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FUNCTIONAL ANALYSES OF HUMAN iPS CELL- AND/OR PERIPHERAL CD14⁺ CELL-DERIVED AND IMMORTALIZED MYELOID CELL LINES (Mylc).

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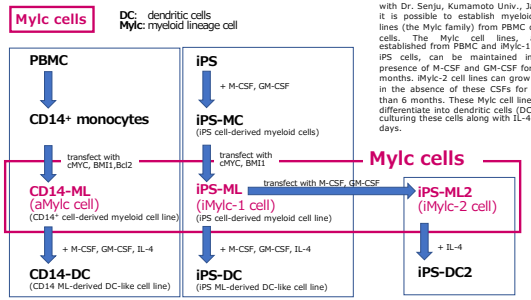
We have a platform to establish immortalized myeloid cell lines (Mylc lines) from human iPS cells and/or primary CD14⁺ cells purified from human peripheral blood mononuclear cells (PBMC) **(slide #1)**. Using this platform, we have established two Mylc lines from different iPS cells and four Mylc lines from different healthy donor-derived CD14⁺ cells **(slide #2)**, and examined the function of these Mylc lines from two perspective, 1) indicator cells for harmful substances and 2) host cells for virus infection. In many kinds of skin-sensitization tests and pyrogen tests, human-derived myeloid cell lines are used as indicator cells. First, we examined whether Mylc lines were utilizable as indicator cells in these tests. We cultured Mylc lines along with endotoxin, non-endotoxin or chemicals **(slide #3)**. Then the amount of inflammatory cytokines (IL-6 and IL-8) in the culture supernatants was measured. Upon the stimulation with endotoxin (LPS) or non-endotoxin (SAC, CpG-A, Resiquimod), Mylc lines produced these cytokines in a dose-dependent manner **(slide #4)**. Mylc lines were more sensitive than the representative human myeloid cell lines, Mono-mac-6 and THP-1 **(slide #5)**. Some harmful chemicals could also induce the production of these inflammatory cytokines **(slide #6)**. All Mylc lines examined could respond to these pyrogens and harmful chemicals. Therefore, the test system using several Mylc lines might be more precise compared with the present tests using one particular cell line. We next examined whether Mylc lines before and after the differentiation into dendritic cell (DC) can be infected with dengue virus type 2 (DV2) 16681 strain. Mylc lines can be differentiated into DCs by culturing Mylc lines along with IL-4 for 3 days. Interestingly, all Mylc lines could be infected with DV2, especially after DC-differentiation (8 out of 8 lines examined) **(slide #7)**. Some of DC-differentiated Mylc lines showed increased sensitivity to DV2 compared with Vero cells that are generally used for dengue virus study **(slide #8)**. Therefore, these results indicate that human Mylc lines may be useful and powerful tool for virus isolation and research. Furthermore, this Mylc lineup including Mylc lines derived from patients would make functional assay and/or screening systems more reliable **(slide #9)**.

1. Platform to establish immortalized myeloid cell lines (Mylc lines)



Using our technologies (collaboration with Dr. Senju, Kumamoto Univ., Japan), it is possible to establish myeloid cell lines (the Mylc family) from PBMC or iPS cells. The Mylc cell lines, aMylc established from PBMC and iMylc-1 from iPS cells, can be maintained in the presence of M-CSF and GM-CSF for 1~2 months. iMylc-2 cell lines can grow even in the absence of these CSFs for more than 6 months. These Mylc cell lines can differentiate into dendritic cells (DCs) by culturing these cells along with IL-4 for 3 days.

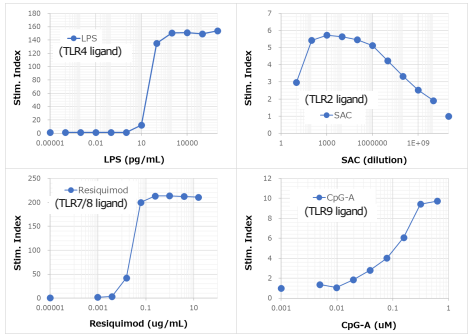
2. List of Mylc lines

iPS-derived	ML (iMylc-1)	ML2 (iMylc-2)
A	K-ML	K-ML2
B	B-ML	
C	A-ML	
E	D-ML	

CD14⁺-derived	ML (aMylc-1)	ML2 (aMylc-2)
PBMC-14	K14-ML	
PBMC-Y	Y14ML	
PBMC-M	M14ML	
PBMC-N	N14ML	
PBMC-A	A14ML	

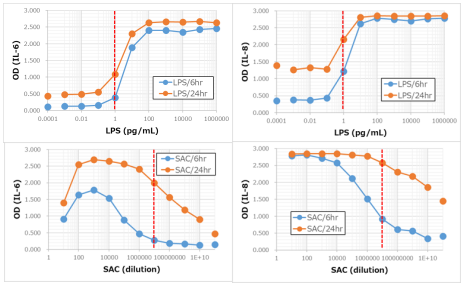
We have established 4 iMylc-1 lines from different iPS cells and 1 iMylc-2 line, and 5 aMylc-1 lines from different healthy donors. These Mylc cell lines can differentiate into dendritic cells (DCs) by culturing these cells along with IL-4 for 3 days. (ex. ML2 ➡ DC2 etc.)

3. Stimulation of iMylc-2 cells with TLR-ligands



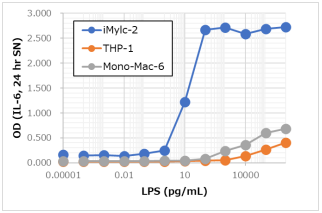
The iMylc-2 cells were cultured with each TLR-ligand for 24 hrs (●). The amount of IL-6 in the SNs were measured. Stimulation Index = (OD of each sample)/(OD of iMylc-2 alone).

4. Comparison of sensitivity of iMylc-2 and PBMC



The iMylc-2 cells were cultured with LPS (top panels) or SAC (bottom panels) for 6 (●) or 24 (●) hrs. The amount of IL-6 (left panels) or IL-8 (right panels) in the SNs were measured. The limit of detection (LOD) by PBMC is indicated by the red dotted lines (data from MAT research). iMylc-2 cells exhibited the biological sensitivity equivalent or more than that of PBMC.

5. Comparison of iMylc-2 with other human-derived myeloid cell lines

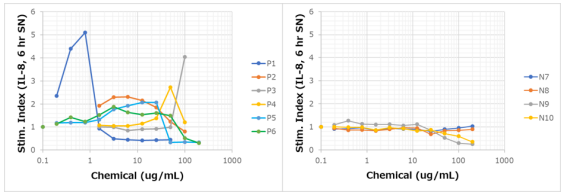


The iMylc-2, THP-1, or Mono-Mac-6 cells ($1x10^4$/well in 96-well plate) were cultured with the titrated amount of LPS for 24 hrs. The supernatants (SNs) from these cultures were harvested and the amount of IL-6 in these SNs were measured by ELISA. The y-axis means the actual OD values in ELISA assay.

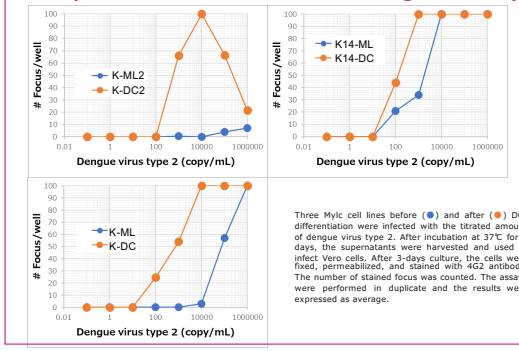
6. Stimulation of iMylc-2 cells with various chemicals

ID	Chemical name	LLNA potency	h-CLAT CD86	h-CLAT CD54	iMylc-2 IL-6	iMylc-2 IL-8
P1	2,4-Dinitrochlorobenzene	extreme	P	P	P	P
P2	4-Phenylenediamine	strong	P	N	N	P
P3	Nickel sulfate hexahydrate	moderate	P	P	P	P
P4	2-mercaptobenzothiazole	moderate	N	P	P	P
P5	R(+) Limonene	weak	N	P	N	P
P6	Imidazolidinyl urea	weak	P	P	N	P
N7	Isopropanol	Non-sensitiser	N	N	N	N
N8	Glycerol	Non-sensitiser	N	N	N	N
N9	Lactic acid	Non-sensitiser	N	N	N	N
N10	4-Aminobenzoic acid	Non-sensitiser	N	N	N	N

The iMylc-2 cells were cultured with the titrated amount of each chemicals for 6 hrs. The amount of IL-6 in the SNs were measured. Stimulation Index = (OD of each sample)/(OD of iMylc-2 alone). Stimulation Index more than 1.5 was regarded as positive (P). The stimulatory chemicals (P1~P6) in Local Lymph Node Assay (LLNA) and h-CLAT assays were also positive in our iMylc-2 assay (see table).

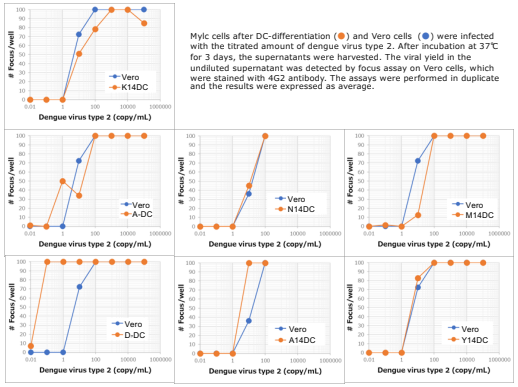


7. Mylc cells can be infected with dengue virus type 2.



Three Mylc cell lines before (●) and after (●) DC-differentiation were infected with the titrated amount of dengue virus type 2. After incubation at 37℃ for 3 days, the supernatants were harvested and used to infect Vero cells. After 3-days culture, the cells were fixed, permeabilized, and stained with 4G2 antibody. The number of stained focus was counted. The assays were performed in duplicate and the results were expressed as average.

8. Comparison of Mylc cells with Vero cells in infection.



Mylc cells after DC-differentiation (●) and Vero cells (●) were infected with the titrated amount of dengue virus type 2. After incubation at 37℃ for 3 days, the supernatants were harvested. The viral yield in the undiluted supernatant was detected by focus assay on Vero cells, which were stained with 4G2 antibody. The assays were performed in duplicate and the results were expressed as average.

9. Advantages of Mylc cells

as indicator cells

- The iMylc-2 cells exhibited the biological sensitivity equivalent or more than that of PBMC.
- In the case of PBMC, the pre-test of each PBMCs is required for the sensitivity. The iMylc-2 cells are ready to use without any pre-tests, functionary stable, easy to prepare large number of cells.
- Using several iMylc-2 cell lines established from different iPS cells, it is possible to enhance the credibility of data in pyrogen detection test.
- In the future, it is also possible to use iMylc-2 cells established from the particular patients.

as host cells in infection

The present

Vero (African green monkey)
C6/36 (mosquito)
Huh7 (human)
A549 (human)
K562 (human)

Problems
- The limited cell lines for virus-isolation
- not always human-derived
- Some viruses are still not isolated.
- New cell lines required for virus-isolation.

MiCAN

Mylc lines
(human iPS-derived : iMylc)
(human PBMC, CD14⁺-derived : aMylc)

MiCAN Mylc
- Human-derived
- Long term-culturable
- Large scale-preparation
- Uniformed quality
- Origin-different, many kinds of Mylc
- Choose the best Mylc for your study