

Sex differences in autoimmune disease: testosterone is associated with a decrease in expression of key anti-viral genes during puberty, which may decrease the risk of autoimmunity in males.

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Introduction: There are well described sex differences in the immune system. It has been shown in both innate and adaptive immunity that females have a more robust response than males. Various autoimmune diseases have a strong sex bias towards females. It is the accepted dogma that oestrogen in females relates to an increased risk of autoimmunity, but evidence to this end is scarce. Interferon alpha (IFN α) is a potent anti-viral innate cytokine, and many autoimmune diseases (juvenile lupus, juvenile dermatomyositis, Sjögrens) display an interferon gene expression signature. Toll like receptors (TLR) and cytoplasmic receptors (RIG, MAVS, MDA5) sense viral RNA and DNA and trigger production of IFN α .

Aim: To investigate whether sex hormones oestradiol or testosterone correlate to gene expression in IFN α production pathways.

Methods: Blood was collected, with informed consent, from healthy, typical volunteers (n=110, age=6-18); Turner's syndrome (n=9, age=13.8-19.6) and transgender volunteers (n=27, age=17.3-19.5) undergoing pubertal blockade and cross-sex hormone treatment. Clinical data and puberty self- assessment were recorded. Peripheral blood mononuclear cells were separated by Ficoll gradient centrifugation. RNA was extracted and measured for gene expression via Nanostring Plex Set. Serum testosterone, oestradiol and oestrone were measured by high performance liquid chromatography/mass spectrometry. Statistical analysis was performed in SPSS using Spearmans rank correlation, and correcting post hoc for multiple testing using Bonferroni method.

Results: In healthy, typical controls, serum testosterone levels correlated negatively with expression of the potent DNA viral sensor TLR9 (spearman's correlation coefficient $r_s=-0.408$, $p=0.001$). In addition, testosterone levels correlated negatively with expression of intracellular cytoplasmic RNA sensors RIG1($r_s =-0.356$ $p=0.015$), MDA5($r_s =-0.419$ $p=0.004$) and MAVS ($r_s =-0.373$ $p=0.011$). Interestingly, testosterone also correlated negatively to gene expression of Line-1 (L1),($r_s=-0.301$ $p=0.037$), an endogenous retroelement that may provide substrate for endogenous IFN α pathway activation. When using the stringent Bonferroni correction for multiple testing however, a corrected p-value of 0.01 represents significance. In healthy controls, there was a significant decrease in L1 ($p=0.001$), and TLR9 ($p<0.001$) after puberty. There was no effect of oestrogen on the expression of these anti-viral genes. When Turners syndrome and transgender volunteers were added to the analysis to provide an inbuilt variation in X chromosome number and sex hormone distribution, the negative correlations with testosterone remained significant.

Conclusion: Testosterone, and not oestrogen, is associated with a downregulation of expression in innate IFN α pathway signalling. This implies that in IFN α related autoimmune diseases, testosterone may be protective in males.