The American Journal of Pathology, Vol. 181, No. 5, November 2012

http://dx.doi.org/10.1016/j.ajpath.2012.07.008

Review

Androgen Receptor Influences on Body Defense System via Modulation of Innate and Adaptive Immune Systems

Lessons from Conditional AR Knockout Mice

Jiann-Jyh Lai,*†‡§¶ Kuo-Pao Lai,*†‡§¶ Weiping Zeng,*†‡§¶ Kuang-Hsiang Chuang,*†‡§¶ Saleh Altuwaijri,*†‡§¶ and Chawnshang Chang*†‡§¶‖

From the George Whipple Laboratory for Cancer Research, the Departments of Pathology,† Urology,‡ Radiation Oncology,§ and the Wilmot Cancer Center,¶ University of Rochester Medical Center, Rochester, New York; and the Sex Hormone Research Center,‖ China Medical University/Hospital, Taichung, Taiwan*

Upon insult, such as infection or tissue injury, the innate and adaptive immune systems initiate a series of responses to defend the body. 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. Removal of such suppression causes thymic enlargement and excessive export of immature 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. ***(Am J Pathol 2012, 181:1504–1512; http://dx.doi.org/10.1016/j.ajpath.2012.07.008)***

The immune system includes both innate and adaptive immune responses. Once insult to the body (eg, infection) is initially encountered, the innate system acts within minutes, followed some hours later by early induced responses conducted by recruited effector cells, such as neutrophils and macrophages, in a nonspecific manner.[1,2] In most cases, infections are eliminated by the innate immune system. When pathogens breach the first line of immune defense, however, the adaptive immune system is activated with antigen-specific effectors (eg, T and B cells), and the generation of memory cells ensures a long-lasting and prompt response to recurrent infection with the same pathogens. On the other hand, the context of the activated innate immunity also shapes the outcome of adaptive immune responses.[3]

Androgen and androgen receptor (AR) signals control the development and function of both male and female reproductive systems.[4–6] The *AR* gene is located on the X chromosome in both the human and the murine genome. AR is a prototypical nuclear receptor, containing an N-terminal domain, a ligand-binding domain, a DNA-binding domain, and a C-terminal domain.[7] After binding of its ligands, testosterone or 5α-dihydrotestosterone, AR translocates into the nucleus and binds to androgen responsive elements on the promoters or enhancers of target genes, thereby turning on their expression.[8] The expression of AR has been detected in various immune cell lineages, including neutrophils, mast cells, macrophages, B cells, and T cells,[9–11] implying the involvement of androgen and its receptor (androgen/AR) in regulating the development and function of the immune system.

Supported by the NIH (NIH-NIDDK R01-DK73414), the George Whipple Professorship Endowment, and the Taiwan Department of Health Clinical Trial and Research Center of Excellence grant DOH99-TD-B-111-004 (China Medical University, Taichung, Taiwan, to C.C.).

Accepted for publication July 18, 2012.

J.J.L. and K.P.L. contributed equally to this work.

CME Disclosure: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interest to disclose.

Address reprint requests to Chawnshang Chang, Ph.D., 601 Elmwood Ave., Box 626, Rochester, NY 14642. E-mail: chang@urmc.rochester.edu.

It has long been suspected that, in both animals and humans, the male sex hormones may modulate the development and function of the immune system. Males are at higher risk of developing sepsis, acute respiratory distress, and multiorgan failure after soft-tissue traumatic hemorrhagic shock and thermal injury, in part because of immune suppression and abnormal activation of neutrophils.[12] On the other hand, males are less prone to autoimmune diseases. Of more than 70 chronic disorders categorized as autoimmune diseases, many affect predominantly women.[13,14] Various studies have uncovered important immune regulatory functions of androgen/AR (as discussed below), and such nonclassical roles of androgen/AR outside the reproductive system are important for understanding the pathogenesis of these immunological conditions.

Recent studies using conditional AR knockout (ARKO) mice, with knockout of AR in selective immune cells, opened a new era for investigating the nonclassical roles of androgen/AR involved in immune regulation; these studies also suggest potential for the development of new therapeutic strategies for treating immune-related diseases.[6,15] In this review, we summarize the roles of androgen/AR in both the innate and adaptive components of the immune system (specifically neutrophils, macrophages, T cells, and B cells), as well as new evidence from studies using conditional ARKO mice. We also address the potential influences of androgen/AR on immune-related diseases.

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 chemokines and cytokines to attract and activate monocytes/macrophages.[16,17]

Originating from hematopoietic stem cells and progenitors, neutrophils in the bone marrow are composed of a precursor 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.[10] Both band and segmented neutrophils express high levels of nuclear and cytoplasmic AR.[10] 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.[10] Young women who suffer from polycystic ovarian syndrome with hyperandrogenism have higher neutrophil counts, which can be normalized by treatment with the antiandrogen flutamide, suggesting that androgen/AR signals can promote neutrophil production.[18]

Consistent with this notion, we found almost 90% reduction of 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.[19] Tfm is a natural single nucleotide deletion mutation at the first exon of the *AR* gene that results in expression of unstable AR transcripts.[20,21] 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,[22,23] 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.[19] Further analysis of neutrophil lineages showed that myelocytes/metamyelocytes and 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.[19] 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).[19]

Mechanistically, AR appears to stimulate neutrophil production 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).[19] Such AR-PIAS3 interaction does not rely on ligand binding, and could be considered an 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.[19] Other lines of evidence using *in vitro* and transgenic mouse studies have demonstrated that Stat3 plays an essential role in G-CSF-dependent granulopoiesis.[24–26] 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.[27–29]

Neutrophils are a key component of the innate immune system in clearing bacterial pathogen infection. As a result of neutrophil reduction, the ability of ARKO mice to survive pathogenic bacteria challenge is severely compromised.[19] 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.[19]

It has long been suspected that chronic inflammation can promote prostate tumorigenesis and progression. Several lines of evidence show that mice lacking proinflammatory cytokines are resistant to carcinogenesis, tumor invasion, and angiogenesis.[30,31] Because it is important for sustaining proinflammatory cytokine expression (TNF-α, IL-1β, IL-6) in neutrophils and macrophages,[19,32] AR may play a pivotal role in remodeling the tumor-promoting microenvironment 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.[33] 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.[33]

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.[34] 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.[17,35] 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.[36] Interestingly, AR expression has been detected in the monocyte/macrophage population,[37] suggesting that AR might modulate macrophage functions involved in these inflammatory conditions.

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.[36,38] Castration of animals or blockade of androgen action by antiandrogen (flutamide) accelerated wound healing and suppressed macrophage recruitment to the wounds.[37,39] 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 flutamide treatment. Conversely, *in vitro* studies found that lipopolysaccharide-induced TNF-α production in macrophages was enhanced by testosterone treatment.[37] 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,[40,41] 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 Cre-loxP system to generate conditional ARKO mice found that mice lacking AR had accelerated wound healing, and such acceleration could not be reversed by 5α-dihydrotestosterone restoration, suggesting that AR signals, rather than androgen itself, are critical to suppression of wound healing.[32] 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.[32] 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,[42] antiandrogens,[43] and kinases.[44,45] Similarly, as already noted, AR might go through androgen-independent signals to promote neutrophil differentiation.[19] 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 in wound 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-α expression via the following mechanisms: i) increasing circulating inflammatory monocyte population, ii) increasing recruitment of monocytes by promoting their chemotaxis through up-regulation of CCR2 expression, and iii) increasing TNF-α expression at the transcriptional level in macrophages.[32] Although the results from bone marrow transplantation imply androgen-independent AR func-

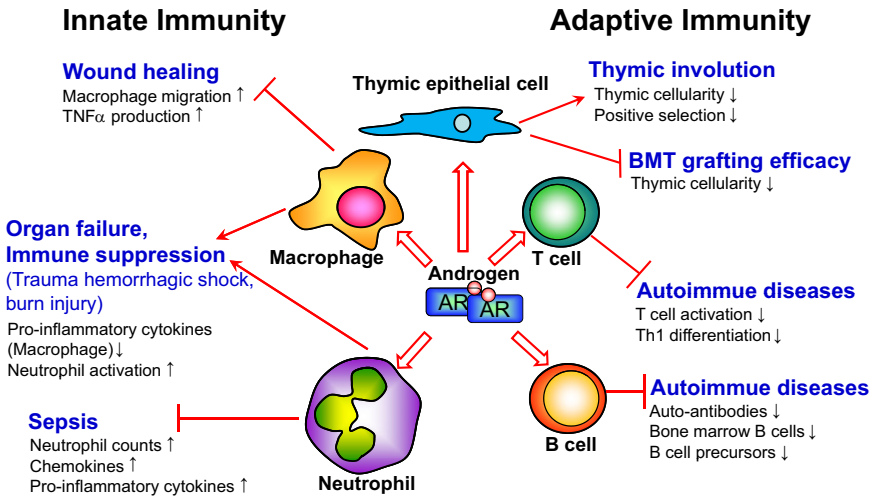

Figure 1. Roles of AR in the innate and adaptive immune compartments, including macrophages, neutrophils, thymic epithelial cells, T cells, and B cells. The model incorporates phenotypic observation and associated diseases from conditional ARKO mice with AR deletion in macrophages (cutaneous wound healing, organ failure, and immune suppression), neutrophils (organ failure, immune suppression, and sepsis), thymic epithelial cells (thymic involution and bone marrow transplantation grafting efficacy), T cells (autoimmune diseases), and B cells (autoimmune diseases).

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.[32]

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[46] and asthma.[47] 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.

Role of Androgen/AR Signals in the Adaptive Immune System

Unlike macrophages and neutrophils, which play a central role in the innate immune system,[48] T and B cells represent 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.[21]

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,[49,50] 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.[51] 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 thymic enlargement by androgen replacement in castrated animals.[52,53] Further supporting the role of androgen/AR in the negative regulation of thymic development, studies by Fitzpatrick et al,[53] Olsen et al,[54] and Lai et al (unpublished data) in ARKO and Tfm mice, as well as castrated mice, found thymic enlargement and increase of thymic cellularity in these mice, which could be due to more proliferating and less apoptotic thymocytes.

The net outcome of castration-induced reversal of thymic involution is increased output of naïve CD4 and CD8 single-positive T cells.[54–56] Although neonatal castration in a rat model led to increased incidence of autoimmune diseases,[57] 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.

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 thymocytes and thymic stromal cells have been extensively analyzed. Although some earlier ligand-binding studies indicated that thymocytes do not express AR,[58,59] later studies have demonstrated AR expression in thymocytes by flow cytometry and immunoblotting in mice and ligand binding assays in humans.[60,61] AR expression in thymocytes can 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 $CD3^{low}CD8^{+}$ immature thymocytes.[61] AR expression in the thymic stroma was also demonstrated.[53] Within the thymic stroma, AR is expressed predominantly in the cortical and medullar thymic epithelial cells.[53]

Reciprocal bone marrow transplantation between normal male mice and Tfm male mice was used to determine whether AR expression in thymocytes or thymic epithelial cells 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 mice to Tfm mice developed enlarged thymus and were insensitive to androgen.[53] These findings, together with the evidence for AR expression in thymic stroma, suggest that AR expression in thymic stroma, 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 thymic stroma (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.[62] cTECs play a crucial role in positive selection.[63] After positive selection, the double-positive thymocytes 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 tissue-restricted self-antigens in these cells.[64] 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 TGFβ1, IL-6, and CD80/CD86, but more of IL-7 and CCL21, compared with wild-type thymic stromal cells on 5α-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 thymocytes was increased,[65] 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.

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.

Table 1. Immune-Related Phenotypes in Tissue-Specific ARKO Mice

Characteristic	Castration	Tfm	G-ARKO	T-ARKO
Mutation/Cre promoter		Natural *AR* mutant with AR mRNA instability	β-actin promoter	Proximal region of *lck* promoter
Specific tissue/cell	Systemic androgen level decreased	General	General	Thymocyte and T cell
Phenotype				
T-cell development	Thymus enlargement	Thymus enlargement	Thymus enlargement	Normal thymus size
B-cell development/ function	Increased immature B-cell population	Immature B-cell population increased	Immature B-cell population increased; autoantibodies increased	NA
Neutrophil development/ function	Moderate reduction of neutrophil counts	Neutropenia	Neutropenia and susceptible to bacterial infections	NA
Wound healing	Accelerated cutaneous wound healing	NA	Accelerated cutaneous wound healing	NA
Related human disease	Autoimmune disease; wound healing	Autoimmune disease; septicemia; wound healing	Autoimmune disease; septicemia; wound healing	Autoimmune disease

(*table continues*)

The mice from castration treatment, Tfm mutation, G-ARKO, T-ARKO, B-ARKO, M-ARKO, TEC-ARKO (K-ARKO), and F-ARKO are included for comparison. Also included is usage of specific Cre promoter, deficiency of AR in selective cells/tissue types, phenotypes, and related human diseases.
NA, not applicable.

Administration of androgens to female mice alleviated rejection of skin grafts and alleviated CD4 T cell–mediated autoimmune diseases such as experimental autoimmune encephalomyelitis, whereas castration in male mice exacerbated this autoimmune disease.[66–68] The effects of androgen/AR on peripheral T cell response are twofold: 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.[55] 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,[21] whereas others have shown weak expression of AR mRNA in peripheral T cells.[69] 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.[70,71] Thus, androgen/AR appears to suppress T cell proliferation by diminishing IL-2 signaling in T cells.

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 peripheral CD4 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.[69] 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 T-helper effector cells induced by TCR stimulation.[69] Androgen treatment also decreased IL-12–induced IFN-γ production by CD4 T cells through inhibition of Stat4 activation.[72] Although the study was performed on resting CD4 T cells that were previously activated by concanavalin A, one may extrapolate a role of androgen-mediated inhibition of Stat4 activation in dampening Th1 differentiation from naïve CD4 T cells as well, because IL-12 is a potent inducer of Th1 cell differentiation.[73]

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 observed in hemorrhagic shock that androgen/AR suppressed IFN-γ and IL-2 expression by splenocytes in male mice, whereas female mice maintained strong production of these two cytokines.[74]

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-

Table 1. *Continued*

B-ARKO	M-ARKO	TEC-ARKO (K-ARKO)	F-ARKO
CD19 promoter	*Lysozyme M* promoter	Bovine *keratin-5* promoter	*Fsp1* promoter
B cell	Myeloid cell	Epithelial cell	Fibroblast
NA	NA	Thymus enlargement	Normal thymus size
Immature B-cell population increased; autoantibodies increased	NA	NA	NA
NA	Normal granulopoiesis in bone marrow; moderate reduction of neutrophil counts in circulation	NA	NA
NA	Accelerated cutaneous wound healing	Wound healing rate similar to wild-type but re-epithelialization rate decreased	Wound healing rate similar to wild-type but re-epithelialization rate decreased
Autoimmune disease	Wound healing	Autoimmune disease; wound healing	Wound healing

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 in autoimmunity and in autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, and therefore B cells have been one of the primary targets for autoimmune therapy.[75] Moreover, profound sexual dimorphism in autoimmune diseases implies that sex hormones might involve the regulation of B-cell responses and/or their development.[76]

B-cell development is negatively regulated by androgen/AR. Castration of male mice caused splenic enlargement, due primarily to the increase of B cells.[52,61] 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.[9] Similar increases of B cells in bone marrow and spleen were also observed in Tfm mice.[77] 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.[78] The increase of B-cell progenitors in the bone marrow led to the 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.[77,78]

Both bone marrow stroma and B cells express AR,[10] and both cell types could be the targets of androgen/AR in regulating B-cell lymphopoiesis. In bone marrow chimeric studies between Tfm and normal mice, the expression of intact AR on bone marrow stroma was critical for the inhibition of B-cell lymphopoiesis by androgen/AR.[79] On the other hand, mice with either general or B cell–specific knockout of the AR gene showed enhanced B-cell lymphopoiesis, demonstrating that B cells are direct targets of androgen.[15] However, the B cell–specific ARKO 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 increased proliferation and reduced apoptosis.[15] Mechanistically, the expression of several key proapoptotic molecules, such as Fas/FasL and caspase-3/8, were reduced in ARKO B cells.[15] In contrast, elevated cell proliferation was observed in ARKO B cells, accompanied by up-regulation of p65 and Bcl-2 expression.[15] These findings came mainly from primary culture of immature B cells cultured 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.[15]

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.[15] 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 such consequences could be found in hypogonadal men with rheumatoid arthritis and/or systemic lupus erythematosus[80,81] and in prostate cancer patients who received androgen ablation therapy and subsequently developed rheumatoid arthritis at higher rates.[82] Conversely, androgen replacement therapy of hypogonadal men with rheumatoid arthritis ameliorated clinical symptoms and reduced the concentrations of IgM rheumatoid factors.

Concluding Remarks

The study of immune regulation by androgen/AR has attracted long-lasting interest. The recent availability of cell lineage-specific ARKO mice has opened new avenues of research.[6,15,19,32] 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 (Figure 1; Table 1). For neutrophils, androgen/AR plays a positive role for both development and function. These effects appear to be beneficial for hosts, to survive bacterial challenge.[19] Although androgen/AR enhances proinflammatory cytokine production by macrophages in wound healing,[32] 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.[15]

Further studies applying these valuable mouse models could not only advance our knowledge and understanding of the communication between androgen/AR and the immune system, but could also suggest novel strategies to modulate the immune system under clinical conditions in which regulation of immune responses by androgen/AR plays critical roles.

Acknowledgment

We thank Karen Wolf for manuscript preparation and critical review.

References

1. Janeway CA Jr, Medzhitov R: Innate immune recognition. Annu Rev Immunol 2002, 20:197–216

2. Medzhitov R, Janeway C Jr: Innate immunity. N Engl J Med 2000, 343:338–344
3. Hoebe K, Janssen E, Beutler B: The interface between innate and adaptive immunity. Nat Immunol 2004, 5:971–974
4. Palacios S: Androgens and female sexual function. Maturitas 2007, 57:61–65
5. Hu YC, Wang PH, Yeh S, Wang RS, Xie C, Xu Q, Zhou X, Chao HT, Tsai MY, Chang C: Subfertility and defective folliculogenesis in female mice lacking androgen receptor. Proc Natl Acad Sci USA 2004, 101:11209–11214
6. Yeh S, Tsai MY, Xu Q, Mu XM, Lardy H, Huang KE, Lin H, Yeh SD, Altuwaijri S, Zhou X, Xing L, Boyce BF, Hung MC, Zhang S, Gan L, Chang C: Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci USA 2002, 99: 13498–13503
7. Chang CS, Kokontis J, Liao ST: Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 1988, 240:324–326
8. Brinkmann AO, Blok LJ, de Ruiter PE, Doesburg P, Steketee K, Berrevoets CA, Trapman J: Mechanisms of androgen receptor activation and function. J Steroid Biochem Mol Biol 1999, 69:307–313
9. Viselli SM, Reese KR, Fan J, Kovacs WJ, Olsen NJ: Androgens alter B cell development in normal male mice. Cell Immunol 1997, 182:99–104
10. Mantalaris A, Panoskaltsis N, Sakai Y, Bourne P, Chang C, Messing EM, Wu JH: Localization of androgen receptor expression in human bone marrow. J Pathol 2001, 193:361–366
11. Chen W, Beck I, Schober W, Brockow K, Effner R, Buters JT, Behrendt H, Ring J: Human mast cells express androgen receptors but treatment with testosterone exerts no influence on IgE-independent mast cell degranulation elicited by neuromuscular blocking agents. Exp Dermatol 2010, 19:302–304
12. Deitch EA: Multiple organ failure. Pathophysiology and potential future therapy. Ann Surg 1992, 216:117–134
13. Jacobson DL, Gange SJ, Rose NR, Graham NM: Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol 1997, 84:223–243
14. Whitacre CC: Sex differences in autoimmune disease. Nat Immunol 2001, 2:777–780
15. Altuwaijri S, Chuang KH, Lai KP, Lai JJ, Lin HY, Young FM, Bottaro A, Tsai MY, Zeng WP, Chang HC, Yeh S, Chang C: Susceptibility to autoimmunity and B cell resistance to apoptosis in mice lacking androgen receptor in B cells. Mol Endocrinol 2009, 23:444–453
16. Pham CT: Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol 2006, 6:541–550
17. Rittirsch D, Flierl MA, Ward PA: Harmful molecular mechanisms in sepsis. Nat Rev Immunol 2008, 8:776–787
18. 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. Hum Reprod 2005, 20:2457–2462
19. Chuang KH, Altuwaijri S, Li G, Lai JJ, Chu CY, Lai KP, Lin HY, Hsu JW, Keng P, Wu MC, Chang C: Neutropenia with impaired host defense against microbial infection in mice lacking androgen receptor. J Exp Med 2009, 206:1181–1199
20. Gaspar ML, Meo T, Bourgarel P, Guenet JL, Tosi M: A single base deletion in the Tfm androgen receptor gene creates a short-lived messenger RNA that directs internal translation initiation. Proc Natl Acad Sci USA 1991, 88:8606–8610
21. Olsen NJ, Kovacs WJ: Effects of androgens on T and B lymphocyte development. Immunol Res 2001, 23:281–288
22. Eaton VS, Blackmore TK: Nilutamide-induced neutropenia. BJU Int 2001, 88:801–802
23. McDonnell ND, Livingston RB: Severe reversible neutropenia following treatment of prostate cancer with flutamide. J Urol 1994, 151: 1353–1354
24. Redell MS, Tsimelzon A, Hilsenbeck SG, Tweardy DJ: Conditional overexpression of Stat3alpha in differentiating myeloid cells results in neutrophil expansion and induces a distinct, antiapoptotic and prooncogenic gene expression pattern. J Leukoc Biol 2007, 82:975–985
25. McLemore ML, Grewal S, Liu F, Archambault A, Poursine-Laurent J, Haug J, Link DC: STAT-3 activation is required for normal G-CSF-dependent proliferation and granulocytic differentiation. Immunity 2001, 14:193–204
26. 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. J Exp Med 1999, 189:683–692
27. 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, 105:3512–3520
28. Kimura A, Kinjyo I, Matsumura Y, Mori H, Mashima R, Harada M, Chien KR, Yasukawa H, Yoshimura A: SOCS3 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling. J Biol Chem 2004, 279:6905–6910
29. Croker BA, Metcalf D, Robb L, Wei W, Mifsud S, DiRago L, Cluse LA, Sutherland KD, Hartley L, Williams E, Zhang JG, Hilton DJ, Nicola NA, Alexander WS, Roberts AW: SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis. Immunity 2004, 20:153–165
30. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA, Apte RN: IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci USA 2003, 100:2645–2650
31. Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, Kollias G, Balkwill F: Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis [Erratum appeared in Nat Med 1999, 5:1087]. Nat Med 1999, 5:828–831
32. Lai JJ, Lai KP, Chuang KH, Chang P, Yu IC, Lin WJ, Chang C: Monocyte/macrophage androgen receptor suppresses cutaneous wound healing in mice through enhancing local TNF-alpha expression. J Clin Invest 2009, 119:3739–3751
33. De Larco JE, Wuertz BR, Furcht LT: The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clin Cancer Res 2004, 10:4895–4900
34. Gordon S, Taylor PR: Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005, 5:953–964
35. Geissmann F, Jung S, Littman DR: Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 2003, 19:71–82
36. Martin P, Leibovich SJ: Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 2005, 15:599–607
37. Ashcroft GS, Mills SJ: Androgen receptor-mediated inhibition of cutaneous wound healing. J Clin Invest 2002, 110:615–624
38. Szpaderska AM, DiPietro LA: Inflammation in surgical wound healing: friend or foe? Surgery 2005, 137:571–573
39. Gilliver SC, Ashworth JJ, Mills SJ, Hardman MJ, Ashcroft GS: Androgens modulate the inflammatory response during acute wound healing. J Cell Sci 2006, 119:722–732
40. Rahman F, Christian HC: Non-classical actions of testosterone: an update. Trends Endocrinol Metab 2007, 18:371–378
41. 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. J Androl 2006, 27:671–678
42. Yeh S, Miyamoto H, Shima H, Chang C: From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proc Natl Acad Sci USA 1998, 95:5527–5532
43. Yeh S, Miyamoto H, Chang C: Hydroxyflutamide may not always be a pure antiandrogen. Lancet 1997, 349:852–853
44. 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 [Erratum appeared in Proc Natl Acad Sci USA 2007, 104:17554]. Proc Natl Acad Sci USA 2006, 103:14477–14482
45. Guo Z, Dai B, Jiang T, Xu K, Xie Y, Kim O, Nesheiwat I, Kong X, Melamed J, Handratta VD, Njar VC, Brodie AM, Yu LR, Veenstra TD, Chen H, Qiu Y: Regulation of androgen receptor activity by tyrosine phosphorylation [Erratum appeared in Cancer Cell 2007, 11:97]. Cancer Cell 2006, 10:309–319
46. Liu PY, Death AK, Handelsman DJ: Androgens and cardiovascular disease. Endocr Rev 2003, 24:313–340
47. Pergola C, Dodt G, Rossi A, Neunhoeffer E, Lawrenz B, Northoff H, Samuelsson B, Rådmark O, Sautebin L, Werz O: ERK-mediated regulation of leukotriene biosynthesis by androgens: a molecular basis

for gender differences in inflammation and asthma. Proc Natl Acad Sci USA 2008, 105:19881–19886
48. Gomez CR, Boehmer ED, Kovacs EJ: The aging innate immune system. Curr Opin Immunol 2005, 17:457–462
49. Flores KG, Li J, Sempowski GD, Haynes BF, Hale LP: Analysis of the human thymic perivascular space during aging. J Clin Invest 1999, 104:1031–1039
50. Goldberg GL, Alpdogan O, Muriglan SJ, Hammett MV, Milton MK, Eng JM, Hubbard VM, Kochman A, Willis LM, Greenberg AS, Tjoe KH, Sutherland JS, Chidgey A, van den Brink MR, Boyd RL: Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. J Immunol 2007, 178: 7473–7484
51. Henderson J: On the relationship of the thymus to the sexual organs: I. The influence of castration on the thymus. J Physiol 1904, 31:222–229
52. Fitzpatrick F, Lepault F, Homo-Delarche F, Bach JF, Dardenne M: Influence of castration, alone or combined with thymectomy, on the development of diabetes in the nonobese diabetic mouse. Endocrinology 1991, 129:1382–1390
53. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ: Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. Endocrinology 2001, 142:1278–1283
54. 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. J Immunol 2005, 175:2982–2993
55. Roden AC, Moser MT, Tri SD, Mercader M, Kuntz SM, Dong H, Hurwitz AA, McKean DJ, Celis E, Leibovich BC, Allison JP, Kwon ED: Augmentation of T cell levels and responses induced by androgen deprivation. J Immunol 2004, 173:6098–6108
56. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, Blazar BR, Millar JL, Malin MA, Chidgey AP, Boyd RL: Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol 2005, 175:2741–2753
57. Radojevic K, Arsenovic-Ranin N, Kosec D, Pesic V, Pilipovic I, Perisic M, Plecas-Solarovic B, Leposavic G: Neonatal castration affects intrathymic kinetics of T-cell differentiation and the spleen T-cell level. J Endocrinol 2007, 192:669–682
58. McCruden AB, Stimson WH: Androgen receptor in the human thymus. Immunol Lett 1984, 8:49–53
59. Grossman CJ, Nathan P, Taylor BB, Sholiton LJ: Rat thymic dihydrotestosterone receptor: preparation, location and physiochemical properties. Steroids 1979, 34:539–553
60. Kovacs WJ, Olsen NJ: Androgen receptors in human thymocytes. J Immunol 1987, 139:490–493
61. Viselli SM, Olsen NJ, Shults K, Steizer G, Kovacs WJ: Immunochemical and flow cytometric analysis of androgen receptor expression in thymocytes. Mol Cell Endocrinol 1995, 109:19–26
62. Gray DH, Ueno T, Chidgey AP, Malin M, Goldberg GL, Takahama Y, Boyd RL: Controlling the thymic microenvironment. Curr Opin Immunol 2005, 17:137–143
63. Starr TK, Jameson SC, Hogquist KA: Positive and negative selection of T cells. Annu Rev Immunol 2003, 21:139–176
64. Derbinski J, Schulte A, Kyewski B, Klein L: Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol 2001, 2:1032–1039
65. Vacchio MS, Williams JA, Hodes RJ: A novel role for CD28 in thymic selection: elimination of CD28/B7 interactions increases positive selection. Eur J Immunol 2005, 35:418–427
66. Palaszynski KM, Loo KK, Ashouri JF, Liu HB, Voskuhl RR: Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol 2004, 146:144–152
67. 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. J Immunol 1997, 159:3–6
68. Graff RJ, Lappé MA, Snell GD: The influence of the gonads and adrenal glands on the immune response to skin grafts. Transplantation 1969, 7:105–111
69. Bebo BF Jr, Schuster JC, Vandenbark AA, Offner H: Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. J Immunol 1999, 162:35–40
70. Angele MK, Schwacha MG, Ayala A, Chaudry IH: Effect of gender and sex hormones on immune responses following shock. Shock 2000, 14:81–90
71. Messingham KA, Shirazi M, Duffner LA, Emanuele MA, Kovacs EJ: Testosterone receptor blockade restores cellular immunity in male mice after burn injury. J Endocrinol 2001, 169:299–308
72. Bao M, Yang Y, Jun HS, Yoon JW: Molecular mechanisms for gender differences in susceptibility to T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Immunol 2002, 168:5369–5375
73. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM: Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 1993, 260:547–549
74. 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, 16:22–30
75. Edwards JC, Cambridge G: B-cell targeting in rheumatoid arthritis and other autoimmune diseases. Nat Rev Immunol 2006, 6:394–403
76. Fish EN: The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 2008, 8:737–744
77. 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. J Immunol 1998, 161:27–34
78. Ellis TM, Moser MT, Le PT, Flanigan RC, Kwon ED: Alterations in peripheral B cells and B cell progenitors following androgen ablation in mice. Int Immunol 2001, 13:553–558
79. Olsen NJ, Gu X, Kovacs WJ: Bone marrow stromal cells mediate androgenic suppression of B lymphocyte development. J Clin Invest 2001, 108:1697–1704
80. Olsen NJ, Kovacs WJ: Case report: testosterone treatment of systemic lupus erythematosus in a patient with Klinefelter's syndrome. Am J Med Sci 1995, 310:158–160
81. Spector TD, Perry LA, Tubb G, Silman AJ, Huskisson EC: Low free testosterone levels in rheumatoid arthritis. Ann Rheum Dis 1988, 47:65–68
82. Pope JE, Joneja M, Hong P: Anti-androgen treatment of prostatic carcinoma may be a risk factor for development of rheumatoid arthritis. J Rheumatol 2002, 29:2459–2462