

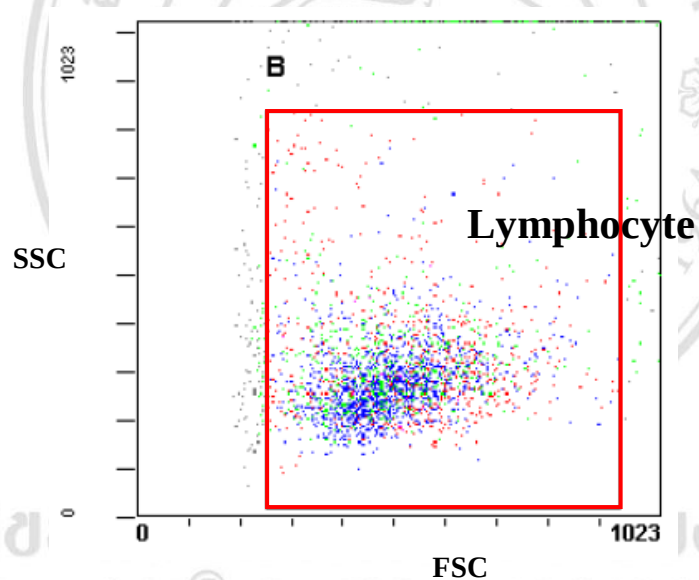
APPENDIX A

Flow cytometry analysis

Th17 determination by flow cytometry

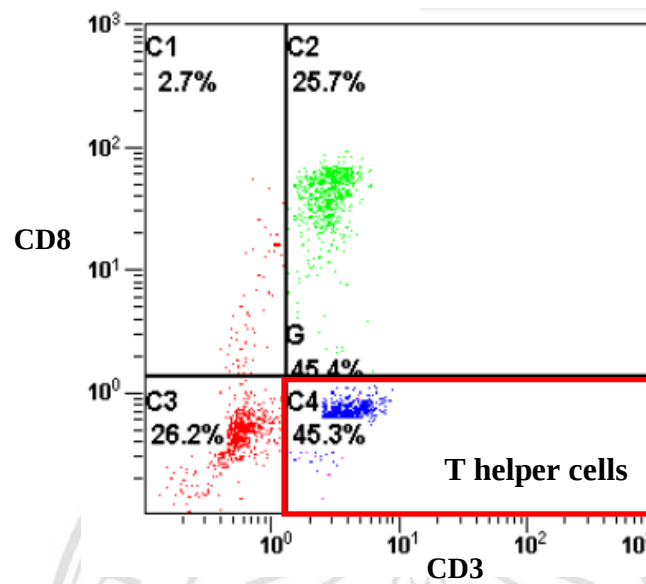
Gating strategy

1. Gating lymphocyte population from the cell size in forward scatter (x bar) and granularity in side scatter (y bar) plot

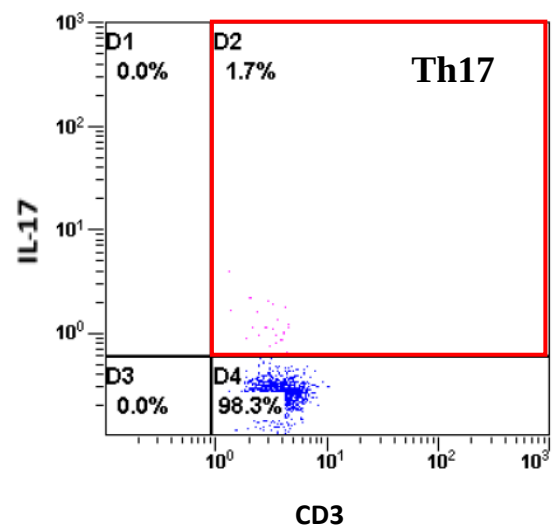
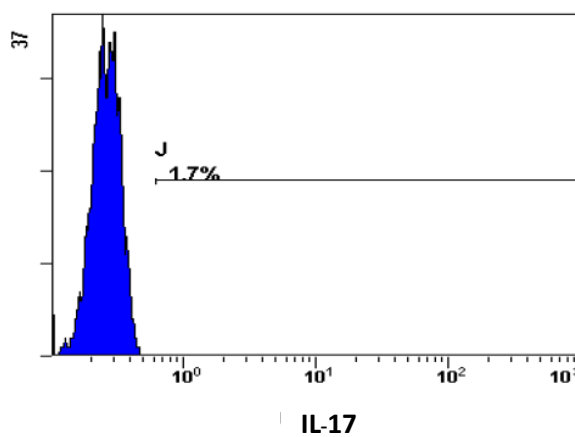


ลิขสิทธิ์
Copyright© by Chiang Mai University
All rights reserved

2. Gating CD 4 lymphocyte population from CD3⁺CD8⁻ T cells population



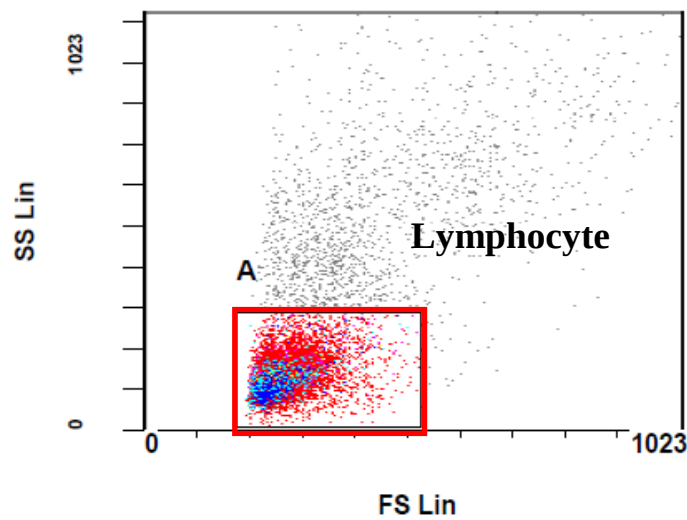
3. Investigated IL17 expression on histogram or dotted plot by using unstained cells as a negative control



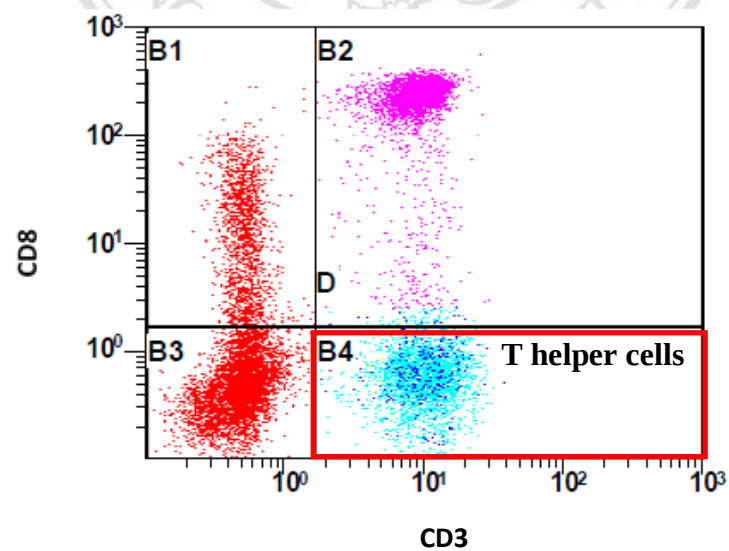
Treg determination by flow cytometry

Gating strategy

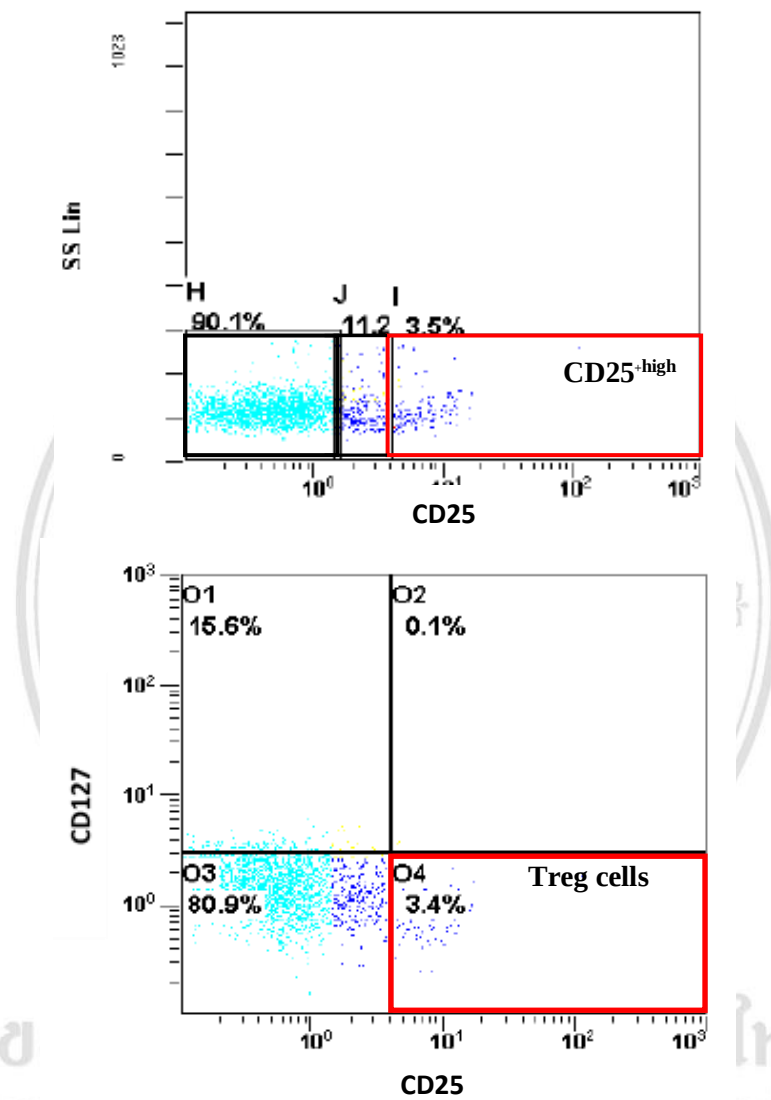
1. Gating lymphocyte population from the cell size in forward scatter (x bar) and granularity in side scatter (y bar) plot



2. Gating CD 4 lymphocyte population from CD3⁺CD8⁻ T cells population



3. Investigated Treg expression (CD25^{high} and CD1127⁺) on dotted plot by using unstained cells as a negative control



ลิขสิทธิ์
Copyright © by Chiang Mai University
All rights reserved

APPENDIX B

Chemicals and Instruments

1. Chemicals

Chemicals	Source
ANA test kit - Evans blue dye - Glycerol - Slides coated with HEp-20-10 cells and primate liver - Tween 20	Euroimmune, Luebeck, Germany
Amphotericin B	Capricorn scientific, Ebsdorfergrund, Germany
Brefeldin A	Biolegend, San Diego, USA
Dimethyl sulfoxide (DMSO)	SigmaAldrich, St. Louis, USA
Fetal bovine serum (FBS)	Capricorn scientific, Ebsdorfergrund, Germany
Ficoll hypaque	IsoPrep, Robbins Scientific Corporation, Sunnyvale, USA
Heparin	Leo pharmaceutical, Ballerup, Danemark
Ionomycin	SigmaAldrich, St. Louis, USA
Paraformaldehyde	SigmaAldrich, St. Louis, USA
Penicillin Streptomycin (Pen Strep)	SigmaAldrich, St. Louis, USA

Chemicals	Source
Phorbol 12-myristate 13-acetate (PMA)	InvivoGen, San Diego, USA
Phosphate buffered saline (PBS) pH 7.2	Euroimmune, Luebeck, Germany
RPMI 1640 Medium	Gibco, Life technologies, Carlsbad, USA
Saponin	AMRESCO, Ohio, USA

2. Instruments

Instruments	Source
15 and 50 mL centrifuge tube	Corning, NY, USA
25 cm ³ T-flask	Corning, NY, USA
24- well plate	Corning, NY, USA
Analytical balance	Scaltec, Dania, USA
Autoclave	Tomy, Japan
Automatic pipette	Labmate HTL, Warszawa, Poland
Carbon dioxide incubator	Shel lab, Cornelius, OR, USA
Centrifuge	Kokusan, Shizuoka Japan Eppendorf, Rotselaar, Belgium
ClassII biological safety cabinet	ESCO. Horsham, USA
Flowcytometer	Beckman coulter, Pasadena, USA
Flowcytometer tube (5mL)	FALCON, Brookings, USA
Fluorescence microscope	Olympus, Tokyo, Japan
Hotplate Stirrer	Thermolyne Nuova, Hampton, NH, USA

Instruments	Source
Inverted microscope	Zeiss Axiovert, Oberkochen, Germany
Light microscope	Nikon, Tokyo, Japan
Microcentrifuge	Hettich. Germany
pH meter	Toledo instruments, Shanghai, China
Plastic tube (5mL)	Hycon plastic, Manchester, NH, USA
Serofuge	Hettich, Germany
Vortex mixer	Gemmy Industrial Corp, Taipei, Taiwan
Water bath	Thermoline, NSW, Australia

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

APPENDIX C

Antibodies

Antibodies	Source
FITC anti-human CD3 (lot: B193130, B211008)	BioLegend. San Diego, USA
FITC anti-human IgG	Euroimmune, Luebeck, Germany
PE anti-human CD8 (lot: B185799, B209311)	BioLegend. San Diego, USA
PE/Cy7 anti-human CD25 (lot: B210940)	BioLegend. San Diego, USA
PerCP/Cy5.5 anti-human IL-17A (lot: B179831, B215531)	BioLegend. San Diego, USA
PerCP/Cy5.5 anti-human CD127 (lot: B200769)	BioLegend. San Diego, USA

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

APPENDIX D

Reagents Preparation

1. Phosphate buffer saline (PBS), pH 7.2

EUROIMMUNE PBS powder 1 pack
Dissolved in deionized distilled water and adjusted volume to 1,000 mL
adjusted to pH 7.2

2. RPMI medium

DMEM powder 1 pack
NaHCO₃ 2.0 gm
Dissolved in deionized distilled water and adjusted volume to 1,000 mL.
Filtered through 0.2 µm Millipore membrane filter and stored at 4°C.

3. Completed RPMI culture medium

Penicillin (10,000 units/mL)/Streptomycin (10,000 units/mL) 1.00 mL
Amphotericin B 1.00 mL
Fetal bovine serum (FBS) 10.00 mL
Incompleted RPMI added up to 100 mL and checked sterility before used.

4. PMA

PMA powder 5 mg
DMSO 1 mL

Store in aliquots at -20°C

5. Ionomycin

PMA powder	1	mg
DMSO	1	mL

Store in aliquots at -20°C

6. Brefeldin A

Brefeldin A Solution	5	mg/mL in DMSO
----------------------	---	---------------

Store in aliquots at -4°C

7. 10% Saponin stock

Saponin powder	1	mg
PBS	10	mL

Stored at 4°C.

8. 1 % Paraformaldehyde

Paraformaldehyde	1	gm
PBS, pH 7.2	100	mL

Heat at 56°C until dissolved

Filtered with 0.2 µm Millipore filter, stored at 4°C.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

CURRICULUM VITAE

Author's Name	Miss Kanyanee Promsawan
Date of birth	April 5, 1991
Place of Birth	Lamphun Province, Thailand
Education	2009 Certificate of high school, Chakkham Khanathon school, Lamphun, Thailand 2013 Bachelor of Science (Medical Technology), Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved