Naïve B-cell output in healthy and HIV-infected children from South Africa and the UK

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Introduction

- HIV directly affects B-cells causing polyclonal hypergammaglobulinemia⁵ with a reduction of memory B-cells in the periphery.¹
- We have previously outlined a mathematic model for determining thymic output of T cells during HIV infection.^{2, 3}
- B-cell development shares some features with T-cells, especially in terms of the somatic recombination of the Ig loci.
- Anti-retroviral treatment (ART) can restore thymic output, and likely to explain the successful T-cell reconstitution when ART is initiated in children⁴.
- In this study, we aimed to quantify healthy ranges of naïve B-cell output and levels. Additionally, we sought to determine the dynamics of naïve B-cell output in ART (anti-retroviral therapy)-treated HIV-positive children in South Africa and the UK.**

Equation 1: Thymic or Naïve B – cell output (cells day⁻¹) $\theta(t) = \frac{y \times N \times \tau}{\Delta(c - \tau)}$

Method

Sample cohorts

- Child Wellness Clinic (n=288 HIV-uninfected South African children, 2 weeks – 12 years)
- The Children with HIV and Early Antiretroviral Therapy (CHER) trial (n=218 HIV-infected South African children, 7 weeks - 8 years)
- GOSH (Great Ormond Street Hospital) (n=16 HIV-uninfected British children, 6 - 18 years)

Data collection and analysis

- Peripheral blood mononuclear cells (PBMCs) isolated from blood using Lymphoprep gradient separation
- Immunophenotyping and Ki67 quantification determined using LSRII or FACSCanto flow cytometer
- HIV-antibody status determined using the Alere Determine® rapid test
- TREC/KREC content quantified by RT-PCR

Mathematical modeling of naïve B cell output (Equation 1)⁵

- y: the fraction of naïve T- or B-cells expressing Ki67 at time t
- τ (tau): the TREC/ KREC content of naïve T-cells or PBMCs in the periphery.
- N: size of the naïve T- or B-cell pool, estimated by multiplying the # of naïve T- or B-cells per μl of blood by the volume of blood (μl) [10⁴[0.97 x Log(body weight in kg)+4.93]], then dividing this figure by 0.02.
- c: TREC/KREC content of pre-naïve T/ B-cells in the peripheral pool (c = 0.6)⁵
- Δ: the average division time for a naïve T/B-cell (Δ = 0.52 per day)¹. For the naïve B-cell model c and Δ are assumed to be the same as for naïve T-cells as the value for naïve B-cells is not known

Results

In healthy children, naïve B-cell output changes with age, exhibiting a steep increase and peak in the first year of life (median 8x10⁸ naïve B-cells/day), followed by a rapid decline by 4 years of age (median 7.2x10⁷ naïve B-cells/day) and plateau at 12 years.

Figure 1: Naïve B-cell output non-linear regression curves across age from healthy South African children (CWC, n=288).

The naïve B-cell output in ART-40W and ART-96W were compared at 60 weeks, 96 weeks and 248 weeks of the CHER trial. Children from ART-XW stop ART following X weeks of continuous treatment. At 60 weeks, ART-96W had significantly higher naïve B-cell output compared to ART-40W.

Figure 3: The effect of ART-interruption on naïve B-cell output.

Naïve B-cell output was significantly higher (Mann-Whitney T-test, p=0.013) in children from Africa (Median, 1.01 x 10⁸ cells/day) when compared to children from the UK (Median, 8.54 x 10⁶ cells/day)

Figure 5: Naïve B-cell output in healthy UK (black) and African children (red)

HIV infected children had higher naïve B-cell output than their HIV-uninfected counterparts overall (Welch's t-test p<0.0001), particularly up to the age of 2 when they were on ART (p=0.01).

Figure 2: Age-specific naïve B-cell output (median) non-linear regression curves of HIV-uninfected children (CWC, blue, n=180) compared to HIV-infected children (ART-96W, red, n=111 samples in 70 individuals) until 5 years of age.

Uninfected children (CWC) (Fig 4A) and ART-96W children (Fig4B) showed an increase in thymic output and naïve B-cell output in the first year of life. Thymic output was then maintained, while there was a sharp decrease in B-cell output.

Figure 4: Regression of thymic output (red) and naïve B-cell output (blue) of Uninfected (A) and ART-96W (B) children

Summary

- Comparison with British children revealed higher naïve B-cell outputs in South African children, indicating a potential feedback mechanism might exist to drive bone marrow output.
- The analyses in HIV-infected children from the CHER trial indicate that ART may increase and or maintain naïve B-cell output.
- Further work is needed to understand how best to preserve naïve B-cell output in children infected with HIV and in conditions in which B-cell dynamics could be impaired.

References

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