Impact of sex-steroid hormones on B cell class-switch recombination is dependent on sex chromosomes

Hannah Peckham1,2, Anna Radziszewska1,2, Nina M De Gruijter1,2,4, George Robinson1,2, Lucia Martin-Gutierrez1,2, Oluwatomisin Nettey1, Gary Butler3, Elizabeth C Jury1,2, Elizabeth C Rosser1,2, Coziana Curtin1,2,3,4

1 – Centre for Adolescent Rheumatology Versus Arthritis at UCL, UCLH and GOSH, London, UK 2 – Centre for Rheumatology, University College London, London, UK 3 – University College London Hospital, London, UK 4 - UCL Great Ormond Street Institute of Child Health, London, UK

Background

Heightened humoral immune responses in females are well-documented, and have been postulated to contribute to the female sex-bias seen in autoimmune disorders such as Systemic Lupus Erythematosus (SLE) and the increased morbidity from infections such as COVID-19 in males. Class-switch recombination (CSR) is a key process whereby B cells ‘switch’ their immunoglobulin ‘class’ from early IgM/D to IgG/A/E (Fig.1). Notably, IgG+ B cells and antibodies are vital for infection clearance, but also make up a major component of the damage-causing autoantibodies seen in autoimmune rheumatic diseases. This process is thought to be affected by both the sex hormones-oestrogen and testosterone, as well as by genes carried on the X sex chromosome.

Methods

Peripheral blood samples were collected from cis-male (XY; n=43) and –female (XX; n=62) volunteers (14-31 years), and trans-male (XX; n=25) and trans-female (XY; n=23) volunteers (15-19 years) on GnRH-analogue (‘puberty blockers’), +/- testosterone (‘T’) or oestradiol (‘E’) treatment, respectively. PBMC/serum phenotyping was performed using flow cytometry and LEGENDPlex™ immunoassay. Sorted CD19+ cells from a representative subset (n=22) were sent for RNAseq analysis. Ordinary one-way ANOVA/ Kruskal-Wallis/ Mann-Whitney u-test used as appropriate. Mean + SD. Significance determined as p<0.05.

Results I: Healthy cis-males have a lower proportion of class-switched (IgG+) B cells than age-matched cis-females, and a higher ratio of IgG4 serum antibodies compared to IgG1 antibodies

Representative flow cytometry plots and scatter plots showing: (A) differences between cis-female (n=62) and -male (n=43) healthy control percentages of class-switched (CD27+ IgD-) CD19+ B cells. (B) IgG+ B cells in cis-females (n=19) compared to cis-males (n=8) (C) Serum immunoglobulin measured by 8-Plex "LegendPlex", n=10/group.

Results II: Oestriadiol blockade in trans-males (XX) sees a decrease in switched B cells compared to cis-females (XX). Additional testosterone makes no further impact.

Levels of CD27+IgD- B cells in (A) cis-females (n=53) compared to trans-males on puberty hormone blockers (“BLK”; n=15) +/- gender-affirming testosterone treatment (*+ T; n=10) (B) IgG+ B cells in trans-males (n=14 BLKs, 5 on T) compared to cis-females (n=19) (C) Serum IgG4:1 ratios, as measured by LegendPlex (n=10/group).

Results IV: AICDA (Activation-induced cytidine deaminase, an enzyme essential for CSR DNA mutations) expression was decreased in cis-males compared to cis-females.

AICDA gene expression measured by RNAseq in (A) cis-females (n=6) vs cis-males (n=4) (B) cis-females vs trans-males on testosterone (n=5) (C) cis-males vs trans-females on oestradiol (n=7).

Conclusions

Oestrogen differentially affected B cell CSR on XX and XY chromosomal backgrounds, potentially by manipulation of AICDA expression. Further work is implicated to establish the mechanisms behind this.