO and idiopathic POI, and the relationship between the immune and the neuroendocrine systems, explaining the anti-inflammatory properties of α-MSH, and its possible future implications in the treatment of autoimmune ovarian diseases.

Primary ovarian insufficiency & AO

Primary ovarian insufficiency is the preferred term for the condition that was previously referred to as premature menopause or premature ovarian failure [15,16,101]. POI is defined as a syndrome [17] considered to be present when a woman who is less than 40 years old has had 'disordered' menses (amenorrhea, oligomenorrhea, polymenorrhea, or metrorrhagia) for 4 month or more, with two serum follicle-stimulating hormone (FSH) levels (obtain at least 1 month apart) in the menopausal range [16]. POI is not a permanent condition, after women have received the diagnosis of POI, 50% of cases have ovarian function, and approximately 5 to 10% of women conceive and deliver a child [16,18]. POI affects approximately 1–5% of women, although its incidence is growing due to the increase in the survival rate of cancer patients treated with chemo- and/or radio-therapy [19]. In the presence of a normal karyotype, POI affects one in 100 of women before the age of 40 years and this incidence decreases by a factor of ten in each previous decade; hence, one in 1000 of women before the age of 30 years and one in 10,000 of women before the age of 20 years [20,21].

The causes of POI are very heterogeneous: iatrogenic as in the case of surgery, radiotherapy or chemotherapy; infectious or chromosomal aberrations [20–22]. However, two of the most commonly known etiologies are the autoimmune form and the abnormal increase in the expansion of the CGG triplet repeat of fragile X in the *FMR1* gene [19,23]. Autoimmune POI, better defined as AO, requires the demonstration of circulating StCA directed against steroidogenic enzymes, such as 21β-hydroxylase (21OH), 17α-hydroxylase (17OH) and cytochrome P450 side-chain cleavage (P450scc) [4,18,24,25]. AO is found in 4–5% of POI cases [26]. Frequently, AO is found to be part of the polyglandular autoimmune syndrome and can be associated principally to adrenal autoimmunity [4,18,24,25] and to others, such as thyroid autoimmunity [23]. POI may be linked to the expression of HLA-DR3 and DR4. Such HLA class II associations are common risk factors for autoimmune diseases [27]. As in other autoimmune diseases, the absolute count and percentage of peripheral blood T-lymphocytes CD4+ has been found to be increased in patients with POI [4,25]. Decreased levels of CD8+/CD57+ cytotoxic T cells, and an increase in the CD4+:CD8+ ratio were also described. POI patients may also have increased circulating CD19+ CD5+ B cells, often associated with autoimmunity [4,27].

In humans, ovary function is primarily regulated by a pituitary–ovarian axis comprised of activins and inhibins produced mainly in ovarian granulosa cells that act as positive and negative regulators of the pituitary gonadotrophins FSH, and luteininzing hormone (LH), respectively [28]. Activins are homo- or heterodimers of βA and βB chains shared by inhibins that contain a unique α-chain not found in activins. Inhibins antagonize activin-induced FSH production, oocyte maturation, ovulation and fertility by binding antagonistically to activin receptors. The results of some recent papers have led to the formulation of a theory on the pathophysiology of human autoimmune POI: theca cells of antral follicles are selectively destroyed by the autoimmune process, and granulosa cells and primordial and primary follicles are spared with subsequent increase of circulating inhibin levels and preservation of anti-Müllerian hormone (AMH) production. Levels of total inhibin and inhibin B are significantly increased in women with AO compared with women with idiopathic POI or women with natural menopause [4,26,29], and could be responsible for a partial negative feedback on the production of FSH. This hypothesis indirectly supports the selective target of StCAs on theca cells [4,26]. The destruction of theca cells determines the diminished production of androstenedione and, consequently, estradiol [30]. The importance of this theory is that it directs the attention specifically to the destruction of the theca cells and to the fact that vital tissue is preserved for several years after clinical manifestation of the disease [4,26,31]. Normal circulating levels of AMH were found in two thirds of cases of autoimmune ovarian insufficiency with less than 5 years of disease duration [26].

Most patients with POI present secondary amenorrhea or irregular cycles and infertility [22]. More than 75% present signs of estrogen deficiency, such as hot flushes, night sweating, emotional liability and dispareunia due to vaginal dryness. A small number of cases present primary amenorrhea [16,22]. Occasionally menses stop abruptly, most commonly, there is a prodrome of oligomenorrhea, polymenorrhea or dysfunctional uterine bleeding [16]. Some patients have had previous pregnancies, mainly those with secondary amenorrhea. They can also present reduced bone density compared with women of the same age, with an increase in the risk of osteoporosis and spontaneous fractures in women that have suffered POI since they were young [22]. POI has also been reported in patients previously treated with oral contraceptives. Additionally, their risk of mortality is twice as high than normal for each age group, with an increase in the risk of cardiovascular disease and its consequences [21]. Autoimmune POI in association with adrenal insufficiency can occur in polyglandular syndromes (APS), type 1 and type 2 [25]. APS type 1 is an autosomal recessive syndrome, resulting from mutations in the *AIRE* gene on chromosome 21, which may be involved in Treg development. These patients present in their childhood with mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency, 60–70% of women with this syndrome develop autoimmune ovarian insufficiency. The association of AO and hypoparathyroidism is almost exclusively observed within APS1. APS2 is defined by adrenal insufficiency and hypothyroidism, and is associated with POI in approximately 10% of cases [3,15,18,25,32].

Once pregnancy has been ruled out, although the list of potential causes of secondary amenorrhea is long, the majority of cases are accounted for by four conditions: polycystic ovary syndrome, hypothalamic amenorrhea, hyperprolactinemia and POI [33]. The evaluation of the decrease in ovarian function must first undergo the dosage of basal FSH (along with thyroid-stimulating

hormone and prolactin) and estradiol (E2) [22]. When the values of FSH are initially high, the levels of FSH and E2 must be evaluated on at least one other occasion, after 4–6 weeks [22,34]. Any FSH value of greater than 10 mUI/ml (except during the midcycle preovulatory LH surge) must be considered suggestive of a decrease in ovarian function [22]. The age of the patient must also be taken into account, lower values of FSH are predictive of POI in younger women [35]. FSH levels higher than 30 mUI/ml indicate POI. Values of E2 lower than 50 pg/ml are found in women with nonfunctional ovarian follicles. Levels of FSH persistently higher than LH together with low levels of E2 evidence the lack of follicular activity [22]. It is also recommended to obtain the karyotype of any women with POI symptoms, and assessing permutations on the gene *FMR1*. Bone density studies are also important [22]. The detection of autoantibodies against steroidogenic enzymes is at present the gold standard for accurate identification of women with AO [4,18,26].

Women with POI should be encouraged to maintain a lifestyle that optimizes bone and cardiovascular health, including engaging in regular weight-bearing exercise, maintaining an adequate intake of calcium (1200 mg daily) and vitamin D (at least 800 IU daily), eating a healthy diet to avoid obesity, and undergoing screening for cardiovascular risk factors, with treatment of any identified risk factors [16]. There are many treatments for POI and the end purpose of all of them is pregnancy. The list of therapies includes clomiphene citrate, gonadotropins, estrogens, GnRH analogues, oral contraceptives, corticosteroids, a combination of these, or, if none of these work, egg donation [22,34]. Recent studies reported dehydroepiandrosterone as another alternative for POI treatment [36]. A reasonable regimen would be 100 μg of transdermal estradiol and 10 mg of oral medroxyprogesterone acetate daily for the first 12 days of each month. Women should keep a menstrual calendar and have a pregnancy test promptly in the case of late menses [16]. It is highly important to consider a multidisciplinary management of the patient [22,34].

Experimental AO: animal models
Both human and animal disease show several similarities, such as similar histological distribution of the ovarian lymphocytic infiltration, the production of ovary autoantibodies and a reduced natural killer cell activity [20]. Different murine experimental models have been established to provide a closer view of the physiopathology of human AO, understand the possible mechanisms implicated in autoimmune POI, and also to explain other probably autoimmune causes of female infertility. EAO can be induced by different mechanisms; in the present article we are going to talk about the experimental models used in the last few years: immunization with well-defined ovarian antigens such as ZP3; neonatal thymectomy in mice; and CD4+-targeted attack against inhibin-α.

Immunization with heterologous ZP antigens or purified ZP3 antigens
The zona pellucida (ZP) is the acellular matrix surrounding developing and ovulated oocytes, and is also found in atretic follicles. Autoantibodies against the ZP have been described as a cause

of infertility in humans. In women with unexplained infertility these antibodies were seen in 5.6% of the cases, whereas in the normal controls positivity was seen in only 1.7% [25]. ZP proteins are preserved across mammals. ZP3 is the most important ZP glycoprotein and acts as a sperm receptor. From a clinical point of view, autoimmunity against the ovary can be directed against the somatic component (mainly granulosa and theca cells) or against the germinal component of the ovarian follicle (oocyte or ZP) and can be associated with autoimmune somatic disorders [3,5]. Immunizing animal models with ZP proteins demonstrated that anti-ZP antibodies could cause ovarian insufficiency and infertility [8], and block the binding sites between the ZP and sperms [37]. Calongos *et al.* showed that anti-ZP antibodies impaired folliculogenesis and oogenesis in preantral mice follicles [8]. Bidirectional communication between the oocyte and granulosa cells is fundamental for fertilization and the development of the embryo [38]. The processes of granulosa cells in the ZP are responsible for this communication. These antibodies with direct activity on the structure of the ZP alter the development of gap junctions between the oocyte and granulosa cells, causing follicular atresia and preventing oocyte maturation and fertilization [8]. Anti-ZP antibodies were proposed in infertile women as probable causes of idiopathic POI. Some works examined the effect of anti-ZP antibodies on sperm-ZP binding by hemizona assay. Anti-ZP antibodies against sera from some POI patients showed significant blocking effects on sperm-ZP binding [37]. There is no final evidence that anti-ZP antibodies are the responsible of some cases of human idiopathic POI, but it is clear that these antibodies are present in serum of this patients, also the effect of these antibodies have been demonstrated in experimental models, but the relationship between them and development of the disease in humans may need to be investigated further.

Neonatal thymectomy
One of the experimental models for AO is the induction of thymectomy in neonatal normal mice (approximately postnatal day 3). In consequence, thymectomyzed animals suffer autoimmune diseases (e.g., thyroiditis, Type I diabetes, oophoritis). The neonatal thymectomy-induced mouse model of AO has two important similarities with human AO. First, both cases have some defect in the immune system that allows the development of organ-specific autoimmunity. Second, both have some ovarian target under attack. Owing to its strong analogy with the human disease, murine experimental post-thymectomy AO may provide a closer insight to the pathogenesis of autoimmune primary ovarian insufficiency in women. In this experimental model, the inflammation is characterized by T-cell infiltrations in the affected organs and the development of organ-specific antibodies in the serum [9]. In euthymic animals, autoimmune disease is not observed by the presence of CD4+ T cells with regulatory or suppressor activity (Tregs) [1]. There are two types of CD4+ Tregs, 'natural' Tregs (nTregs) and induced Tregs (iTregs), which are defined by where they develop. nTregs develop in the thymus during the course of positive and negative selection, while iTregs develop in the periphery from conventional CD4+ T cells following antigenic

stimulation under a variety of conditions. nTregs comprise a small population, only approximately 5–10% of peripheral CD4+ T cells, however, their existence is vital. nTregs migrate from the thymus into the periphery after postnatal day 3, thus thymectomy of mice at this time results in lethal autoimmunity due to the lack of peripheral Tregs [39]. Thymectomy between postnatal days 1 and 4 determines the presence of T cells that escape the regulatory mechanisms of the thymus. In euthymic animals, Tregs are found in the thymus and spleen after postnatal day 5. However, new studies showed that although there are no Tregs in both these organs before postnatal day 5, they are present in lymph nodes (LNs) [9]. These authors also showed the presence of disease-specific Tregs capable of preferentially suppressing a particular type of autoimmune disease over another in the same host. This study determined the presence of disease-specific Tregs in the LN of B6AF1 d3tx mice (that mainly develop AO and dacryoadenitis [DA]) in postnatal weeks 6–8. The capacity of these cells to suppress AO and/or DA was studied. It was demonstrated that treatment with Treg cells from ovarian LN suppresses AO but not DA, and Tregs from lacrimal gland LN preferentially reduced the severity of DA but not AO, suggesting they are strategically positioned for disease control. Furthermore, the severity of the autoimmune pathology increased after a few weeks in animals that were administered anti-CD25 antibodies. Therefore, endogenous Tregs located in LN in d3tx mice are responsible for producing an attenuated form of the autoimmune pathology rather than allowing its maximum expression, but are insufficient to fully control the disease [9].

CD4+ targeted attack against inhibin-α

Inhibin is the negative regulator of FSH release. Immunization of SWX/J female mice with the p215–234 sequence of inhibin-α activates CD4+ T cells and induces a biphasic form of EAO involving an early phase of enhanced fertility and a delayed phase of POI. Affected mice showed high serum levels of inhibin-α-neutralizing antibodies that prevented the inhibin-mediated down-regulation of activin-induced pituitary FSH release. The loss of activin/FSH downregulation lead to prolonged metaestrus-diestrus, superovulation, increased numbers of mature follicles, increased offspring, accelerated depletion of primordial follicles and, ultimately, primary infertility. Therefore, inhibin-α-targeted experimental AO is initiated by CD4+ Th1 T cells that stimulate B cells to produce inhibin-α-neutralizing antibodies, which is directly capable of mediating POI and transferring disease into naive recipients. This experimental model allowed the authors to show how primary infertility may develop in the context of elevated FSH levels and high levels of antibodies directed to an ovarian protein, closely mimicking the hallmark features of human POI [10].

α-MSH: characterization, anti-inflammatory properties & EAO

α-MSH: characterization

The immune system must be activated to establish homeostasis and this requires multiple interactions with the neuroendocrine system. Among the neuroendocrine mediators, neuropeptides are

expressed and released first, but not exclusively, by the nervous system and have deep effects on the immune system. Their wide distribution suggests the complexity of interactions, different functions and mechanisms in which they are involved. The biochemical dialogue shared between the CNS and immune system is an example of one of these interactions. The CNS regulates the immune innate response through highly complex mechanisms. Neuropeptides and different synaptic contacts are involved in these mechanisms [40]. Neuropeptides are a group of substances involved in cell-to-cell communication, acting as hormone messengers, neurotransmitters or immunomodulators. Each is different in composition, function and size, ranging from approximately three amino acids to 40 amino acids. One of the most studied peptides is α-MSH. The proopiomelanocortin hormone (POMC) is the precursor of α-MSH and its cleavage is made in pairs of basic amino acids. Cleavage is performed intracellularly by proconvertases (PCs), a family of serin proteins; PC1 and PC2 are the two endoproteases involved in the release of mature α-MSH. In the CNS, the precursor mainly gives rise to α-MSH and β-endorfin. Proinflammatory cytokines as well as corticotrophin-releasing hormone (CRH) are the prototypical stimuli regulating POMC expression and the processing of this prohormone in both central and peripheral tissues [40]. α-MSH is a tridecapeptide (molecular weight: 1664.91), binds to melanocortin receptors (MC-Rs) that belong to the superfamily of G protein-coupled receptors with seven transmembrane domains [41]. Five MC-R subtypes have been described (MC-1R–MC-5R). Ligand stimulation of all MC-Rs leads to the activation of adenylate cyclase with the subsequent increase in intracellular second messenger AMPc; however, other signaling events, such as calcium fluxes or activation of MAPKs, also take place [40]. On the other hand, MC-1R, expressed mainly in melanocytes, is also present in immune cells including neutrophils, monocytes, dendritic cells (DCs), endothelial cells and B lymphocytes [42].

α-MSH has fascinated different researchers; it has been well known for several years, first for its role in skin pigment regulation, then for its activity on behavior [40] and, at present, the anti-inflammatory effect of the peptide has received particular attention.

Anti-inflammatory properties of α-MSH

The concentration of α-MSH in normal human circulation is low, but it can increase in certain inflammatory disorders [41]. The anti-inflammatory effect of α-MSH *in vitro* is efficient in picomolar doses (this result is in accordance with the levels of the peptide findings in human circulation) and is linked to MC-1R.

The central administration of α-MSH 1–13 inhibited the peripheral inflammatory actions of all the mediators tested: IL-1β; Il-8; LTB-4 and PAF. These findings are consistent with previous observations on the anti-inflammatory effect of α-MSH 1–13. α-MSH is also capable of inducing IL-10, a cytokine with strong immunosuppressive activity [42], and suppressing the expression of ICAM-1 induced by proinflammatory stimuli, such as IFN-γ; lipopolysaccharide or TNF-α [41]. Other surface molecules modulated by α-MSH are CD86 and CD40, needed for antigen presentation on monocytes and DCs.

α-MSH: *in vivo* studies

One of the first findings on the peptide and inflammation came from the pioneering work of Catania and co-workers on fever and the effect of α-MSH [42].

Antipyretic effect

Fever was induced by central administration of endogenous or exogenous pyrogens and the efficacy of the peptide to decrease the fever was examined. Intracerebral injections of α-MSH suppressed the effect of the pyrogen. The antipyrogen effect is mediated by MC-Rs, mainly MC-3R and MC-4R. These receptors are expressed in autonomic sites in the hypothalamus and brainstem [41]. Intravenous injections of α-MSH were also effective in reducing fever, indicating that the peptide may cross the blood–brain barrier.

Experimental autoimmune encephalomyelitis

The strong anti-inflammatory effect of α-MSH in the CNS was evidenced in attempts to treat experimental autoimmune encephalomyelitis (EAE), the most common animal model used for multiple sclerosis. The researchers used a special delivery system for α-MSH [43]. EAE was induced in mice by myelin antigens – that is, myelin basic protein, causing paralysis and sometimes demyelinization of the CNS. Transference of the peptide-transduced cells into animals with EAE produced a decrease of inflammatory cell infiltrates within the CNS, an increase of IL-10 and a decrease of IL-2. This indicates a novel therapeutic approach for treating autoimmune diseases in the CNS [43].

Systemic inflammation

α-melanocyte-stimulating hormone also proved to be effective in models of systemic inflammation (e.g., sepsis, acute respiratory distress). Systemic administration of α-MSH in a model of peritonitis/endotoxemia induced by cecal ligation and puncture improved the survival rate of the mice. It is interesting to note that the effect of α-MSH was similar to that of gentamicin and, furthermore, the use of both drugs together increased the survival rate of the animals. The anti-inflammatory effect of the peptide was also tested in experimental organ fibrosis, where α-MSH showed antifibrotic properties. Mice were injected during 3 weeks with bleomycin. The authors were able to demonstrate that the peptide reduced the amounts of collagen type I compared with the animals receiving only bleomycin. The peptide increased the levels of two enzymes, sodium dismutase 2 and heme oxygenase-1, both of which are related with oxidative stress defence and tissue protection [44].

Ocular Inflammation

There is vast experience on treating ocular inflammation with α-MSH. Animal models like uveitis or retinitis are commonly used for studying human autoimmunity inflammatory eye diseases. Uveitis can be induced by immunizing mice with human interphotoreceptor retinoid-binding protein peptide emulsified with Freud's adjuvant and *M. tuberculosis* antigen. Intravenous injections of α-MSH administered 10 and 12 days after immunization suppressed the mean scores of uveitis. It seems that α-MSH

might act by inducing Tregs. However, the mechanisms by which the α-MSH in the aqueous humor plays a role as an endogenous immunomodulator is still not clear as the doses used in these studies were supraphysiological [40].

Acute pancreatitis

An important anti-inflammatory activity of α-MSH was demonstrated in a model of acute pancreatitis. Experimental pancreatitis can be induced by injecting cerulein. This drug increases exocrine secretion of the pancreas, but administration of α-MSH before the cerulein injection reduced the levels of amylase, pancreatic weight and inflammation. The epithelial exocrine glands expressed MC-5R, although it is still unclear which type of receptor is involved [45,46]. Studies on knockout mice must be carried out to clarify the role of MC-Rs in pancreas physiology.

Organ damage: α-MSH & post-lesion repair

α-melanocyte-stimulating hormone exhibited a protective effect on damaged organs [47–49]. It seems that this effect is compatible with its anti-inflammatory effect and modulation of NF-κB. The peptide also improves neurological and electrophysiological response in spinal cord function [41,50,51].

Experimental ischemia

α-MSH is effective in experimental ischemia. Several studies were carried out regarding the role of α-MSH in ischemic organ damage. Forslin *et al.* demonstrated that the peptide suppressed intracerebral TNF-α protein after transient global ischemia, indicating that it not only suppressed brain temperature but also reduced cerebral levels of proinflammatory cytokines that were apparently involved in the mechanism of α-MSH ischemic brain damage [52].

α-MSH: *in vitro* studies

Many *in vitro* studies indicate that the peptide may provide protection against neuronal toxicity. Rat dorsal root ganglia (DRG) cultures were partially protected from the growth-inhibitory effect of cisplatin, a neurotoxic drug. Using the immortalized hypothalamic tumor cell line GT-1-1 with NDP-MSH, the authors demonstrated that MC-4R is involved in the inhibition of apoptosis through MAPK since HSO24 abolished the effect. In an *in vitro* study of neurotoxicity, Jo *et al.* showed that administration of cyclosporine A induced the expression of the Fas/Fas ligand system in kidney-2 cells, but α-MSH reduced apoptosis and attenuated the enhanced levels of Fas/Fas ligand [40].

α-MSH & experimental AO

Considering the anti-inflammatory faculties of the peptide and its influence on ovarian function, we studied the effect of α-MSH on EAO. EAO was induced by immunization with ovarian antigen using a modified Jankovic model: five to six fresh rat ovaries were homogenized with complete Freund's adjuvant (CFA). The ovary–adjuvant mixture was injected into the food pads of both legs (100 mg of fresh tissue/rat in 0.25 ml). First, we observed that during the evolution of the disease the release of nitric oxide (NO) and progesterone from granulosa cultured cells, as well as

the inflammatory cell infiltrates in ovaries, were modified. To study the effect of α-MSH we used an analogue of the peptide, [Nle4-D-Phe7] α-MSH, because it is more stable and is known to inhibit the inflammation of peripheral tissues [42]. Although α-MSH did not modify progesterone release from granulosa cells on day 0, it did reduce the release of NO in cells from estrus and diestrus rats [11]. Other authors have previously described an inhibitory effect of the peptide on inducible NO synthase transcription, and a similar effect might occur in granulosa cells where the level of nitrites was reduced. During the development of EAO, the production of nitrite and progesterone elicited a different pattern from that observed on day 0. EAO rats showed a period of constant diestrus ranging from approximately 7–14 days after immunization. Between days 7 and 14, NO significantly increased in estrus rats and this could be correlated with a reduction in progesterone concentration. Our findings could also be related to the decrease in plasmatic estradiol observed in rats, and are in agreement with results by other authors showing that iNOS localized in granulosa cells was highly expressed when steroidogenesis was virtually absent. The increase in NO was inversely proportional to the levels of progesterone, similar to the report showing a concentration-dependent inhibition of progesterone synthesis in the presence of NO donors. This suggests that the NO generated might be able to inhibit steroidogenesis and mediate the cytotoxic action of IL-1β on ovarian cells. Therefore, in this study we were able to show that α-MSH could modulate NO production during part of the development of the EAO in a culture of rat granulosa cells [11].

In a previous report we also demonstrated an interaction between the secretion of LH and α-MSH. Thereafter, in order to study the effect of α-MSH on EAO and the modification it could induce on progesterone and LH release, we hypothesized that the peptide was able to modify LH and progesterone release and consequently reverse or block the illness. Administration of α-MSH to EAO rats during proestrus and diestrus decreased the levels of LH to values similar to control rats. By contrast, no significant differences were observed in the levels of progesterone, suggesting that the peptide had no effect on this hormone. This apparent discrepancy might be due to the fact that prolactin hormone is the main luteotrophic hormone in rats. Although α-MSH significantly reduced LH levels in EAO rats, this effect was not enough to block or reduce the illness, indicating that elevated levels of progesterone in proestrus are important for inducing EAO. Several studies have shown that a variety of peptides and cytokines are involved in ovarian regulatory mechanisms. Our laboratory demonstrated that α-MSH was able to stimulate progesterone release in a preovulatory granulosa cell culture; this effect was blocked by the presence of IL-1β and TNF-α [53]. Although our experimental model, that allowed as to demonstrate the anti-inflammatory effect of α-MSH on EAO, does not represent exactly the physiopathology of the human autoimmune POI, it could be considered as a first step for the design of experiments to show that α-MSH, or its analogs, could be useful for the treatment of POI; not only for its anti-inflammatory effect but also for its action on ovulation.

Expert commentary

POI is one of the diseases responsible for infertility in young women. It is important to note that it is a syndrome with many possible etiologies and, therefore, different strategies in the diagnosis and treatment should be considered. The autoimmune form has been highly studied in the last years. The diagnosis of this form includes the presence of circulating StCAs, high serum inhibin, circulating FSH lower than in POI of other etiologies and AMH levels in a normal range preserved for years, and the concomitance with adrenal autoimmunity must also be considered. This form is the only one where the relationship between antibodies and the development of the disease has been proven. There are other antibodies being studied as probable causes of idiopathic POI, but their clinical relevance is still controversial. Further studies must be carried out to understand the probable relationship between other forms of autoimmunity and the development of POI. Murine experimental models allow researchers a closer understanding of the different mechanisms that are involved in the physiology and pathophysiology, to then be able to extrapolate the results to useful tools for the understanding of humans. In recent years, the integrative vision of the organism has enabled the best understanding of the complex mechanisms that are involved. Beginning to think that 'everything might be regulated by everything' will allow scientists to understand the immense complexity of our organism; the relationship between neuropeptides, such as α-MSH, and the immune system is one of the examples that supports this issue.

Five-year view

Perspectives of α-MSH on human immune-mediated inflammatory disease

α-melanocyte-stimulating hormone has shown to have promising anti-inflammatory effects and can be considered a possible therapeutic drug for AO. Recent *in vivo* studies with NDP-MSH in humans confirmed no major adverse effects of the peptide. Due to its low toxicity, α-MSH has a safe profile compared with the immunomodulating drugs currently used [54]. However, the route of administration is an important issue because α-MSH has a very short action; this short effect is due to the presence of aminopeptidases, prolylendopeptidase, trypsin and so on [40]. Thus, in order to increase its efficacy and half-life it is necessary to find a stable analogue that is more resistant to enzymes. KPV, a tripeptide derived from α-MSH, could be an attractive candidate in the future as it has similar effects to α-MSH. On the other hand, there may be other peptides with a similar capacity to α-MSH; an interesting peptide to be considered is neuropeptide isoleucine glutamic acid, which has similar functions to α-MSH [55–58]. Further studies must be carried out on oophoritis and α-MSH to determine the possibility of developing a treatment with this peptide.

Key issues

- The most important function of the immune system is to discriminate between self and nonself.
- The immune homeostasis is lost in autoimmune diseases. The human ovary can be the target of an autoimmune attack in various circumstances, including several organ-specific or systemic autoimmune diseases.
- Autoimmune oophoritis is one of the main causes of primary ovarian insufficiency (POI) and it is demonstrated by the presence of circulating steroid cell antibodies directed against steroidogenic enzymes.
- Frequently, autoimmune oophoritis is found to be part of the polyglandular autoimmune syndrome and can be associated principally to adrenal autoimmunity.
- Antibodies directed to other targets are proposed as possible contributors to infertility in idiopathic POI.
- Different murine experimental models have been established to provide a closer view of the physiopathology of human autoimmune oophoritis, understand the possible mechanisms implicated in autoimmune POI, and also to explain other probable autoimmune causes of infertility in women.
- In recent years, the importance of bidirectional communication between immune and neuroendocrine systems has been established.
- Activation of the immune system is necessary to establish homeostasis and requires multiple interactions with the neuroendocrine system.
- Neuropeptides are a group of substances involved in cell-to-cell communication, acting as hormone messengers, neurotransmitters or immunomodulators.
- α-melanocyte stimulating hormone (MSH) is a tridecapeptide derived from proopiomelanocortin.
- α-MSH has profound anti-inflammatory properties that were demonstrated in several studies *in vivo* and *in vitro*.
- In a model of experimental autoimmune oophoritis made in our laboratory it was demonstrated that α-MSH is able to reduce the nitric oxide production in granulose cells culture.
- There is a great possibility of using α-MSH in the future for treating human-mediated inflammatory immune diseases. Based on its anti-inflammatory effect and on recent results in humans showing that treatment with NDP-MSH had no major adverse effects, it may be good alternative for the treatment of autoimmune diseases.

References

Papers of special note have been highlighted as:
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