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

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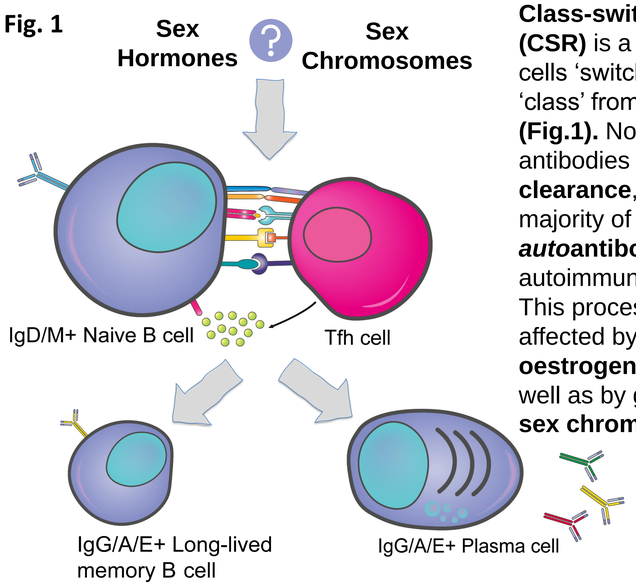
1 Background

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.**

Fig. 1



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 the majority of the damage-causing ***auto*antibodies** 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.**

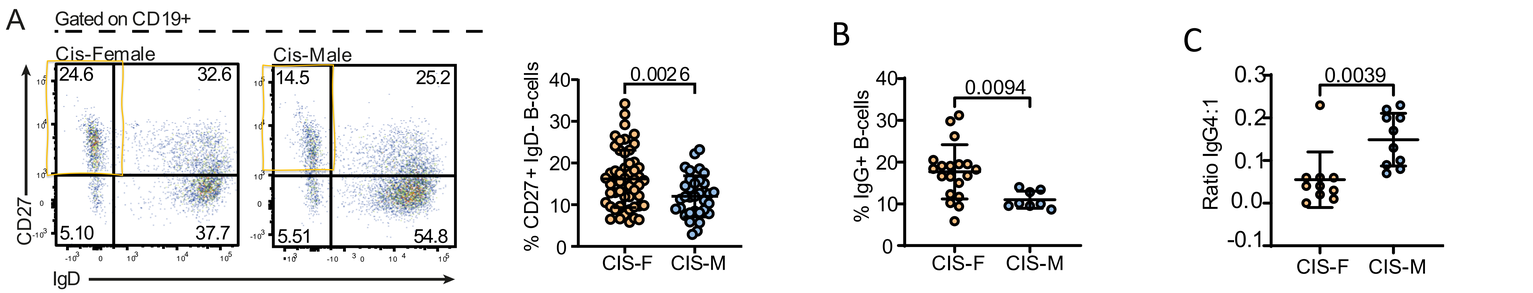
This study uses a unique cohort of healthy young cisgender and transgender volunteers to investigate the relative effects of hormones and chromosomes on CSR…

2 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 $p<0.05$

XX Vs XY

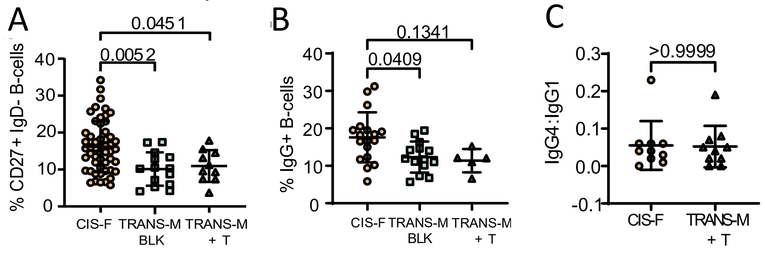
3 Results I: Healthy cis-males have a lower proportion of class-switched (IgG+) B cells than age-matched cis-females, and a higher ratio of IgG4 serum antibodies compared to IgG1 antibodies



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.

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

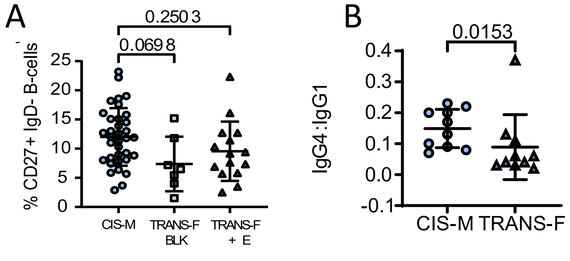
XX



*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).*

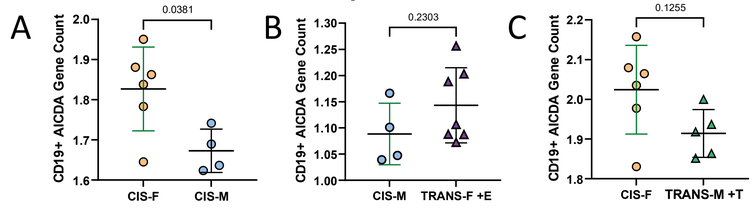
5 Results III: Gender-affirming oestradiol treatment in trans-females (XY) does not increase class-switched B cell proportions to those seen in cis-females. *Blocking testosterone and supplementing oestradiol is associated with a decrease in IgG4:1 serum antibody ratio, an effect not seen upon hormonal manipulation on the XX background.*

XY



*Levels of CD27+IgD- B cells in **(A)** cis-males (n=43) compared to trans-females on puberty hormone blockers ("BLK"; n=7) +/- gender-affirming oestradiol treatment ("+ E"; n=15). **(B)** Serum IgG4:1 ratios, as measured by LegendPlex (n=10/group).*

6 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).

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.

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