HIV specific IgM memory B-cells dominate in seronegative early-treated children

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Background

HIV specific memory responses of a vertically HIV-infected child is shaped by exposures to viral antigens, which can be directly related to the time of initiation of an effective ART. Several evidences suggested that a suppression of viral replication during a period of relative immunological immunity, might critically hamper the establishment and expansion of virus-specific immune responses. Moreover, humoral HIV-1 specific immune responses were not detected in the majority of HIV-1 infected infants treated within few months of age. Early initiation of ART in infants can suppress viremia but it can generally dampen HIV-specific immune responses. In the last years several authors have shown a clear correlation between the timing of ART initiation, duration of Ag exposure and percentage of HIV infected children who do not mount HIV specific antibody responses (seronegative patients, SN). The paucity of HIV-1 specific immune responses in these children could represent an important limitation during periods of viral breakthrough secondary to poor medication adherence, drug resistance or in the perspective of a therapeutic vaccine.

In this study we investigated whether HIV specific B-cells persist in SN patients, and what are the associated gene signatures after re-engaging the virus.

Materials and Methods

Study participants

Twenty eART treated children (<1 year) with a long-term viral control (>2 years) were enrolled at Children Hospital Bambino Gesù. Both ELISA and WB revealed that 6 patients (30%) were seronegative. Patient’s characteristics, are shown in Table 1. To detect HIV-1 specific cells a PE-gp140 protein was used.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Seronegative</th>
<th>Seropositive</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>9 (45)</td>
<td>18 (90)</td>
<td>*</td>
</tr>
<tr>
<td>Timing of eART</td>
<td>7.33 (2.6)</td>
<td>22.11 (5.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Time in eART</td>
<td>8.43 (3.8)</td>
<td>6.78 (1.6)</td>
<td>0.04</td>
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<tr>
<td>TABLE 1: Comparison of total, naïve, memory and PD cells between seronegative and seropositive patients.</td>
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HIV-1 envelope protein gp140 was obtained from European Research Institutes for Poverty Related Disease (EUPRIRD) and a conjugation kit was used to obtain a label gp140-A-R-PE.

Cryopreserved PBMCs were thawed and stimulated with a mix of three different pools of ENV peptides and CD40L for 16 hours at 37°C. Following incubation, cells were stained for different surface markers. The gating strategy reported here, was used to sort cells by FACSAria (BD). Total B (CD19+ IgD+) and four additional subsets of specific B cell (IgM+ gp140+, IgM+ gp140b+ or non-specific B cell IgM- gp140+, IgM- gp140b+) for the gp140+ HIV-1 antigen.

100 cells were sorted in PCR buffer containing probes for Gene expression assays. Pre-amplification was performed in order to analyze gene expression by 96.96 Fluidigm (96 assays x 96 samples). Data analysis was performed in polynomially activated MAIA and L2-PRIMEX to simultaneously detect IgM and IgG memory responses to gp160, gp120, gp41 and p17.

Results

Does HIV-1 specific B-cell memory persist in seronegative children?

We analyzed that gp140+ B cells are present in SN patients despite their seronegativity, and no differences were found in terms of total CD19+, IgM+ and IgG+gp140+B cells when compared to SP.

The distribution of Ag specific cells was further analysed according to the expression of surface molecules CD27 and CD22.

Where do HIV-B cells reside?

We further investigated the frequency of gp140+ IgM+ or IgG+ among different B cell subsets categorized through the expression of CD27 and IgG. Analysis revealed that the percentage of HIV-specific IgG cells in unswitched memory (COP17- IgD+) was higher in SN compared to SP (p=0.002). This was not the case of IgG+ IgD+ Ag-specific B cells that showed comparable distributions between the groups.

IgM secreting cells dominate the specific response in seronegative children towards HIV antigens

Functional characteristics of HIV specific B cells of our patients analysed by flowcytometry revealed that SN patients, rather than SP present a predominant ability to secrete IgM. On the other hand, no differences emerged when IgG forming spot were tested.

What is the fate of an IgM+ HIV-specific cell when the virus is re-encountered?

96 genes were selected to discriminate for transcriptional pathways directing Ag-specific cells towards plasma cell differentiation or germinal center reaction after Ag re-encountering. ANOVA analysis detected gene expression differences, here outlined as Differentially Expressed Genes (DEGs), comparing gene expression of stimulated and unstimulated samples. Volcano plot on the right show DEGs of IgM+gp140+ cells for both SP and SN patients.

Following stimulation, SP patients upregulated PROM1 (Blimp1), while SN did not, probably suggesting an impairment in plasma cell differentiation of IgM+gp140+B cells. PROM1 appears differently expressed in HIV-specific IgM B-cells compared to their non-specific counterparts, which showed a higher expression level (data not shown).

NFKB1, resulted significantly induced in SP and not in SN patients further suggesting such impairment. Moreover, genes involved in immune activation and lymphocyte proliferation pathways (CD69, CAMKIV, CAV1, IL21R, IL24, CD38) were found up-regulated after Ag-stimulation only in SP. POZ, inhibitor of B cell activation was downregulated exclusively in SP patients.

The lower ability of SN to induce positive regulation was further suggested by the downregulation of TRIM Domain Containing Adaptor Protein (TIRAP), a gene involved in TLR signalling pathway. In line with this, after antigen stimulation, SN upregulate IL2, perhaps due to propensity to limit a pro-inflammatory activation of Ag-specific IgM cells after HIV re-exposure.

Conclusions

Our results demonstrated that:

- HIV-specific responses persist in SN.
- IgM memory B-cells dominate the HIV-specific response in SN.
- Plasma cell differentiation profile is hampered in SN compared to SP patients after in-vitro stimulation.

These information can be used to drive cell memory differentiation in SN children.