Lymphocyte Proliferations Performed in Cytometry Tubes Containing Lyophilized Lectins

P. Jinoch, M. Prouza
EXBIO Praha, Vestec u Prahy, Czech Republic

Introduction

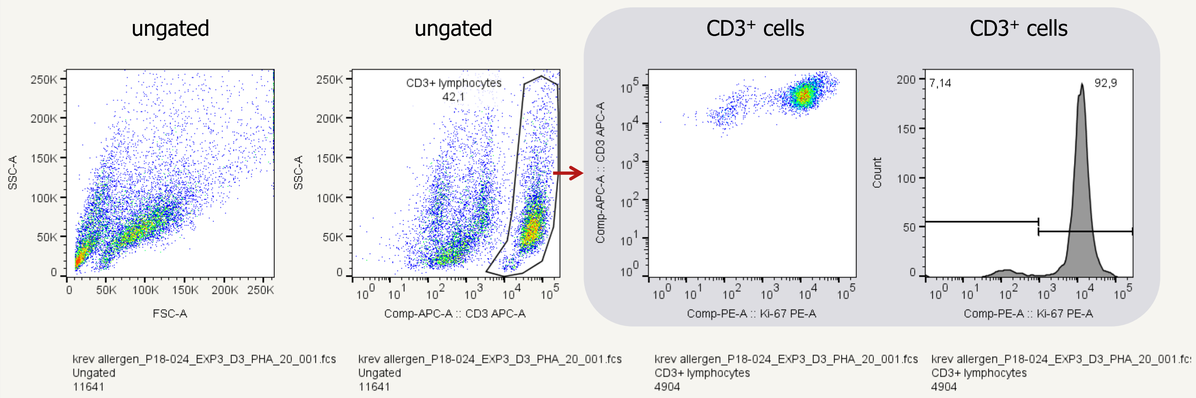
Evaluation of lymphocyte proliferative responses to mitogens (legume lectins) is used in diagnosis of immune deficiencies and to monitor the reconstitution of immune functions after bone marrow transplantation.
The stimulation and detection is performed differently across laboratories. Repeatedly the intracellular detection of Ki-67 protein was reported as a reliable quantitative indicator of proliferation in PHA stimulated lymphocytes.
It is required during cell mitosis for the maintenance of spatial separation and mobility of chromosomes. The expression of Ki-67 vary during the cell cycle, it starts after the beginning of S phase and reaches its maximum in M phase. It can also found in cells in G1 phase that are returning from mitosis. Because flow cytometry deals mostly with logarithmic scales of fluorescence, those variations in expression are less important in the discrimination of Ki-67 negative and Ki-67 positive cells.

Objective

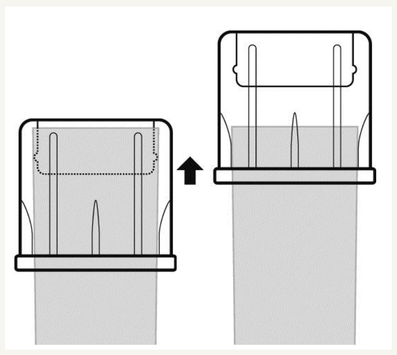
To develop a flow cytometry Ki-67 based lymphocyte proliferation assay and to complement it with single test tubes that would contain stabilized mitogens. The tubes should be compatible with all the specimen processing steps (cell culture, antibody staining) as well as with its analysis in flow cytometer.
The tube approach will minimize the risk of a specimen misslabeling, avoid the „edge" effects of multiple well plates and decrease the costs for the rarely performed laboratory test.

Results I

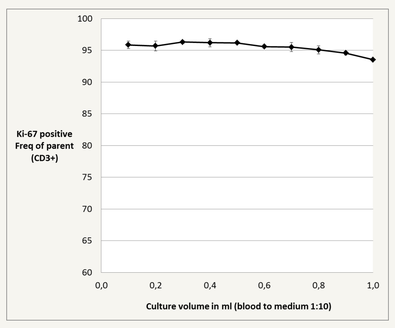
Ki-67 detection set up (CD3 APC, Ki-67 PE, both detected with IC staining, stimulated whole blood)



Single test tube design (PP dual position caps, 12 x 75 mm PS tubes, 1:10 diluted whole blood)



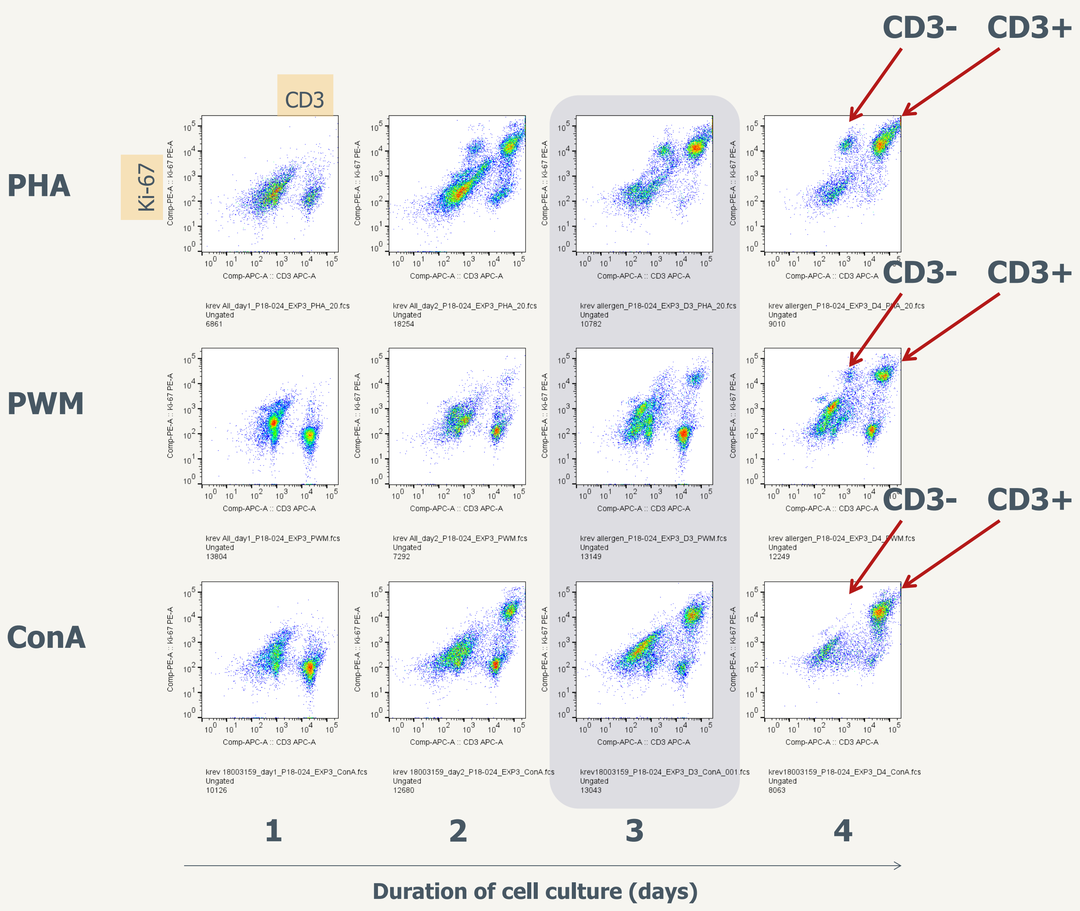
Culture volume (PHA stimulation, 3 days)



Results II

(Non)exclusivity of lectin stimulations (T- vs B- lymphocytes)

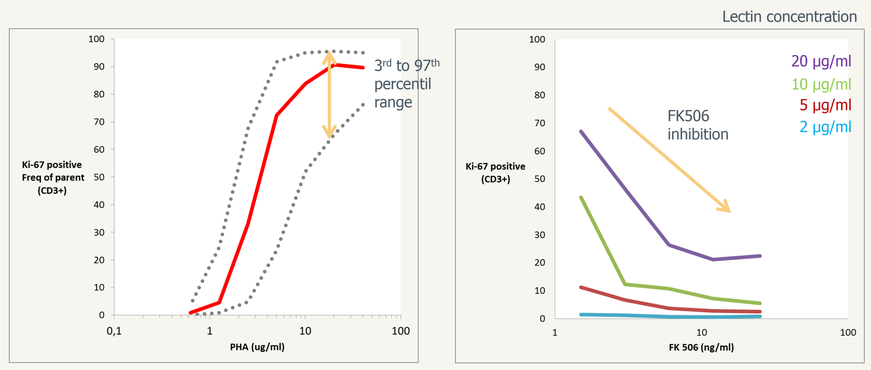
Whole blood stimulated with 20 µg/ml of lectins, response measured in time, ungated report
Assumed actions of mitogens: PHA T-lymphocytes, ConA T-lymphocytes, PWM B-lymphocytes



Results III

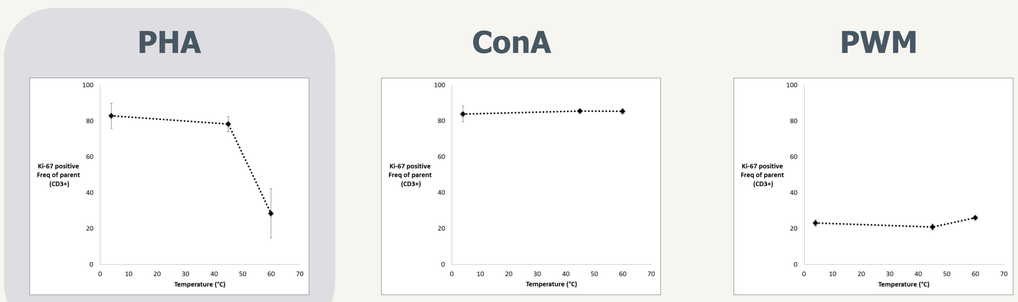
Interpersonal variability and in vitro response inhibition with FK-506

- **LEFT COLUMN** Blood specimens from 10 blood donors were stimulated with various concentrations of PHA, ConA, PWM for 3 days, graphs represent 3rd (dotted) – 50th (in red) – 97th (dotted) percentile of the response
- **RIGHT COLUMN** Blood from a single donor was stimulated with various concentration of PHA, ConA, PWM for 3 days in culture medium with immunosuppressant FK506 at concentrations of 3, 6, 12, 25 ng/ml



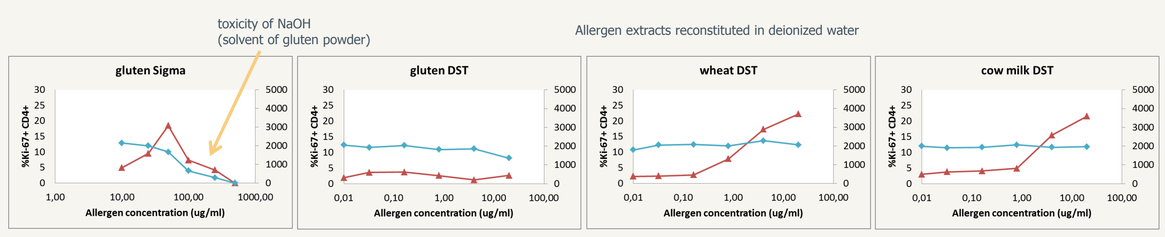
Results IV

PHA is susceptible to degradation (10 µg lectin tubes, preincubated for 3 days at 5, 45, 70 °C)



Results V

Delayed type of hypersensitivity (7 days culture, population $CD3^+CD4^+$, food allergens in tubes)



Numbers on analyzed **CD4** lymphocytes in **blue**, frequency of **Ki-67** positive in **red**

Conclusion

- The single test tube approach and Ki-67 staining can be easily adopted by a laboratory that seeks a simple and straightforward method to determine the proliferative responses of lymphocytes.
- The reported exclusivity of different lectins for stimulation of either T- or B- lymphocyte subsets was not confirmed. Interpretations of PWM stimulations can not be done without the lymphocyte phenotyping.
- A large interpersonal variation to PHA was observed, especially in concentrations that are usually used by laboratories (5-10 µg/ml). PHA was very susceptible to thermal degradation in contrast to ConA and PWM. The preservation of PHA for storage requires protective sugars (not shown).
- The assay can be used to monitor effect of an immunosuppressant in vitro.
- Ki-67 prove to be very sensitive marker of antigen specific stimulations in tube whole blood long term cultures. The use of lyophilized allergens may find its use in the proliferation assays related to the diagnosis of delayed type of hypersensitivity to food and drugs.