T cell polyfunctionality
An important immune correlate of vaccine efficacy

Delphine SAUCE[1], Guy GOROCHOV[1] and Martin LARSEN[1]

[1]Centre d'Immunologie et des Maladies Infectieuses (CIMI Paris U1135), Sorbonne Universités, UPMC Univ Paris 06, Paris, France

Background

> Understanding the factors that delineate the efficacy of T cell responses towards pathogens is crucial for our ability to develop potent therapies against infectious diseases. Multidimensional evaluation of T cell functionality at the single-cell level enables exhaustive analysis of combinatorial functional properties, hence polyfunctionality.

> We recently invented an algorithm that quantifies polyfunctionality, the Polyfunctionality Index.[1] Importantly, quantitative assessment of T cell polyfunctionality correlates with T cell efficacy measured as the capacity to kill target cells *in vitro* and control infection *in vivo*.[2]

> Here we apply our approach to data from an HIV vaccine trial. Our analysis suggests that induction of a synergistic network of $CD4^+$ T-cell subsets is implicated in HIV-protection. Accordingly, we provide evidence that vaccine-induced protection is associated with increased frequencies of CD40L expressing Th2 cells (P=0.036) and IL-2 secreting Th17 cells (P=0.022).[3]

> Our approach represents a generalizable methodology to objectively evaluate the impact of polyfunctionality on T cell efficacy, and a software package is now available to ease its implementation (www.FunkyCells.com).

[1] LARSEN *et al.* PLoS One 2012
[2] BOYD *et al.* PLoS One 2015
[3] SAUCE *et al.* Sci Rep 2016
[4] LIN *et al.* Nat Biotech 2015

Objectives

> The present study was undertaken to clarify to which extend T cell polyfunctionality may predict vaccine efficacy, measured as HIV seroconversion in the well-characterized HIV-vaccine trial, RV144. The secondary objective was to identify, which functional cell subsets display predictive properties.

Methods

> We employed the polyfunctionality index on a dataset derived from the RV144 HIV-vaccine trial,[4] selected for it's unique ability to evaluate the importance of polyfunctionality with regards to vaccine efficacy.

> HIV-specific $CD4^+$ T cells were analyzed for their capacity to secrete a panel of effector molecules, IFN-γ, IL-4, IL-17A, TNF-α, IL-2 and CD40L.

> We finally evaluated logistic regression models of T cell polyfunctionality as independent variable and vaccine efficacy as dependent (predicted) variable. using the polyfunctionality index algorithm.[1] Polyfunctionality index parameters (q and phi) were adjusted to optimize model fit (maximum likelihood). Adjusted parameters were interpreted to identify effector functions most strongly associated with vaccine efficacy.

RESULTS

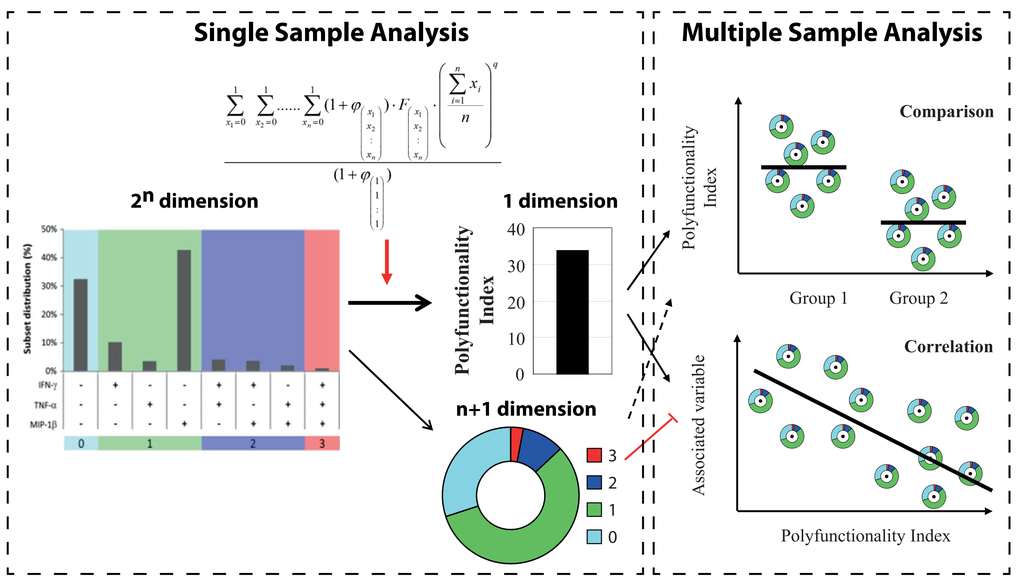

Figure 1. Quantification of cellular polyfunctionality.
Multiparametric single-cell analysis generates very large combinatorial datasets. E.g. for 3 bimodal parameters 8 so-called boolean combination gates can be identified objectively. The complexity of such boolean datasets (2^n dimensions) can be reduced by deducing the frequency of cells positive for 0, 1, 2 up to n simultaneous parameters (n+1 dimensions). Whereas such polyfunctionality profiles have been instrumental for our understanding of cellular polyfunctionality, they do not provide a quantification of polyfunctionality and they are not compatible with classic statistics. We have therefore invented a novel algorithm, which quantifies polyfunctionality (polyfunctionality index), compatible with classical statistical techniques, such as comparative and correlative statistics.

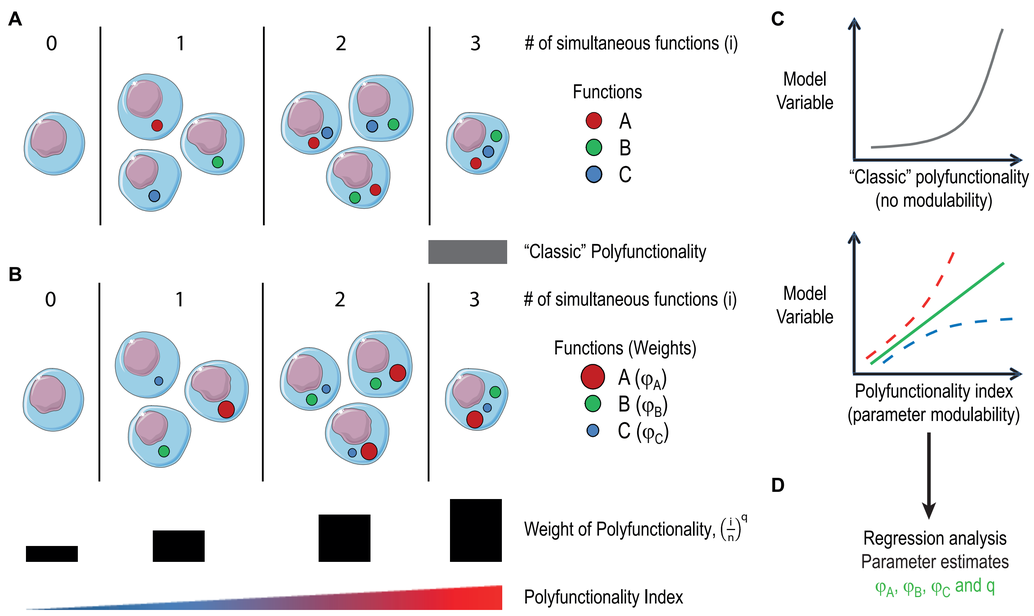

Figure 2. The principles of single-cell polyfunctionality analysis for modelling associated variables, such as T cell efficacy.
3 (n) bimodal effector molecules (A, B and C) were measured at the single-cell level identifying $2^3 = 8$ distinct combinatorial cell subsets, which can be stratified according to the number of simultaneous functions (i) at the single-cell level. **A)** "Classical" polyfunctionality analysis generally assess only cells positive for a defined minimal number of simultaneous effector molecules (i.e. 3 simultaneous functions). **B)** Contrarily, the polyfunctionality index considers all 8 functional subsets and ingeniously parameterizes the influence of individual ($\varphi_A > \varphi_B > \varphi_C$) as well as combined (q) functionalities. **C)** Polyfunctionality assessed with the polyfunctionality index algorithm is therefore modulable, contrary to "classic" polyfunctional analysis, enabling a more optimal fit (green line) of model variables. **D)** Using regression analysis it is feasible to obtain proper parameter estimates (φ_A, φ_B, φ_C and q), which have biological significance. Indeed, interpretation of such parameters enables an objective evaluation of the influence of individual as well as combinatorial functions on the predictive capacity of polyfunctionality with regards to a desired model variable, such as T cell efficacy.

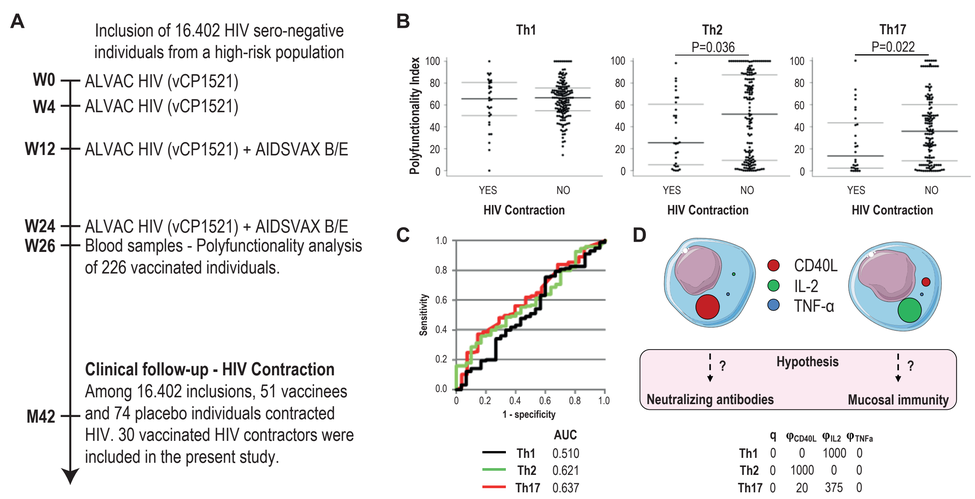

Figure 3. Th2 and Th17 cells are associated with HIV protection.
A) Schematic time line representing the vaccination protocol, sampling and clinical follow-up. **B)** The functional parameters, CD40L, IL-2 and TNF-α, were analyzed within HIV-stimulated $CD4^+$ T-cells expressing IFN-γ (Th1), IL-4 (Th2) and IL-17A (Th17), respectively. Binary logistic regression models of the dependent variable HIV protection versus 3 independent variables, qφ-adjusted PI for Th1 (black, q = 0, $\varphi_{CD40L} = 0$, $\varphi_{IL2} = 1000$, $\varphi_{TNFa} = 0$), Th2 (green, q = 0, $\varphi_{CD40L} = 1000$, $\varphi_{IL2} = 0$, $\varphi_{TNFa} = 0$) and Th17 (red, q = 0, $\varphi_{CD40L} = 20$, $\varphi_{IL2} = 375$, $\varphi_{TNFa} = 0$) cell subsets were established. The qφ-adjusted PI of Th1, Th2 and Th17 cell subsets were compared between volunteers stratified according to HIV contraction in the 42 months follow-up period post vaccination. **C)** Receiver operating characteristic (ROC) curves for qφ-adjusted PI for each Th-subset are displayed. Prediction models based on the polyfunctionality of Th1, Th2 and Th17 cell subsets included 185, 180 and 165 of 226 vaccinated volunteers with detectable Th subset HIV-specific $CD4^+$ T-cell responses, respectively. **D)** Proposed biological interpretation of adjusted PI parameters. The median and 25th/75th percentiles are marked with black lines. Group comparisons were conducted with a non-parametric Mann-Whitney test. The area under curve (AUC) metric of each prediction model is indicated.

Conclusion

> Our study demonstrates the capacity of the polyfunctionality index to objectively evaluate the impact of polyfunctionality and identify functional parameters associated with vaccine efficacy.

> We show that CD40L-expressing Th2 cells and IL-2-secreting Th17 cells are associated with immune-protection to HIV. We hypothesize that these cell subsets may be associated with efficient induction of neutralizing antibodies and mucosal immunity.

> Vaccinated individuals, which did not contract HIV during the follow-up period, display a bimodal frequency distribution of CD40L-expressing Th2 cells and IL-2-secreting Th17 cells. Although HIV-contraction was used as a measure of immune-protection, we propose that individuals with low frequency of these T cell subsets are likely not protected, but rather never encountered HIV. A prolonged clinical follow-up is urgently required to validate or discard this hypothesis.

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