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COMMENTARY

Sex-bias in CD8$^+$ T-cell stemness and exhaustion in cancer

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Sex differences in the immune system are well documented in a wide range of organisms including sea urchins, fruit flies, mice and humans.[4] In humans, females generally elicit stronger innate and adaptive immune responses than males, which protects them better from infections and augments vaccine-efficacy. At the same time, however, it renders women more susceptible to autoimmune diseases and reduces the efficacy of immunotherapeutic interventions.[1,4] In contrast, treatments to suppress immune responses in the context of autoimmune diseases appear to work better in males.[1,4] Sex differences in the immune system can be genome encoded or hormone mediated, where the former occur at all stages of life, while hormone driven differences appear only post-puberty.[4] Multiple genes with functions in the immune system, including *TLR7*, *FOXP3*, *CD40L*, *IL2RG*, *XIAP* and *BTK*, are X-linked and reactivation of the usually silent second X chromosome in females promotes sex differences in the immune system.[5] Sex hormones also play a central role in establishing immunological sex differences by acting directly on immune cells or indirectly by shaping the tissue niches they reside in.[6,7] For example, we have shown that sex hormones act on adipose tissue to control the distribution, phenotype and function of tissue-resident regulatory T cells in a sex-specific manner.[7] Further supporting the role of sex hormones in the immune system, two recent studies, published in *Immunity* and *Nature*, have revealed how androgen receptor (AR) signalling can skew CD8$^+$ T-cell differentiation and limit their anti-tumour function.[8,9]

CD8$^+$ T cells are critical to fight against intracellular pathogens such as viruses and bacteria. They are also efficient in recognising and killing malignant cells and thus play a fundamental role in tumour immune surveillance and control.[10] When CD8$^+$ T cells recognise cognate peptide bound to MHC-I molecules on the surface of infected or tumour cells, they get activated, clonally expand and differentiate into effector cells that produce inflammatory cytokines, including interferon gamma (IFN-γ) and tumour necrosis factor (TNF), and cytolytic molecules such as perforin and Granzyme B (GzmB; Figure 1),[10] which together contribute to the killing of pathogen infected and tumour cells. Prolonged antigenic stimulation, however, as in chronic infection or cancer, leads to the progressive dysfunction of CD8$^+$ T cells, a process known as 'exhaustion'. T-cell exhaustion is marked by the downregulation of cytokine production and continuous expression of co-inhibitory receptors, most notably programmed cell death 1 (PD-1; Figure 1), which is associated with poorer prognosis in cancer.[10] Immunotherapeutic approaches such as immune checkpoint blockade (ICB) therapy using antibodies that block

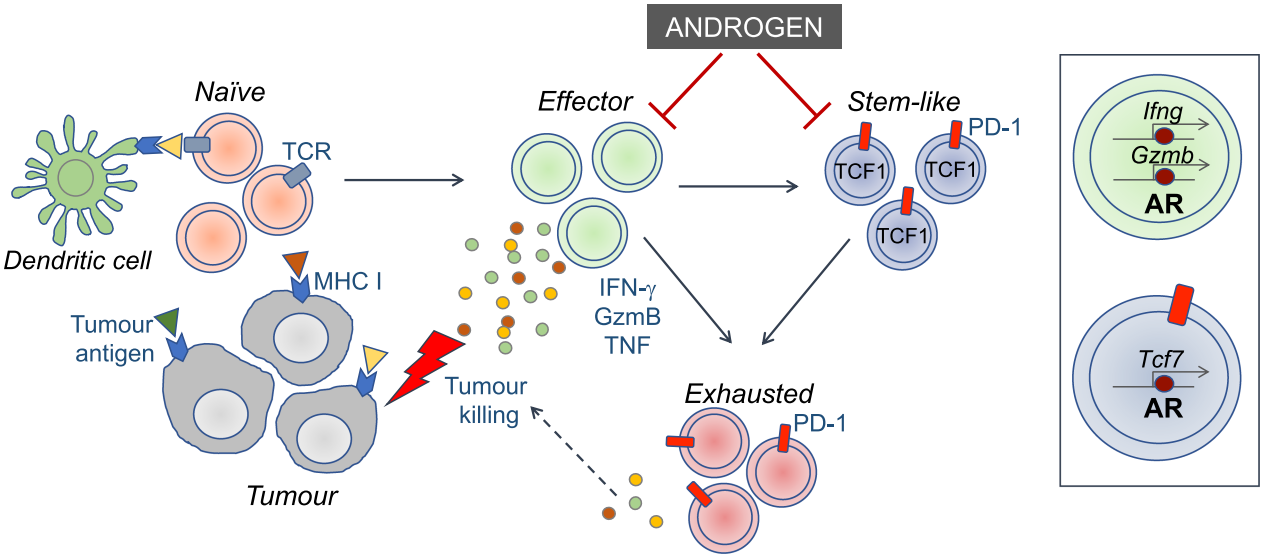

Figure 1. Impact of androgen receptor (AR) signalling on the differentiation of intratumoural CD8$^+$ T cells. Naïve CD8$^+$ T cells upon tumour antigen encounter proliferate and differentiate into effector cells that produce IFN-γ, TNF and GzmB. Upon prolonged antigen stimulation as in tumours, CD8$^+$ T cells acquire an exhausted phenotype, marked by low expression of cytotoxic molecules and elevated expression of the inhibitor molecule PD-1. Tumour-reactive CD8$^+$ T cells also contain stem-like cells, which require expression of TCF1 and act as precursors for exhausted CD8$^+$ T cells. AR signalling suppresses expression of TCF1 to restrain the differentiation of stem-like CD8$^+$ T cells while also repressing *Ifng* and *Gzmb*, thereby limiting the differentiation of effector T cells (in the box).

interactions between PD-1 and its ligand are aimed at reverting exhaustion and restoring the effector function of CD8$^+$ T cells. Exhausted CD8$^+$ T cells are heterogenous and contain a population of cells with self-renewing stem-like capacity marked by the expression of PD-1 and the transcription factor TCF1. These cells act as precursors for exhausted T (TPEX) cells and are essential to maintain the exhausted CD8$^+$ T-cell pool during chronic infection or cancers (Figure 1).[11] Critically, the therapeutic response to ICB is mediated by TPEX cells, and in keeping with this notion, enrichment of TPEX cells in tumours correlates with favourable response to ICB therapy.[11] Given the contribution of precursor and exhausted CD8$^+$ T cells to anti-tumour immunity, there is immense interest in understanding the cell intrinsic and extrinsic factors that regulate stemness and exhaustion of T cells.

Sex bias in incidence and mortality has been reported in several human cancers.[3] To test the immunological mechanisms underpinning this observation, Yang *et al.*[8] used three distinct murine cancer models (MC38 colon cancer, B16-SIY melanoma and DEN hepatocellular carcinoma), all of which showed higher tumour burden and fewer tumour-infiltrating T cells in males. Not only were CD8$^+$ T cells reduced in male tumours, but they also produced lower amounts of IFN-γ, TNF and GzmB and showed increased expression of PD-1 compared to females. Exacerbated exhaustion of CD8$^+$ T cells coincided with a reduction of TCF1$^+$ stem-like TPEX cells. Notably, ablation of CD8$^+$ T cells but not CD4$^+$ T cells normalised tumour growth, indicating that CD8$^+$ T cells were mediating the sex bias in anti-tumour immunity. To understand how biological sex influences the differentiation of CD8$^+$ T cells, the authors analysed scRNAseq data from murine colon tumours, which revealed high expression of AR in tumour infiltrating CD8$^+$ T cells. To assess its T-cell intrinsic function, the authors then ablated AR in three different types of TCR transgenic CD8$^+$ T cells, which were then adoptively transferred to male mice that carried tumours engineered to express their cognate antigens. Strikingly, AR deficiency increased the expansion, proliferative potential and anti-tumour functions of CD8$^+$ T cells and led to the expansion of stem-like TPEX cells. To understand which genes are regulated by AR signalling in CD8$^+$ T cells, chromatin accessibility of male and female tumour infiltrating CD8$^+$ T cells and male AR-deficient CD8$^+$ T cells was assessed. In female CD8$^+$ T cells, the *Tcf7* gene locus (encoding TCF1) showed

higher accessibility than those in males. Consistent with a direct role of male sex hormones in regulating gene expression, deletion of AR in male $CD8^+$ T cells increased the accessibility of *Tcf7* locus in $CD8^+$ T cells (Figure 1). Castration experiments further confirmed the role of AR signalling in dampening $CD8^+$ T-cell function as evidenced by smaller tumours in castrated male mice than in controls. Notably, Yang *et al*. could confirm their findings in patient studies. Indeed, analysis of human colorectal cancer and skin cutaneous melanoma samples revealed lower frequencies of $CD8^+$ T cells in males than in females, and phenotypic as well as transcriptomic analyses of male $CD8^+$ T cells revealed a positive correlation between AR signalling genes and expression of exhaustion markers. In keeping with these findings, AR^{hi} $CD8^+$ T cells showed elevated expression of PD-1. Overall these results suggest that androgen modulates $CD8^+$ T-cell differentiation in tumours in an evolutionarily conserved manner.[8]

In the second study, Guan *et al*.[9] show how AR signalling affects the efficacy of PD-1 targeting ICB therapies. The authors chose metastatic castration-resistant prostate cancer patients that were first treated with enzalutamide (AR blocker) and subsequently responded favourably or failed to respond to pembrolizumab (anti-PD-1) treatment.[9] scRNAseq of lymphocytes isolated from tumour biopsies revealed an enrichment of a cytotoxic gene signature in $CD8^+$ T cells among the responders, while Hsp90, which facilitates AR function, was expressed higher in $CD8^+$ T cells from patients who did not respond to anti-PD1 blockade. Further analyses showed that AR expression correlated positively with resistance to anti-PD-1 blockade, while it negatively correlated with the cytotoxic signature suggesting a suppressive role for AR in $CD8^+$ T-cell function. To gain mechanistic insights into function of AR in $CD8^+$ T cells, the authors used two different murine prostate tumour models where they combined androgen deprivation therapy (ADT) with anti-PDL-1 treatment or a combination of anti-PDL-1 and enzalutamide. Notably, only combination therapy resulted in tumour regression, confirming the inhibitory role of AR signalling in checkpoint blockade response. $CD8^+$ T cells in mice that received ADT and enzalutamide when treated with anti-PDL-1 showed elevated expression of TNF, IFN-γ and GzmB expression. In contrast, expression of these molecules were substantially lower in single agent-treated mice, suggesting that $CD8^+$ T cells mediated the response to androgen and checkpoint blockade. This was confirmed by depletion of $CD8^+$ T cells, which led to loss of tumour control. At the molecular level, Guan *et al*. showed that *Ifng* and *Gzmb* gene loci were enriched for androgen response elements and were able to bind AR as shown by chromatin immunoprecipitation and qPCR. DNA binding of AR was inhibited by enzalutamide, confirming the direct interaction of AR to *Ifng* and *Gzmb* regulatory elements and supporting a direct role for androgen signalling in repressing these genes (Figure 1). The authors also performed transcriptomic profiling of *in vitro* activated AR-deficient and sufficient $CD8^+$ T cells and showed enrichment of genes associated with cytotoxicity in AR-ablated cells. Overall, they conclude that AR^{hi}IFN-γ^{lo} $CD8^+$ T cells are enriched in tumours that are resistant to checkpoint blockade, whereas enrichment of AR^{lo}IFN-γ^{hi} cells is associated with favourable response.[9] Together these studies uncover the impact of androgen on $CD8^+$ T-cell function, tumour progression and therapeutic response and show how this ultimately leads to sex-bias in the incidence and severity of cancers.

Supporting evidence for the negative role of AR in cancer therapy, also comes from other studies. Vellano *et al*.[12] showed that in human primary and metastatic melanoma, females responded better to BRAF/MEK inhibitors. Consistent with this observation, blocking AR improved the efficacy of BRAF/MEK inhibitors in males in murine melanoma models. Kwon *et al*.[13] working with a model of urothelial carcinoma, observed stronger tumour growth and fewer tumour infiltrating cytotoxic T cells in male mice. In contrast to Yang *et al*.,[8] however, Kwon *et al*. observed transactivation of the *Tcf7* locus by AR and an expansion of TPEX cells. Thus, while it is clear that androgen negatively impacts on anti-tumour immunity, how precisely sex hormones regulate $CD8^+$ T-cell function, remains to be studied in more detail. Furthermore, it will be important to assess whether AR signalling is modulating $CD8^+$ T-cell transcriptional programmes differently in distinct tissue microenvironments or even in the periphery. Overall, these findings demonstrate once more the profound sex differences in immunity and

reinforce the need to incorporate both males and females in preclinical studies and carefully consider the inclusion of both men and woman in clinical trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Tabinda Hussain: Conceptualization; writing – original draft; writing – review and editing. **Axel Kallies:** Conceptualization; writing – original draft; writing – review and editing. **Ajithkumar Vasanthakumar:** Conceptualization; writing – original draft; writing – review and editing.

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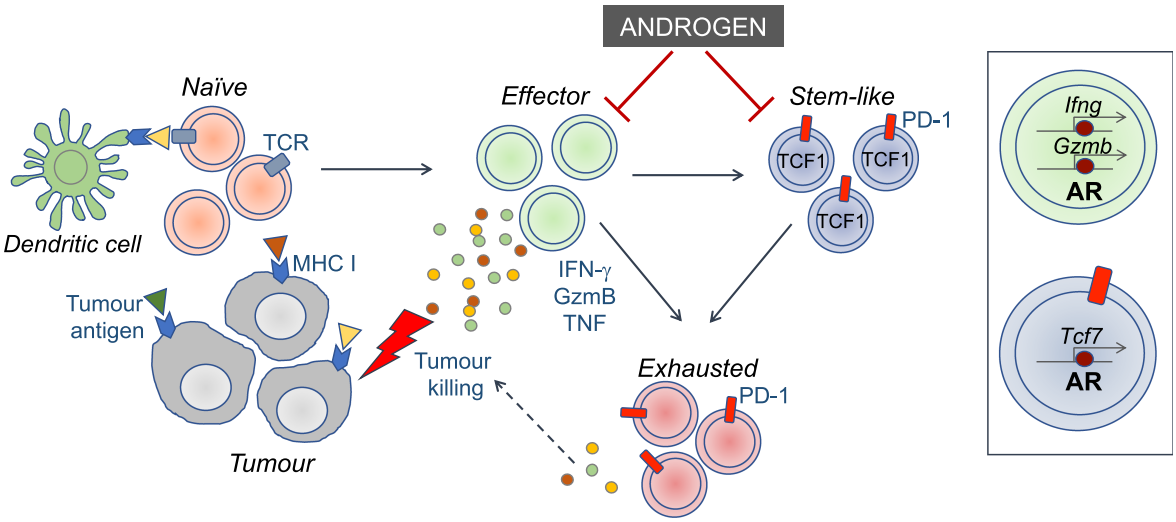

This commentary article highlights two recently published studies, which for the first time revealed the immunological underpinnings of sex-bias in cancer incidence and mortality. These studies showed that the androgen receptor restrains anti-tumour immunity in males by repressing cytotoxic genes in $CD8^+$ T cells.