APPENDICES

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

Chemicals/Materials	Source
Absolute methanol	J.T.Baker, Philipsburg, NJ, USA
Ammonium chloride	Merck, Darmstadt, Germany
Ammonium oxalate	Riedel, Germany
Amphotericin B	Squibb, NS, USA
Bovine serum albumin fraction V	Sigma, St. Louis, MO, USA
Chloroquine diphosphate	Sigma, St. Louis, MO, USA
Citric acid-1-hydrate	Merck, Darmstadt, Germany
DEAE-Dextran	Sigma, St. Louis, MO, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
Dipotassium ethelene diamine tetra-acetic acid	Fluka, Buchs, Switzerland
Disodium hydrogen phosphate	Fisher, Leics, UK
Ethelenediamine tetra-acetic acid	BDH, Poole, England
FACS™ lysing solution	Becton Dickinson, San Jose, CA, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC	Sigma, St. Louis, MO, USA

FITC conjugated rat $F(ab')_2$ anti-mouse Igs	Dako, Glostrup, Denmark
Foetal calf serum	Biochrcom, Leonorenstr, Germany
Formaldehyde solution min. 37%	Merck, Darmstadt, Germany
Gentamicin	Roussel, London, UK
Gentian violet powder	Bangkoknovelty, Bangkok, Thailand
Giemsa's stain powder	BDH, Poole, England
Glacial acetic acid	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Glycine	Fisher, Leics, UK
Heparin	Leo, Ballerup, Denmark
Hydrochloric acid fuming 37%	Merck, Darmstadt, Germany
Minimum Essential Medium	Gibco, Grand Island, NY, USA
Optilyse® B lysing solution	Immunotech, Marseille, Frane
Paraformaldehyde	Fluka, Buchs, Switzerland
PE conjugated goat $F(ab')_2$ anti-mouse IgG	Immunotech, Marseille, Frane
PerCP conjugated CD45 mAb (Anti-Hle-1)	Becton Dickinson, San Jose, CA, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Potassium hydrogen carbonate	Asia Pacific, Australia
Potassium hydroxide pellets	May&Baker, Dagenham, England
Protein A sepharose® CL-4B	Pharmacia Biotech, CA, USA
Rat anti-mouse IgM coupled sepharose 4B	Zymed, San Francisco, CA, USA

Simultest™ CD3/CD4	Becton Dickinson, San Jose, CA, USA
Simultest™ CD3/CD8	Becton Dickinson, San Jose, CA, USA
Simultest™ Control γ1/γ1	Becton Dickinson, San Jose, CA, USA
Simultest™ LeucoGATE	Becton Dickinson, San Jose, CA, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydroxide	Eka, Nobel, Sweden
10% Sodium hypochlorite	OV, Chiangmai, Thailand
Tris (hydroxymethyl) aminomethane	Merck, Darmstadt, Germany
Wright's stain powder	BDH, Poole, England

Appendix B: List of antibodies, DNAs and cell line used in this study

Monoclonal antibodies	**Isotype**
MT4	IgM
MT14/3	IgG1

DNAs	**Name**
cDNA encoding CD4	CD4-DNA
cDNA encoding CD14	CD14-DNA

Animal cell line	**Cell type**	**Origin**
COS cells	SV 40 transformed cell line	Kidney, African green monkey

Appendix C: List of instruments used in this study

Instrument-Model	Source
Analytical balance; PB303-S	Mettler Toledo, Switzerland
pH meter; AG CH-8953	Precisa, Switzerland
High speed micro refrigerated centrifuge; MRX-150	Tomy, USA
Spectrophotometer; UV-1201	Shimadzu, Japan
Savant speedvac systems	Savant Inc., USA
Microdialyzer system; 500	Pierce, USA
Refrigerator (4°C)	Toshiba, Thailand
Centrifuge; RT6000D	Sorvall, USA
Waterbath; NB9-102	Thermoline, Australia
Light microscope; CHA	Olympus, Japan
Flow cytometer; FACSCalibur	Becton Dickinson, USA
Refrigerator (-20°C)	Sanyo, Thailand
Biological safety cabinet class II; Nu-400-400E	Nuaire, USA
CO_2 incubator; TC2323	Sheldon, USA
Microcentrifuge; Biofuge pico	Kendro, USA
Inverted microscope; CX40	Olympus, Japan
Fluorescence microscope; BX-40	Olympus, Japan
Incubator (30°C-220°C); U-60	Memmert, Germany
Autoclave; HA-3D	Hirayama, Japan

Appendix D: Reagents and buffers preparation

1. Reagents for monoclonal antibody purification

1.1 1N HCl

37% HCl solution	8.3 ml
Distilled water	91.7 ml

Prepare in fume hood by gradually adding HCl solution into distilled water with gentle stirring and store at room temperature

1.2 1N NaOH

NaOH	4.000 g
Distilled water	100 ml

Mix well and store at room temperature

1.3 0.15M Phosphate buffer saline (PBS pH 7.2)

NaCl	8.000 g
KCl	0.200 g
Na_2HPO_4	1.150 g
KH_2PO_4	0.200 g
Distilled water	800 ml

Adjust pH to 7.2 by 1N HCl or 1N NaOH

Adjust volume to 1000 ml

Filter with 0.2 μm millipore filter, store at room temperature

1.4 Elution buffer for IgM (0.2M glycine-HCl pH 2.8)

Glycine	1.500 g
Distilled water	85 ml

Adjust pH to 2.8 by 1 N HCl

Adjust volume to 100 ml

Filter with 0.2 μm millipore filter, store at 4°C

1.5 Elution buffer for IgG (0.1M citric acid pH 3.0)

Citric acid-1-hydrate	2.100 g
Distilled water	70 ml

Adjust pH to 3.0 by 5 N NaOH

Adjust volume to 100 ml

Filter with 0.2 μm millipore filter, store at 4°C

1.6 Neutralizing buffer (2M Tris-HCl pH 8.0)

Tris-base	24.220 g
Distilled water	60 ml

Adjust pH to pH 8.0 by concentrate HCl

Adjust volume to 100 ml, store at 4°C

1.7 Storage buffer (0.05% NaN_3-PBS pH 7.4)

$Na_2HPO_4.12\ H_2O$	0.758 g
KH_2PO_4	0.050 g
NaCl	0.438 g
10% NaN_3 in PBS pH 7.2	250 µl
Distilled water	40 ml

Adjust pH to 7.4 by 1N HCl

Adjust volume to 50 ml

Filter with 0.2 µm millipore filter, store at 4°C

2. Reagents for direct and indirect immunofluorescence staining

2.1 Phosphate buffer saline (PBS pH 7.2)

NaCl	8.000 g
KCl	0.200 g
Na_2HPO_4	1.150 g
KH_2PO_4	0.200 g
Distilled water	800 ml

Adjust pH to 7.2 by 1N HCl or 1N NaOH

Adjust volume to 1000 ml

2.2 1%BSA-0.02%NaN_3 in PBS (1%BSA-PBS- NaN_3)

BSA fraction V	1.000 g
PBS pH 7.2	99.800 ml
10% NaN_3 in PBS pH 7.2	200 µl

Mix well until BSA completely dissolved

Filter with 0.2 µm millipore filter, store at 4°C

2.3 1%Paraformaldehyde in PBS

Paraformaldehyde	5.000 g
PBS pH 7.2	500 ml

Heat at 56°C until dissolved

Filter with 0.45 µm filter, store in glass at 4°C

2.4 PBS containing 0.1% NaN_3 (PBS-0.1% NaN_3)

10%NaN_3 in PBS pH 7.2	5 ml
PBS pH 7.2	495 ml

Mix well, store at room temperature

3. Reagents for DEAE-Dextran transfection

3.1 0.5M EDTA pH 8.0

EDTA	18.610 g
Distilled water	90 ml

Mix well, Adjust pH to 8.0 with 5N KOH

Adjust volume to 100 ml

Sterile by autoclaving, store at room temperature

3.2 0.5mM EDTA-PBS

0.5M EDTA pH 8.0	0.100 ml
PBS pH 7.2	100 ml

Prepare in biosafety cabinet by sterile technique

Mix well, filter with 0.2 μm millipore filter, store at room temperature

3.3 Minimum Essential Medium (MEM)

MEM powder	1 package
$NaHCO_3$	2.200 g
Distilled water	900 ml

Add MEM powder to distilled water with gentle stirring

Rinse out inside of package to remove all traces of powder

Add gentamicin and amphotericin B to final concentration of 40 μg/ml and 2.5 μg/ml, respectively

Mix well and adjust pH to7.2 with 10% glacial acetic acid

Adjust volume to 1000 ml

Filter with 0.2 μm millipore filter, stored at 4°C

3.4 10% FCS-MEM

Foetal calf serum (FCS)	10 ml
MEM	90 ml

Prepare in biosafety cabinet by sterile technique, mix well and store at 4°C

3.5 Turk's solution

Glacial acetic acid	3 ml
1%gential violet	1 ml
Distilled water	100 ml

Mix well, filter with Whatman no.1 and store at room temperature

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

DEAE-Dextran	0.100 g
PBS pH 7.2	10 ml

Mix well, filter with 0.2 μm millipore filter

Aliquot to vials (500 μl/vial) and store at -20°C

3.7 Chloroquine diphosphate stock solution (10 mM)

Chloroquine diphosphate	0.0519 g
PBS pH 7.2	10 ml

Mix well, filter with 0.2 μm millipore filter

Aliquot to vials (500 μl/vial) and store at -20°C

3.8 10% DMSO-PBS

Dimethyl sulfoxide	10 ml
PBS pH 7.2	90 ml

Mix well, filter with 0.2 μm millipore filter and store at room temperature

4. Reagents for red blood cell lysing solution

4.1 1% Ammonium oxalate

Ammonium oxalate	1.000 g
Distilled water	100 ml

Mix well, store at room temperature

4.2 0.83% Ammonium chloride

NH_4Cl	0.830 g
Distilled water	100 ml

Mix well, store at room temperature

4.3 Ammonium chloride Tris buffer

0.17M Tris-base	10 ml
0.16M NH_4Cl	90 ml

Mix well, adjust pH to 7.2 by 1N HCl or 1N NaOH

Store at room temperature

4.4 Hypotonic ammonium chloride

NH_4Cl	0.332 g
$KHCO_3$	0.040 g
EDTA	0.002 g
Distilled water	35 ml

Mix well, adjust pH to7.2 with 1N HCl

Adjust volume to 40 ml, store at room temperature

4.5 0.83%ammonium chloride containing 3%formaldehyde (3%formaldehyde- NH_4Cl)

0.83% NH_4Cl (2x)	10 ml
37% Formalin	1.640 ml
Distilled water	8.360 ml

Mix well, store at room temperature

4.6 PBS containing 0.83%ammonium chloride and 3%formaldehyde (3%formaldehyde-NH_4Cl-PBS)

NH_4Cl	0.166 g
PBS pH 7.49	18.360 ml
37% Formalin	1.640 ml

Mix well, store at room temperature

5. Reagents for differential white blood cell count

5.1 Wright-Giemsa stain

Wright's stain powder	3.000 g
Giemsa's stain powder	0.330 g
Glycerol	30 ml
Absolute methanol	970 ml

Gradually add glycerol and absolute methanol in to stain powder

Mix well until stain powder completely dissolved, filter with Whatman no.1

Collect in brownish bottle, left in 37°C incubator for a week and daily mix

5.2 Haden's phosphate buffer pH 6.4 stock solution

KH_2PO_4	6.630 g
Na_2HPO_4	2.560 g
Distilled water	1000 ml

Mix well, store at room temperature

Working solution dilute with distilled water at dilution 1:10

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