APPENDICES

Appendix A: List of the chemicals and materials used in this study

Chemicals/Materials	Source
Acetone	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Ammonium persulfate	Sigma, St. Louis, MO, USA
Amplicillin	Sigma, St. Louis, MO, USA
Aprotinin	Sigma, St. Louis, MO, USA
Bisacrylamide	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Chemilumnescent reagent	Pierce, Rockford, IL, USA
Chloroquine diphosphate	Sigma, St. Louis, MO, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
DEAE-Dextran	Sigma, St. Louis, MO, USA
Developer and replenisher	Kodak, NY, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
Dulbecco's Modified Eagle Medium	Gibco, Grand Island, NY, USA
Ethyl alcohol	Merck, Darmstadt, Germany
Ethylenediaminetetraacetic acid	Fluka, Buchs, Switzerland
Fetal bovine serum	Gibco, Grand Island, NY, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC-conjugated sheep F(ab')2 anti-mouse Igs	Silenus, Boronia, Victoria, Australia
Gentamicin	Russel, London, UK
Heparin	Leo, Ballerup, Denmark

Iodoacetamide	Sigma, St. Louis, MO, USA
Iscove's Modified Dulbecco's Medium	Gibco, Grand Island, NY, USA
Isopropanol	Merck, Darmstadt, Germany
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Nitrocellulose membrane	PALL, East Hill, NY, USA
Nonidet P-40	Pierce, Rockford, IL, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Fermentas, MA, USA
Protein G separose	Zymed Laboratories, Inc., CA, USA
RPMI Medium 1640	Gibco, Grand Island, NY, USA
Skimmed milk	Difco laboratories, Detroit,MI, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany

Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
Sreptavidin-HRP	Zymed, South San Francisco, CA
TEMED	BioRad Laboratories, Griffin
Tris-base	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland

Appendix B: List of antibodies used in this study

Monoclonal antibody name (specificity)	Isotype
MT4 (anti-CD4)	IgM
MT4/2 (anti-CD4)	IgM
MT4/3 (anti-CD4)	IgG2a
MT4/4 (anti-CD4)	IgM
MT99/3 (anti-CD99)	IgG2a
FE1H10 (un-defined)	IgM
13M-1F (anti-bacteriophage)	IgG2a
Thal N/B (anti-hemoglobin)	IgG1
Hb1a (anti-hemoglobin)	IgM
M6-1E9 (anti-CD147)	IgG2a
OKT-3 (anti-CD3)	IgG1
MT8 (anti-CD8)	IgM

Appendix C: List of instruments used in this study

Instrument-Model	**Source**
2-20 µl Autopipette	Bio-rad, USA
20-200 µl Autopipette	Bio-rad, USA
100-1000 µl Autopipette	Bio-rad, USA
40-350 µl multichanel autopipette	Socorex, Switzerland
AKTA® prime	Amersham, USA
Analytical balance	Mettler Toledo, Canada
Autoclave	Huxey, Taiwan
CO_2 incubator	Thermo electron coporation, USA
Eletrophoresis and Electrotransfer unit	Amersham, USA
Flow cytometer-FACSort	Beckton Dickinson, USA
Fluorescent microscope	Olympus, Japan
Inverted microscope	Olympus, Japan
Laminar flow	Nuaire, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
pH meter	Precisa, Switzerland
Refrigerated centrifuge	Sorvall, Germany
Rotator	Technomara, Switzerland

Appendix D: Reagents and buffers preparation

1. Reagents for cell culture

1.1 Incomplete IMDM medium

IMDM powder	1	pack
$NaHCO_3$	3.024	g
ddH_2O	800	ml
Stirred until dissolve		
Gentamycin (40 µg/ml)	1	ml
Dissolved in ddH_20 and adjusted volume to	1000	ml
Filtrated through 0.2 µm millipore membrane filter		
Added Fungizone (5 mg/ml)	500	µl
Mixed and stored at 4°C		

1.2 Complete IMDM medium

Incomplete IMDM medium	90	ml
Heat inactivated fetal bovine serum	10	ml
Checked sterility before used		

1.3 Incomplete DMEM medium

DMEM powder	1	pack
$NaHCO_3$	3.7	g
HEPES	2.603	g
ddH_2O	800	ml
Stirred until dissolved		
0.34% 2-ME	1	ml
Dissolved in ddH_20 and adjusted volume to	1000	ml

Filtered through 0.2 μm millipore membrane filter, stored at 4°C

1.4 Complete DMEM medium

Incomplete DMEM medium	89.5	ml
Fetal bovine serum	10	ml
Pen/Strep	500	μl
Checked sterility before used		

1.5 Incomplete RPMI medium

RPMI powder	1	pack
$NaHCO_3$	2	g
HEPES	3.57	g
ddH_2O	800	ml
Stirred until dissolved		
Gentamycin (40 μg/ml)	1	ml
Dissolved in ddH_20 and adjusted volume to	1000	ml
Filtered through 0.2 μm millipore membrane filter		
Added Fungizone (5 mg/ml)	500	μl
Mixed and stored at 4°C		

1.6 Complete RPMI medium

Incomplete RPMI 1640 medium	90	ml
Fetal bovine serum	10	ml
Checked sterility before used		

2. Reagents for DEAE-Dextran transfection

2.1 0.5 mM EDTA-PBS

PBS pH 7.2	100	ml
0.5 M EDTA pH 8.0	100	µl

Filtrated through 0.2 µm millipore membrane filter

Stored at room temperature

2.2 DEAE-Dextran stock solution (10 mg/ml)

DEAE-Dextran (M.W. 500,000)	0.1	g
PBS pH 7.2	10	ml

Filtrated through 0.2 µm millipore membrane filter

Aliquot to vials and stored at -20°C

2.3 Chloroquine diphosphate stock solution (10 mM)

Chloroquine diphosphate	0.103	g
PBS pH 7.2	20	ml

Filtrated through 0.2 µm millipore membrane filter

Aliquot to vials and stored at -20°C

2.4 10% DMSO-PBS

Dimethyl sulfoxide	10	ml
PBS pH 7.2	90	ml

Filtrated through 0.2 µm millipore membrane filter

Stored at room temperature

3. Reagents for SDS-PAGE

3.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15	g
Deionized distilled water	80	ml
Adjusted pH to 8.8 by concentrate HCl		
Adjusted final volume to	100	ml
Filtrated through 0.2 μm millipore membrane filter		
Stored at 4ºC		

3.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0	g
Deionized distilled water	80	ml
Adjusted pH to 6.8 by concentrate HCl		
Adjusted final volume to	100	ml
Filtrated through 0.2 μm millipore membrane filter		
Stored at 4ºC		

3.3 2x non-reducing buffer

0.5 M Tris HCl pH 6.8	2.5	ml
87% glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	5.16	ml
1% Bromphenol blue	40	μl
Mixed well, aliquot and stored at -20ºC		

3.4 2x reducing buffer

0.5M Tris HCl pH 6.8	2.5	ml
87% glycerol	2.3	ml
Sodium dodecyl sulfate	0.4	g
Distilled water	4.16	ml
2-ME	1	ml
1% Bromphenol blue	40	µl

Mixed well, aliquot and stored at -20ºC

3.5 1X Running buffer

Tris base	3.028	g
Glycine	14.413	g
Sodium dodecyl sulfate	1.0	g
Distilled water	1000	ml

Mixed well, prepared before used

3.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60	g
Bis-acrylamide	1.6	g
ddH_2O	200	ml

Mixed throughly and filtrated through 0.2 µm millipore membrane filter

Kept in dark at 4ºC

3.7 Slab gel

	separating gel			stacking gel
	12.5%	10%	7.5%	4%
Distilled water	3.2 ml	4 ml	4.85 ml	1.5 ml
30% Monomer	4.2 ml	3.3 ml	2.5 ml	332.5 µl
4X Separating gel buffer	2.5 ml	2.5 ml	2.5 ml	-
4X Stacking gel buffer	-	-	-	625 µl
10% SDS (in distilled water)	100 µl	100 µl	100 µl	25 µl
10% APS (in distilled water)	50 µl	50 µl	50 µl	12.5 µl
TEMED	10 µl	10 µl	10 µl	5 µl

3.8 10% APS

Ammonium persulfate	0.1	g
Distilled water	1	ml

Mixed well, aliquot and stored at -20ºC

3.9 10% SDS

Sodium dodecyl sulfate	10	g
Distilled water	100	ml

Mixed well, aliquot and stored at -20ºC

3.10 1X Blotting buffer

Tris-base	1.515	g
Glycine	7.205	g
Sodium dodecyl sulfate	0.5	g
Distilled water	350	ml

Mixed well

Methanol	100	ml
Adjusted final volume to	500	ml

Filtrated through 0.2 μm Millipore membrane filter

Stored at room temperature

3.11 0.025% Coomassie brilliant blue R250

Coomassie brilliant blue R250	0.125	g
Methanol	200	ml
Acetic acid	35	ml

Adjusted volume to 500 ml by ddH_2O

Stored at room temperature

4. Reagents for Immunoprecipitation

4.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN_3)

Tris base	3.03	g
NaCl	2.922	g
EDTA (M.W. 292.25)	0.292	g
NaN_3	0.1	g
Distilled water	200	ml

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

4.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF)	100	μl
(100 mM in acetone)		

Iodoacetamide (0.5M in distilled water)	100	µl
Aprotinin (1 mg/ml in PBS)	100	µl
10% NP-40	1	ml
Tris-lysis buffer pH 8.2	8.7	ml
Pepstatin A	10	µl

Mixed well, aliquot to vial and stored at -20ºC

4.3 1 mM Glycine in PBS

Glycine	0.0375	g
PBS pH 7.2	500	ml

Stored at 4ºC

4.4 5 mM Biotin in PBS

Sulfo-NHS-LC-biotin	0.00278	g
PBS pH 7.2	1	ml

Freshly prepared

5. Reagents for indirect immunofluorescent staining

5.1 1X Phosphate buffer saline (PBS)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2PO_4	0.2	g
Distilled water	900	ml

Adjusted pH to 7.2 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature

5.2 1%BSA-0.02%NaN_3 in PBS

Bovine serum albumin fraction V	10	g
PBS pH 7.2	1000	ml
10% NaN_3 in PBS	2000	µl

Mixed well until BSA completely dissolved, stored at 4ºC

5.3 1%Para-formaldehyde in PBS

Para-formaldehyde	5	g
PBS pH 7.2	500	ml

Heat at 56ºC until dissolved

Filtrated through 0.2 µm millipore membrane filter, stored at 4ºC

5.4 10X Sheath Fluid

NaCl	160	g
KCl	4	g
Na_2HPO_4	23	g
KH_2PO_4	4	g
Sodium azide (NaN_3)	20	g
Distilled water	1800	ml

Adjusted pH to 7.2 by 5N NaOH

Filtrated through 0.2 µm millipore membrane filter

Adjusted volume to	2000	ml

Stored at room temperature

5.5 10%NaN_3-PBS

NaN_3	10	g
1XPBS	100	ml

5.6 Red blood cells lysis buffer

1XPBS	2665	µl
Diethylene glycol	300	µl
37% formaldehyde	135	µl

6. Reagents for IgM purification

6.1 Binding buffer (20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5)

1 M Na_2HPO_4	5.8	ml
1 M NaH_2PO_4	4.2	ml
$(NH_4)_2SO_4$	52.856	g
ddH_2O	400	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm millipore membrane filter

Kept at 4ºC, degas for 30 min before used

6.2 4X Binding buffer (100 ml) for dilute ascitic fluid

1 M Na_2HPO_4	4.6	ml
1 M NaH_2PO_4	3.36	ml
$(NH_4)_2SO_4$	42.284	gm
ddH_2O	70	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 100 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm millipore membrane filter

Kept at 4ºC, degas for 30 min before used

6.3 Eluting buffer (20 mM sodium phosphate pH 7.5)

1 M Na_2HPO_4	11.6	ml
1 M NaH_2PO_4	8.4	ml
ddH_2O	800	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 1000 ml with ddH_2O

Mixed thoroughly and filtrated through 0. 2 μm millipore membrane filter

Kept at 4ºC**,** degas for 30 min before used

6.4 Regeneration buffer

1 M Na_2HPO_4	5.8	ml
1 M NaH_2PO_4	4.2	ml
Isopropanol	150	ml
ddH_2O	200	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 μm millipore membrane filter

Kept at 4ºC**,** degas for 30 min before used

7. Reagents for IgG purification

7.1 Binding buffer (20 mM sodium phosphate buffer, pH 7.0)

1 M Na_2HPO_4	11.6	ml
1 M NaH_2PO_4	8.4	ml
ddH_2O	800	ml

Adjusted the pH to 7.0 with 5 N NaOH

Adjusted the volume to 1000 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm millipore membrane filter

Kept at 4ºC, degas for 30 min before used

7.2 Eluting buffer (0.1 M Glycine-HCl, pH 2.7)

Glycine	3.753	g
ddH_2O	350	ml

Adjusted the pH to 2.7 with conc.HCl

Adjusted the volume to 500 ml with ddH_2O

Mixed thoroughly and filtrated through 0. 2 µm millipore membrane filter

Kept at 4ºC, degas for 30 min before used

7.3 Neutralizing buffer (1 M Tris-HCl, pH 9.0)

Tris base	12.114	g
ddH_2O	60	ml

Adjusted the pH to 9.0 with conc. NaOH

Adjusted the volume to 100 ml with ddH_2O

Mixed thoroughly and filtrated through 0.2 µm millipore membrane filter

Kept at 4ºC, degas for 30 min before used

8. Reagent for cell lysate preparation

8.1 0.5M Iodoacetamide (2 ml)

Iodoacetamide (FW185 (C_2H_4INO))	0.185	g
ddH_2O	2	ml

Aliquot 100 µl/vial, stored at -20ºC

8.2 Phenylmethyl-sulfonylfluoride (PMSF) 2 ml

PMSF (FW174.5 ($C_2H_7FO_2S$)		0.03484 g
Acetone	2	ml

Aliquot 100 µl/vial, stored at -20ºC

8.3 Aprotinin 1 mg/ml

Reconstitute aprotinin (lyophilized form) with ddH_2O	5	ml

Aliquot 100 µl/vial, stored at -20ºC

8.4 2mM Pepstatin A in DMSO

Pepstatin A (M.W. 685.9)	0.0014 g	
DMSO	1	ml

Mixed well, aliquot 10 µl/vial, stored at -20ºC

CURRICULUM VITAE

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Presentation

Supaporn Khamchun and Watchara Kasinrerk. Different epitope on CD4 molecule expressed on lymphocytes and monocytes indicating by a specific monoclonal antibody. The 1st CMU graduate Research Conference, Chiang Mai University. Chiang Mai, Thailand. November 2009.