Signal enhancement permits reliable detection of low-frequency CD4 memory T cells secreting IFN-γ, IL-4, IL-5, and IL-17

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INTRODUCTION: One of the major deficiencies of all current T cell assays (tetramer, ICS, ELISPOT, proliferation) is that they frequently produce false-negative data. This is the case, for example, when one attempts to detect the antigen-specific T cells months or years after vaccinations, as with those elicited by vaccination with tetanus toxoid (TT). While the antigen-specific memory T cells are clearly present in the vaccinated subjects mediating long-term immunity, in most individuals the antigen either does not induce a detectable recall response in PBMC at all, or borderline results are obtained. In particular the detection of T cell subsets other than IFN-γ producers has been challenging. When little-to-no response is detected in PBMC when testing for the recall response, the possible interpretation of such data is either that the frequency of the memory T cells has dropped below the detection limit (being around 1 in 100,000 for ELISPOT), or that the memory cells are present in a dormant state that does not permit their detection in standard functional assays, in which case signal enhancers could help detect the memory T cells.

METHODS: Serum-free CTL-Test™ Plus Medium has been formulated to contain T cell signal enhancers for the detection of dormant memory cells. In a systematic study, this enhanced medium was compared to the corresponding non-enhanced medium, CTL-Test™. The recall response to TT and CEF peptides was tested in 42 human donors measuring IFN-γ, IL-2, IL-4, IL-5, and IL-17 production by the memory T cells in ex vivo ELISPOT assays, without in vitro expansion. The respective media alone served as the negative control for assessing the spontaneous background cytokine production. The cytokines were detected with the respective ImmunoSpot® Test Kits, and the ELISPOTs were counted with an ImmunoSpot® S6 Core reader.

RESULTS: TT recalls CD4 cells, CEF peptides CD8 cells *(Figure 1)*. Up to 10-fold increased TT-induced spot counts were seen with the CTL-Test™ Plus Medium for most of the subjects tested; the signal was enhanced for detection of the Th1 (IFN-γ, IL-2), Th2 (IL-4, IL-5), and the Th17 (IL-17) subsets of CD4 cells *(Figure 2)*. For CD8 cells activated by the CEF peptide pool, or by individual CEF peptides, CTL-Test™ Medium led to higher spot counts in a subset of the PBMC donors, but unlike for CD4 cells, this effect was not consistent for the entire test collective *(Figure 3)*. CTL-Test™ Medium did not significantly change the medium background for either of the cytokines measured (data not shown).

CONCLUSIONS: CTL-Test™ Plus Medium leads to specific signal enhancement for the detection of TT-specific CD4 cells of Th1, Th2, and Th17 lineages, without increasing the medium background. Notably, about half of the PBMC donors who show no or borderline responses to TT in the non-enhanced medium, displayed a highly-significant recall response to TT in the CTL-Test™ Plus Medium. In ongoing studies, similar results were obtained for other protein antigens that recall CD4 cell memory. By increasing the signal-to-noise ratio, CTL-Test™ Plus Medium holds the promise to facilitate the reliable ex vivo detection of antigen-specific Th1, Th2, and Th17 memory cells.

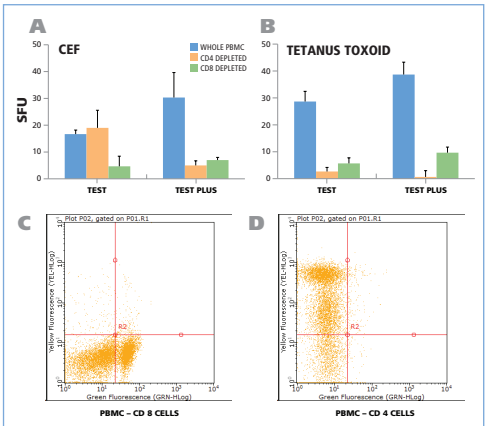

Figure 1: **The tetanus toxoid (TT) and CEF peptide pool-induced recall response is CD4 and CD8 cell-mediated, respectively.** PBMC were isolated and tested for the CEF peptide pool **(A)** or the TT-induced **(B)** recall response in an IFN-γ ELISPOT assay using both media, CTL-Test™ and CTL-Test™ Plus. The antigen-induced spot counts are shown in blue bars with the SD for triplicate wells. The same PBMC were also tested in triplicates after depletion of CD8 cells, or CD4 cells, as specified by the orange and green bars, respectively. The medium control was less than 5 spots/well under all conditions (not shown). The extent of CD8 and CD4 cell depletion are shown in C and D, respectively.

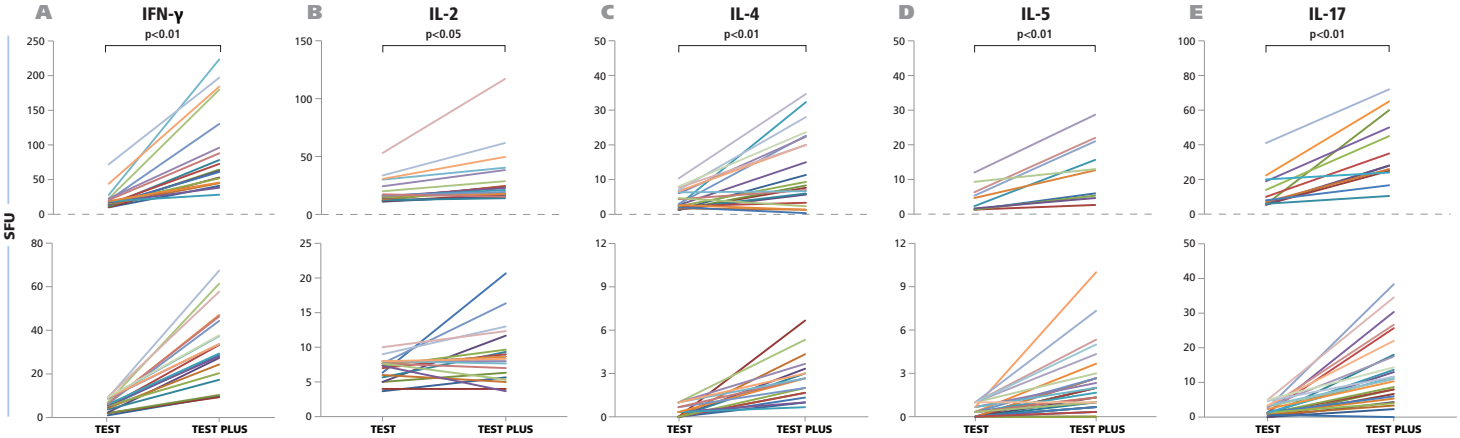

Figure 2: **Enhancement of the TT recall response in CTL-Test™ Plus Medium is seen for IFN-γ-, IL-2-, IL-4-, IL-5-, and IL-17-producing memory CD4 cells.** Cryopreserved PBMC of 42 healthy donors were thawed and tested in the specified media for the TT-induced recall response. The cells were tested at 250,000 PBMC per well directly after thawing, without resting (as resting did not improve the test results, data not shown). The recall response of each PBMC donor is represented by a different colored line connecting the average value (n=3) of spot numbers in the two media, Test and Test Plus. In each panel, results for the specified cytokine recall response are shown with low responders in the graphs below and high responders in graphs above. The two groups were compared using Student's t-test. The medium control was less than 10 spots, typically zero, for all donors and cytokines.

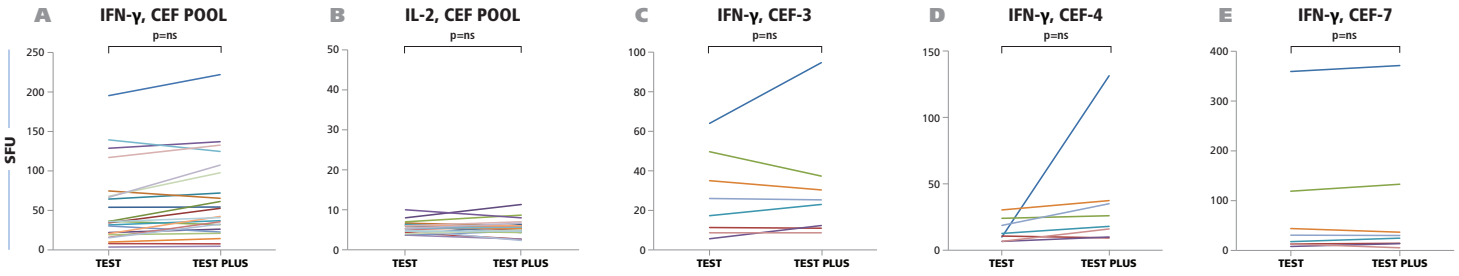

Figure 3: **The CEF recall response in CTL-Test™ Plus Medium.** For the test procedure and representation of data, the legend of Figure 2 applies except for the recall antigen used and cytokine detected that are specified in each panel. Since CEF peptides elicit only IFN-γ and IL-2 production in CD8 cells, only these data are shown.

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