

# **Pubertal females produce an enhanced interferon-alpha anti-viral response compared to males, which is associated with X chromosome number, and not sex hormones.**

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**Introduction:** Very little is known about the development of the immune system during puberty. Autoimmune diseases, like juvenile onset systemic lupus erythematosus (jSLE), have an unexplained female bias and a higher incidence after puberty. IFN alpha (IFN $\alpha$ ) is a potent antiviral cytokine, and jSLE has a strong IFN $\alpha$  transcriptional signature. Toll like receptors 7 and 9 (TLR7/9) sense viral RNA and DNA respectively, and trigger plasmacytoid dendritic cells (pDC) to produce IFN $\alpha$ .

**Objectives:** To discover whether sex differences in IFN $\alpha$  production by pDC are present in young people, and influenced by puberty, sex hormones or sex chromosomes.

**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. Cells were stimulated with TLR7 agonist, R848, or TLR9 agonist, CPGODN2216, before assessing for the production of IFN $\alpha$  by pDC by flow cytometry. Serum testosterone, oestradiol and oestrone were measured by high performance liquid chromatography/mass spectrometry. Statistical analysis was performed using SPSS via univariable and multivariable linear regression.

**Results:** In cis-gender healthy volunteers, with TLR7 stimulation, on average 9.3% more pDC produced IFN $\alpha$  in females (p=0.03) and 6.3% more after puberty, independent of sex (p=0.043). Adding Turners syndrome and transgender volunteers, allowed for a model that controlled for the effect of sex hormones, and X chromosome number. This showed that, regardless of hormonal environment, two X chromosomes are associated with on average 10.9% more pDC producing IFN $\alpha$  after TLR7 stimulation specifically (p=0.002). There were no sex or pubertal differences if cells were stimulated with TLR9 agonist.

**Conclusion:** These data show for the first time that, in young people, female derived pDCs produce more IFN $\alpha$  than male pDCs upon TLR7 stimulation. Puberty is associated with an increase in pDC producing IFN $\alpha$ , regardless of sex. In addition, possessing two X chromosomes is associated with a higher production of IFN $\alpha$  regardless of hormone levels. These findings are specific to TLR7 induced IFN $\alpha$  production, which is interesting as TLR7 is coded for on the X chromosome. A novel collaboration between endocrinology and rheumatology has enabled us to provide novel insights into the development of the immune system over puberty, but also into the risk profile of patients with immune disorders with sex bias, such as jSLE.