The role of Androgens, in Immune regulation, inImmuneModulatory effects, in Inhibitory effects on T- and B-cell development and Cancer and Autoimmune diseases Treatment and Prevention

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Abstract: Androgens are important sex steroid hormones for women as well as men. Androgens exert suppressive effects on both humoral and cellular immune responses and seem to function as natural anti-inflammatory hormones.

Recent studies from immune cell-specific androgen receptor (AR) knockout mice demonstrated that androgen and its receptor (androgen/AR) play significant roles in both immune regulations.

In the innate immunity, androgen/AR is required for generation and proper function of neutrophils; androgen/AR also regulates wound healing processes through macrophage recruitment and proinflammatory cytokine production. In adaptive immunity, androgen/AR exerts suppressive effects on development and activation of T and B cells.

Altogether, androgen/AR plays distinct roles in individual immune cells, and targeting androgen/AR may help in treatment and management of immune-related diseases.

This article will update the clinical effects and benefits of androgen and further clarify the documented advances which support the substantial therapeutic benefits of androgen for infectious disease, cancer, and autoimmune conditions will be addressed

Key Word: Androgens,Immune regulation, Immune Modulation,Inhibitory effects, Innate and adaptive immune, Cancer, and Autoimmunity

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1. Introduction

Androgens act through the androgen receptor (AR), from the NR3C4 gene located on chromosome X. Intracellular ARs are present in bone marrow stromal cells(1), thymocytes(2),and immature dendritic cells (DCs)(3),(4).Splenic T cells and macrophages express a membrane form of AR(5).The action of androgens on immune function may vary depending on the type of androgen used, the dose administered, and the timing of administration(6).For example,some studies report immunosuppressive effects(7),(8),(9),whereas endogenous androgens are also thought to be immunostimulatory(10).Testosterone, the primary and best known androgen, has been implicated as a regulator of the immune response to viruses, vaccines, host tissue, and cancer. Despite the relevance of these pleiotropic effects, the mechanisms underlying the activity of testosterone on the immune system are not well understood. Testosterone may suppress the expression of the pro-inflammatory cytokines TNF-α, interleukin (IL)-1β and IL-6 and potentiate the expression of the anti-inflammatory cytokine IL-10(11). Testosterone inhibits Th1 differentiation by up-regulating type 1 protein tyrosine phosphatase (Ptpn1) in both mice and humans(12), reduces the proliferation and differentiation of lymphocytes(13),(14), (15), and may suppress immunoglobulin production, in particular IgA(10).Supraphysiological doses of testosterone may inhibit the cytotoxic activity of natural killer (NK) cells(16),(17),(18).Overall, these data strongly support an immunosuppressive role for androgens although, since their effects may vary considerably depending on the level of exposure, the potential role of these hormones in gender-specific immune function is still unknown.

2. Androgen Physiology

The sex hormones dehydroepiandrostenedione (DHEA) and its sulfated form, DHEAS, androstenedione(A4), testosterone (T), dihydrotestosterone (DHT), estrone (E1), and estradiol (E2) are produced in the adrenal glands, the gonads, and in numerous peripheral sites. Although adrenal androgen secretion increases in response to adrenocorticotropic hormone (ACTH), androgens do not influence ACTH secretion, and ACTH plays a primarily permissive role in adrenal androgen physiology. In fact, a specific regulator of adrenal androgen secretion has been proposed, but, so far, has eluded isolation (19).Ovarian androgen secretion increases following stimulation of the theca cells by luteinizing hormone (LH). However, unlike the situation in men in which testosterone inhibits the secretion of LH, either directly or through aromatization to estradiol, there is no known feedback regulatory loop controlling androgen secretion in women, at least not at the levels normally observed. Most of the circulating androgens are bound to sex hormone-binding globulin (SHBG) or albumin. SHBG has a high affinity for the biologically active sex hormones DHT, T, A4, E2, and E1, whereas DHEA and DHEAS exhibit little or no binding(20).The role of SHBG in the regulation of sex hormone action has yet to be fully elucidated. It is thought to regulate the concentration of circulating steroid hormones, act as a reservoir for ready-made hormones, and serve as a partner for

nongenomic steroid action. Between 0.5 and 7.5% of the androgens in women exist in the free or unbound state and are available to freely act upon cells(21).Unlike SHBG, albumin has a low affinity for sex hormones; therefore, the albumin-bound steroids may be readily available to the tissues. The free and albumin-bound fractions of sex steroid hormones together are termed *bioavailable*. Although serum measurements of the sex steroid hormones are generally relied upon to diagnose androgen excess or deficiency, most of the androgens produced in women are made in peripheral tissues, which contain the enzymes to convert DHEAS to DHEA (which can then be transformed into A4 and then T) or 5α-reductase (where T may be further converted to the potent androgen DHT). Alternatively, in selected tissues such as liver, skin, fat, muscle, kidney and bone, T can undergo aromatization to E2, which in turn may be converted to E1 in those tissues that contain 17β-hydroxysteroid dehydrogenase. These locally produced hormones can act on neighboring cells in a paracrine fashion or on the cell of origin in an intracrine manner. The extent of the intracrine process can be appreciated by measurements of androgen metabolites, such as androsteroneglucuronide, androstane-3α,17β diolglucuronide, androstane-3β,17β-diolglucuronide, and androsterone sulfate(22).

3. Biological aspects and genetic background of androgens

Sex hormone concentrations, evaluated particularly in patients with rheumatoid arthritis before glucocorticoid therapy, were frequently found to be altered, especially in men and premenopausal women(23).In particular, low gonadal testoster- one/dihydrotestosterone and adrenal androgens dehydroepian- drosterone and its sulphate levels, as well as reduced androgen/ estrogen ratio, were detected in the body fluids (blood, synovial fluid, smears, saliva) of male and female RA patients, supporting the possible pathogenic role for the decreased levels of the immunosuppressive androgens(24).The pathway of sex steroid synthesis involves the sequential degradation of cholesterol to progestin, then androgens (i.e., testosterone) and finally estrogens (i.e., 17b-estradiol). This pathway is found in both genders, and circulating plasma concentrations of sex hormones are representative of the relative conversion of androgens and estrogens. It is the ratio of androgens to estrogens that creates a male and not a female milieu. Sex hormones can exert local actions (paracrine) in the tissues in which they are formed or enter the circulation. Several physiological, pathological and therapeutic conditions may change the sex hormone milieu and/or peripheral conversion. These include the menstrual cycle, pregnancy, postpartum period, menopause, chronic stress, inflammatory cytokines, use of corticosteroids, oral contraceptives and steroid hormonal replacements ± each of which induces altered androgen/ estrogen ratios and related effects(25).Recently, considerable interest has focused on endocrine disruption ± a new area of endocrinology concerned with chemicals that mimic hormones, in particular sex steroids(26).Chemicals that mimic estrogens (the so-called estrogenic xenobiotics) have been the main focus of the research. By blocking androgen action, exposure to these anti-androgens may evoke changes similar to those associated with estrogen exposure(27).Furthermore, genetic polymorphism affecting thelevels or function of androgens and estrogens may lead to an imbalance in the complex hormonal-immune system interaction and might contribute to the etiology of RA. Estrogen synthase (CYP19) locus is the cytochrome p450 that catalyzes the conversion of C19 androgens to C18 estrogens. In RA, a linkage to this locus has been described in sibling-pair families having older age at the onset of disease (> 50 years)(28).CYP19 polymorphisms that lead to higher levels of CYP19 or a higher enzyme activity lead to reduced levels of androgens, and presumably would make such older subjects hypoandrogenic and

more susceptible to RA. The increase in RA incidence that occurs in older ages as androgen production declines in both genders, in addition to the low gonadal and adrenal androgens (that correlate to disease activity) in RA patients, as well as the decline in the female excess with age and the rarity of the disease in young males, add interest to the observed CYP19 polymorphism(29).Recently, Huang et al.(30),indicated a relationship between CYP17 genotypes and the age at onset of rheumatoid arthritis in female patients. The CYP17 gene, coding for the cytochrome P450c17a, mediates both steroid 17a-hydroxylase and 17,20-lyase activities which represent the RA = rheumatoid arthritis key points in human steroidogenesis. A single base change in the 5' promoter region of the CYP17 creates an additional Sp-1-type promoter site that might cause increased expression. These authors found a new recognition site presented as two alleles (A1 and A2). Interestingly, they observed that female RA patients with the A2 allele tended to develop the disease at a younger age than those without, and having the A2 allele was a protective factor against older age onset of female RA. The results of the study suggest that the A2 allele is related to early onset and the A1 allele to late onset. In fact, the A2 allele, being an expression of increased CYP17 activities, is thought to be linked to elevated production of both estrogens and androgens through increased transcription(30).Given that androgens function generally as immunosuppressors, whereas estrogens function as immunostimulants,, and that having the A2 allele could modify the onset of RA, Huang et al.(30),suggest that the effects of the androgen increase induced by the A2 presence might not be biologically influential in the fertile age (i.e., younger RA female patients), which is characterized by high estrogens (immunostimulant). However, the same induction of increased production of androgens (immunosuppressive) might become an influential resisting factor in older women, who are characterized by physiologically reduced estrogens(31).The conclusions of Huang's study induced us to reevaluate the results of an investigation we published 15 years ago of statistically higher concentrations of androgens ± particularly testosterone,androstenedione and DHEAS ± in the serum of postmenopausal women affected by RA when compared to age-matched healthy controls(32),(33).A role for CYP17-altered activity is presently under investigation. In view of the possible role played by androgens in the pathogenesis of the rheumatic diseases, the association between repeat lengths of CAG microsatellites and the androgen receptor gene in RA patients was recently studied (34).Shorter CAG repeats of the androgen receptor gene, presenting high levels of transactivation activity, were found to be related to younger age onset of male RA, further suggesting the possible role of androgens as a modulating factor in autoimmunity. Finally, an association between HLA phenotype and serum testosterone levels was identified(35),in particular the demonstration of low testosterone levels in men with HLAB15, DR2, DR5 haplotytes. A recent study has confirmed that major histocompatibility complex phenotypes influence serum testosterone concentration(36).

4. Mechanisms of action of androgens on immune cells

Effects of androgens on B lymphocytes The effects of testosterone have been tested on the productionof immunoglobulin M by an Epstein-Barr virus-transformedhuman B cell line (SKW6-CL4)(37).Testosterone at bothphysiological and supraphysiological levels, as expected be-cause of the absence of functional receptors, does not influence either IgM production or the proliferation of the human SKW6- CL4 cells. In contrast, putative contradictory data were reportedin another study on the effects of testosterone on human peripheral blood mononuclear cells ± namely, a dose-depen- dent inhibition of IgG and IgM production by cells from

normalmales and females(38).The magnitude of the suppressive effect on isolated B cells was much lower than on whole PBMCs. Inaddition, testosterone treatment reduced monocyte interleukin- 6 productions compared with controls but did not appear to directly affect isolated B or T cells. A follow-up study on PBMCs from patients with systemic lupus erythematosus confirms that testosterone suppresses both IgG anti-dsDNA antibody and total IgG production(39).Antibody production in B lympho- cytes in that study was suppressed by testosterone, although the magnitude of its effect on B cells was lower than on PBMCs. Similar to normal monocytes, testosterone reducedIL-6 production. Moreover, exogenous IL-6 partially restored the testosterone-induced decrease in antibody production by PBMCs. Thus, these data indicate that testosterone may modulate susceptibility to human autoimmune diseases, at least indirectly through actions on monocytes. The end result is decreased B cell activity. 17b-estradiol treatment of male and female mice, as notedearlier, induces earlier and substained expression of IgG anti- dsDNA antibodies compared to controls, whereas orchidectomyor administration of dihydrotestosterone to orchidectomizedmale mice has minimal effects on the production of these antibodies(40).Effects of androgens on T lymphocytes the role of androgens in T cells is also complex andinadequately studied in both humans and animals. Directexposure of murine T cells to DHT reduces the amount of IL-4,IL-5 and interferon-gamma produced after activation with anti-CD3 without affecting the production of IL-2(41).The authorsobserved differences in the production of IL-2, IL-4 and IFN-gamma between males and females at a given age. Both IL-4and IFN-gamma production is elevated in females.More recently, another experimental study observed that testosterone exerts a protective effect on experimental auto-immune encephalomyelitis. The data suggested that this protective effect resulted from the induction of a Th2 bias in autoantigen-specific T lymphocytes(42).Enhanced IL-10production by the autoantigen-specific T lymphocytes may explain these observations. This study was the first to demonstrate the ability of testosterone to shift an autoanti- gen-specific T lymphocyte response toward the Th2 phenotype, in vivo, coupled with an observed effect on a clinicalautoimmune disease [Figure 3]. Effects of androgens on monocytes/macrophagesHuman macrophages appear to contain the key enzymes of steroidogenesis, as shown at least by their capability to create, in the short term, the active metabolites of testosterone(43).In particular, macrophages are endowed with 5a-reductase en-zymes that catalyze the formation of DHT from testosterone, the more biologically active metabolite, after a relatively short exposure (24 hours). In cultured human macrophages, the rate and amount of conversion of testosterone into active metabolic products (i.e., DHT) is very close to that observed in classicaltarget cells for androgen activity, such as human prostate cancer cells. Recent studies have shown that both physiologic (10-8 M) and pharmacologic (10-6 M) concentrations of testosterone inhibit IL-1b secretion by PBMCs obtained from RA patients(44).In addition, physiologic concentrations of testosteroneinhibit IL-1 synthesis in primary cultured human synovial macrophages(45).In related studies, DHT was found to repress the expression and activity of the human IL-6 gene promoter in human fibroblasts, thus supporting the concept of anti-inflammatory/immunosuppressive effects of androgens.

5. Androgen/AR in the Innate Immune System

Roles of Neutrophil AR in Defending the Body

Granulopoiesis is a dynamic process leading to the production of 120 billion granulocytes daily in humans; its synthesis capacity can be increased at least 10-fold in response to certain stress conditions, including infection. Neutrophils are the most abundant type of granulocyte; eosinophils and basophils are much rarer. Upon insult, neutrophils arrive on site within minutes and usually peak at 12 to 24 hours, establishing the first line of immune defense. In addition to their major function for the phagocytosis of invading infections/pathogens or damaged tissues, neutrophils are able to secrete several chemokinesand cytokines to attract and activate monocytes/macrophages (46),(47), Originating from hematopoietic stem cells and progenitors,neutrophils in the bone marrow are composed of aprecursor pool and a storage pool. Peripheral blood neutrophils, which are postmitotic, consist of a free circulating pool and a marginal pool. AR is universally expressed in neutrophil lineages from the proliferative precursors (myeloblasts, promyelocytes, and myelocytes) to mature neutrophils (metamyelocytes, band forms, and neutrophils), with no difference in AR expression patterns between males and females(48). Both band and segmented neutrophils express high levels of nuclear and cytoplasmic AR(48).In myelocytes, AR is stained positively in the cytoplasm and shows perinuclear dot staining. Similarly, AR is localized in the cytoplasm of myeloblasts, but without perinuclear dot staining.(48).Young women who suffer from polycystic ovarian syndrome with hyperandrogenismhave higher neutrophil counts, which can be normalized by treatment with the antiandrogenflutamide, suggesting that androgen/AR signals can promote neutrophil production (49).Consistent with this notion, we found almost 90% reductionof neutrophil counts in general ARKO (G-ARKO) mice, compared with wild-type mice, and a similar reduction was also found in male mice carrying the testicular feminization (Tfm) mutation in the *AR* gene.(50).Tfm is a natural single nucleotide deletion mutation at the first exon of the *AR* gene that results in expression of unstable AR transcripts (51),(52).Castration of male mice also resulted in neutrophil reduction; however, the effect of castration was less dramatic than in ARKO or Tfm mice. These results suggest that AR confers a direct and more profound effect than androgen to control neutrophil production. Although neutropenia was observed in some prostate cancer patients treated with antiandrogens,(53),(54).castration in humans does not always result in neutropenia but may instead lead to only a mild reduction of neutrophil counts, suggesting that AR, but not androgen, is more critical for neutrophil homeostasis(50).Further analysis of neutrophil lineages showed that myelocytes/ metamyelocytesand mature neutrophils, but not myeloblasts and promyelocytes, were significantly reduced in ARKO mice. Additionally, restoring the AR expression in the ARKO granulocyte-macrophage progenitors rescued neutrophil production, implying that AR is essential in regulating neutrophil differentiation(50).Further studies also suggested that AR regulates primarily the transition between proliferation of precursors (myeloblasts,promyelocytes, and myelocytes) and maturation of neutrophils (metamyelocytes, band forms, and neutrophils)(50).Mechanistically, AR appears to stimulate neutrophilproduction by enhancing granulocyte colony-stimulating factor (G-CSF) signaling. This is achieved by activating ERK1/2 and also by sustaining Stat3 activity via diminishing the inhibitory binding of protein inhibitor of activated STAT protein 3 (PIAS3)(50).Such AR-PIAS3 interaction does not rely on ligand binding, and could be consideredan androgen-independent regulation. Notably, the major induction of Stat3 reporter activity was caused by AR expression and was only slightly enhanced by adding androgens,suggesting that androgens are not essential for the function of AR in promoting Stat3 activity(50).Other lines of evidence using *in vitro* and transgenic mouse studies have demonstrated that Stat3 plays an essential role in G-CSF-dependent granulopoiesis(55),(56),(57).Moreover, several reports have shown that mice

with knockout of suppressor of cytokine signaling 3 (SOCS-3), in which Stat3 activity is persistent, developed severe neutrophilia(58),(59),(60). Neutrophils are a key component of the innate immunesystem in clearing bacterial pathogen infection. As a result of neutrophil reduction, the ability of ARKO mice to survive pathogenic bacteria challenge is severely compromised(50).In addition to reduced neutrophil counts,functional defects of neutrophils also contributed to the susceptibility to septic challenges in the ARKO mice.Although the residual neutrophils in ARKO mice retained the normal capacity for phagocytosis and respiratory burst, they produced less proinflammatory cytokines IL- 1β, IL-6, and TNF-α and of chemokines CCL2, CCL3,CCL4, CXCL1, CXCL4, and CXCL7(50).It has long been suspected that chronic inflammationcan promote prostate tumorigenesis and progression.Several lines of evidence show that mice lacking proinflammatorycytokines are resistant to carcinogenesis, tumorinvasion, and angiogenesis (61),(62).Because it is important for sustaining proinflammatory cytokine expression (TNF-α, IL-1β, IL-6) in neutrophils and macrophages(50),(63).AR may play a pivotal role in remodeling the tumorpromotingmicroenvironment via release of proinflammatory cytokines from neutrophils. Neutrophils can be attracted by IL-8 released from castration-resistant prostate cancer cells; they then migrate toward the tumor lesion and release enzymes (eg, MMP-9, collagenase) to remodel the extracellular matrix of nearby tissues (64).Thus, neutrophils could serve as enhancers of cancer cell migration through remodeling of the extracellular matrix. Moreover, the reactive oxygen species produced by neutrophilic oxidases to kill invading organisms have the potential to interact with tumor cells, attenuating their apoptotic cascade and increasing their mutation rate and malignancy(64).

6. Roles of Macrophage AR in Regulating Inflammatory Responses

Immediately after neutrophil activation, monocytes are recruited from the circulation into inflamed tissues in response to chemokines secreted from neutrophils and damaged tissues (65)The infiltrated monocytes subsequently differentiate into macrophages, which in turn become the master regulators of the inflammatory response. The hallmark functions of macrophages include not only phagocytosis of the infecting microbes, dead neutrophils, and tissue debris, but also the production of proinflammatory cytokines and growth factors to regulate the succeeding immune responses and tissue regeneration(66).In addition, macrophages can present antigens to T cells, and thus macrophage activity shapes the successive T cell activation and bridges innate immunity and the adaptive immune response. Increasing evidence shows that macrophages play an important role in regulating tissue regeneration and inflammatory responses during wound healing and traumatic-hemorrhagic shock(67).Interestingly, AR expression has been detected in the monocyte/macrophage population (68),suggesting that AR might modulate macrophage functions involved in these inflammatory conditions.

7. Macrophage AR Promotes Inflammation in Cutaneous Wound Healing

In the early phase of cutaneous wound healing, macrophages are among the major inflammatory cells recruited to the injured tissues. Although inflammation is necessary for removing tissue debris, an excess of it hinders wound healing(67),(69). Castration of animals or blockade of androgen action by antiandrogen (flutamide) accelerated wound healing and

suppressed macrophage recruitmentto the wounds(68),(70).In a mouse model of wound healing,the proinflammatory cytokine TNF-α, but not IL-6, at the site of injury was down-regulated by castration or flutamidetreatment. Conversely, *in vitro* studies found that lipopolysaccharide-induced TNF-α production in macrophages was enhanced by testosterone treatment(68).These data suggest that androgens might regulate inflammatory responses to suppress wound healing. Notably, increasing evidence from *in vitro* studies shows that androgens can also go through non-AR pathways to regulate cellular activities (71),(72).and therefore using castration or antiandrogens to decrease androgen levels cannot clarify whether the androgen effects are through AR or non-AR pathways to inhibit wound healing. Interestingly, a recent report using the CreloxP system to generate conditional ARKO mice found that mice lacking AR had accelerated wound healing, and such acceleration could not be reversed by 5_ dihydrotestosteronerestoration, suggesting that AR signals, rather than androgen itself, are critical to suppression of wound healing(63).Using reciprocal bone marrow transplantation, it was found that AR deficiency in the infiltrating cells, and not in mesenchymal cells, was responsible for the accelerated wound healing in ARKO mice. AR in the wild-type donor bone marrow cells, even in the low-testosterone environment of ARKO recipients, could still suppress wound healing, suggesting that AR can go through androgen-independent pathways to suppress wound healing(63).Although the detailed mechanisms of these androgen-independent AR effects in wound healing remain unclear, these results are in accord with earlier studies showing that AR could also be activated by other factors in addition to androgens, such as estrogens (73),antiandrogens(74), and kinases(75),(76).Similarly, as already noted, AR might go through androgen-independent signals to promote neutrophil differentiation(50).Further studies are warranted, to determine whether any of these mechanisms are involved in suppression of wound healing. Taken together, these studies reveal the novel concept that AR, but not androgen, is critical for wound healing suppression. The essential roles of monocyte/macrophage AR inwound healing suppression were further corroborated in a study using myeloid-specific ARKO mice (M-ARKO), which found that AR in the infiltrating monocytes/macrophages executed its suppressive roles in cutaneous wound healing by enhancing local TNF-αexpression via the following mechanisms: i) increasing circulating inflammatory monocyte population, ii) increasing recruitment of monocytes by promoting their chemotaxisthrough up-regulation of CCR2 expression, and iii) increasing TNF-α expression at the transcriptional level in macrophages(63).Although the results from bone marrow transplantation imply androgen-independent AR func- tions in the suppression of wound healing, it is noteworthy that the AR regulation of TNF-α and CCR2 expression is androgen-dependent and directly regulates the promoter activities of these two proteins(63).These findings demonstrate the essential roles of AR in the macrophage-associated inflammatory regulation toward the pathological conditions (such as wound healing) and raise an important question, whether monocyte/ macrophage AR may also play essential roles in other inflammatory diseases that demonstrate sex differences, such as atherosclerosis(77),and asthma (78).A positive answer may then point toward development of a potential therapeutic approach via targeting AR in treatment and management of these sexually dimorphic inflammatory diseases.

8. Role of Androgen/AR Signals in the Adaptive Immune System

Unlike macrophages and neutrophils, which play a central role in the innate immune system (79).T and B celsrepresent the central players in adaptive immune response. The activation of T

and B cells requires proper antigen presentation, cell-cell interaction (engagement of T cell receptor TCR, or B cell receptor BCR, and costimulation), and specific environmental cytokine profiles.Studies using castration and Tfm mouse models suggest that androgen/AR signals are involved in both differentiation and activation of T cells and B cells, and might contribute to the sexual dimorphism of autoimmune diseases(52).

9. Role of Androgen/AR in the Development and Activation of T Cells
Androgen/AR Signals in T Cell Development

The thymus is the major organ in which naïve T cells develop. Several studies have shown that T cell generation from thymus is significantly reduced after puberty (80),(81).Suggesting that sex hormones may play critical roles in maintaining the homeostatic T cell populations and repertoires; it was more than a century ago that castration of male animals was found to result in thymic enlargement (82).The effects of castration on the reversal of thymic involution have since been analyzed in great detail. The role of androgen on thymic involution was confirmed by the reversal of thymicenlargement by androgen replacement in castrated animals(83),(84).Further supporting the role of androgen/AR in the negative regulation of thymic development, studies by Fitzpatrick et al(84). Olsen et al (85),and Lai et al (unpublished data) in ARKO andTfm mice, as well as castrated mice, found thymic enlargement and increase of thymiccellularity in these mice, which could be due to more proliferating and less apoptotic thymocytes. The net outcome of castration-induced reversal of thymicinvolution is increased output of naïve CD4 and CD8 single-positive T cells(85),(86),(87).Although neonatal castration in a rat model led to increased incidence of autoimmune diseases (88),comparison of thymic and peripheral TCR repertoires showed no noticeable differences between castrated and normal mice (Lai et al, unpublished data). The latter observation appears to argue against a role of androgen/AR signals in the negative selection of thymocytes. The aforementioned reduction of thymocyte apoptosis in both ARKO and castrated mice may indicate that there is no role for androgen/AR signals in enhancing negative selection.

10. Importance of AR Expression in the Thymic
Epithelium for Thymocyte Development

To determine the target cells involved in androgen/AR effects on thymopoiesis, the expression of AR in thymocytesand thymic stromal cells have been extensively analyzed. Although some earlier ligand-binding studies indicated that thymocytes do not express AR (89),(90),later studies have demonstrated AR expression in thymocytesby flow cytometry and immunoblotting in mice and ligand binding assays in humans(2),(91). AR expression in thymocytescan also be detected by RT-PCR (Lai et al, unpublished data). Flow cytometry analysis showed that AR is expressed in all thymocyte subpopulations defined by the expression of CD3, CD4, and CD8, with the highest expression in CD3lowCD8 immature thymocytes(91).AR expressionin the thymicstroma was also demonstrated(84). Within the thymicstroma, AR is expressed predominantly in the cortical and medullar thymic epithelial cells (84). Reciprocal bone marrow transplantation between normalmale mice and Tfm male mice was used to determinewhether AR expression in thymocytes or thymic epithelialcells is

important for androgen/AR effects on thymic development.Bone-marrow chimeric mice with bone marrow grafts from Tfm mice to normal mice showed normal response to androgen and thymic involution. In contrast, chimeric mice receiving bone marrow from normal miceto Tfm mice developed enlarged thymus and were insensitiveto androgen (84). These findings, together with the evidence for AR expression in thymicstroma, suggest that AR expression in thymicstroma, rather than thymocytes, is responsible for inhibition of T cell development by androgen/AR signals. Using G-ARKO mice, we have performed similar bone marrow chimeric studies and confirmed the importance of AR expression in thymicstroma (Lai et al, unpublished data). Based on the residing compartment, thymic epithelial cells can be further divided into cortical thymic epithelial cells (cTECs) and medullar thymic epithelial cells (mTECs). These two populations have distinct functions in thymocyte development (92).cTECs play a crucial role in positive selection (93).After positive selection, the doublepositivethymocytes encounter other stromal cells (including macrophages, dendritic cells, and mTECs) in the medullar compartment for negative selection. The role of mTECs in central tolerance has been of great interest since the discovery of promiscuous expression of tissuerestrictedself-antigens in these cells (94).Thus, mTECs can directly induce or, through cross-presentation of self-antigens, promote negative selection of self-reactive T cells. We have established TEC-ARKO mice by using bovine keratin-5 promoter Cre to delete AR in thymic epithelial cells and found that AR was ablated in both cTEC and mTEC populations (Lai et al, unpublished data). Upon further breeding of ARKO with TCR transgenic mice, results suggested that AR plays an essential role in regulating positive selection. In contrast to TEC-ARKO mice, T cell–specific ARKO (T-ARKO) mice with AR deletion in thymocytes revealed normal size and cellularity of thymus (Lai et al, unpublished data). These results are in accord with findings in bone marrow transplantations showing that AR in thymic epithelial cells plays a more crucial role than thymocytes in thymic development. To investigate the potential targets of androgen/AR in thymocyte development, we demonstrated that ARKO thymic stromal cells expressed less mRNA of TGF_β1, IL-6, and CD80/CD86, but more of IL-7 and CCL21, compared with wild-type thymic stromal cells on 5_-dihydrotestosterone treatment (Lai et al, unpublished data). Interestingly, androgen/AR can up-regulate CD80 promoter activity through direct promoter binding (Lai et al, unpublished data). Notably, it has been shown that in CD28 or B7 (CD80/CD86) knockout mice, the selection of mature CD4 and CD8 single-positive thymocyteswas increased (95), suggesting that CD28-B7 engagement serves as a negative regulator for positive selection. This may explain, at least in part, how androgen/AR signaling inhibits positive selection of thymocytes.

11. Effect of Androgen/AR on Peripheral T Cell Function

In addition to effects in development, the effect of androgen/AR on T cell–mediated immune responses has been recognized in many models of cellular immunity. Administration of androgens to female mice alleviatedrejection of skin grafts and alleviated CD4 T cell–mediatedautoimmune diseases such as experimental autoimmuneencephalomyelitis, whereas castration in malemice exacerbated this autoimmune disease (96),(42),(97).Theeffects of androgen/AR on peripheral T cell response aretwo-fold: first, androgen/AR suppresses T cell proliferation; second, androgen/AR modulates the balance of Th1 and Th2 responses. In terms of T cell proliferation, T cells from castrated mice proliferated more vigorously *in vitro*than T cells from normal mice regardless of the modes of T cell activation, although the effect seemed to be transient. Because the enhancement of T cell activation could occur in the absence of

antigen-presenting cells or any other accessory cells, androgen/AR appeared to exert a direct effect on T cells. This is an issue of debate, however, given that some studies have found no AR expression in peripheral T cells,whereas others have shown weak expression of AR mRNA in peripheral T cells (98).In models of burn injury and hemorrhagic shock in which delayed-type hyper-responsiveness and T cell proliferation were suppressed, blockade of androgen/AR restored T cell response, which was accompanied by increased IL-2 production and IL-2R expression in T cells (99),(100).Thus, androgen/AR appears to suppress T cell proliferation by diminishing IL-2 signaling in T cells.

12. Regulation of Th1 and Th2 Responses by Androgen/AR

The inhibitory action of androgen/AR on T cell proliferation may be a contributing factor for the lower incidence of autoimmune diseases in males than in females. In addition, evidence has accumulated to support a role of androgen/ AR in regulating the functional differentiation of peripheralCD4 T cells, which may have even greater implications for the sexual dimorphism of autoimmunity. Organ-specific autoimmune diseases such as experimental autoimmune encephalomyelitis are typically associated with biased Th1 immune responses to autoantigens. In a study using experimental autoimmune encephalomyelitis, autoantigen-specific T cells from immunized male animals were less capable of transferring disease, compared with T cells from female animals (98).Analyses of cytokine profiles of the autoreactive T cells showed higher IFN-γ production in female than in male mice. In *in vitro* cultures, the presence of androgen diminished both the differentiation of Th1 cells from naïve precursors and the production of IFN-γ by Thelpereffector cells induced by TCR stimulation(98). Androgen treatment also decreased IL-12–induced IFN-γ production by CD4 T cells through inhibition of Stat4 activation (101).Although the study was performed on resting CD4 T cells that were previously activated by concanavalinA, one may extrapolate a role of androgen-mediated inhibitionof Stat4 activation in dampening Th1 differentiation from naïve CD4 T cells as well, because IL-12 is a potent inducer of Th1 cell differentiation (102).Finally, it should be pointed out that the Th1 and Th2 divergence of immune response between males and females is not restricted to autoimmunity. It was also observedin hemorrhagic shock that androgen/AR suppressed IFN-γand IL-2 expression by splenocytes in male mice, whereas female mice maintained strong production of these two cytokines (103).

13. Role of Androgen/AR Signals in the Development and Activation of B Cells

The major function of B cells is to produce antibodies on stimulation. Most of the B cells respond to foreign anti- gens, such as virus, bacteria, and toxins; however, some autoreactive B cells escape from negative selection during B-cell development and are released into the periphery. These autoreactive B cells are able to produce antibodies against self-antigens and subsequently result inautoimmunity and in autoimmune diseases, such as systemiclupus erythematosus and rheumatoid arthritis, and therefore B cells have been one of the primary targets forautoimmune therapy (104).Moreover, profound sexual dimorphism in autoimmune diseases implies that sex hormones might involve the regulation of B-cell responses and/or their development (105).B-cell development is negatively regulated by androgen/ AR.

Castration of male mice caused splenic enlargement, due primarily to the increase of B cells(83),(91).Further studies found that B cells were increased not only in the spleen but also in the bone marrow of castrated mice.The B cell increase could occur in thymectomized mice, and therefore it was independent of the effect of castration on T cells.Similar increases of B cells in bone marrow and spleen were also observed in Tfm mice (106).Closer examination of the B-cell lineage in the bone marrow of castrated mice revealed the most dramatic increase in the late pro-B population, and to lesser degrees in the pre-B- and immature B-cell populations (107).The increase of B-cell progenitors in the bone marrow led tothe increase of the peripheral B-cell pool, with altered composition. Specifically, the proportion of newly emigrated immature B cells was found to be up to 45% of total circulating B cells in castrated and Tfm mice, although these cells represent only 10% to 15% in normal mice (106), (107).Both bone marrow stroma and B cells express AR,and both cell types could be the targets of androgen/ARin regulating B-cell lymphopoiesis. In bone marrow chimericstudies between Tfm and normal mice, the expressionof intact AR on bone marrow stroma was critical forthe inhibition of B-cell lymphopoiesis by androgen/AR (108).On the other hand, mice with either general or B cell–specific knockout of the AR gene showed enhanced Bcelllymphopoiesis, demonstrating that B cells are directtargets of androgen (35).However, the B cell–specificARKO mice, showed a milder effect on B-cell lymphopoiesis, compared with the general ARKO mice. The full effect of androgen on B-cell lymphopoiesis therefore depends on its action on both B cells and the stroma. In both the general and the B cell–specific ARKO mice, the pre-B- and immature B-cell populations were increased in the bone marrow, apparently due to both increasedproliferation and reduced apoptosis (35).Mechanistically, the expression of several key proapoptotic molecules, such as Fas/FasL and caspase-3/8, were reduced in ARKO B cells (35).In contrast, elevated cell proliferation was observed in ARKO B cells, accompanied by upregulationof p65 and Bcl-2 expression (35).These findingscame mainly from primary culture of immature B cellscultured in a medium (MesenCult) containing very low levels of androgen. The above-mentioned changes in gene expression could therefore be due primarily to androgen-independent pathways, although androgen-dependent regulation could not be completely ruled out in this study(35).The enlarged B-cell pool could potentially increase the frequency of autoreactive B cells in castrated or AR deficient mice. In fact, in both the general and the B cell–specific ARKO mice, levels of anti–double-stranded DNA antibodies were significantly elevated (35).Thus, removal of the brake on B-cell lymphopoiesis imposed by androgen/ AR could lead to autoimmune diseases in which autoantibodies play pathogenic roles. Examples of suchconsequences could be found in hypogonadal men withrheumatoid arthritis and/or systemic lupus erythematosus(109),(110).and in prostate cancer patients who received androgen ablation therapy and subsequently developed rheumatoid arthritis at higher rates(111)Conversely, androgen replacement therapy of hypogonadal men with rheumatoidarthritis ameliorated clinical symptoms and reduced the concentrations of IgM rheumatoid factors.

14. Conclusion

The study of immune regulation by androgen/AR has attracted long-lasting interest.All the data discussed here seem to indicate that gonadal androgens (testosterone and DHT) exert their modulatory effects via both a direct influence on cytokine production by activated monocytes/macrophages (inhibition of IL-1, IL-6 and tumor necrosis factor-alpha production)

and an indirect influence on cytokine production by activated T cells (inhibition of IL-4, IL-5 and IFN-gamma production). Androgens (DHEAS and DHEA) may exert a direct effect on cytokine production by T cells (increase of IL-2 and IFN-gamma synthesis).

The physiological effects of estrogens in women indirectly reflect a role for androgens, for if there were no androgens, there would be no estrogens.In addition to serving as an important prohormone for estrogen synthesis in the bone, androgens directly influence osteoblastic and osteoclastic function. The importance for androgens in female sexual desire has been well demonstrated; studies of postmenopausal women with low sexual desire and low T levels demonstrate an increase in desire and sexual activity when their T levels are increased to within the reference range for premenopausal women.

Up-to-date studies using these animal models and other approaches have shown that androgen/AR exerts different regulatory effects on the immune system, which may also be influenced by the pathophysiological conditions of the hosts. For neutrophils, androgen/AR plays a positive role for both development and function.

Although androgen/AR enhances proinflammatory cytokine production by macrophages in wound healing,it inhibits cytokine production after traumatic-hemorrhagic shock and burns. For the adaptive arm of the immune system, androgen/AR has generally inhibitory effects on T- and B-cell development. In the periphery, androgen/ AR dampens T cell activation and inhibits Th1 differentiation. These inhibitory effects of androgen/AR on B and T cells may help lower the risk of autoimmunity in males.

Further studies require in both animal and clinical trials to advance our knowledge and understanding androgen/AR and the immune system, as well as novel strategies to modulate the immune system under clinical conditions in which regulation of immune responses by androgen/ AR plays critical roles.

15. Reference

1. Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, et al. Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. Journal of Clinical Investigation. 1995;95(6):2886.
2. Kovacs WJ, Olsen N. Androgen receptors in human thymocytes. The Journal of Immunology. 1987;139(2):490-3.
3. Butts CL, Bowers E, Horn JC, Shukair SA, Belyavskaya E, Tonelli L, et al. Inhibitory effects of progesterone differ in dendritic cells from female and male rodents. Gender medicine. 2008;5(4):434-47.
4. Martocchia A, Stefanelli M, Cola S, Falaschi P. Sex steroids in autoimmune diseases. Current topics in medicinal chemistry. 2011;11(13):1668-83.

5. Benten WPM, Lieberherr M, Stamm O, Wrehlke C, Guo Z, Wunderlich F. Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Molecular Biology of the Cell. 1999;10(10):3113-23.
6. Ortona E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. Sex-based differences in autoimmune diseases. Annali dell'Istituto Superiore di Sanità. 2016;52(2):205-12.
7. Fujii H, Nawa Y, Tsuchiya H, Matsuno K, Fukumoto T, Fukuda S, et al. Effect of a single administration of testosterone on the immune response and lymphoid tissues in mice. Cellular immunology. 1975;20(2):315-26.
8. Olsen NJ, Kovacs WJ. Gonadal Steroids and Immunity*. Endocrine reviews. 1996;17(4):369-84.
9. Ortona E, Delunardo F, Maselli A, Pierdominici M, Malorni W. Sex hormones and gender disparity in immunity and autoimmunity. Italian Journal of Gender-Specific Medicine. 2015;1(2):45-50.
10. Calabrese LH, Kleiner SM, Barna BP, Skibinski CI, Kirkendall DT, Lahita RG, et al. The effects of anabolic steroids and strength training on the human immune response. Medicine and science in sports and exercise. 1989;21(4):386-92.
11. Cutolo M, Wilder RL. Different roles for androgens and estrogens in the susceptibility to autoimmune rheumatic diseases. Rheumatic Disease Clinics of North America. 2000;26(4):825-39.
12. Kissick HT, Sanda MG, Dunn LK, Pellegrini KL, On ST, Noel JK, et al. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. Proceedings of the National Academy of Sciences. 2014;111(27):9887-92.
13. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. Nature Reviews Endocrinology. 2013;9(1):56-62.
14. Sthoeger Z, Chiorazzi N, Lahita R. Regulation of the immune response by sex hormones. I. In vitro effects of estradiol and testosterone on pokeweed mitogen-induced human B cell differentiation. The Journal of immunology. 1988;141(1):91-8.
15. Trigunaite A, Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system. Cellular immunology. 2015;294(2):87-94.
16. Callewaert DM, Moudgil VK, Radcliff G, Waite R. Hormone specific regulation of natural killer cells by cortisol Direct inactivation of the cytotoxic function of cloned human NK cells without an effect on cellular proliferation. FEBS letters. 1991;285(1):108-10.
17. Marshall-Gradisnik SM, Weatherby RP, Deakin GB, Coutts RA, Meir RA, Connellan PA, et al. Natural killer cell activity following six weeks of strength training in healthy young males with/without testosterone enanthate administration. Journal of Exercise Science and Fitness. 2008;6(2):106-14.
18. Brittenden J, Heys S, Ross J, Eremin O. Natural killer cells and cancer. Cancer. 1996;77(7):1226-43.
19. Parker L, Odell W. Evidence for existence of cortical androgen-stimulating hormone. American Journal of Physiology-Endocrinology and Metabolism. 1979;236(6):E616.
20. Rivera-Woll L, Papalia M, Davis S, Burger H. Androgen insufficiency in women: diagnostic and therapeutic implications. Human reproduction update. 2004;10(5):421-32.
21. Cameron DR, Braunstein GD. Androgen replacement therapy in women. Fertility and sterility. 2004;82(2):273-89.
22. Labrie F, Luu-The V, Labrie C, Bélanger A, Simard J, Lin S-X, et al. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocrine Reviews. 2003;24(2):152-82.

23. Masi AT, Feigenbaum S, Chatterton R, Cutolo M. Integrated hormonal-immunological-vascular (HIV triad) systems interactions in the rheumatic diseases. Clinical and experimental rheumatology. 1994;13(2):203-16.
24. Cutolo M, Castagnetta L. Immunomodulatory Mechanisms Mediated by Sex Hormones in Rheumatoid Arthritisa. Annals of the New York Academy of Sciences. 1996;784(1):237-51.
25. Cutolo M, Sulli A, Villaggio B, Seriolo B, Accardo S. Relations between steroid hormones and cytokines in rheumatoid arthritis and systemic lupus erythematosus. Annals of the rheumatic diseases. 1998;57(10):573.
26. Sohoni P, Sumpter J. Several environmental oestrogens are also anti-androgens. Journal of endocrinology. 1998;158(3):327-39.
27. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite p, p'DDE is a potent androgen receptor antagonist. 1995.
28. John S, Myerscough A, Eyre S, Roby P, Hajeer A, Silman AJ, et al. Linkage of a marker in intron D of the estrogen synthase locus to rheumatoid arthritis. Arthritis & Rheumatism. 1999;42(8):1617-20.
29. Masi AT, Da Silva JAP, Cutolo M. Perturbations of hypothalamic-pituitary-gonadal (HPG) axis and adrenal androgen (AA) functions in rheumatoid arthritis. Baillière's clinical rheumatology. 1996;10(2):295-332.
30. Huang J, Ushiyama T, Inoue K, Mori K, Hukuda S. Possible association of CYP17 gene polymorphisms with the onset of rheumatoid arthritis. Clinical and experimental rheumatology. 1998;17(6):721-4.
31. Cutolo M. The role of the hypothalamus-pituitary-adrenocortical and-gonadal axis in rheumatoid arthritis. Clinical and experimental rheumatology. 1997;16(1):3-6.
32. Stanford JL, Noonan EA, Iwasaki L, Kolb S, Chadwick RB, Feng Z, et al. A polymorphism in the CYP17 gene and risk of prostate cancer. Cancer Epidemiology Biomarkers & Prevention. 2002;11(3):243-7.
33. Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S. Sex hormone status in women suffering from rheumatoid arthritis. The Journal of rheumatology. 1986;13(6):1019-23.
34. Kawasaki T, Ushiyama T, Ueyama H, Inoue K, Mori K, Ohkubo I, et al. Polymorphic CAG repeats of the androgen receptor gene and rheumatoid arthritis. Annals of the rheumatic diseases. 1999;58(8):500-2.
35. Cutolo M, Accardo S. Sex hormones, HLA and rheumatoid arthritis. Clinical and experimental rheumatology. 1990;9(6):641-6.
36. Larsen B, King C, Simms M, Skanes V. Major histocompatibility complex phenotypes influence serum testosterone concentration. Rheumatology. 2000;39(7):758-63.
37. Mori H, Sawairi M, Itoh N, Hanabayashi T, Tamaya T. Effects of sex steroids on immunoglobulin M production by Epstein-Barr virus-transformed B-cell line SKW6-CL4. Journal of in vitro fertilization and embryo transfer. 1991;8(6):329-35.
38. Kanda N, Tsuchida T, Tamaki K. Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. Clinical & Experimental Immunology. 1996;106(2):410-5.
39. Kanda N, Tsuchida T, Tamaki K. Testosterone suppresses anti-DNA antibody production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Arthritis & Rheumatism. 1997;40(9):1703-11.
40. Verthelyi D, Ahmed SA. 17 beta-estradiol, but not 5 alpha-dihydrotestosterone, augments antibodies to double-stranded deoxyribonucleic acid in nonautoimmune C57BL/6J mice. Endocrinology. 1994;135(6):2615-22.

41. Araneo BA, Dowell T, Diegel M, Daynes RA. Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and gamma-interferon, but not IL-2 by activated murine T cells. Blood. 1991;78(3):688-99.
42. Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. The Journal of Immunology. 1997;159(1):3-6.
43. Cutolo M, Villaggio B, Barone A, Sulli A, Accardo S, Granata OM, et al. Primary Cultures of Human Synovial Macrophages Metabolize Androgensa. Annals of the New York Academy of Sciences. 1996;784(1):534-41.
44. Li Z, Danis V, Brooks P. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. Clinical and experimental rheumatology. 1992;11(2):157-62.
45. Cutolo M, Accardo S, Villaggio B, Barone A, Sulli A, Balleari E, et al. Androgen metabolism and inhibition of interleukin-1 synthesis in primary cultured human synovial macrophages. Mediators of inflammation. 1995;4(2):138-43.
46. Pham CT. Neutrophil serine proteases: specific regulators of inflammation. Nature Reviews Immunology. 2006;6(7):541-50.
47. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. Nature Reviews Immunology. 2008;8(10):776-87.
48. Mantalaris A, Panoskaltsis N, Sakai Y, Bourne P, Chang C, Messing EM, et al. Localization of androgen receptor expression in human bone marrow. The Journal of pathology. 2001;193(3):361-6.
49. Ibáñez L, Jaramillo AM, Ferrer A, de Zegher F. High neutrophil count in girls and women with hyperinsulinaemic hyperandrogenism: normalization with metformin and flutamide overcomes the aggravation by oral contraception. Human Reproduction. 2005;20(9):2457-62.
50. Chuang K-H, Altuwaijri S, Li G, Lai J-J, Chu C-Y, Lai K-P, et al. Neutropenia with impaired host defense against microbial infection in mice lacking androgen receptor. The Journal of experimental medicine. 2009;206(5):1181-99.
51. Gaspar M-L, Meo T, Bourgarel P, Guenet J-L, Tosi M. A single base deletion in the Tfm androgen receptor gene creates a short-lived messenger RNA that directs internal translation initiation. Proceedings of the National Academy of Sciences. 1991;88(19):8606-10.
52. Olsen NJ, Kovacs WJ. Effects of androgens on T and B lymphocyte development. Immunologic research. 2001;23(2-3):281-8.
53. Eaton V, Blackmore T. Nilutamide-induced neutropenia. BJU international. 2001;88(7):801-2.
54. McDonnell N, Livingston RB. Severe reversible neutropenia following treatment of prostate cancer with flutamide. The Journal of urology. 1994;151(5):1353-4.
55. Redell MS, Tsimelzon A, Hilsenbeck SG, Tweardy DJ. Conditional overexpression of Stat3α in differentiating myeloid cells results in neutrophil expansion and induces a distinct, antiapoptotic and pro-oncogenic gene expression pattern. Journal of leukocyte biology. 2007;82(4):975-85.
56. McLemore ML, Grewal S, Liu F, Archambault A, Poursine-Laurent J, Haug J, et al. STAT-3 activation is required for normal G-CSF-dependent proliferation and granulocytic differentiation. Immunity. 2001;14(2):193-204.
57. Hermans MH, Antonissen C, Ward AC, Mayen AE, Ploemacher RE, Touw IP. Sustained receptor activation and hyperproliferation in response to granulocyte colony-stimulating factor

(G-CSF) in mice with a severe congenital neutropenia/acute myeloid leukemia–derived mutation in the G-CSF receptor gene. The Journal of experimental medicine. 1999;189(4):683-92.
58. Jenkins BJ, Roberts AW, Najdovska M, Grail D, Ernst M. The threshold of gp130-dependent STAT3 signaling is critical for normal regulation of hematopoiesis. Blood. 2005;105(9):3512-20.
59. Kimura A, Kinjyo I, Matsumura Y, Mori H, Mashima R, Harada M, et al. SOCS3 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling. Journal of Biological Chemistry. 2004;279(8):6905-10.
60. Croker BA, Metcalf D, Robb L, Wei W, Mifsud S, DiRago L, et al. SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis. Immunity. 2004;20(2):153-65.
61. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. Proceedings of the National Academy of Sciences. 2003;100(5):2645-50.
62. Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, et al. Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis. Nature medicine. 1999;5(7):828-31.
63. Lai J-J, Lai K-P, Chuang K-H, Chang P, Yu I-C, Lin W-J, et al. Monocyte/macrophage androgen receptor suppresses cutaneous wound healing in mice by enhancing local TNF-α expression. The Journal of clinical investigation. 2009;119(12):3739.
64. De Larco JE, Wuertz BR, Furcht LT. The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clinical Cancer Research. 2004;10(15):4895-900.
65. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nature Reviews Immunology. 2005;5(12):953-64.
66. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003;19(1):71-82.
67. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends in cell biology. 2005;15(11):599-607.
68. Mills SJ, Ashworth JJ, Gilliver SC, Hardman MJ, Ashcroft GS. The sex steroid precursor DHEA accelerates cutaneous wound healing via the estrogen receptors. Journal of investigative dermatology. 2005;125(5):1053-62.
69. Szpaderska AM, DiPietro LA. Inflammation in surgical wound healing: friend or foe? Surgery. 2005;137(5):571-3.
70. Gilliver SC, Ashworth JJ, Mills SJ, Hardman MJ, Ashcroft GS. Androgens modulate the inflammatory response during acute wound healing. Journal of cell science. 2006;119(4):722-32.
71. Rahman F, Christian HC. Non-classical actions of testosterone: an update. Trends in Endocrinology & Metabolism. 2007;18(10):371-8.
72. Sun YH, Gao X, Tang YJ, Xu CL, Wang LH. Androgens Induce Increases in Intracellular Calcium Via a G Protein—Coupled Receptor in LNCaP Prostate Cancer Cells. Journal of andrology. 2006;27(5):671-8.
73. Yeh S, Miyamoto H, Shima H, Chang C. From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proceedings of the National Academy of Sciences. 1998;95(10):5527-32.
74. Yeh S, Miyamoto H, Chang C. Hydroxyflutamide may not always be a pure antiandrogen. The Lancet. 1997;349(9055):852-3.
75. Gao H, Ouyang X, Banach-Petrosky WA, Gerald WL, Shen MM, Abate-Shen C. Combinatorial activities of Akt and B-Raf/Erk signaling in a mouse model of androgen-

independent prostate cancer. Proceedings of the National Academy of Sciences. 2006;103(39):14477-82.
76. Guo Z, Dai B, Jiang T, Xu K, Xie Y, Kim O, et al. Regulation of androgen receptor activity by tyrosine phosphorylation. Cancer cell. 2006;10(4):309-19.
77. Liu P. Death A. Handelsman D. Androgens and cardiovascular disease Endocr Rev. 2003;24:313-40.
78. Pergola C, Dodt G, Rossi A, Neunhoeffer E, Lawrenz B, Northoff H, et al. ERK-mediated regulation of leukotriene biosynthesis by androgens: a molecular basis for gender differences in inflammation and asthma. Proceedings of the National Academy of Sciences. 2008;105(50):19881-6.
79. Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. Current opinion in immunology. 2005;17(5):457-62.
80. Flores KG, Li J, Sempowski GD, Haynes BF, Hale LP. Analysis of the human thymic perivascular space during aging. The Journal of clinical investigation. 1999;104(8):1031-9.
81. Goldberg GL, Alpdogan Ö, Muriglan SJ, Hammett MV, Milton MK, Eng JM, et al. Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. The Journal of Immunology. 2007;178(11):7473-84.
82. Henderson J. On the relationship of the thymus to the sexual organs: I. The influence of castration on the thymus. The Journal of physiology. 1904;31(3-4):222.
83. FITZPATRICK F, LEPAULT F, HOMO-DELARCHE F, BACH J-F, DARDENNE M. Influence of Castration, Alone or Combined with Thymectomy, on the Development of Diabetes in the Nonobese Diabetic Mouse*. Endocrinology. 1991;129(3):1382-90.
84. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ. Androgen Receptors in Thymic Epithelium Modulate Thymus Size and Thymocyte Development 1. Endocrinology. 2001;142(3):1278-83.
85. Heng TS, Goldberg GL, Gray DH, Sutherland JS, Chidgey AP, Boyd RL. Effects of castration on thymocyte development in two different models of thymic involution. The Journal of Immunology. 2005;175(5):2982-93.
86. Roden AC, Moser MT, Tri SD, Mercader M, Kuntz SM, Dong H, et al. Augmentation of T cell levels and responses induced by androgen deprivation. The Journal of Immunology. 2004;173(10):6098-108.
87. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. The Journal of Immunology. 2005;175(4):2741-53.
88. Radojević K, Arsenović-Ranin N, Kosec D, Pešić V, Pilipović I, Perišić M, et al. Neonatal castration affects intrathymic kinetics of T-cell differentiation and the spleen T-cell level. Journal of endocrinology. 2007;192(3):669-82.
89. McCruden A, Stimson W. Androgen receptor in the human thymus. Immunology letters. 1984;8(1):49-53.
90. Grossman CJ, Nathan P, Taylor BB, Sholiton LJ. Rat thymic dihydrotestosterone receptor: preparation, location and physiochemical properties. Steroids. 1979;34(5):539-53.
91. Viselli SM, Olsen NJ, Shults K, Steizer G, Kovacs WJ. Immunochemical and flow cytometric analysis of androgen receptor expression in thymocytes. Molecular and cellular endocrinology. 1995;109(1):19-26.
92. Gray DH, Ueno T, Chidgey AP, Malin M, Goldberg GL, Takahama Y, et al. Controlling the thymic microenvironment. Current opinion in immunology. 2005;17(2):137-43.

93. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annual review of immunology. 2003;21(1):139-76.
94. Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nature immunology. 2001;2(11):1032-9.
95. Vacchio MS, Williams JA, Hodes RJ. A novel role for CD28 in thymic selection: elimination of CD28/B7 interactions increases positive selection. European journal of immunology. 2005;35(2):418-27.
96. Palaszynski KM, Loo KK, Ashouri JF, Liu H-b, Voskuhl RR. Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Journal of neuroimmunology. 2004;146(1):144-52.
97. Graff RJ, Lappé MA, Snell GD. The influence of the gonads and adrenal glands on the immune response to skin grafts. Transplantation. 1969;7(2):105-11.
98. Bebo BF, Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. The Journal of Immunology. 1999;162(1):35-40.
99. Angele MK, Schwacha MG, Ayala A, Chaudry IH. Effect of gender and sex hormones on immune responses following shock. Shock. 2000;14(2):81-90.
100. Messingham K, Shirazi M, Duffner L, Emanuele M, Kovacs E. Testosterone receptor blockade restores cellular immunity in male mice after burn injury. Journal of Endocrinology. 2001;169(2):299-308.
101. Bao M, Yang Y, Jun H-S, Yoon J-W. Molecular mechanisms for gender differences in susceptibility to T cell-mediated autoimmune diabetes in nonobese diabetic mice. The Journal of Immunology. 2002;168(10):5369-75.
102. Hsieh C-S, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T Cells Through IL-12. Science. 1993;260:547.
103. Angele MK, Knöferl MW, Ayala A, Bland KI, Chaudry IH. Testosterone and estrogen differently effect Th1 and Th2 cytokine release following trauma-haemorrhage. Cytokine. 2001;16(1):22-30.
104. Edwards JC, Cambridge G. B-cell targeting in rheumatoid arthritis and other autoimmune diseases. Nature Reviews Immunology. 2006;6(5):394-403.
105. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nature Reviews Immunology. 2008;8(9):737-44.
106. Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. The Journal of Immunology. 1998;161(1):27-34.
107. Ellis TM, Moser MT, Le PT, Flanigan RC, Kwon ED. Alterations in peripheral B cells and B cell progenitors following androgen ablation in mice. International immunology. 2001;13(4):553-8.
108. Olsen NJ, Gu X, Kovacs WJ. Bone marrow stromal cells mediate androgenic suppression of B lymphocyte development. The Journal of clinical investigation. 2001;108(11):1697-704.
109. Olsen NJ, Kovacs WJ. Testosterone Treatment of Systemic Lupus Erythematosus in a Patient With Klinefelter's Syndrome. The American journal of the medical sciences. 1995;310(4):158-60.
110. Spector T, Perry L, Tubb G, Silman A, Huskisson E. Low free testosterone levels in rheumatoid arthritis. Annals of the rheumatic diseases. 1988;47(1):65-8.

111. Pope JE, Joneja M, Hong P. Anti-androgen treatment of prostatic carcinoma may be a risk factor for development of rheumatoid arthritis. Journal of rheumatology. 2002;29(11):2459-62.