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Short Communication

Impaired immune response to *Candida albicans* in cells from Fanconi anemia patients

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ABSTRACT

Fanconi anemia (FA) is a genetic disorder characterized by bone marrow failure and cancer predisposition. Several studies show alterations of the immunological status of FA patients including defects in peripheral blood lymphocyte subsets, serum immunoglobulin levels, and inflammatory cytokines. However scanty information is available on the response of FA cells to specific infectious antigens. In this work we examined the response of FA cells to different immunological stimuli and found a defective response of IL-1β, TNF-α and IL-17 to *Candida albicans* stimulation thus pointing to a potentially impaired response to fungal infections of FA patients.

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1. Introduction

Fanconi anemia is a genetic disease characterized by marrow failure and high tumor risk, primarily acute myeloid leukaemia and head and neck squamous cell carcinomas [1]. Chromosome instability and DNA repair defect of FA cells can justify the tendency to develop cancers of FA cells. However other cellular and metabolic defects have been observed in this disease such as altered red-ox and calcium homeostasis, defects in the respiratory and energy metabolism and altered expression and processing of cell structural protein [2]. In addition to this, previous studies showed alterations of the immunological profile of FA patients. In particular, reduction of B and NK lymphocytes, of T regulatory suppressive cells and alterations of the serum levels of immunoglobulin and numerous cytokines including TNF-α, IL-6, TGF-β, IL-10, IL-1β have been described [3–6]. Amongst cytokines, TNF-α plays an important role in the pathogenesis of marrow failure since FA cells over-express TNF-α to which they in turn exhibit increased sensitivity that finally translates in an increased rate of hematopoietic cell death [7].

Clinical observations suggest that FA patients have an increased susceptibility to viral infections due to HPV and SV40 [8,9], higher incidence of invasive fungal infection during allogeneic Hematopoietic Stem Cell Transplantation (HSCT) [10] and that *Candida albicans* was frequently present in the oral carcinoma samples of FA patients [11].

Most information on immunological defects of FA mainly come from studies on serum collected from peripheral blood (PB) of patients or on immortalized cell lines whereas little is known on the capacity of FA T-lymphocytes to react when incubated with specific infectious antigens.

In this study we evaluated the immunological response of FA peripheral blood mononuclear cells (PBMC) to viral, fungal and bacterial stimuli, assessed the proliferative capacity of primary PB T-cells and measured the cytokine production in order to identify potential abnormalities.

2. Methods

2.1. Patients and controls

FA patients referred to our unit entered this study that was approved by the Ethics Committee (Institutional Review Board) of G. Gaslini Children's Institute. As a control group, we used age

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matched healthy children who were sampled during hospitalizations for minor surgery or trauma. Controls were free of infections, autoimmune and inflammatory diseases. Informed consent was obtained from patients, controls and/or their relatives during the work-up process according to the procedures approved by the Institutional Review Board of our Institute.

2.2. Proliferation assay

PBMC were separated by Ficoll gradient centrifugation. The proliferative response was performed by dye dilution assay by flow cytometry, as previously described [12]. For the proliferation assay, duplicate cultures of 4×10^5 cells in 200 μl/well were seeded in round-bottom microtiter plates without stimulus and with Phytohemagglutinin (PHA) as positive control, bodies of heat-killed *C. albicans* (Ca) [13], Tetanus Toxoid (TT) and Hemagglutinin protein (Flu). After 5 days of culture, cells were collected and labeled with CD3 and CD8 fluorochrome conjugated monoclonal antibodies and acquired cytometer. The results were expressed as stimulation index (SI) which is the ratio between percent of Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) dim stained cells in stimulated samples and percent of CFSE dim stained cells in unstimulated samples.

2.3. Cytokine level measurement

Supernatants harvested from PBMC cultures after 5 days were used to determine levels of TNF-α, IL-1β, IL-17A and IFN-γ by a flow cytometry bead-based immunoassay (CBA Array, BD) according to manufacturer's instructions.

2.4. Statistical analysis

Comparisons between patients and controls were done with the non-parametric Mann–Whitney U test; comparisons between paired data were done by means of the Wilcoxon test.

3. Results and discussion

Eleven diepoxybutane (DEB) positive FA patients were recruited to the study. Median age was 7.6 years (range 0.4–22), 6 patients had severe bone marrow failure (BMF) as expressed by transfusion dependency of platelets and red blood cells and 5 patients had only mild BMF with cytopenia but not transfusion dependency. One patient was on androgen therapy while the remaining were not on any medical therapy. All samples were collected pre HSCT. All patients received tetanus vaccination during the first year of life.

In order to evaluate the in vitro antigen-specific immune response of PB T cells, we measured $CD3^+$, $CD3^+/CD8^+$ and $CD3^+/CD8^-$ lymphocyte proliferation and cytokine release from PBMC cells cultured in the presence of different recall stimuli as Ca, Flu and TT or PHA as positive control. Previous literature show contradictory results on the lymphoproliferative response to different antigens in FA patients [14,15]. In our work the antigen-specific proliferative ability, as assessed by stimulation index (SI), of $CD3^+$, $CD3^+/CD8^+$ and $CD3^+/CD8^-$ T cells from FA patients was superimposable to the SI index of the same cell subsets from healthy donors after stimulation with the same recall stimuli (Fig. 1). Hence, while lymphocyte proliferation does not seem to be responsible of the immune defect observed in FA, an important role could be played by altered cytokine expression resulting from a modified equilibrium of lymphocyte subpopulations [4] and from a reduced suppressive activity of T regulatory cells [16].

In our samples we analyzed cytokine production in the presence of recall antigens and quantified the pro-inflammatory cytokines IL-1β and TNF-α, reported to play a pathogenic role in FA [3–6], in cell culture supernatant.

Baseline IL-1β (Fig. 2A) and PHA stimulated IL-1β (Supplementary Fig. 1A) levels were not significantly different in FA and healthy controls. On the contrary, the production of IL-1β in response to *C. albicans* was significantly lower in FA than controls (Fig. 2A).

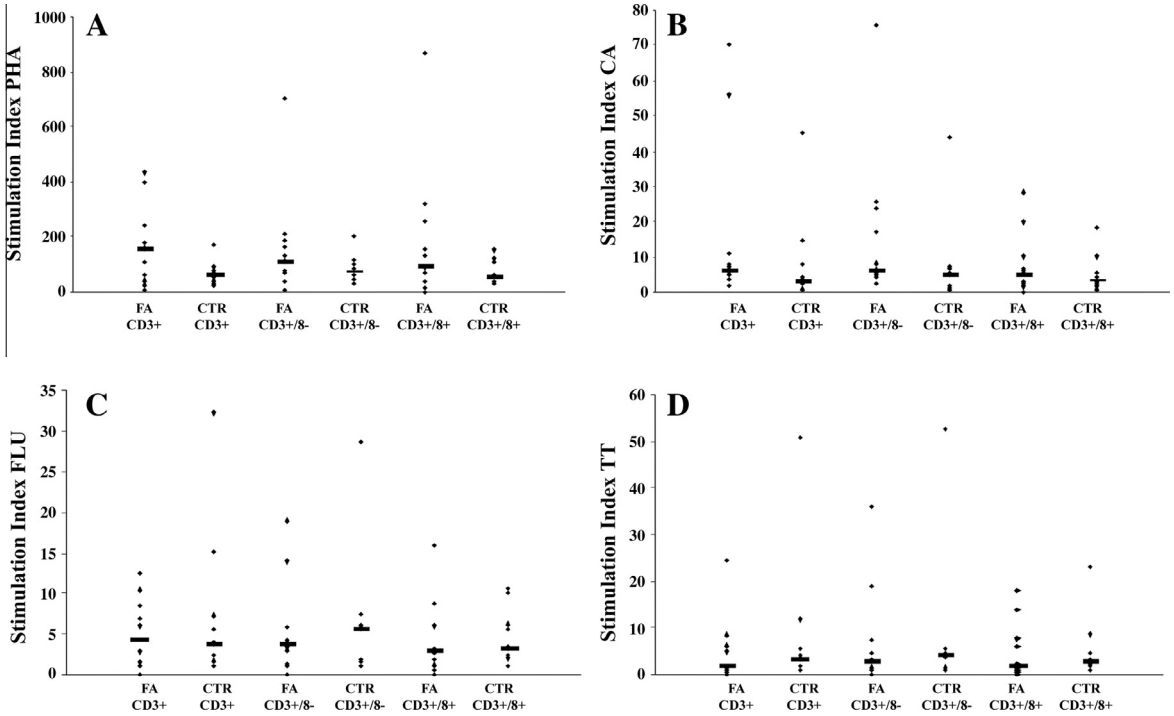

Fig. 1. $CD3^+$, $CD8^+$ and $CD3^+/CD8^-$ lymphocyte proliferation from culture of PBMC of FA patients and healthy controls (CTR) in the presence of different recall stimuli. Median values (–) and individual patient data points (♦) are shown. A. Phytohemagglutinin (PHA), B. *Candida albicans* (CA), C. Toxoid titanic (TT), D. Hemagglutinin protein (FLU).

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