Materials and Methods

Study population

Plasma samples were collected from 76 patients with acute P. vivax infections (AC) at Wuhe County Hospital, Guzhen County Hospital, The Frist City Hospital, Bengbu city, Anhui province, China. The diagnosis of P. vivax malaria infection was based on the examination of Giemsa-stained thick blood films. Polymerase chain reaction (PCR) with species-specific primers was performed on DNA isolated from the blood samples to further verify P. vivax infections.

Blood samples were collected from additional 32 peoples residing in the same *P. vivax*-endemic area. These subjects serving as "immune controls" (IC) did not have acute *P. vivax* infections at the time of blood collection as determined by both microscopy. A further 20 healthy adults living in Bengbu city area without previous malaria exposure or antibodies to malaria parasites were recruited to serve as "naïve controls" (NC). The clinical characteristics of the subjects are listed in Table 1. This study was approved by Ethical Approval Committee of Biomedical Institute of Anhui Medical University. Informed consents were obtained from each individual before a blood sample was taken.

Parasite cultures and antigen preparations

P. vivax-infected red blood cells (iRBC) purified from infected blood was used as crude antigens for coating. Briefly, *P. vivax* infected blood was depleted of white blood cells by filtering through a sterile column of CF11 cellulose (Whatman®, Maidstone, UK) and the red blood cells were washed with RPMI-1640 by centrifugation at 1190 g for 5 minutes. The parasites were cultured for 24 – 30 hours at 5% hematocrit in McCoy's 5A medium (GIBCO,

Carlsbad, USA) supplemented with 25% human AB serum. *P. vivax* parasites were maintained in an incubator containing 5% CO_2, 5% O_2 and 90% N_2 until matured to schizont stage (≥6 nuclei). The late stage iRBC were enriched by centrifugation using 60% Percoll (GE Healthcare, Uppsala, Sweden) at 1190 g for 10 minutes. The enriched iRBC pellets were sonicated for 40 seconds at 150 watts and the protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, USA). The vials were then aliquoted and stored at -70°C until use. Uninfected RBC were processed similarly as above and the protein concentration equal to the malaria antigens was stored at -70°C to be used as a negative control.

Protein expression and purification

PVMSP1 (19)

Briefly, blood filtered paper from isolated P. vivax infected patients was used to get genomic DNA then PCR was used to get MSP1(19) gene by using designed primers in Table. Sequences were cut by using BamHI and XhoI and verified. Verified sequences were cloned into pET28a vector. Protein was expressed in E. coli BL21 by culturing in LB medium. IPTG was used for protein induction and cell pellet was collected and sonicated. Protein was purified by using native condition as described by manufacturer's protocol [Qiagen, USA]. Supernatant was collected and protein was purified by Ni-NTA beat (bind to 6His-tag). Finally, protein was eluted and checked expression by SDS-PAGE.

PVAMA1

PCR was used to get PVAMA-1 gene by using designed primers in Table. Sequences were cut by using NdeI and XhoI enzymes and verified. Verified sequences were cloned into pET22b vector. Protein was expressed in E. coli BL21 by culturing in LB medium. IPTG was

used for protein induction and cell pellet was collected and sonicated. Protein was purified by using denaturing condition as described by manufacturer's protocol [Qiagen, USA]. Supernatant was collected and protein was purified by Ni-NTA beat (bind to 6His-tag). Finally, protein was eluted and checked expression by SDS-PAGE.

Results

Naturally anti-*P. vivax* IgG antibody responses after infected with *P. vivax* comparing between Thai and Chinese *P. vivax* infected patients

Anti-Crude PV

There was significantly difference of base line level of IgG among naïve controls against *P. vivax* crude antigen between Chinese (0.07) and and Thai (0.03) (MD=0.07, 95%CI=0.04-0.1, P<0.001). After infection by *P. vivax*, the significant elevation of IgG was found both Chinese (0.85) and Thai (0.14) infected patients. However, *P. vivax* infected patients from Chinese was found the increasing in the level of IgG higher than Thai infected patients. In similar to naïve control, the immune controls among Chinese patients (0.8) had higher level of IgG against *P. vivax* crude antigen than Thai patients (0.07).

Anti-*P. vivax* MSP-1(19)

Base lines of mean level of total IgG to PvMSP1(19) among healthy controls of Thai and Chinese population living outside (naïve controls) and inside (immune controls) endemic area was compared. We found that there was higher levels mean of total IgG among Thai (0.03) than Chinese naïve controls (0.3) (P<0.001). Moreover, the level of antibodies in peoples living in *P. vivax* endemic area was significant different between Thai (0.31) and China population (0.06) (P<0.001). We found two samples of Thai healthy control had IgG to PvMSP1(19) as high as *P. vivax* infected patients as shown in above figure, so we excluded this samples to calculate the value. Among both population, the mean level of total IgG to PvMSP-1(19) was significantly

increased during acute *P. vivax* infection in both of Thai (0.8) (P<0.001) and Chinese (0.5) (P<0.001). Interestingly, we found the significantly higher of these levels among Thai than China P. vivax infected patients (P=0.02).

Anti-*P. vivax* AMA-1

The mean level of total IgG against PvAMA-1 among healthy controls living outside the P. vivax endemic area was very low both of Thai (0.1) and Chinese population (0.03). However, there was the significant higher among Thai than Chinese (P<0.000). There was higher level among Thai (0.2) than Chinese immune comtrols (0.1) (P<0.001). We also found non significant difference between healthy donors among Thai peoples living inside and outside endemic areas (P>0.05), which was contrasted with Chinese (P<0.001). During acute P. vivax infection, there was significant higher comparing with naïve controls ((Thai P=<0.001, Chinese P<0.0.001). However, among *P. vivax* patients population, we found no significant different between Thai (0.3) and China (0.2) population (P=-0.15). We also found a sample of P. vivax infected patient had obviously high level of IgG to PvAMA-1, so we did not use this data to calculate.

Cross-reactivity between *P. vivax* and *P. falciparum* antigens; comparative study between Chinese and Thai *P. vivax* infected patients

To examine the cross-reactivity between *P. vivax* and *P. falciparum* antigens, the *P. vivax* and *P. falciparum* crude antigens were used as coated antigen on the plate. The median percentage of base line level of IgG in naive controls among Chinese was very low (0.003) and significantly lower than that of Thai (0.04) donors (MD=0.07, 95%CI=0.04-0.1, P<0.001). Moreover, we also found the significant higher level of IgG against *P. falciaprum* antigens in

immune controls among Thai (0.18) than Chinese (0.01) (MD=0.6, 95%CI=0.45-0.75). After infection with *P. vivax*, patients have had higher level of IgG against *P. falciparum* (Chinese=0.07, Thai=0.36).

Discussion

In this study, we have evidence that Thai villagers having had *P. vivax* infection and living in the endemic area do not produce high level of crude *P. vivax*-specific IgG [14]. However, in an assay with proteins extracted from the parasites. Since this has been tested with the complex proteins in the crude extract from *P. vivax*-infected erythrocytes, we have confirmed the presence of antibodies to *P. vivax* antigens by testing with recombinat *P. vivax* proteins, i.e. $PvMSP1_{19}$ and PvAMA1, and compared the level of natural antibodies between two *P. vivax* endemic areas, Chinese and Thai. The species specific antibodies could tell us the cross-reactivity between *P. falciparum* and *P. vivax* which lead to the examination of epidemiological status in those areas.

Various immunoglubulins are produced and IgG is the most important [15], Previously, a passive transfer of immune IgG to Gambian children provides protection [16]. The immunity against blood stage of *P. falciparum* infection is associated with class and subclass of IgG antibody [17]. Similarly, IgG1 and IgG3 are predominant among *P. vivax*-infected patients with history of malaria [18]. Recent study has shown anti-*P. vivax* merozoite surface protein 1 (MSP-1) IgG among subjects with distinct degrees of malaria exposure in endemic area. The IgG1 and IgG3 against *P. vivax* are higher among the subjects with a 1 year-exposure period than the group with >19 years of exposure [19]. PvMSP119 is shown to induce immunity in non-human primates [6].

Our study showed the significant lower of base line level of anti-crude *P. vivax* IgG among Chinese than Thai. We found the higher level of anti-crude *P. vivax* IgG in Chinese than Thai immune controls. Moreover, this level was very high in similar to that showed in the acute *P. vivax* infection. This could be the *P. vivax* endemicity in China was more than Thai, even these two area are hypo-endemic area.

However, in contrast to what we found in anti-crude *P. vivax* IgG, the base line level of anti-MSP-1 and anti-AMA-1 IgG was significantly lower in naïve controls of China than Thai. This could be hypothesized that the IgG among Thai donors can recognize a part of peptide of MSP-1 or AMA-1, therefore they can develop IgG against these peptides. Another possible reason could be all of these malaria naïve donors have had malaria experiences, but asymptomatic conditions among Thai healthy donors, therefore, they can develop and maintain the low level of antibodies in their circulation.

Crude *P. falciparum* antigens was used in this study to determine the species specific antibody and the cross-reactivity. We found the very low level of base line IgG antibody among both of healhy controls and immune controls of Chinese donors. After infected with *P. vivax*, Chinese patients also develop a bit of *P. falciparum* antibodies. These results were in contrast to those found in Thai. The higher level of base line of anti-*P. falciparum* IgG was found among healhy and immune controls. Moreover, development of anti-P. falciparum IgG in Thai *P. vivax* infected patients were much more higher than Chinese. This could be suggested the accuracy of species specific of total IgG. Moreover, his could be told us the higher risk or incidence of *P. falciparum* infection in Thailand was more than China. This also could be told us the *P. falciparum* infection experience in their life during staying in malaria endemic area. Therefore,

Thai patients living in malaria endemic area could develop *P. falciparum* antibody and maintain this level in their body.

Acknowledgements

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Reference

1. Sachs J, Malaney P: **The economic and social burden of malaria.** *Nature* 2002, **415**(6872):680-685.

2. Krotoski WA, Krotoski DM, Garnham PC, Bray RS, Killick-Kendrick R, Draper CC, Targett GA, Guy MW: **Relapses in primate malaria: discovery of two populations of exoerythrocytic stages. Preliminary note.** *Br Med J* 1980, **280**(6208):153-154.

3. Oliveira TR, Fernandez-Becerra C, Jimenez MC, Del Portillo HA, Soares IS: Evaluation of the acquired immune responses to Plasmodium vivax VIR variant antigens in individuals living in malaria-endemic areas of Brazil. *Malar J* 2006, 5:83.

4. Rodrigues MH, Rodrigues KM, Oliveira TR, Comodo AN, Rodrigues MM, Kocken CH, Thomas AW, Soares IS: **Antibody response of naturally infected individuals to recombinant Plasmodium vivax apical membrane antigen-1.** *Int J Parasitol* 2005, **35**(2):185-192.

5. Wickramarachchi T, Illeperuma RJ, Perera L, Bandara S, Holm I, Longacre S, Handunnetti SM, Udagama-Randeniya PV: Comparison of naturally acquired antibody responses against the C-terminal processing products of Plasmodium vivax Merozoite Surface Protein-1 under low transmission and unstable malaria conditions in Sri Lanka. *Int J Parasitol* 2006.

6. Rosa DS, Iwai LK, Tzelepis F, Bargieri DY, Medeiros MA, Soares IS, Sidney J, Sette A, Kalil J, Mello LE *et al*: Immunogenicity of a recombinant protein containing the Plasmodium vivax vaccine candidate MSP1(19) and two human CD4+ T-cell epitopes administered to non-human primates (Callithrix jacchus jacchus). *Microbes Infect* 2006, 8(8):2130-2137.

7. Troye-Blomberg M, Perlmann P: **T cell functions in Plasmodium falciparum and other malarias.** *Prog Allergy* 1988, **41**:253-287.

8. Bolad A, Berzins K: Antigenic diversity of Plasmodium falciparum and antibody-mediated parasite neutralization. *Scand J Immunol* 2000, 52(3):233-239.

9. WHO: **Report of a WHO informal consulation. Report WHO/CDC/RBM/2001.33.** In: *The use of antimalarial drugs.* Geneva: World Health Organization; 2001.

10. Rodrigues MH, Cunha MG, Machado RL, Ferreira OC, Jr., Rodrigues MM, Soares IS: Serological detection of Plasmodium vivax malaria using recombinant proteins corresponding to the 19-kDa C-terminal region of the merozoite surface protein-1. *Malar J* 2003, 2(1):39.

11. Soares IS, da Cunha MG, Silva MN, Souza JM, Del Portillo HA, Rodrigues MM: Longevity of naturally acquired antibody responses to the N- and C-terminal regions of Plasmodium vivax merozoite surface protein 1. *Am J Trop Med Hyg* 1999, 60(3);357-363.

12. Soares IS, Oliveira SG, Souza JM, Rodrigues MM: Antibody response to the N and C-terminal regions of the Plasmodium vivax Merozoite Surface Protein 1 in individuals living in an area of exclusive transmission of P. vivax malaria in the north of Brazil. *Acta Trop* 1999, 72(1):13-24.

13. Soares IS, Levitus G, Souza JM, Del Portillo HA, Rodrigues MM: Acquired immune responses to the N- and C-terminal regions of Plasmodium vivax merozoite surface protein 1 in individuals exposed to malaria. *Infect Immun* 1997, 65(5):1606-1614.

14. Jangpatarapongsa K, Sirichaisinthop J, Sattabongkot J, Cui L, Montgomery SM, Looareesuwan S, Troye-Blomberg M, Udomsangpetch R: **Memory T cells protect against Plasmodium vivax infection**. *Microbes Infect* 2006, **8**(3):680-686.

15. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S: **Antigen presentation and T cell stimulation by dendritic cells**. *Annu Rev Immunol* 2002, **20**:621-667.

16. McGregor IA: **The Passive Transfer of Human Malarial Immunity**. *Am J Trop Med*

Hyg 1964, **13**:SUPPL 237-239.

17. Shi YP, Udhayakumar V, Oloo AJ, Nahlen BL, Lal AA: Differential effect and interaction of monocytes, hyperimmune sera, and immunoglobulin G on the growth of asexual stage Plasmodium falciparum parasites. *Am J Trop Med Hyg* 1999, 60(1):135-141.

18. Pinto AY, Ventura AM, Souza JM: [IgG antibody response against Plasmodium vivax in children exposed to malaria, before and after specific treatment]. *J Pediatr (Rio J)* 2001, 77(4):299-306.

19. Morais CG, Soares IS, Carvalho LH, Fontes CJ, Krettli AU, Braga EM: IgG isotype to C-terminal 19 kDa of Plasmodium vivax merozoite surface protein 1 among subjects with different levels of exposure to malaria in Brazil. *Parasitol Res* 2005, 95(6):420-426.

List of Tables

Table 1 Information and Clinical data of *P.vivax* patients, immune and naïve controls [a]

	No.	Sex		Age	Hct	Temperature	Parasitemia
		M	F		(%)	(°C)	(%)
Chinese Naïve controls							
Immune controls							
Acute *P. vivax*							
Thai Naïve controls	27	15	12	32 ± 12 (15 - 70)	41 ± 3 (37 - 48)	37.5 ± 0.5	0
Immune controls	53	40	13	40 ± 15 (16 - 77)	47 ± 7 (34 - 64)	36.8 ± 0.4 (36 - 37)	0
Acute *P. vivax*	52	32	20	32 ± 12 (15 - 70)	41 ± 6 (30 - 63)	37.8 ± 1.3 (34 - 40)	0.4 ± 0.4 (0.02 - 1.2)

Table 2 the PCR primers

Name	Sequences 5'→ 3' (enzymes)(vectors)
PvMSP1(19) Forward	CG GGATCC aat gtg caa act cag tta tta ac (BamHI) (PET28a)
PvMSP1(19) Reverse	CCG CTC GAG gct gga gga gct aca gaa aac (Xho I)
PvAMA Forward	GGAATTC CAT ATG acc gtt gag aga agc aca cg (Nde I) (PET22b)
PvAMA Reverse	CCG CTC GAG tag tag cat ctg ctt gtt cg (XhoI)

Figure legends

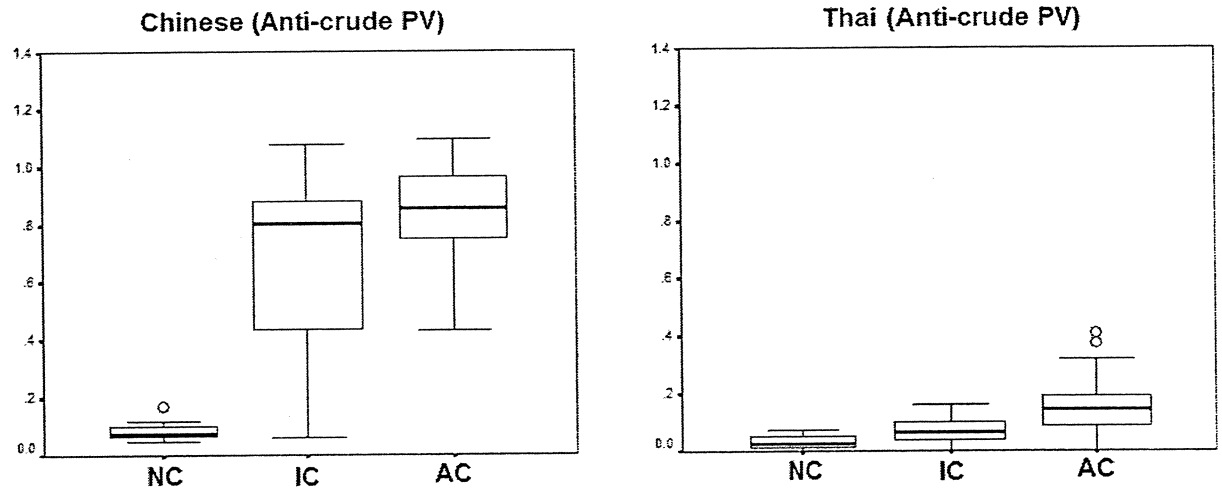

Fig. 1 Absorbance (405 nm) value of IgG antibody reacting with crude *P. vivax* antigens in the naïve controls (NC), immune controls (IC), acute *P. vivax* infection (AC) comparing between Chinese and Thai. Data are shown in median, interquartile ranges (box plots), maximum and minimum (upper-lower lines).

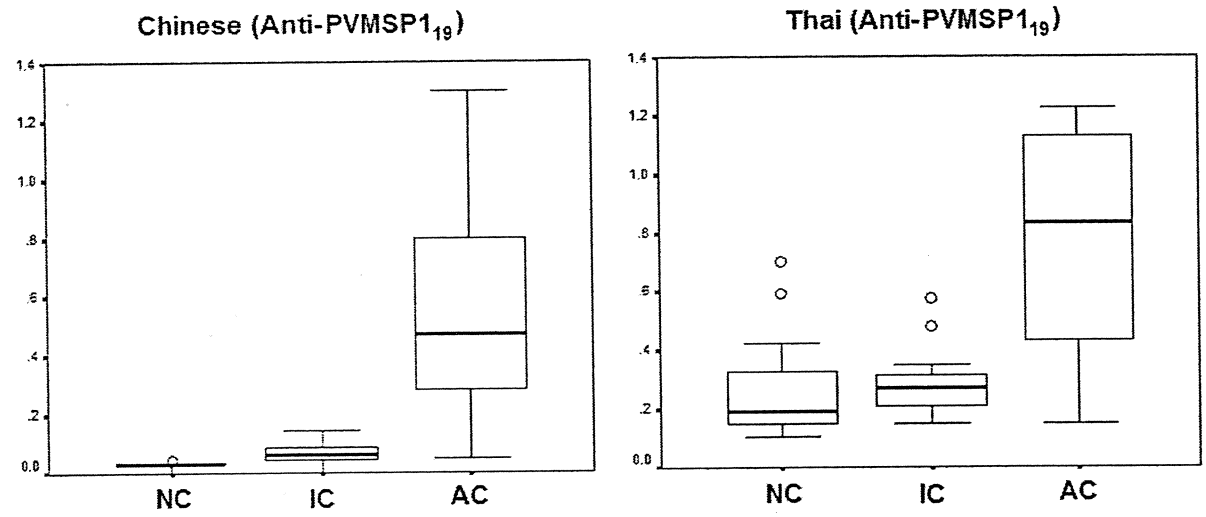

Fig. 2 Absorbance (405 nm) value of IgG antibody reacting with recombinant *P. vivax* MSP-1_{19} protein in the naïve controls (NC), immune controls (IC), acute *P. vivax* infection (AC) comparing between Chinese and Thai. Data are shown in median, interquartile ranges (box plots), maximum and minimum (upper-lower lines).

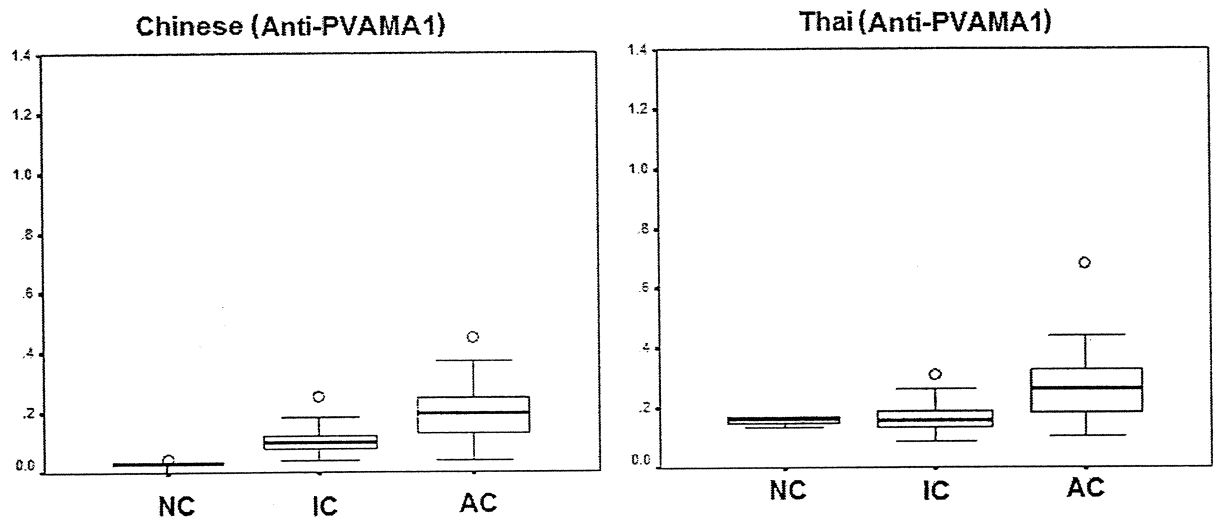

Fig. 3 Absorbance (405 nm) value of IgG antibody reacting with recombinant *P. vivax* AMA-1 protein in the naïve controls (NC), immune controls (IC), acute *P. vivax* infection (AC) comparing between Chinese and Thai. Data are shown in median, interquartile ranges (box plots), maximum and minimum (upper-lower lines).

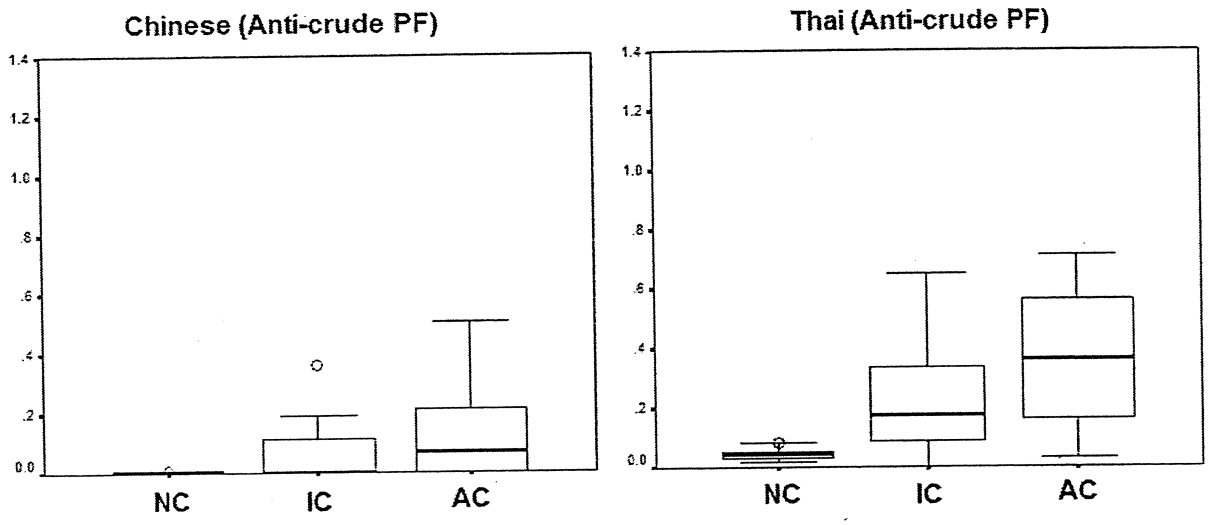

Fig. 4 Absorbance (405 nm) value of IgG antibody reacting with crude *P. falciparum* antigen in the naïve controls (NC), immune controls (IC), acute *P. vivax* infection (AC) comparing between Chinese and Thai. Data are shown in median, interquartile ranges (box plots), maximum and minimum (upper-lower lines).

Killing mechanism of *Plasmodium vivax* parasites by γδ T cells

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Introduction

In Thailand, major malaria endemic areas are located along the Thai-Myanmar and Thai-Cambodian borders. Malaria prevalence in these areas is increased indicating the potential risk of drug resistance. In certain area of Srakaew, *P. vivax* infection is >90% of the infected cases. In most cases, villagers have immunity to the parasites, which may help reducing disease severity. However, this does not prevent subsequent infections. One of the major interests is that how host responds in order to eliminate the parasites, and how possible can the immune response protect host from the infection and severe manifestations. Measuring level of the antibody to *P. vivax* parasite among indigenous villagers of endemic area will be a good parameter for evaluation of the baseline immunity. Cell-mediated immune response, having specificity to a particular antigen of *P. vivax* parasite, elicited during the repeated episodes of infection will determine how efficient the patients can protect themselves from the disease.

T cell activation by malaria parasites plays an important role in elicitation of protective immunity against the disease. Various T cell populations categorized by a number of phenotypic markers are responsible for different functions. Function of various mononuclear leukocytes determines effectiveness of the cell-mediated response to malaria. Depending on the antigen presentation and stimulation of the specific parasite proteins, particular T cell phenotypes will expand. As shown earlier that expansion of gamma/delta T cells play roles in innate immunity to malaria infection (Stevenson and Riley 2004). Our previous study has shown a similar increase of gamma/delta T cells in acute and convalescent *P. vivax* infection (Jangpatarapongsa et al. 2006). However, function of these T cells is not verified, whether they are for or against the *P. vivax* parasite. Antigen-specific T cells recruited during *P. vivax* infection can either eliminate or promote the parasites. There is an immune suppression caused by the presence of *P. falciparum*

parasite during acute infection (Hisaeda et al. 2005). However, there is limited information about how patients primarily respond to acute *P.vivax* infections, and much less understanding about how immune surveillance would be in a carrier having hypnozoites in the liver.

Antigen recognition by human T cells is mediated through two types of T cell receptor (TCR) consisting of alpha/beta and gamma/delta chain which are associated with CD3 surface molecules. The vast majority of lymphocytes in the peripheral blood (>90%) express the alpha/beta TCR whereas about 6-8% bear the gamma/delta TCR. gamma/delta T produce various cytokines and act as MHC restricted and non-restricted cytotoxic effector cells (Haas et al. 1993). gamma/delta T cells are elevated in acute or convalescent *P. falciparum* infection (Bordessoule et al. 1990; Ho et al. 1990; Roussilhon et al. 1990; Ho et al. 1994). An increase of gamma/delta T cells is in non-immune *P. vivax* patients during paroxysms (Perera et al. 1994), and gamma/delta-memory T cells in acute and convalescent period (Jangpatarapongsa et al. 2006). Activation of gamma/delta T cells but not alpha/beta T cells from malaria naïve donors inhibits blood stage parasite replication (Elloso et al. 1994; Troye-Blomberg et al. 1999) and induces production of pro-inflammatory cytokines (Troye-Blomberg et al. 1999). Recent study has shown that gd T cells can inhibit the growth of *P. falciparum* in vitro by cytolytic pathways suggesting that these cells have protective function involving both cytotoxic and regulatory mechanisms against malaria infection. However, the direct killing mechanism of these cells during *P. vivax* infection has not yet been investigated. This studies aims to study and compare the killing mechanism of γδ T cells against two major malaria species: *P. falciparum* and *P. vivax.*

Materials and methods

Isolation and activation peripheral blood mononuclear cells (PBMC)

Venous blood from five healthy donors was collected in heparinized tubes and PBMC were separated by gradient centrifugation using Lymphoprep™ (AXIS-Shied PoC AS, Oslo, Norway) according to the manufacturer's recommendations. The PBMC pellet were resuspended in RPMI 1640 supplemented with 10%fetal calf serum (FCS), and then plated in 24-well plates at a concentration of 1×10^6/ml together with 30 mg/ml Isopentenylpyrophosphate (IPP) for 2 weeks with addition of 20 U/ml rhIL-2 every 3 days exclusion. At day 14, cells were washed twice in TCM, counted, phenotyped by flow cytometry and used as effector cells in the parasite reinvasion/growth inhibition assays. PBMC at the same amount of gamma delta were used to compared. % growth inhibition of the parasite was calculated according to the following equation (Farouk et al. 2004): %parasite growth inhibition = (% parasitemia in control – % parasitemia in test) / (% parasitemia in control) ×100.

Parasite cultures

P. falciparum (AMB47) and *P. vivax* infected blood collected from endemic area wa used for intact parasites in the studies. *P. vivax* infected blood was depleted of white blood cells by filtering through a sterile column of CF11 cellulose (Whatman®, Maidstone, UK) and the red blood cells were washed with RPMI-1640 by centrifugation at 1190 g for 5 minutes. Both parasites were adjusted to be 2% parasitemia, 5% Hematocrit (Hct) before co-culturing with PBMC or γδ T cells. Co-cultured parasites were maintained in an incubator containing 5% CO2, 5% O_2 and 90% N_2. Uninfected RBC was processed similarly as above was used as a base line.

***In vitro* stimulation of PBMC**

PBMC ($1x10^5$, $1x10^6$ and 2 x 10^6 cells/well) in RPMI-1640 supplemented with 25 mM HEPES, 1.8 mg/ml D-glucose, 2 mM glutamine, 40 mg/ml of gentamicin and 10% heat-inactivated FCS were cultured for 3 days at 37°C in a humidified chamber with 5% CO_2, 5% O_2 and 90% N_2 in the presence of Intact *P. falciparum* or *P. vivax* parasites at a 2% parasitemia and 5% Hct. Medium alone, $1x10^6$ cells/well of NRBC extracts or were used as negative control and base line level, respectively. All experiments were performed in duplicates. After activation, the cells were harvested and stained for the gamma deta T cells and interested markers.

Intracellular staining and flow cytometric (FCM) analysis

For surface marker by the three-color FCM analysis, harvested cells were stained with a combination of fluorochrome-conjugated monoclonal antibodies (mAbs): RPE-Cy5-labeled anti-CD3 (Caltag, Burlingame, USA) and RPE-anti-gamma9 (Immunotech, Marseille, France) and FITC-labeled anti-CD69 or FITC-labeled anti-CD25 for 30 min at 4°C and washed with phosphate-buffered saline (PBS). Cells were fixed with 1% paraformaldehyde and data acquisition and analysis on FACSCalibur using the CELLQUEST software (Becton Dickinson, San Jose, USA).

For intracellular, after staining with RPE-Cy5-labeled anti-CD3 (Caltag, Burlingame, USA) and RPE-anti-gamma9 (Immunotech, Marseille, France), the cells were fixed with 1% paraformaldehyde and washed with a permeabilizing solution. After incubation in the permeabilizing buffer for 20 min at room temperature, the cells were incubated with FITC-labeled anti-CD107a and then washed with PBS.

Results

Growth inhibition of *P. falciparum* parasites by γδ T cells

The median percentage of parasites growth inhibition was increased everyday when intact *P. falciparum* parasites were co-cultured with PBMC (Fig. 1A) and gamma delta T cells (Fig. 1B). the % growth inhibition was dose dependence manner. At day 2, we found the higher of growth inhibition in $1x10^6$ cells/well of gamma delta T cells than PBMC. However, the growth inhibition was not difference if we put more amounts of cells co-culturing with parasites.

Activation of γδ T cells by *P. falciparum* parasites

The median level of $CD3^+gamma9^+$ T cells in PBMC was decreased increased at day 2 stimulation (Fig 2A). However, gamma delta T cells were maintained and increased after day 2 stimulation with intact *P. falciparum* parasites (Fig. 2B). Moreover, the highest level of $gamma9^+$ T cells was intact parasites co-culturing with 10^5 cells/well. The high amount of cells resulted in decreasing of gamma9 T cells activation. The similarly results were shown in both PBMC and enriched gamma9 T cells. Therefore, the activation of gamma9 T cells was dose dependent manner.

Early activation of γδ T cells by *P. falciparum* parasites

Normally, $CD69^+$ molecule was expressed at the early stage of activation. The gamma9 T cells activation level from Fig. 1 was also confirmed the cells activation status. In concordance with gamma9 T cells activation, we found the early activation marker ($gamma9^+CD69^+$ T cells)

were expressed at day 2 in PBMC (Fig 3A), and day 1 in gamma delta T cells after activation with intact *P. falciparum* parasites (Fig. 3B). Moreover, the early activated marker was increased when amount of gamma delta T cells was increased (Fig 3B).

Activation of lysosomal-associated membrane protein-1 (LAMP-1) intracellularly expression in γδ T cells by *P. falciparum* parasites

Lysosome associated membrane protein-1 (LAMP-1 or CD107a) are major protein components of the lysosomal membrane. Protein components of the lysosomal membrane also mediate a number of essential functions of this compartment, including the acidification of the lysosomal lumen, transport of amino acids, fatty acids, and carbohydrates resulting from the hydrolytic degradation (Eskelinen 2006). In addition, lysosomal membrane proteins may be involved in the interaction and fusion of the lysosomes with themselves as well as with other cell components, including endosomes, phagosomes, and the plasma membrane (Fukuda 1991).

In this study, we determined the cytolytic activities by quantification of the levels of gamma9^{+}CD107a^{+} T cells expressed intracellular of gamma delta T cells (Fig. 4). In concordance with the activated marker (CD69^{+}), the median percentage of gamma9^{+}CD107a^{+} T cells was increased in gamma delta T cells co-culturing with intact *P. falciparum* parasites which was higher than was found in PBMC. Interestingly, the expression of CD107a+ was highest in lower amount of cells (10^{5} cells/well).

Gamma/delta T cells in acute *P. vivax* infected patients

Gamma/delta T cells are activated during acute *P. vivax* infection and the high level is maintained after treatment (Fig.5). We hypothesize that the gamma/delta T cells can eliminate the parasites and therefore control parasitemia to subside the disease severity.

Activation of γδ T cells by *P. vivax* parasites

At the first donors, the percentage of $CD3^+gamma9^+$ T cells expressing on lymphocytes was increased after PBMC co-culturing with intact *P. vivax* parasites (Fig. 6). However, the level of $CD3^+gamma9^+$ T cells was increased after enriched gamma delta T cells was co-cultured with intact P. vivax parasites at day 2 and maintained at day 3. We also found the increasing of $CD3^+gamma9^+$ T cells was increased at high amount of cells, suggesting that this level was dose dependence.

Early and late activation of γδ T cells by *P. vivax* parasites

The gamma9 T cells activation was also confirmed the cells activation status. In similar to what we found in *P. falciparum*, $CD3^+gamma9^+$ T cells of the PBMC co-culturing with intact *P. vivax* parasites also expressed $CD69^+$ at early stage (day 1) (Fig. 7A). Moreover, we found the early activation marker ($gamma9^+CD69^+$ T cells) was expressed at day 1 in gamma delta T cells after activation with intact *P. vivax* parasites (Fig. 7B). Moreover, the early activated marker was increased when amount of gamma delta T cells was increased (Fig 7A,B).

In contrast to early stage activation, the $CD25^+$ expressing on $CD3^+gamma9^+$ T cells was expressed at day 3 activation in PBMC co-culturing with intact *P. vivax* parasites (Fig. 7C).

However, the level of $CD3^{+}gamma9^{+}CD25^{+}$ T cells in gamma delta T cells co-culturing with *P. vivax* parasites was expressed at the first day stimulation (Fig. 7D). The activation was also dose dependence manner.

Activation of lysosomal-associated membrane protein-1 (LAMP-1) intracellularly expression in γδ T cells by *P. vivax* parasites

In comparison with what we found in *P. falciparum*, we also determined the cytolytic activities expressing of $CD107a^{+}$ intracellularly of gamma delta T cells in PBMC or gamma delta T cells co-culturing with intact P. vivax parasites (Fig. 8). The level of $CD3^{+}gamma9^{+}CD107a^{+}$ T cells was increased at day 0 and rapidly decreased at day later (Fig. 8A). In contrast to what we found in PBMC, the level of $gamma9^{+}CD107a^{+}$ T cells was increased in gamma delta T cells co-culturing with intact *P. vivax* parasites at day 2 and rapidly decreased at day later.

Acknowledgements

We thank all staff at the Mae Sot and Mae Kasa Malaria Clinics, the Department of Entomology, AFRIMS, Bangkok, and the Malaria Training Center in Saraburi, Thailand for collection of the samples. This work was partly supported by the Thailand Research Fund (BRG498009). KJ was a research fellow supported by The Commission on Higher Education of Thailand and the Fogarty International Center (FIC), National Institutes of Health (NIH) and by a grant (D43-TW006571) to LC and RU from FIC, NIH.

References

Bordessoule, D., P. Gaulard, et al. (1990). "Preferential localisation of human lymphocytes bearing gamma delta T cell receptors to the red pulp of the spleen." J Clin Pathol **43**(6): 461-4.

Elloso, M. M., H. C. van der Heyde, et al. (1994). "Inhibition of Plasmodium falciparum in vitro by human gamma delta T cells." J Immunol **153**(3): 1187-94.

Eskelinen, E. L. (2006). "Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy." Mol Aspects Med **27**(5-6): 495-502.

Farouk, S. E., L. Mincheva-Nilsson, et al. (2004). "Gamma delta T cells inhibit in vitro growth of the asexual blood stages of Plasmodium falciparum by a granule exocytosis-dependent cytotoxic pathway that requires granulysin." Eur J Immunol **34**(8): 2248-56.

Fukuda, M. (1991). "Lysosomal membrane glycoproteins. Structure, biosynthesis, and intracellular trafficking." J Biol Chem **266**(32): 21327-30.

Haas, W., P. Pereira, et al. (1993). "Gamma/delta cells." Annu Rev Immunol **11**: 637-85.

Hisaeda, H., K. Yasutomo, et al. (2005). "Malaria: immune evasion by parasites." Int J Biochem Cell Biol **37**(4): 700-6.

Ho, M., P. Tongtawe, et al. (1994). "Polyclonal expansion of peripheral gamma delta T cells in human Plasmodium falciparum malaria." Infect Immun **62**(3): 855-62.

Ho, M., H. K. Webster, et al. (1990). "Increased gamma delta T cells in acute Plasmodium falciparum malaria." Immunol Lett **25**(1-3): 139-41.

Jangpatarapongsa, K., J. Sirichaisinthop, et al. (2006). "Memory T cells protect against Plasmodium vivax infection." Microbes Infect **8**(3): 680-6.

Perera, M. K., R. Carter, et al. (1994). "Transient increase in circulating gamma/delta T cells during Plasmodium vivax malarial paroxysms." J Exp Med **179**(1): 311-5.

Roussilhon, C., M. Agrapart, et al. (1990). "T lymphocytes bearing the gamma delta T cell receptor in patients with acute Plasmodium falciparum malaria." J Infect Dis **162**(1): 283-5.

Stevenson, M. M. and E. M. Riley (2004). "Innate immunity to malaria." Nat Rev Immunol **4**(3): 169-80.

Troye-Blomberg, M., S. Worku, et al. (1999). "Human gamma delta T cells that inhibit the in vitro growth of the asexual blood stages of the Plasmodium falciparum parasite express cytolytic and proinflammatory molecules." Scand J Immunol **50**(6): 642-50.

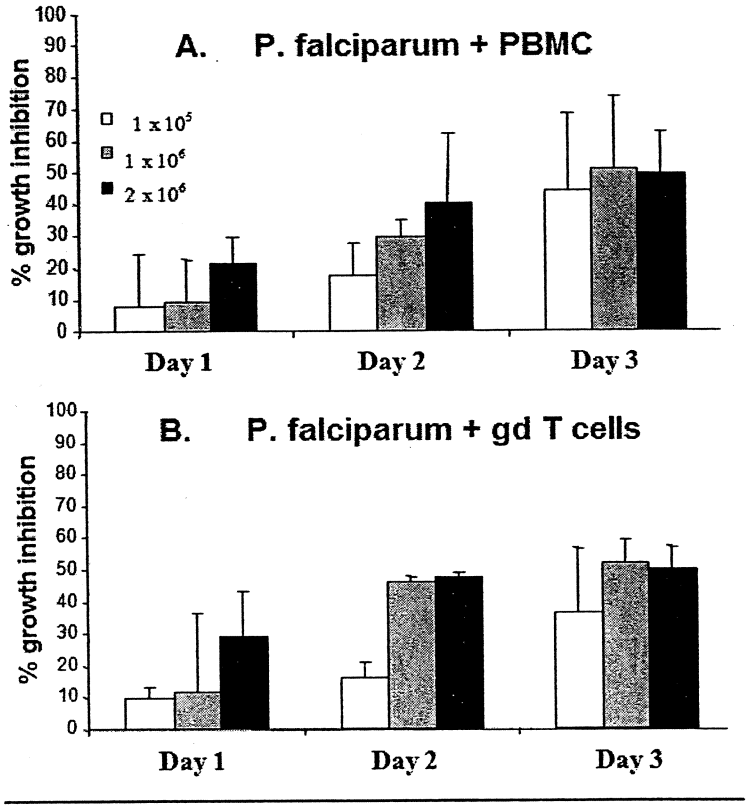

Fig. 1. % *P. falciparum* parasites growth inhibition after co-culturing with PBMC (A) or γδ T cells (B) at different cell number from $1x10^5$, $1x10^6$ and $2x10^6$ cells/well. parasitemia was counted everyday from day 0 until day 3. Data represent median percentage (blocks) ± SD (vertical bars) from five experiments.

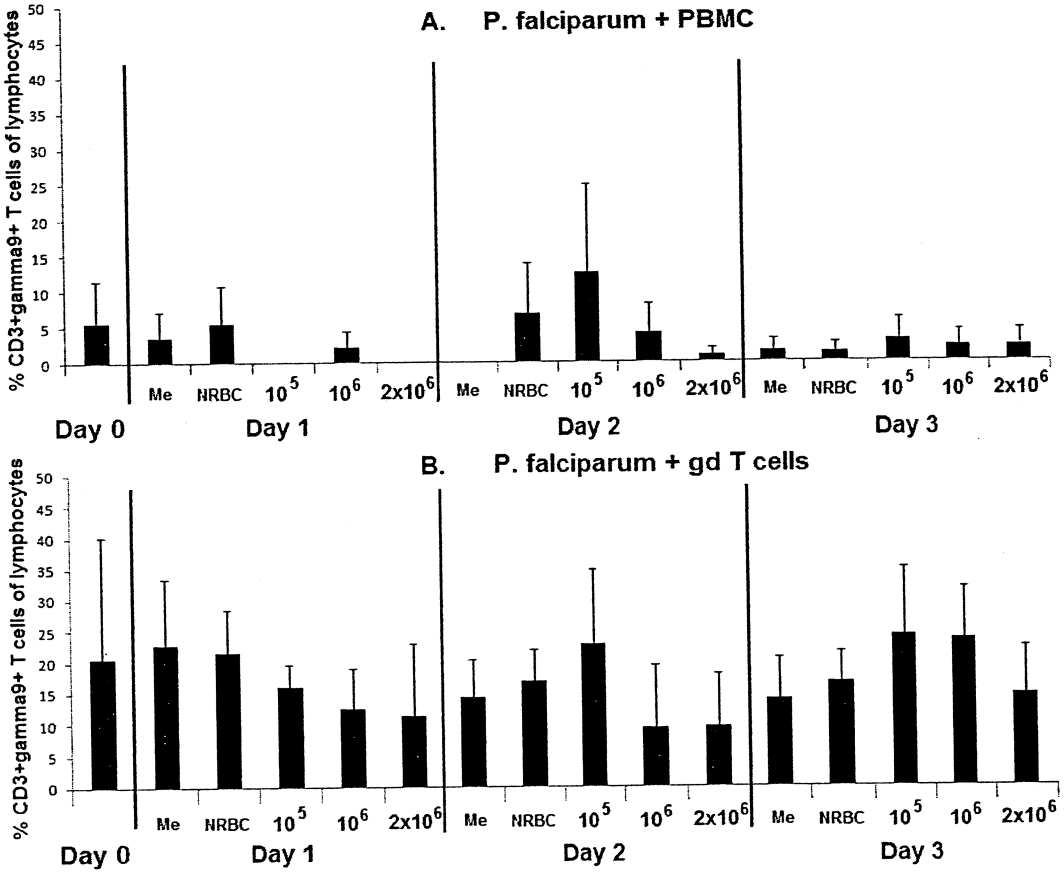

Fig. 2 % $CD3^{+}gamma9^{+}$ T cells of lymphocytes shown by flow cytometry. *P. falciparum* at 2% parasitemia, 5% Hct was co-culturing with **PBMC (A)** or **γδ T cells (B)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks) ± SD (vertical bars) from five experiments.

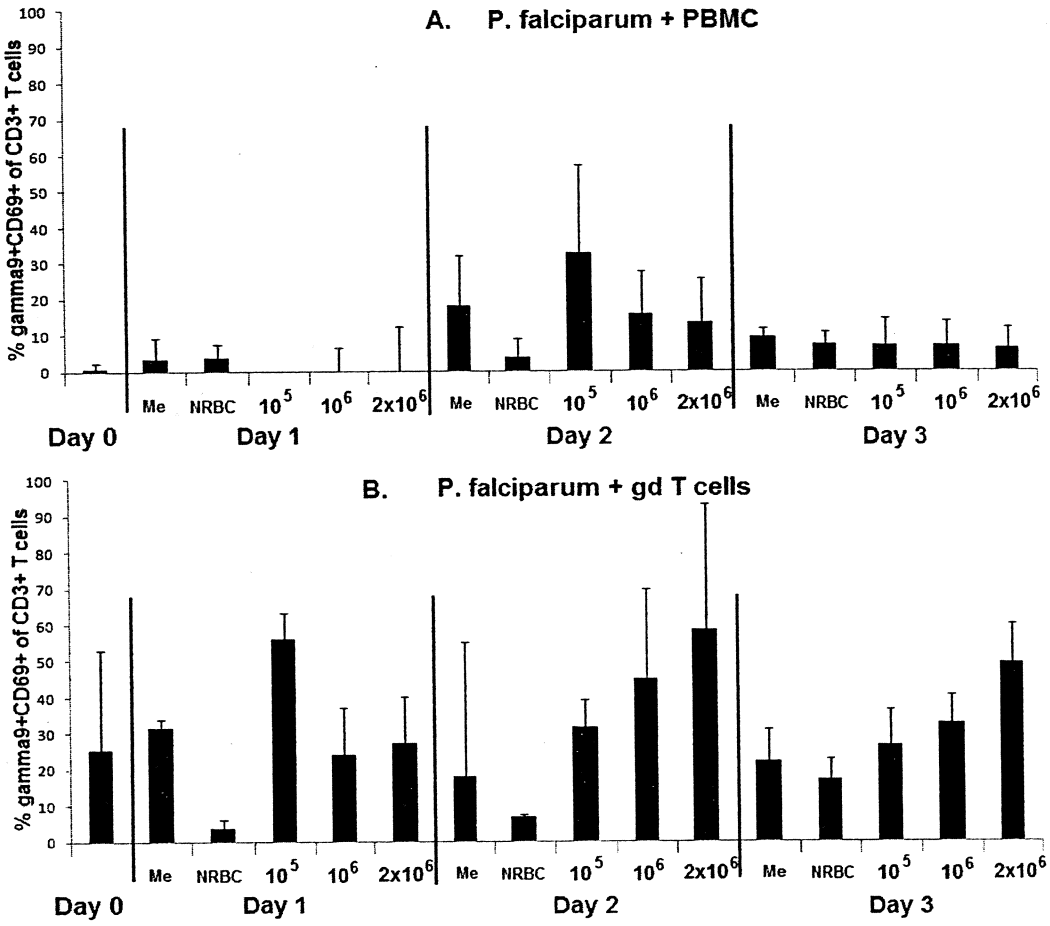

Fig. 3 % gamma9$^+$CD69$^+$ T cells of CD3$^+$ shown by flow cytometry. *P. falciparum* at 2% parasitemia, 5% Hct was co-culturing with **PBMC (A)** or **γδ T cells (B)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks) ± SD (vertical bars) from five experiments.

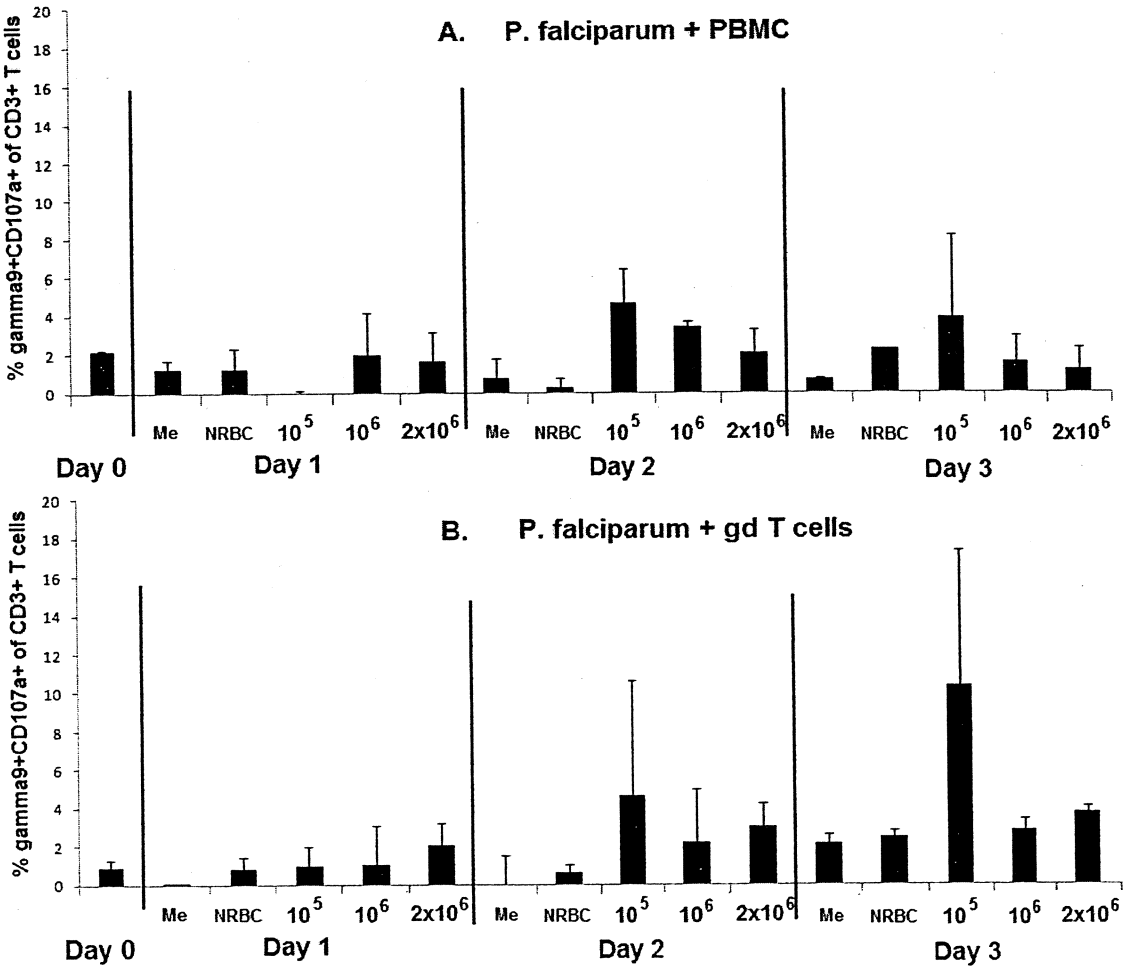

Fig. 4 % gamma9$^+$CD107a$^+$ T cells of CD3$^+$ shown by flow cytometry. *P. falciparum* at 2% parasitemia, 5% Hct was co-culturing with **PBMC (A)** or □□ **T cells (B)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks) ± SD (vertical bars) from five experiments.

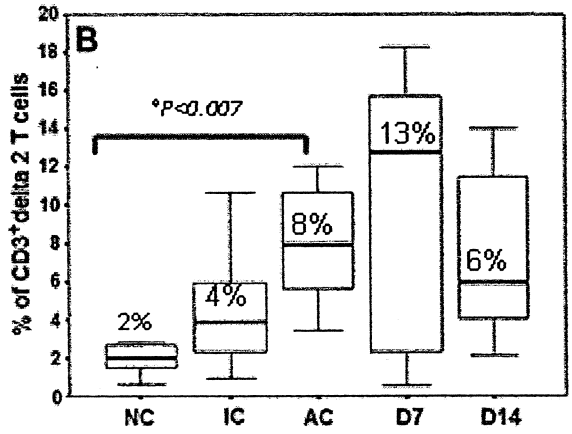

Fig. 5 Gamma/delta T cells shown by flow cytometry. Naïve controls (NC)(N=27), malaria-exposed donors (IC)(N=25), acute *P. vivax* infection (AC)(N=17), the day 7 (D7), and day 14 (D14) after treatment. Median, interquartile ranges (box plots), maximum and minimum (upper-lower lines) are shown.

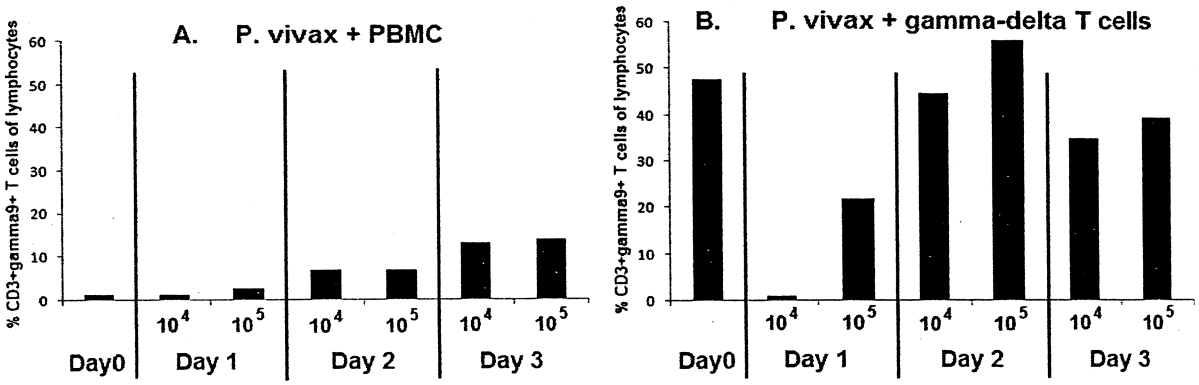

Fig. 6 % $CD3^+$ $gamma9^+$ T cells of lymphocytes shown by flow cytometry. Intact *P. vivax* parasites at 2% parasitemia, 5% Hct were co-culturing with **PBMC (A)** or **γδ T cells (B)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks) ± SD (vertical bars) from five experiments.

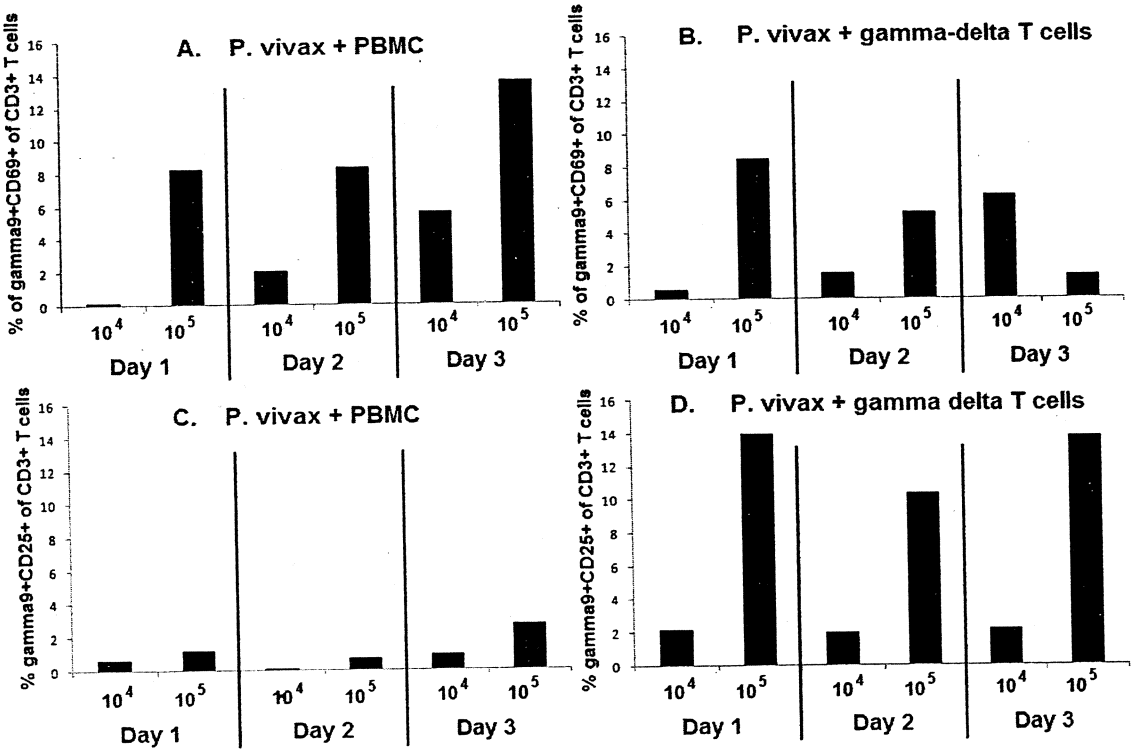

Fig. 7 % gamma9$^+$CD69+ (A,B) and **CD25$^+$ T cells (C,D)** of CD3$^+$ shown by flow cytometry. Intact *P. vivax* parasites at 2% parasitemia, 5% Hct were co-culturing with **PBMC (A,C)** or **γδ T cells (B,D)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks)

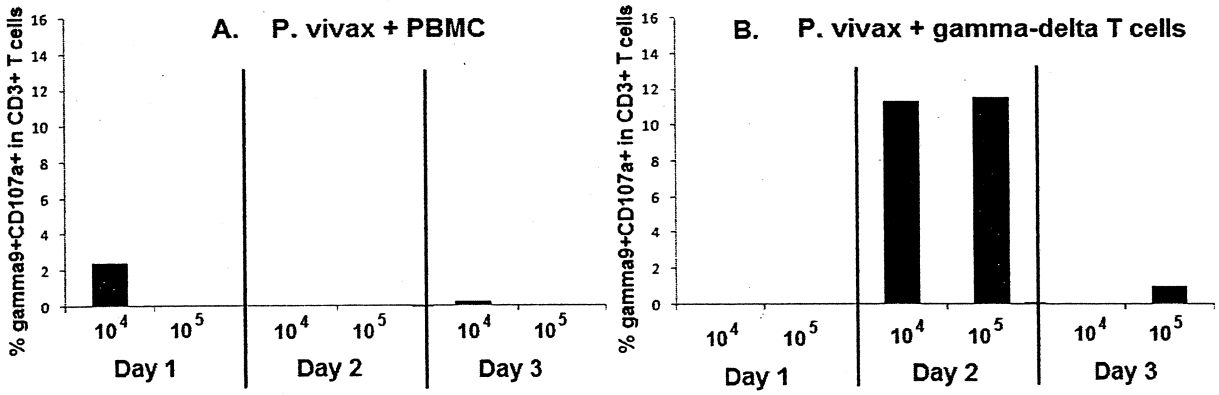

Fig. 8 % gamma9$^+$CD107a$^+$ T cells of CD3$^+$ shown by flow cytometry. Intact *P. vivax* parasites at 2% parasitemia, 5% Hct were co-culturing with **PBMC (A)** or **γδ T cells (B)** at different cell number 10^5, 10^6 and $2x10^6$ cells per well. Medium alone (Me) was used as negative controls and normal red blood cells (NRBC) was used as base lines levels. Data represent median percentage (blocks)

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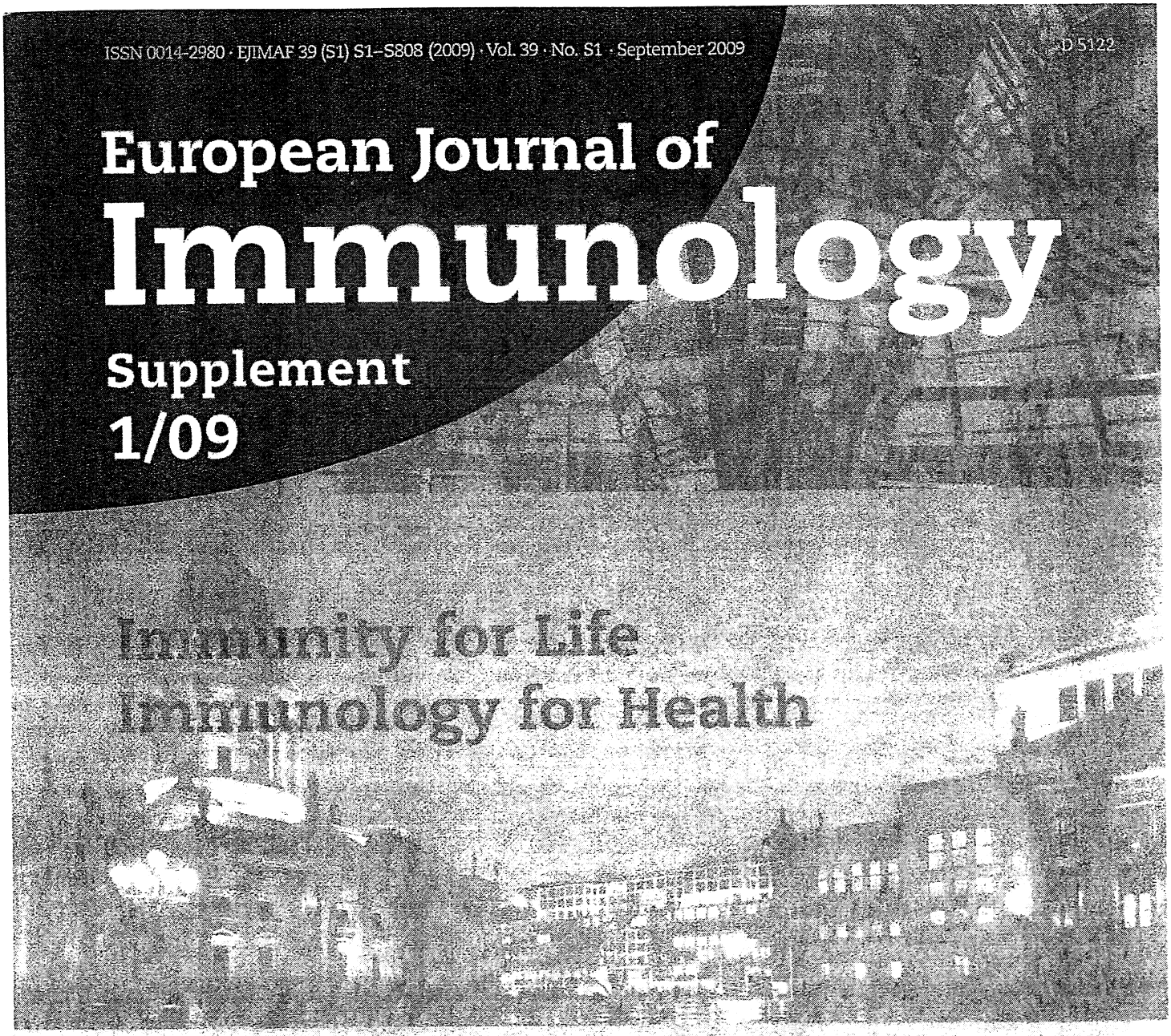

Abstracts

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PA13/60 KILLING MECHANISM OF PLASMODIUM VIVAX PARASITES BY GAMMA-DELTA T CELLS

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Objectives & methods: An increasing of gamma-delta T cells during acute *P. vivax* infection and convalescent period has been reported. Moreover, the activ of gamma-delta T cells leads to the inhibition of blood stage *P. falciparum* parasites *in vitro*. To determine the killing mechanisms of *P. vivax* parasites by ga delta T cells comparing with what has been found in *P. falciparum*, the gamma-delta T cells were enriched by Isopentenylpyrophosphate (IPP) from naïve P Different number of gamma-delta T cells and normal PBMC were incubated with intact of *P. vivax* parasites and protein extract of *P. vivax* parasites, recomb $PvMSP1_{19}$ and PvAMA1 proteins. Gamma-delta T cells was daily determined the cytokine and granzyme intracellular releasing by Flow cytometry until day turing.
Results: Among the enriched gamma-delta T cells, the percentage of cells expressing $CD69^+$ and $CD25^+$ was elevated after co-culturing with intact and th teins of *P. vivax* parasites. The overall gamma-delta T cells showed proliferation at day 3 after the co-cultivation. Moreover, the gamma-delta T cells expressing gamma$^+$ and $CD107a^+$ (lysosomal associated membrane proteins: LAMP-1) elevated from the first day of PBMC collection after co-culturing with the intac *P. vivax* antigens. This level was correlated with the significantly decreasing number of parasites and the increasing percentage of parasite growth inhibition
Conclusion: Our results showed the activation of gamma-delta T cells during *P. vivax* infection *in vitro*. This suggests that gamma-delta T cells could be stimul by *P. vivax* parasites and these actively activated gamma-delta T cells could kill the parasites via mechanism of granzyme and cytokines at the early stage of cell vation. This study provides more understanding in activation of the innate immunity during acute malaria infection which may lead to the selection of appro malaria proteins as vaccine candidates in the future.

PA13/61 ANALYSIS OF INVARIANT NKT CELLS OF PATIENTS WITH ATOPIC DERMATITIS BY FLOW CYTOMETRY

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Objectives: Several evidence suggest that invariant NKT cells (iNKT) connect innate and acquired immune system. They are able to produce both Th1 and cytokines after stimulation. Atopic dermatitis (AD) is a chronic inflammatory skin disease. Th1-like and Th2-like cytokines have been implicated in the pathoj sis of AD, but there are controversial data on their role in AD.
Methods: The frequency and absolute number of iNKT cells in mononuclear cells (PBMCs) of peripheral blood of patients with atopic dermatitis (AD) (n=43) healthy controls (n=13) were determined by flow cytometry using anti-CD3 and monoclonal antibody specific for the CDR3 loop of the invariant TCR α cha iNKT cells (clone:6B11). Furthermore, after PMA/ionomycin stimulation for 4 hours, intracellular IFNγ and IL-4 cytokines were detected in CD4+CD8- , CD4- (DN), CD4-CD8+ and CD4+CD8+ subsets of iNKT cells by five colour flow cytometry in patients with AD (n=10) and healthy controls (n=10).
Results: Both frequency and absolute number of iNKT cells were significantly lower in patients with AD ($P< 0.01$) compared to healthy controls. The frequ of DN subpopulation was significantly lower in AD patient ($P< 0.01$). There was a positive correlation between the frequency of DN cells and iNKT cells bo AD patients ($R=0.726$ and $P< 0.001$) and healthy controls ($R=0.693$ and $P< 0.001$). In the intracellular IFNγ level there were no significant difference in a the iNKT subsets of AD patients, however the intracellular IL-4 level was significantly higher in DN subpopulation of iNKT cells of AD patients compared to he controls ($P< 0.05$).
Conclusion: The frequency, the number of iNKT cells and the cytokine producing capacity of the CD4/CD8 iNKT subsets are different in peripheral blood obta from AD patients compared to healthy controls.
Our result suggest that the DN iNKT cell subset can serve as a source of IL-4 that promotes the Th2 differentiation in AD patients and might play a role in the pa genesis of this disease.

PA13/62 ISOLATION OF MURINE INTRAHEPATIC IMMUNE CELLS EMPLOYING A MODIFIED PROCEDURE FOR MECHANICAL DISRUPTION AND FUNCTIONAL CHARACTERIZATION OF THE B, T AND NATURAL KILLER T CELLS OBTAINED

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Introduction: Intrahepatic immune cells (IHIC) are known to play central roles in immunological responses mediated by the liver, and isolation and phenot characterization of these cells is therefore of considerable importance.
Aims: In the present investigation, we developed a simple procedure for the mechanical disruption of mouse liver that allows efficient isolation and phenot characterization of IHIC. These cells are compared with the corresponding cells purified from the liver after enzymatic digestion with different concentratio collagenase and DNase.
Results: The mechanical disruption yielded viable IHIC in considerably greater numbers than those obtained following enzymatic digestion. The IHIC isol employing the mechanical disruption were heterogeneous in composition, consisting of both innate and adaptive immune cells, of which B, T, natural killer (I NK T cells, granulocytes and macrophages were the major populations (constituting 37.5%, 16.5%, 12.1%, 7.9%, 7.9% and 7.5% of the total number of cells re ered respectively). The IHIC obtained following enzymatic digestion contained markedly lower numbers of NK T cells (1.8%). The B, T and NK T cells among I isolated employing mechanical disruption were found to be immunocompetent, i.e. they proliferated in vitro in response to their specific stimuli (lipopolysac ride, concanavalin A and alpha-galactosylceramide respectively) and produced immunoglobulin M and interferon-gamma.
Conclusions: Thus, the simple procedure for the mechanical disruption of mouse liver described here results in more efficient isolation of functionally compe IHIC for various types of investigation.

PA13/63 THE FUNCTION AND CHARACTERISTICS OF SUPERANTIGEN SEB-ACTIVATING $CD8^+$NKT CELLS

Y. Chen[1], Y.L. Guo[1], J. Zhong[2], S.L. Zhang[2]
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Nature Killer T cells (NKT) are a special T cell population with co-expresses NK and T cell surface markers. Murine NKT cells include $CD4^+$ NKT and $CD4^-CD8^-$ cells. $NK1.1^+$ NKT cells may release large amounts of IL-2, IL-4, IFN-γ and IL-10 after they are activated. It has been reported that α-Galactorsykeramide (α-Gal a glycolipid, may induce proliferation of NKT cells with the role of immune regulation by stimulating mouse spleen cells. This study demonstrated that supera gen staphylococcal enterotoxin B (SEB) , a kind of peptide, can activate the NKT cells with the function of immune tolerance. The response ability of SEB-activa effect cells to ConA, LPS and IL-2 had significantly decreased compared with that of normal lymphocytes. The effect cells exerted an inhibitory effect for response of normal lymphocytes to ConA and IL-2. There was a significantly increase in the percent of $CD8^-NK1.1^+$ and $TcRV\beta8^+NK1.1^+$ NKT cells identified fi the SEB-activated cells. Based on the cell distribution detected in the upper part of the FACS picture, expression of CD69 molecule existed in 68.95% of the c from large-scale selection. The percent of $CD8^+NK1.1^+$ and $TcRV\beta8^-NK1.1^+$ NKT cell subsets in the giant lymphocytes were enhanced to 84.0 and 38.9 fo respectively. Under a light microscope at x400 magnification, the SEB-activating lymphocytes in size were larger than not only the ConA-activated cells but also adherent macrophages with an increase of 5 fold observed under a microscope. There were a few granules seen in cytoplasm. The value of cytoplasm vs nuclei less than 1.0 and they are non-adherent cells. The differentiation pathway of the SEB-activating $CD8^+$ and $TcRV\beta8^-$NKT cells was not relative to a NK source. T were produced directly from T cell population and were considered as a subsets of T lymphocytes. Our results suggest that the superantigen SEB can act on $CD8^+$NKT cell and $TcRV\beta8^+$NKT cells. And the two NKT cell subsets may play a critical role in SEB mediated tolerance.

PA13/64 INTESTINAL INTRAEPITHELIAL γδ T CELL SHAPE THEIR CELLULAR ENVIRONMENT THROUGH TCR-DEPENDENT PRODUCTION OF CHEMOKINES AND CYTOKINES

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γδ T cells in the intestinal intraepithelial compartment (γδ iIEL) show an intrinsic activated phenotype. We hypothesised that their T cell receptor γδ (TCRγδ implicated in the activation of γδ iIEL. Because the TCR γδ ligands in mice are not well described, monoclonal antibodies (mAb) directed against the γδ TCR the clone GL3 which binds the δ subunit of TCR γδ, are important tools to specifically activate γδ T cells. Using cytometric Indo-1AM measurement, we could de calcium flux of intestinal and peripheral γδ T cells from TCRd-H2BeGFP reporter mice. Stimulation with anti-γδ clone GL3 or anti-CD3 clone 2C11 elicited ac tion of γδ T cells suggesting that TCR γδ and CD3 molecules in γδ T cells are functional and signalling competent.

sion was found in the B-cell zone after low-dose injection only. A much stronger T-cell proliferation was induced after high-dose injection of SRBC. This newly formed T cells did not respond to the second encounter with SRBC in the footpad. This unresponsiveness remained even after transferring the T cells into a naïve host and challenging them again with SRBC.
Conclusion: We conclude that the absent DTH reaction after high-dose injection of SRBC is not due to lower numbers of T cells. We hypothesise that the newly formed T cells are either unable to migrate into the skin or are regulatory T cells which suppress the DTH reaction in the skin.

V55 LIVER SINUSOIDAL ENDOTHELIAL CELLS INDUCE ANTI-INFLAMMATORY CD4$^+$ T CELLS SUPPRESSING MURINE AUTOIMMUNE HEPATITIS

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Objectives: Cellular mechanisms that maintain the intrahepatic immune balance are crucial in viral or autoimmune liver diseases and for allograft acceptance. For naive CD8$^+$ T cells, liver sinusoidal endothelial cells (LSEC) have been shown to act as non-professional antigen presenting cells and thus induce tolerance by inhibition of cytotoxicity. In this study we investigated consequences of CD4$^+$ T cell priming by LSEC.
Methods: Priming by LSEC was investigated in bone marrow chimeric mice expressing MHC class II exclusively on non-hematopoietic cells. We studied the cytokine expression of LSEC primed CD4$^+$T cells (T_{LSEC}) and determined the stability of their phenotype *in vivo* by adoptive transfer into congenic mice and immunogenic antigen application. Investigating suppressive capacities of T_{LSEC} we performed an *in vitro* suppression assay. The ability of T_{LSEC} to influence proinflammatory reactions *in vivo* was analyzed in a model of T cell-mediated autoimmune hepatitis. Hepatic inflammation was monitored by ALT levels and histologic analyses. The migration pattern of T_{LSEC} was investigated by an *in vivo* homing assay.
Results: We demonstrated that LSEC induce proliferation of naive CD4$^+$ T cells *in vitro*. Although the expression of CD45RB was downregulated in T_{LSEC}, these cells did not produce effector cytokines. This phenotype of T_{LSEC} remained stable *in vivo*. The *in vivo* migration pattern of T_{LSEC} was different from cells activated by professional antigen presenting cells isolated from the spleen, since they showed enhanced homing into lymph nodes and the intestine while they were also present in the liver. Interestingly, T_{LSEC} negative for CD25 and Foxp3, suppressed the proliferation of naive CD4$^+$ T cells *in vitro*. They did neither support a DTH reaction nor a hepatic inflammation and were even able to suppress hepatitis.
Conclusion: Priming of naive CD4$^+$ T cells by LSEC leads to an anti-inflammatory phenotype here referred to as T_{LSEC}. Thus liver sinusoidal endothelia may directly contribute to limiting hepatic inflammation and supporting local tolerance towards exogenous antigens or endogenous self-antigens.

V56 SPECIFIC T-CELL RESPONSES IN LESIONS AND PERIPHERAL BLOOD FROM PATIENTS WITH BURULI ULCER

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[1]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany, [2]University of Munich, Munich, Germany, [3]University Medical Centre Groningen, Groningen, Netherlands, [4]Kumasi Centre for Collaborative Research, Kumasi, Ghana

Buruli ulcer disease (BUD), caused by *Mycobacterium (M.) ulcerans*, is a neglected bacterial infection of the poor in remote rural areas. BUD is a mutilating disease leading to severe disability; it is the third most common mycobacterial infection in immunocompetent people after tuberculosis and leprosy most endemic in West Africa.
There is some evidence that a T helper type 1-mediated immune response is protective against *M. ulcerans* but the role and distribution of antigen-specific T cells in BUD lesions is hardly defined. In addition, analysis and diagnosis of specific T-cell immunity against *M. ulcerans* is hampered by concomitant infection with other atypical mycobacteria, *M. tuberculosis*, and/or *M. bovis BCG* vaccination.
Here we determine the T-cell distribution of two ulcerative lesions and peripheral blood from a BUD patient using a quantitative PCR method for analyses of T-cell receptor Vβ (TCR-BV) chains and compare the antigen-specific T-cell response in peripheral blood of children infected with *M. ulcerans* or other mycobacteria using *in vitro* restimulation with mycobacterial lysates and intracellular cytokine analyses.
TCR-BV chain analyses in two lesions from a BUD patient reveal differential expression of certain chains depending on the distance from the lesion center. Skewed TCR-BV chains are different between two distinct lesions from the same BU patients. This suggests that T cells, which infiltrate the BUD lesions, are oligoclonally expanded but a predominantly infiltrating subtype could not be identified.
Antigen-specific T-cell cytokine analyses of peripheral blood mononuclear cells from patients with BUD (n = 26) and other mycobacterial infections (n = 9) reveal IFN-γ, IL-2, and TNF-α secretion after stimulation with lysates and purified protein derivates from a non-toxic *M. ulcerans* strain, *M. tuberculosis*, and *M. avium*. Despite crossreactivity against different mycobacterial lysates, ratios of specific T cells (percentage of *M. ulcerans*-specific T cells/*M. tuberculosis*- specific T cells in individual donors) were significantly different between the BUD patients and donors with other mycobacterial infections. Therefore concomitant measurement of T-cell cytokine expression after restimulation with different mycobacterial lysates can help to distinguish early infection with *M. ulcerans* from infection with other mycobacteria.

V57 *PLASMODIUM VIVAX* ALTERS IMMUNOSUPPRESSION CAUSED BY *PLASMODIUM FALCIPARUM* INFECTIONS

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Objectives: *Plasmodium falciparum* infection causes transient immunosuppression during parasitemic stage. However, immune response during simultaneous infection with both *P. vivax* and *P. falciparum* has not been investigated. In particular, it is not clear whether host immune response to malaria will be different when compare an infection with single malaria species versus mixed malaria species.
Methods: Human blood mononuclear cells from mixed *P. vivax-P. falciparum* infection were characterized by flow cytometry for the immunomodulatory role of T cells. In addition, antibodies to parasite-derived proteins and to PfMSP-1_{19} and PvMSP-1_{19} recombinant proteins were determined.
Results: We found that CD3$^+$delta 2$^+$-TCR T cells, T-killer cell phenotype, were significantly higher in the acute-mixed *P. vivax-P. falciparum* infection compared with either single *P. vivax* or *P. falciparum* infection. Interestingly, mixed malaria-infection had the highest antibodies against both *P. vivax* and *P. falciparum* compared with those antibodies obtained from the single malaria infection.
Conclusion: This suggests that co-infection with *P. vivax* could induce effector T-killer cells. In addition, antimalarial antibodies found in the mixed infection could have protective role against disease severity in *P. falciparum* infection as shown by the lower parasitemia in the mixed infection group. These findings imply that *P. vivax* may help resolving the severity of *P. falciparum* infection.

V58 PHENOTYPIC CHARACTERIZATION OF PERIPHERAL BLOOD MEMORY CD8+ T CELL SUBSETS, WITHIN DIFFERENT GROUPS OF PATIENTS SUFFERING AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) IN THE NORTHWEST OF ARGENTINA. FIRST REGIONAL REPORT

C.M. Parodi[1], A. Barrio[1], M.F. García Bustos[1], M.C. Mora[1], F. Ramos[1], J. Beckar[2], S. Monroig[2], B. Ruibal-Ares[3], M.M. E de Bracco[3], M.A. Basombrío[1]
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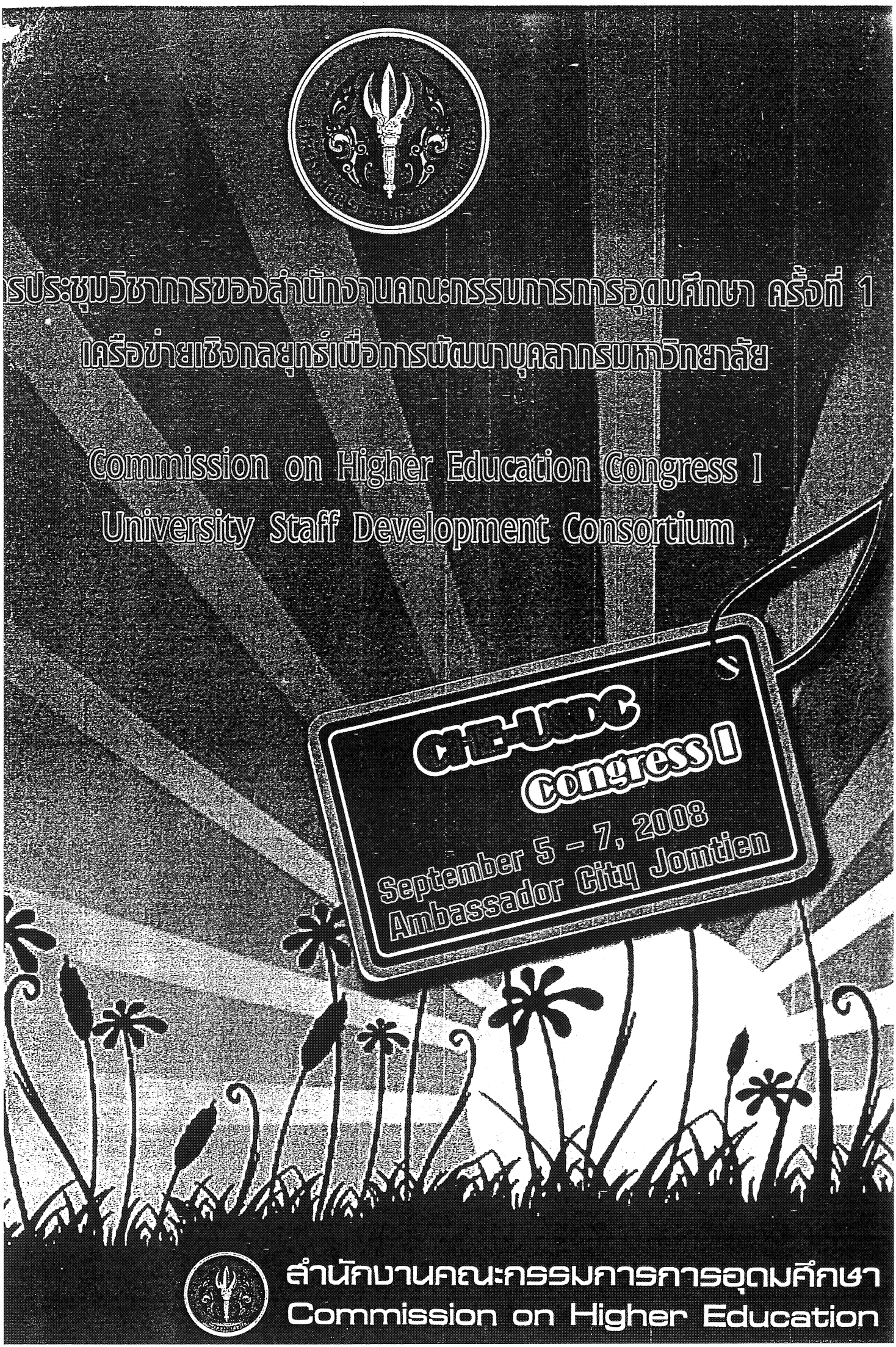
Aim: Phenotypic characterization of CD8+ T peripheral cells from patients suffering ATL is the purpose of this work in order to better understand their role within the pathology outcome.
Methods: Study groups: 1) 9 patients with diagnosis of ATL (infection duration: 5-20 years); 2) 5 ATL patients that received 2 or more complete therapy regimens but suffered frequent relapses (infection duration: 5-20); 3) 2 acute ATL patients (infection duration: < 1 month); 4) 6 healthy subjects. Isolated, cryopreserved and thawed peripheral blood mononuclear cells were stained with: Anti-CD3,-CD8,-CD57,-CD45RO,-CD27,-CD127,-CD45RA,-CD28 -FITC,-PE or -PerCP labelled monoclonal antibodies (BD, Pharmingen). Results were evaluated by flow cytometry (FACScan cytometer, CellQuest software).
Results: Lower percentages of CD127, CD27, CD28 and "early" CD8+ T cells (CD27+, CD28+) were observed in the first two groups of patients compared to the control group. Likewise, increase of "late" (CD27-, CD28-) and CD57+ T cells was observed, indicating the presence of highly differentiated cells. As shown in the table, these differences were more accentuated in group 1. On the other hand, no differences were found between acute ATL patients and the control group.


รประชุมวิชาการของสำนักงานคณะกรรมการการอุดมศึกษา ครั้งที่ 1
เครือข่ายเชิงกลยุทธ์เพื่อการพัฒนาบุคลากรมหาวิทยาลัย
Commission on Higher Education Congress I
University Staff Development Consortium
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September 5 - 7, 2008
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สำนักงานคณะกรรมการการอุดมศึกษา
Commission on Higher Education

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Killing mechanism of *Plasmodium vivax* parasites by γδ T cells

Kulachart Jangpatarapongsa[1,2], Sasithorn Promwan[1],Kirasikan Dabkam[1], Somying Loharangsikul[1], Suradej Hongeng[3], Kesinee Chotivanich[4], Duangporn Polpanich[5], Jetsumon Sattabongkot[6], Jeerapat Sirichaisinthop[7], Liwang Cui[8] and Rachanee Udomsangpetch[1,2]
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Objectives and Method

An increasing of γδ T cells during acute *P. vivax* infection and convalescent period has been reported (Jangpatarapongsa K, *et al.* 2006). Moreover, the activation of γδ T cells leads to the inhibition of blood stage *P. falciparum* parasites *in vitro* (Troye-Blomberg, *et al.* 1999). To determine the killing mechanisms of *P. vivax* parasites by γδ T cells comparing what has been found in *P. falciparum*, the γδ T cells were enriched by Isopentenylpyrophosphate (IPP) from naïve PBMC. The different number of γδ T cells of normal PBMC was incubated with intact *P. vivax* parasites, recombinant $PvMSP1_{19}$ and PvAMA1 proteins. After 5 days co-culturing, γδ T cells was determine the cytokine and granzyme intracellular releasing by Flow cytometry.

Results

The percentage of γδ T cells in lymphocytes was elevated after PBMC co-culturing with intact *P. vivax* parasites. Moreover, the enriched γδ T cells were found the proliferation at day 2 after co-culturing. At day 1 and 3 of γδ T cells stimulation, we found the high level of $CD69^+$ and $CD25^+$ expressing on γδ T cells respectively. This could tell us the early stage of γδ T cells activation among lymphocyte populations by *P. vivax*.

As expected, by comparing with *P. falciparum*, we also determine the cytolytic activities of γδ T cells by the intracellular expression of $CD107a^+$ during co-culturing with intact *P. vivax* parasites. The level of enriched γδ T cells expressing $CD107a^+$ was elevated at day 0 and rapidly decreased on further days. In contrast to what we found in normal PBMC stimulation, the increasing of γδ T cells expressing $CD107a^+$ was increased at day 2 and rapidly decreased on further days.

Conclusion and Discussion

Our results showed the activation of γδ T cells during *P. vivax* infection *in vitro*. We also showed the killing mechanism of γδ T cells against *P. vivax* infection by releasing of granzyme at early stage activation. These suggest that γδ T cells may play role against *P. vivax* parasites at early stage of infection in blood stage.

Publications Output

Jangpatarapongsa K, Chootong P, Sattabongkot J, *et al. Plasmodium vivax* parasites alter the balance of myeloid and plasmacytoid dendritic cells and induction of regulatory T cells. Eur. J. Immunol (accepted).

Xia H, Jangpatarapongsa K, Fang Q. et al. Immune response to *Plasmodium vivax* infection: A study in Thailand and the Central of China (manuscript in preparation).

The financial support from The Commission on Higher Education and Young Scientist and Technologist Programme, NSTDA are gratefully acknowledged.

Program & Abstracts

Commission on Higher Education Congress II

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(CHE - USDC Congress II)

27 - 29 August, 2009

Dusit Thani Pattaya HoTel

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KILLING MECHANISMS OF *PLASMODIUM VIVAX* PARASITES BY GAMMA-DELTA T CELLS

Kulachart Jangpatarapongsa[1,9], Somying Loharungsikul[1], Lertyot Treeratanapiboon[2], Sasithorn Promwan[1], Kirasikarn Dabkam[1], Suradej Hongeng[3], Kesinee Chotivanich[4], Doungporn Polpanich[5], Jetsumon Sattabongkot[6], Jeerapat Sirichaisinthop[7], Liwang Cui[8] and Rachanee Udomsangpetch[1,9]

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ORAL PRESENTATION

Objectives & Methods: An increasing of gamma-delta T cells during acute *P. vivax* infection and convalescent period has been reported. Moreover, the activation of gamma-delta T cells leads to the inhibition of blood stage *P. falciparum* parasites *in vitro*. To determine the killing mechanisms of *P. vivax* parasites by gamma-delta T cells comparing with what has been found in *P. falciparum*, the gamma-delta T cells were enriched by Isopentenylpyrophosphate (IPP) from naïve PBMC. Different number of gamma-delta T cells and normal PBMC were incubated with intact of *P. vivax* parasites and protein extract of *P. vivax* parasites, recombinant $PvMSP1_{19}$ and PvAMA1 proteins. Gamma-delta T cells was daily determined the cytokine and granzyme intracellular releasing by Flow cytometry until day 5 culturing.

Results: Among the enriched gamma-delta T cells, the percentage of cells expressing $CD69^+$ and $CD25^+$ was elevated after co-culturing with intact and the proteins of P. vivax parasites. The overall gamma-delta T cells showed proliferation at day 3 after the co-cultivation. Moreover, the gamma-delta T cells expressing IFN-gamma$^+$ and CD107a+ (lysosomal associated membrane proteins: LAMP-1) elevated from the first day of PBMC collection after co-culturing with the intact and P. vivax antigens. This level was correlated with the significantly decreasing number of parasites and the increasing percentage of parasite growth inhibition.

Conclusion: Our results showed the activation of gamma-delta T cells during P. vivax infection in vitro. This suggests that gamma-delta T cells could be stimulated by P. vivax parasites and these actively activated gamma-delta T cells could kill the parasites via mechanism of granzyme and cytokines at the early stage of cell activation. This study provides more understanding in activation of the innate immunity during acute malaria infection which may lead to the selection of appropriate malaria proteins as vaccine candidates in the future.

Publication Outputs: **Jangpatarapongsa K**, Chootong P, Sirichaisinthop J, Sattabongkot J, Tangpradubkul S, Hisaeda H, Troye-Blomberg M, Cui L, Udomsangpetch R. *Plasmodium vivax* alter the balance of myeloid and plasmacytoid dendritic cells and induction of regulatory T cells. **(2008) *Eur. J. Immunol* Oct;38(10):2697-705**

Xia H, **Jangpatarapongsa K**, Qiang F, Kaiming H, Sattabongkot J, Qi G, Cui L, Li B, Udomsangpetch R. Immune response to *Plasmodium vivax* infection: A study in the Central of China. (In preparation)

Jangpatarapongsa K, Loharungsikul S, Treeratanapiboon L, Promwan S, Dabkam K, Hongeng S, Chotivanich K, Polpanich D, Sattabongkot S, Sirichaisinthop J, Cui L, Udomsangpetch R. Killing mechanism of *Plasmodium vivax* parasites by gamma-delta T cells. (in preparation)

Vivax Malaria Research III+ Conference
Bangkok, Thailand
30 September 2009 – 1 October 2009

AGENDA

30 September 2009 — **Pullman Bangkok King Power Hotel**

0800 – 0930:	Registration, Coffee and Pastry Break	Infinity Room 1 Foyer, Ground Floor
0930 – 1030:	Welcome & review of draft report Dr. John Adams et al. University of South Florida Department of Global Health	Infinity Room 1, Ground Floor
1030 – 1100:	Mid Morning Break	Infinity Room 1 Foyer, Ground Floor
1130 – 1230:	Discussion of draft report	
1230 – 1330:	Lunch	Cuisine Unplugged, Ground Floor
1330 – 1515:	Afternoon Presentations	Infinity Room 1, Ground Floor

Diagnosis, clinical management, pathogenesis

Plasmodium vivax infected red cells cytoadherence to glycosaminoglycan
Dr. Kesinee Chotivanich, Mahidol University

Comparison of _P. vivax_ with _P. falciparum_ symptoms and development of exposure related immunity in the Low Transmission region of the Peruvian Amazon
Oralee Branch, New York University

Cytoadhesion of _Plasmodium vivax_ infected erythrocytes
Laurent Renia, Singapore Immunology Network

Epidemiology, vectors, environmental control

Impact of global malaria programme on _P.vivax_ prevalence in Cambodia 2001-2002
Frederic Ariey, Institut Pasteur

A role for G6PD Mahidol Mutation in Protection Against Malaria in South-East Asia
Rick Paul and Anuvaj Sakuntubhai, Institut Pasteur

VMR3+ is supported by a grant from the Bill and Melinda Gates Foundation

Active surveillance of malaria in military areas of operation (AO) along Northern Thai-Myanmar and Thai-Cambodia
COL Jariyanart Gaywee, Ph.D

Vivax malaria in China
Cao Jun, Ph.D

Situation of vivax malaria in Thailand
Jeeraphat Sirichaisinthop, MD, MPH, Vector Borne Disease Training Center, Bangkok

Geographic and Genetic of Thai *Plasmodium vivax* isolates
Usa Lek-Uthai, Mahidol University

Immunity, preclinical discovery, vaccine development

Mapping epitopes of the *Plasmodium vivax* Duffy binding protein with naturally acquired inhibitory antibodies.
Dr. Patchanee Chootong, Mahidol University

Natural immunity against *P. vivax* infection
Dr. Kulachart Jangpatarapongsa, Mahidol University

Drugs, Resistance, Targets and Development

Potential use of 8 aminoquinolines for malaria elimination on islands
Dennis Shanks, Director, Australian Army Malaria Institute

Drugs for Liver Stages
Dennis Kyle, Professor, University of South Florida, Department of Global Health

Ex vivo studies on fresh and cryopreserved clinical isolates of *Plasmodium vivax*: Practical considerations and its relevance to antimalarial sensitivity studies
Bruce Russell, Singapore Immunology Network

1515 – 1600: Break

1600 – 1745: Afternoon Discussion — Infinity Room 1, Ground Floor

1800 – 1930: Dinner — Infinity Room 2, Ground Floor

VMR3+ is supported by a grant from the Bill and Melinda Gates Foundation

1 October 2009 **Armed Forces Research Institute of Medical Sciences**
Vet Med conference room, Building 5

0800 – 0900: Coffee and Pastry

0900 – 11:30: Discussion

11:30: Meeting Adjournment

VMR3+ is supported by a grant from the Bill and Melinda Gates Foundation