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Aim: Antigen presenting cells (APC) such as monocytes and dendritic cells express both activating and inhibitory receptors on their membrane. One such molecule is the immunoglobulin like transcript 3 (ILT3) which is upregulated in conjunction with inflammatory events and can be shed from the surface of APC into the circulation by metalloproteases or alternative splicing during inflammatory events. Proinflammatory stimuli can induce the shedding of membrane ILT3 into its soluble form in the circulation, potentially through the activity of metalloproteinases like ADAM17. Our aim was to determine whether the increase of soluble ILT3 (sILT3) in the serum is associated with immune activation, as reflected in anti-HLA antibody production.

Methods: We have monitored the presence of Donor Specific Antibodies (DSA) by Luminex with single antigen beads and the quantified soluble ILT3 in sera collected from 35 pediatric recipients of heart transplants and 31 adult heart and/or lung recipients. Soluble ILT3 molecules were captured by anti-ILT3 monoclonal antibodies conjugated to microbeads. After incubation with fluorescein-conjugated monoclonal anti ILT3 antibodies, the fluorescence signal was measured using a BD FACSCanto™ clinical Flow Cytometry System.

Results: Analysis of the data showed a statistically significant association between DSA development and serum ILT3 elevation at the 0.05 level for both the pediatric and adult population of transplant recipients ($p \approx 0.0171$ for pediatric population and $p = 0.035$ for the adult recipients). The data indicates that both humoral and cellular immunity developed following transplantation without necessarily leading to irreversible rejection. Although 10% of the adult recipients showed serum ILT3 elevation but no anti-HLA antibodies at the time of testing, within 3 months they all developed anti-HLA antibodies.

Conclusion: We have developed an assay to invasively monitor sILT3 in the serum of patients. The increase in the amount of sILT3 above the normal threshold is significantly associated with the de novo development of alloantibodies reflecting an ongoing immune attack against the graft. Our data suggests the potential value of sILT3 quantitation for non-invasive monitoring of transplant recipients. However, because membrane ILT3 is an inhibitory molecule, this attack may spontaneously subside when T cell activation is blocked by dimeric forms of ILT3 binding to their T cell ligand.

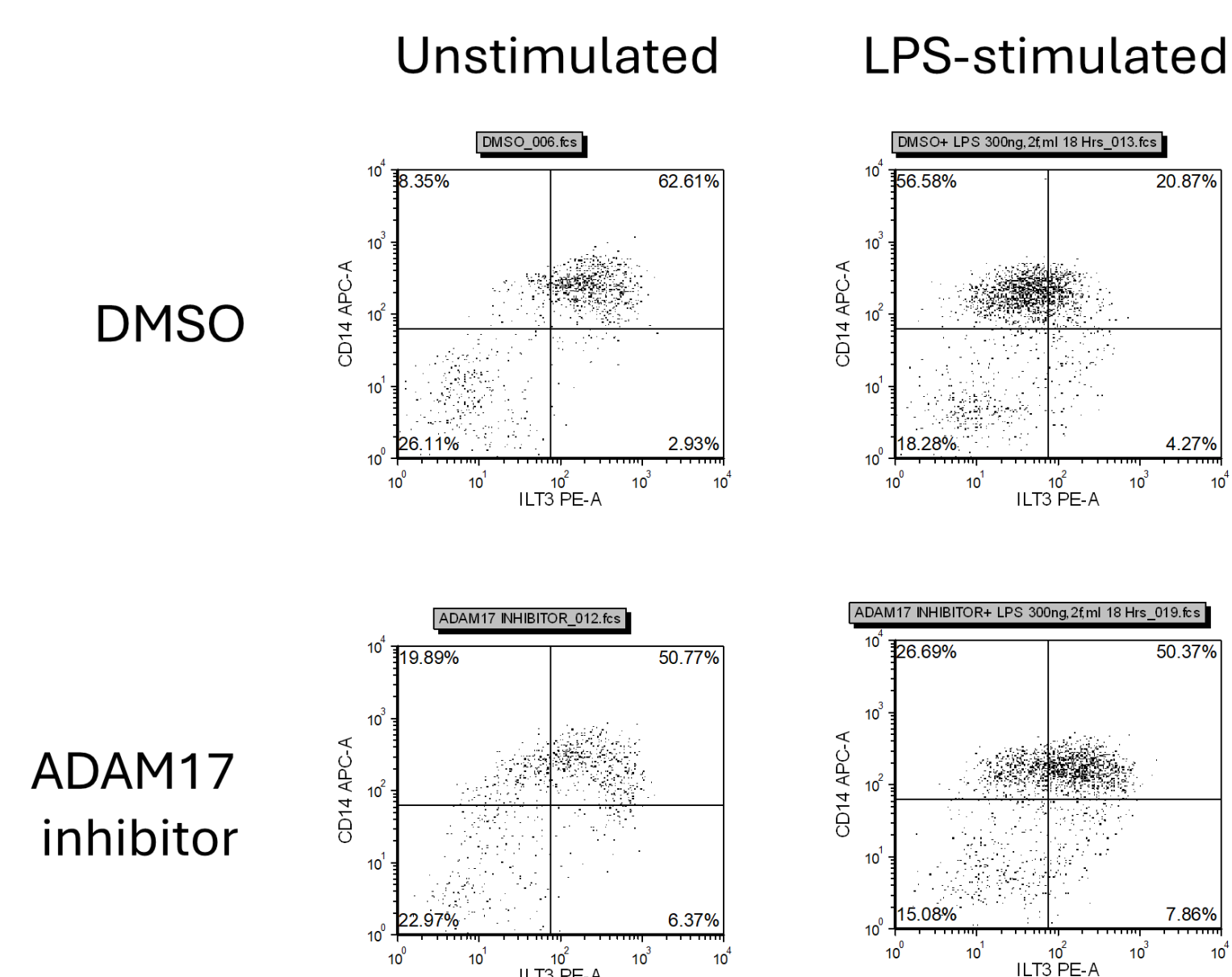


Figure.1 Metalloproteinase ADAM17 inhibitor prevents shedding of ILT3 from CD14 Positive monocytes.

The freshly isolated PBMC from healthy donor were pretreated with ADAM17 inhibitor (or DMSO as control) before exposed to the proinflammatory LPS stimulation. After 18 hours incubation, the surface ILT3 expression was determined by flowcytometry.

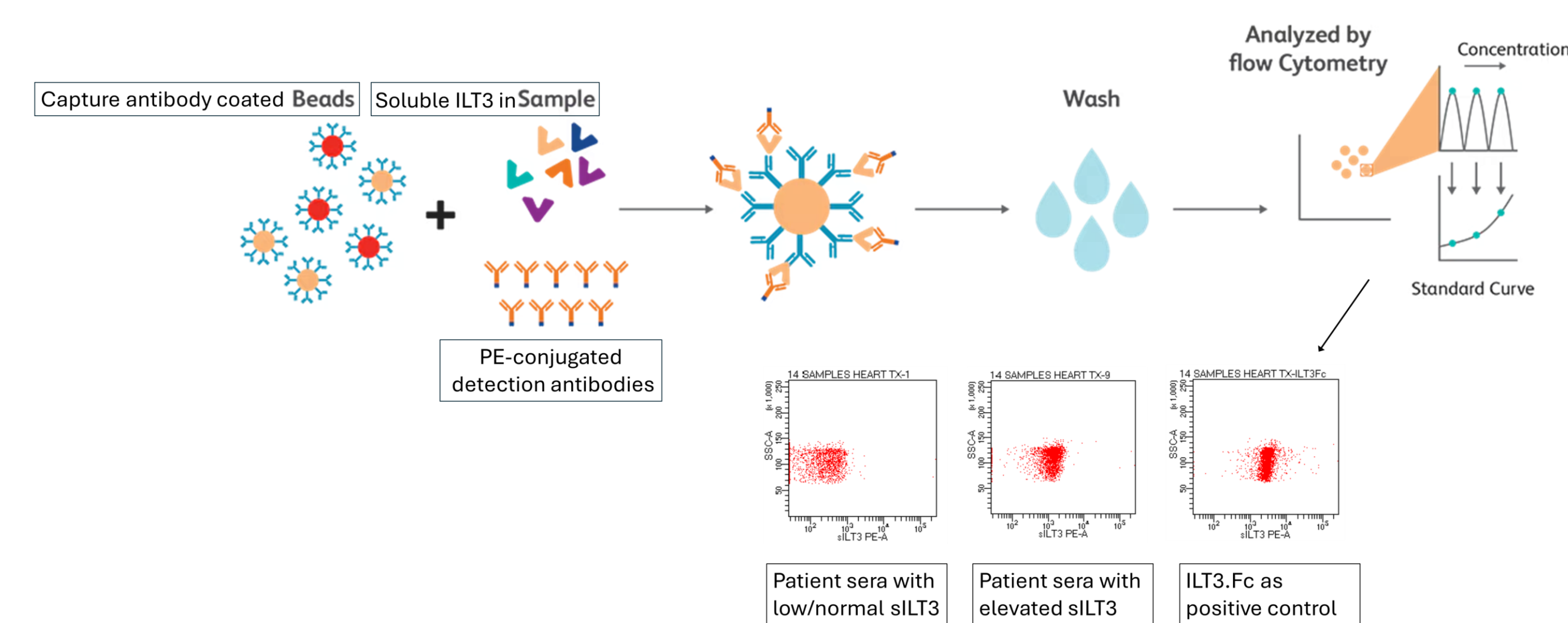


Figure.2 The scheme illustration of the assay of detection soluble ILT3 from patient seras.

The cell free sera were first prepared then incubated with beads coated with capture antibodies for 2 hours at room temperature. After that, the PE conjugated detection antibody was then added and incubated for 1 hour. Before analyzed by flow cytometry the beads were intensively washed. The Mean Fluorescence Intensity (MFI) of the beads were used as indicator of the abundance of sILT3.

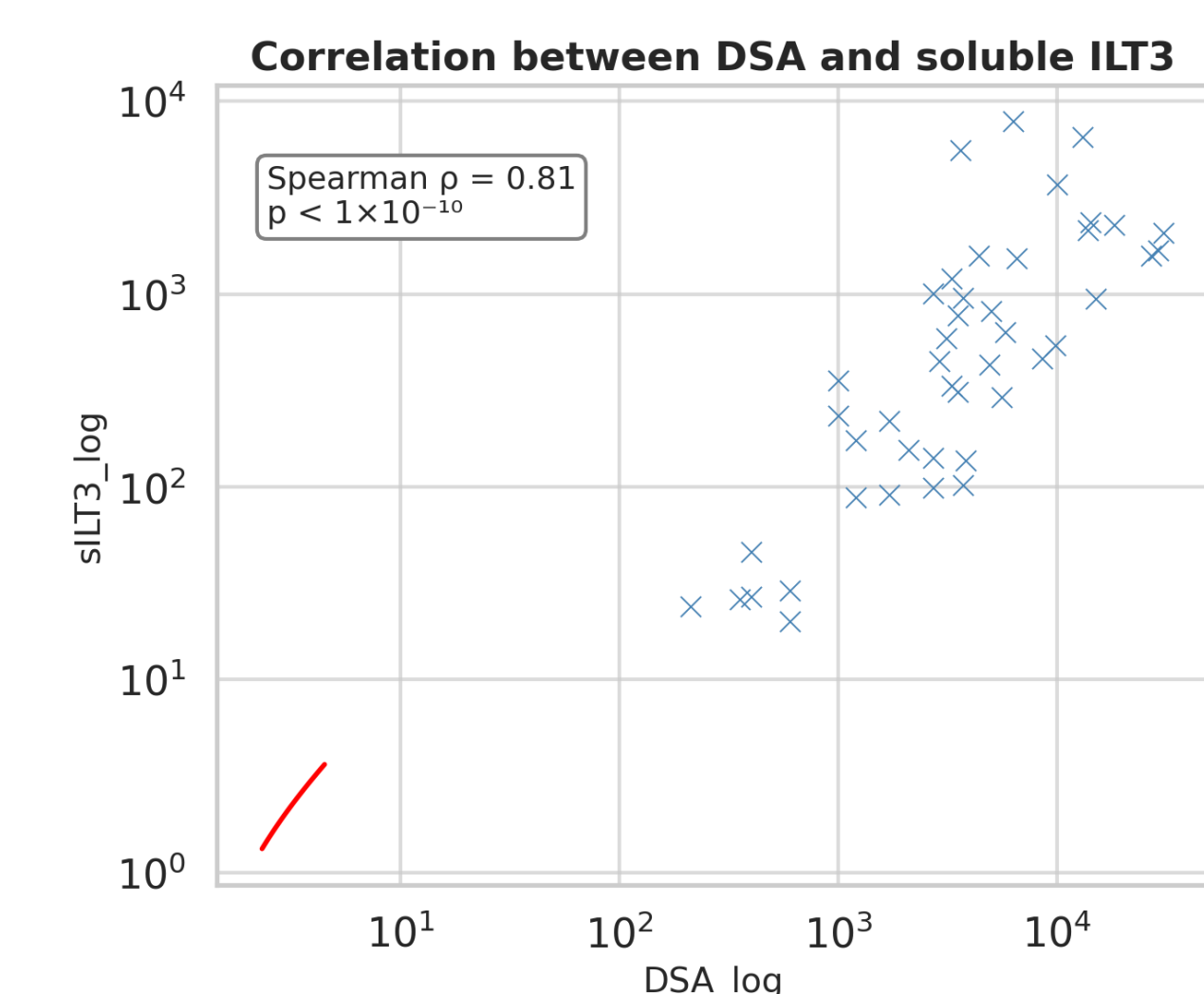


Figure.3 Pearson correlation (log10 transformed), $r = 0.817$, 95% CI = [0.69, 0.90], $p = 1.3 \times 10^{-11}$

There was a highly significant positive correlation between antibody levels (DSA) and soluble ILT3 (sILT3), with correlation coefficients around 0.8 using both Spearman and log-transformed Pearson, and confidence intervals excluding zero.