**////Title: Establishing a Positive Control for CD4 Cells: A Vital Addition to the Research Toolkit**

**////Stand-first**: Measurement of CD4 T cell-mediated immunity requires functional tests to be conducted with viable peripheral blood mononuclear cells, PBMC. Recently, scientists at CTL successfully developed a positive control that not only verifies the functionality of CD4 T cells in PBMC, but also that the antigen-presenting cell compartment is unimpaired in the test sample as well.

**////Body text:**

Monitoring of the immune response to infection and vaccination demands ongoing innovation and methodological development.

The measurement of CD4 T cell-mediated immunity is particularly challenging because it requires that functional tests are performed using live PBMC. Once removed from the body, PBMC start to die and are highly sensitive to the conditions of shipment, isolation, storage, as well as cryopreservation. Therefore, establishing the functional integrity of PBMC is critical for successful CD4 T cell immune monitoring.

Positive control antigens enable the assessment of whether CD4 T cells themselves, and the antigen-presenting cell compartment required for CD4 T cell activation, are functionally unimpaired in the PBMC test sample. Until recently, such positive control antigens had not been identified, and consequently, their inclusion was a critical shortcoming in immune monitoring efforts.

Therefore, Professor Paul Lehmann and his team at CTL set out to generate a positive control for CD4 T cells by identifying protein antigens that required processing prior to antigen presentation and were capable of eliciting strong CD4 T cell recall responses in most humans.

The researchers selected proteins derived from various environmental pathogens to which most healthy humans would likely have been exposed to, and against which they would have developed CD4 T cell immunity by the time they reached adulthood. These antigens were derived from varicella, influenza, parainfluenza, mumps, measles, rubella, and cytomegalovirus, as well as S*treptococcus*, *Mycoplasma*, *Lactobacillus*, *Neisseria*, and *Candida*.

PBMC from 245 healthy donors aged 25–45 years (including individuals from Caucasian, Asian and African-American ethnicities) were tested for the presence of antigen-specific T cells. In subsequent experiments, it was established whether CD4 and/or CD8 T cells were being activated.

Of all antigens tested, only three were found to elicit interferon-γ-producing CD4 T cells in PBMC from the majority of test subjects. These were proteins derived from cytomegalovirus, parainfluenza and influenza viruses. Individually, none of these antigens triggered a recall response in all donors. However, the combination of the three into a single antigen pool, that the researchers named CPI, produced the sought-after result: the PBMC of all 245 participants were CPI-reactive.

Discussing their findings, Dr Lehmann and his colleagues noted that the PBMC samples tested were obtained from adults all of whom were living in Southern California. Further research is required to confirm that the antigen exposure in this cohort also reflects that of other geographical areas. They further noted that infections with cytomegalovirus, parainfluenza virus or influenza virus usually occurs in childhood or early adulthood, and consequently that the CPI antigen combination may not be suitable for studies examining cellular immunity earlier in life.

These exciting findings mark a significant step forward in the field of immune monitoring. The research performed by Dr Lehmann and his colleagues provides the first evidence that universal positive controls can be developed for verifying the functionality of CD4 T cells and of antigen-presenting cells in a PBMC sample. Future immune monitoring efforts of CD4 T cell immune responses will undoubtedly benefit from Dr Lehmann’s innovation in providing a much-needed fitness test for CD4 T cells and the antigen-presenting cells in PBMC test samples.

This SciPod is a summary of the paper ‘A Positive Control for Detection of Functional CD4 T Cells in PBMC: The CPI Pool’, published in the journal Cells. https://doi.org/10.3390/cells6040047

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