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Short communication

CD27 expression of T-cells discriminates IGRA-negative TB patients from healthy contacts in Ghana

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ABSTRACT

IFN-γ release assays (IGRAs) have suboptimal sensitivity for detection of *Mycobacterium tuberculosis* (*Mtb*) infection and cannot discriminate between tuberculosis (TB) patients and healthy -potentially *Mtb* infected- contacts (HCs). In a case-control study, we determined T-cell phenotypes of IGRAs in TB patients (n = 20) and HCs (n = 20) from Ghana. CD27 expression of T-cells was significantly lower in TB patients as compared to HCs independent from *Mtb*-specificity. CD27 expression discriminated both study groups - including TB patients with low or indeterminate IGRA results - effectively. We conclude that CD27 is a promising biomarker for diagnosis of TB patients with inconclusive IGRA results.

IFN-γ release assays (IGRAs) are widely used for detection of T-cell responses against *Mtb* infection. IGRAs are based on short-term *in vitro* stimulation with *Mtb*-specific antigens (mainly Early Secreted Antigen Target (ESAT)-6 and Culture Filtrate Protein (CFP)-10) and subsequent measure of IFN-γ in the supernatant. IGRAs are highly specific but fail to detect *Mtb* infection in subgroups of TB patients from Sub-Saharan Africa [1,2]. In addition, discrimination between active TB disease and healthy -potentially *Mtb* infected- contacts (HCs) is not possible on the basis of IGRA results [3,4].

Previous studies demonstrated the capacity of T-cell phenotyping in combination with IGRA antigen *in vitro* stimulation to distinguish between active TB disease and HCs [5,6]. These studies found higher *Mtb*-specific T-cell proportions with low CD27 expression in TB patients [6]. CD27 is a co-stimulatory receptor expressed on naïve and memory T-cells. During maturation of effector T-cells, CD27 expression decreases progressively in accordance with effector T-cell independency of co-receptor stimulation. In the present study, we determined CD27 expression on T-cells stimulated with IGRA antigens, purified protein derivative (PPD) of *Mtb*, mitogen, and without stimulation in study groups of TB patients and HCs.

1. Material and methods

1.1. Study cohort

This study recruited TB patients (n = 20) (median age 37.5, range 27–74; male/female: 18/2) and HCs (n = 20) (median age 33.5, range 5–61; male/female: 12/8) in 2018 at the Agogo Presbyterian Hospital in Ghana. HCs showed no symptoms of TB but were close relatives living in the same household with TB patients. Diagnosis for TB was based on clinical evaluation, chest X-ray, patient history, and GeneXpert (Cepheid) results. TB patients were included prior to treatment initiation. All study participants were tested using the QuantiFERON (Qiagen) IGRA. Manufacturer's criteria were used for evaluation of QuantiFERON results. The majority of TB patients (n = 13; 65%) had negative (n = 7) or indeterminate (n = 6) IGRA results but GeneXpert results confirmed *Mtb* infection in the vast majority (10 of 13; 77%) of TB patients. GeneXpert negative tuberculosis patients had chest X-ray and clinical symptoms (i.e. blood coughing, weight loss) that were strongly suggestive for TB. All patients diagnosed with TB responded well to treatment. As for TB patients, the vast majority of household contacts had negative

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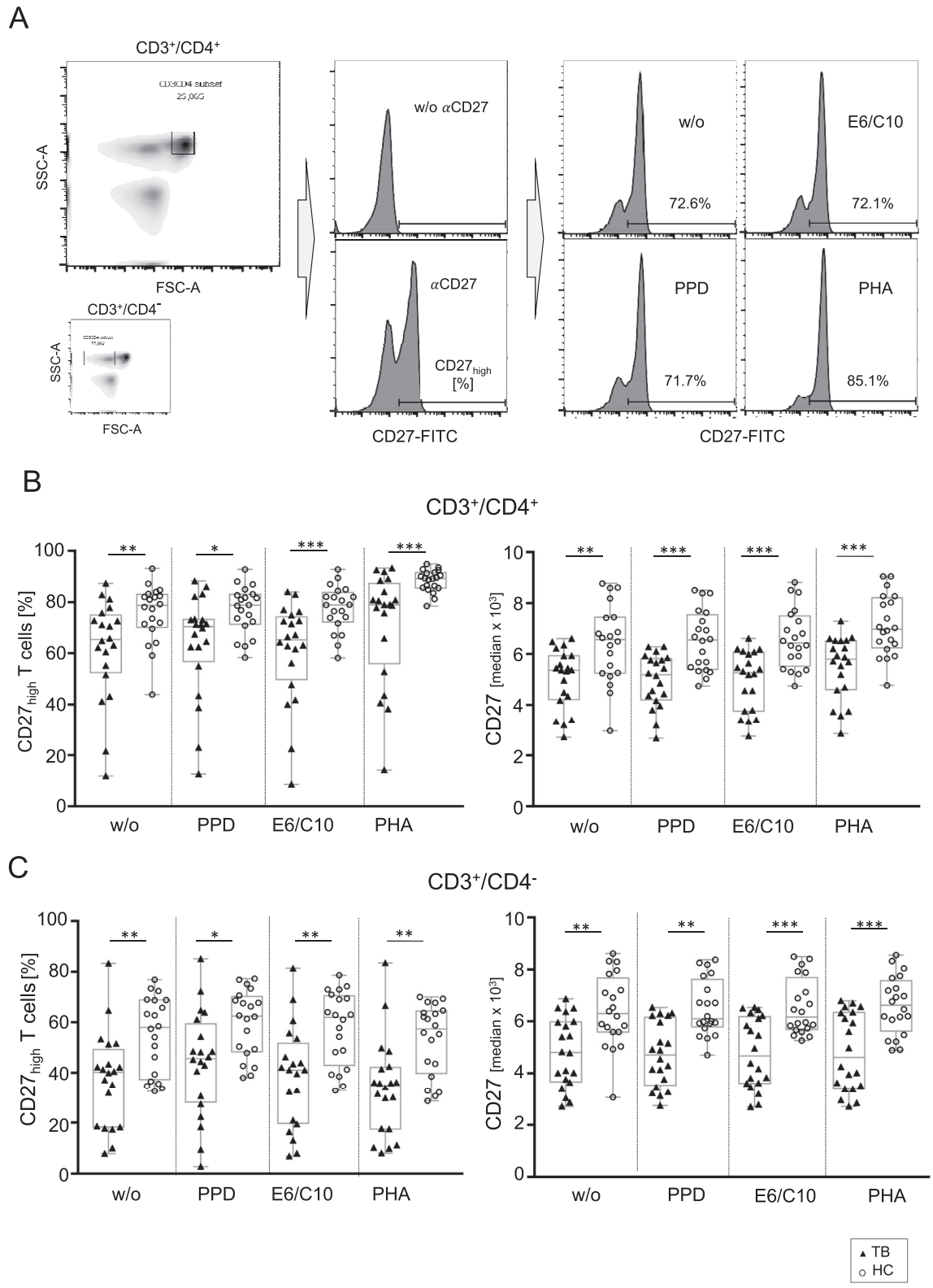

Fig. 1. Lower CD27 expression of $CD4^+$ and $CD4^-$ T-cells from TB patients as compared to HCs. CD27 expression of T-cells of TB patients and HCs after overnight PBMC culture with *Mtb* antigens (i.e. PPD, E6/C10), the mitogen PHA, and without stimulation were determined using a non-CD27 stained control sample as depicted in (A). (**B, C**) Proportions of $CD27_{high}$ T-cells (left graphs) and median CD27 protein expression of $CD27_{high}$ T-cells (right graphs) are shown for $CD3^+/CD4^+$ (**B**) and $CD3^+/CD4^-$ (**C**) T-cells. TB patients are indicated by black triangles and HCs as open circles. Each symbol depicts the mean of duplicates from an individual donor. Nominal p-values for the Mann–Whitney–*U*-test (two-tailed) are given. E6/C10: ESAT-6/CFP-10.

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(70%) or indeterminate (5%) QuantiFERON results. The present study received approval from the Committee on Human Research, Publication and Ethics (CHRPE/AP/023/18; CHRPE/221/14) at the Kwame Nkrumah University of Science and Technology in Kumasi. All study subjects gave written informed consent. For children written informed consent was provided by their parents.

1.2. T-cell phenotyping and intracellular IFN-γ measure by flow cytometry

PBMCs were isolated from heparinized blood by density centrifugation (Ficoll, Biochrom) and 1.2×10^5 cells were cultured with co-stimulatory antibodies α-human CD49d (9F10) (1 μg/ml) and CD28 (CD28.2) (1 μg/ml) as described [5]. PPD (10 μg/ml; Statens Serum Institute), ESAT-6/CFP-10 (2 μg/ml; provided by T. Ottenhoff, Leiden University Medical Center), or PHA (1 μg/ml; Sigma–Aldrich) were used for stimulation (20 h, 37°C, 5% CO_2) in the presence of Brefeldin A (2.5 μg/ml; Sigma–Aldrich). The following antibodies were used: αhuman CD3-APC (UCTH1), CD4-PerCP/Cy5.5 (RPA-T4), CD27-FITC (O323), and IFN-γ-PE (B27) (all BioLegend). Cells were measured using BDAccuri C6 (BD Biosciences) and data were analyzed by FlowJo software (BD Biosciences). Samples not stained with CD27 antibodies were used to set the threshold for CD27 positive T-cells. A representative example for the gating procedure is depicted in Supplementary Figure 1.

1.3. Statistical analyses

Statistical analyses were performed using GraphPad prism Software v7 (Graphpad Software). The non-parametric Mann–Whitney-*U*-test was applied. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the sensitivity and specificity of CD27 expression for discrimination of TB patients (as well as the TB patient's subgroup with negative or indeterminate QFT results) from HCs. Significance was considered below a p-value of 0.05.

2. Results and discussion

We analyzed CD27 expression of $CD4^+$ T-cells from TB patients and HCs cultured with different stimuli. A threshold was set to define $CD27_{high}$ T-cells using an all-minus-one antibody control (Fig. 1A) and proportions were compared between TB patients and HCs. We found significantly lower proportions of $CD4^+/CD27_{high}$ T-cells in TB patients as compared to HCs (Fig. 1B, left graph). Significant differences were detected in samples stimulated with *Mtb*-specific antigens (ESAT-6/CFP-10: $p = 0.0007$; PPD: $p = 0.02$), PHA ($p = 0.0006$), and without stimulation ($p = 0.009$) (Fig. 1B, left graph). Since CD27 differences could indicate changes in the proportions of $CD4^+$ T-cell subsets and/or impaired CD27 expression on individual T-cells, we next compared CD27 protein expression levels on $CD27_{high}$ T-cells. Samples from TB patients had generally

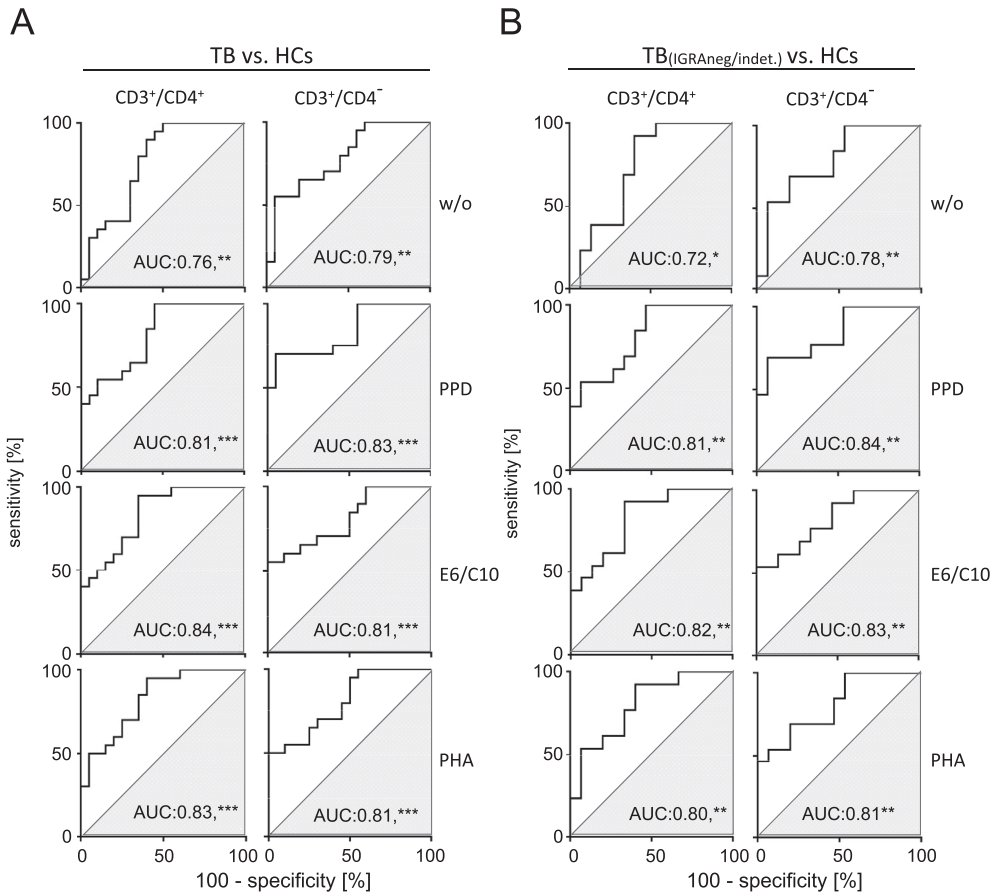

Fig. 2. CD27 expression discriminates between TB patients and HCs. Receiver Operator Characteristic (ROC) analysis for classification of TB patients and HCs using CD27 median expression of stimulated $CD4^+$ and $CD4^-$ T-cells of TB patients and HCs. Area Under Curve (AUC) values for discrimination of (**A**) TB patients from HCs as well as (**B**) QFT negative/indeterminate TB patients and HCs are given. Nominal p-values are shown as * for p<0.05, ** for p<0.01, and *** for p<0.001.

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lower median CD27 levels on $CD4^+/CD27_{high}$ T-cells as compared to HCs (Fig. 1B, right graph). These results indicated impaired CD27 expression on $CD4^+$ T-cells as the potential cause for higher proportions of $CD27_{low}$ T-cells of TB patients. To determine if other T-cell subpopulations showed also differences, we next analyzed CD4 negative T-cells for CD27 expression. Similar differences between study groups were detected for CD4 negative T-cells (Fig. 1C) indicating generally decreased CD27 expression of T-cells from TB patients.

CD27 differences were found without re-stimulation and with PHA suggesting mechanisms independent of *Mtb* antigen specificity. Therefore, we next determined CD27 expression on IFN-γ^+/$CD4^+$ T-cells (Supplementary Fig. 1A). PPD and ESAT6/CFP10 specific $CD27_{low}$ IFN-γ^+ T-cells were more frequent in TB patients as compared to HCs whereas PHA-activated T-cells showed no significant differences (Supplementary Fig. 1B). This indicated a retained $CD27_{low}$ phenotype of *Mtb*-specific T-cells when re-stimulated *in vitro*. This finding was in accordance with previous studies demonstrating lower CD27 expression as a feature of *Mtb*-specific $CD4^+$ T-cells in active TB [5–8]. Our results confirmed lower CD27 expression of T-cells from TB patients but suggested pathognomonic mechanisms independent from *Mtb* specificity. A possible explanation for general (i.e. antigen-specificity independent) effects on the T-cell phenotype could be aberrant serum cytokine levels, which we previously described for TB patients [9]. Future studies are needed to address this important point and to determine if decreased CD27 expression influences T-cell functions in TB patients.

Furthermore, we investigated if differential CD27 expression of T-cells can be used to discriminate TB patients from HCs. ROC analyses showed strong capacity of CD27 expression level of T-cells to distinguish TB patients from HCs. Similar AUC values and significant discrimination was detected for *Mtb* antigens, PHA, and without stimulation (Fig. 2A). A significant subgroup of TB patients in our study group was IGRA-negative or -indeterminate ($n = 13$; 65%) although *Mtb* infection was confirmed for the vast majority of cases (see Methods). Since CD27 differences were seen also in the absence of a *Mtb*-specific stimulation, we assumed that $CD27_{low}$ expression of T-cells could contribute to the diagnosis for this TB patients' subgroup. ROC analyses efficiently discriminated IGRA-negative and -indeterminate TB patients from HCs (Fig. 2B). These results showed that increased proportions of $CD27_{low}$ T-cells characterize patients with active TB and can contribute to TB diagnosis especially in the context of negative or inconclusive IGRA results that are frequent in Sub-Saharan Africa [1,2]. However, interindividual heterogeneity of TB patients/healthy contact responses rendered the efficacy of CD27 expression alone limited for discrimination. These results may reflect heterogeneity of TB pathogenesis e.g. due to confounding environmental and immune genetic factors. Additional studies may therefore analyze the capacity of a group of candidates, including CD27 expression, in combination with learning algorithms (e.g. using support vector machines, linear discriminant analyses, classification/regression trees) for discrimination as it has been described for TB [10].

Since *in vitro* cultured PBMCs without stimulation showed CD27 differences as well, we speculate that *ex vivo* analyzed T-cells from TB patients would also present with lower CD27 expression. Future studies will address this point and the question if functional differences of $CD27_{low}$ T-cells affect TB disease progression.

Conflict of interest

The authors declare to have no conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micinf.2019.07.003.

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