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理事長 中島 章 殿

研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

1．研究者氏名 付 軍

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研究テーマ 熱帯熱，マラリアMSP1の免疫生物学とそのワクチン応用に関する研究

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 (有) ・ 無 （学会名・内容）

第 67 回 日本寄生虫学会大会

固相化ペプチドを用いた熱帯熱マラリア MSP1 のヒトヘルパーTエピトープのスクリーニング

第 9 回 国際寄生虫学会（Ninth International Congress Of Parasitology）

Analysis of T-cell epitopes on MSP1 of *plasmodium falciparum* with SPOT method of simultaneous peptide synthesis on cellulose membrane

(2) 学会誌等に発表した論文 (有) ・ 無 （雑誌名・論文名）

PARASITOLOGY INTERNATIONAL VOL.47 Supplement , August 1998

Analysis of T-cell epitopes on MSP1 of *plasmodium falciparum* with SPOT method of simultaneous peptide synthesis on cellulose membrane

3．今後の研究計画

MSP1 に対する宿主免疫応答がマラリアの感染防御および発病調節に関与する免疫生物学的意義を検討する。今後特に問題となるの以下の点であるので、その問題解決を目指したい。

(1) ワクチン応用が考えられている MSP1 のブロック 17 に対する免疫応答のうち、感染防御と直接関係する宿主応答を明らかにする。特にエピトープの比較検討、抗体のアイソタイプとの相関などをマラリア流行地住民を対象に進める。

(2) MSP1 の分子多型が宿主の免疫応答に及ぼす影響を解析する。これまでの検討で、MSP1 の T 細胞エピトープ、B 細胞エピトープの解析を進めて来ているが、免疫標的蛋白質の分子多型は宿主免疫を不応答状態に誘導する可能性もあるので、その点を実験的に検討したい。

(3) MSP1 のブロック 17 について、免疫応答標的となるエピトープペプチドのモチーフ解析を行い、アナログペプチドによって生ずる免疫応答パターンの修飾を解析する。

以上の解析を通じて、MSP1 をワクチンとして実用化するために課題として残っている問題を解析していく計画である。

4．研究指導者の意見

付　軍にはヒト寄生虫感染の免疫応答の解析のモデルとして、マラリアをテーマとして与えた。従来からマラリア流行地住民のマラリア発症に免疫応答が関与すること、MSP1 に対する抗体産生がその指標となりうることなどの情報があったが、今年度の付の研究によって MSP1 の中でも特定の領域に対する抗体産生低下することが発症と関連があること、マラリアの症状を示した人とそうでない人とでは MSP1 に対する抗体でも標的エピトープに質的な違いがあることなど、この方面の研究に新しい情報を与える結果となったことは値すると考えている。

付　軍は来日以来、経済的に苦しい環境にも関わらず真面目に研究に励み、アルバイトに忙殺されることなく努力してきた。大学の研究室での人間関係、居住地住民との人間付き合いなど、日本留学の機会を自らの国際理解のために大いに活用しており、また私達日本人にとっても良い影響を与えてくれている。このまま日本で十分に研究を進展させることができるように、引き続き本人の経済的支援が得られるように指導教員としても強く希望している。

研究指導者氏名　太田伸生

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

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熱帯熱マラリア感染者の症状発現とメロゾイト表面抗原 MSP1 に対する IgG 抗体産生

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要旨

ソロモン諸島ガダルカナル島は holo-endemic ともいわれるマラリアの濃厚流行地 0 である。マラリア流行地の住民は一定の抵抗性を獲得しており、感染による症状は非流行地住民と比較して一般に軽微であるが、実際の症状発現には住民集団内でも多様性が見られる。我々はマラリアの症状発現に宿主免疫応答の多様性が関与している可能性を調べるために、熱帯熱マラリア（Pf）のスライド陽性住民の MSP1 に対する IgG 抗体産生の差異を測定して、症状の程度と比較した。

ガダルカナル島の Pf 陽性者のうち発熱、頭痛、悪寒、嘔吐を呈したものを有症状群、それ以外を無症状群とした。無症状群の多くは日常の仕事やスポーツを支障なく行っている。この 2 群について、MSP1 の N 末端 6 ブロックに対する IgG を ELISA で測定した。両群間の年令、性の構成比率には差がなかった。マラリア即往のない日本人血清の結果を基に陽性判定を行った場合、有症状群では MSP1 に対する抗体陰性者の頻度が有意に高かった。対照としたポリオウイルスに対する抗体産生では、2 群間でまったく差がなかった。マラリアに対する抗体価は変動するパラメータである可能性があるが、1 年間隔で採取できたガダルカナルの住民血清サンプルの比較では大きな変動は見られず、ソロモンのようなマラリア流行の季節変動が小さい流行地では抗体をある程度安定した免疫学的パラメータと考えてよいと思われた。

以上の結果から、Pf 感染時の MSP1 に対する低い IgG 応答性は症状発現と何らかの関係を持つ可能性が示唆された。この低応答性が症状発現の原因であるのか、逆にその結果であるのかは今後継続して検討する必要がある。この現象の医学生物学的意味づけについては MSP1 の感染防御における機能との関連から考察する予定である。

キーワード：熱帯熱マラリア；IgG ；MSP１；ポリオウイルス。

1. Introduction

The presence of protective immunity to malaria parasites is highly probable in humans, however, details are still controvertial. A number of factors contributing protective immunity are speculated, and age, sex, and/or race including genetic background have been analyzed [1-3]. It is commonly noticed that many people infected with malaria parasites have no clinical symptoms in malaria-endemic area, and 60-70% of parasite-positive inhabitants in endemic areas are thought to be healthy carriers [4]. Guadalcanal, the Solomon Islands, is the most severely endemic place of malaria in the world, and the annual incidence of malaria is reported to be more than 300 per 1,000 population [5]. It is also common that many malaria-positive people in Guadalcanal are free from clinical symptoms. Such observation clearly shows that protective immunity to malaria contains two different concepts; inhibiting infection and protection from disease onset.

Immune responses to malaria antigens seem to be important in both two concepts of protective immunity [6,7]. Previous studies suggest that antibody responses to malaria antigens including MSP1, CSP, and RESA are impaired in symptomatic patients with falciparum malaria compared with asymptomatic individuals [4]. MSP1, major merozoite surface glycoprotein, is one of the most immunodominant malaria antigens during their asexual erythrocytic stage. To analyze in detail the malaria-specific antibody as an immunological parameter in the pathogenesis, we compared IgG antibody responses to recombinant MSP1 molecules between symptomatic and asymptomatic groups in Guadalcanal, both are infected with *Plasmodium falciparum*. We observed that impairment in antibody response in symptomatic group is malaria-specific, however, antigen molecules or epitopes to which immune responses are impaired might be selected. Biological significance of such phenomena will be discussed.

2. Materials and Methods

2.1. Studied subjects

Subjects tested in the present study were people of Guadalcanal, the Solomon Islands, where transmission of malaria is in hyper-endemic state [8]. There is little or no seasonal difference in the endemicity, therefore, people in the island seem to be at high risk for infection with *P. falciparum* whole year through. We collected blood samples by using EDTA as anti-coagulanr from 67 Solomon's donors under the informed consent, all of whom were slide-positive for *P. falciparum* in microscopic observation. Of 67 tested subjects, 44 were random subjects of symptom-free carrier individuals, and 23 were patients with clinical symptoms who visited clinics because of high fever (>38°C), chill, head ache, and/or vomiting. There was no significant difference in the sex ratio and age between the two groups; male-female ratio was 12:11 in symptomatic and 26:18 in asymptomatic group, and mean age was 15.7 (median 14) yr in symptomatic and 22.0 (median 18) yr in asymptomatic group. Mean parasite density (PD) in the symptomatic group was 212 ± 610/200 white blood cells (WBC). It was higher than that in asymptomatic group, 24.1/200 WBC, although not all the asymptomatic subjects were examined for PD. We collected sera from 20 healthy Japanese donors, who were never exposed to malaria, as negative controls.

2.2. Antigens used in ELISA

We measured IgG antibody levels to MSP1 in ELISA. MSP1 used in this study was of MAD 20 allele [9], and are recombinant proteins of the amino-terminal region containing blocks 1 through 6 (M1/6), a monomorphic block 3 (M3) and a dimorphic block 6 (M6) [10]. We adjusted the concentration of each preparation of MSP1 to be 5

μg/ml, and tested each plasma at 100-times dilution. We used horse-radish peroxidase-conjugated goat anti-human IgG as the second antibody (Cappel, Durham, USA), and after we added o-phenylenediamine (Sigma, St. Louis, USA) as a substrate, OD value at 492 nm was measured. We tentatively determined positive responses when OD value is higher than the mean OD value plus 3 SD of the Japanese negative control sera.

2.3. *Neutralizing antibody to polio virus*

As a positive control, netralizing antibodies to type III polio virus were measured by indirect hemagglutination test. People in Guadalcanal were immunized with polio vaccine, and almost all are expected to be antibody-positive. We determined positive responses when titers are x4 or more.

2.4. *Statistical analysis*

Statistical analysis was done by Student's t-test or by $\chi 2$ test.

3. Results and Discussion

We observed impaired IgG antibody production to MSP1 in symptomatic donors compared with that in asymptomatic group. Difference in the mean OD value was statistically significant ($p<0.001$) (Fig.1). Frequency of antibody-negative donors was also significantly higher in the symptomatic group (34.8% vs 2.27%) ($p<0.01$). Such impaired antibody response in the symptomatic group was malaria-specific because we observed almost same mean antibody titers to polio virus in the two groups (Fig.2). We further measured IgG antibody levels to two blocks of the amino-terminal region of

MSP1. Dimorphic M6 was thought to be highly immunogenic for cellular and humoral responses in humans compared with monomorphic M3 [11,12]. Our present observation was consistent with previous study by Frue et al. [12] that M6 was more immunogenic for IgG response than M3, however, the symptomatic group showed deeply suppressed IgG responses only for M6 ($p<0.01$) (Table 1). Furthermore, responsiveness to M3 and M6 was discordant each other in the symptomatic group ($p<0.05$) (Table 1).

The exact explanation for the present observation remains uncertain. It is not clear whether such low antibody response caused higher parasitemia as in symptomatic individuals. Alternatively, a possibility of immune suppression mediated by malaria parasites can not be ruled out [13]. The carboxyl-terminal block of MSP1 is suggested to be directly involved in MSP1-mediated protective immunity [14], while biological roles of the amino-terminal region remain unclear. It is interesting to note that there was no significant suppression in IgG level to M3 (Table 1), because antibodies to M3 were reported to inhibit protective immunity induced by the carboxyl-terminal region of MSP1 [15]. It is needed to analyze more detailed biological roles of the amino-terminal region of MSP1 to discuss the biological roles of those impaired immune responses in symptomatic patients.

Another interesting point to see is whether such impaired antibody response is a temporal or fixed phenomenon. Antibody titers have tight relationship with age of human hosts [1]. In this study, we compared two groups of which age distribution was not significantly different, however, asymptomatic group was older than symptomatic group. We have to consider this factor before getting final conclusion. Fluctuation of antibody level in a malaria endemic area should be also considered [12]. Endemicity in Guadalcanal shows only a faint seasonal difference. In this study, serum samples were collected twice with an interval of nearly one year from some of asymptomatic donors. Mean OD values of those sera were almost stable (Table 2), suggesting that MSP1-

specific IgG seems to be a relatively stable immunological parameter in Guadalcanal. It is possibile to suppose that high PD-caused consumption of specific antibodies in sera of the patient group [16]. It is, however, not likely the case because we observed cases showing weak IgG levels to M6 but high M3-specific IgG, or vice versa. It is rather probable that complicated regulatory mechanisms induced the impaired immune responses to some particular epitopes. Immunogenetic factors could be involved in such regulation [17]. We are planning to test MSP1-specific antibody levels in the symptomatic groups after their recovery from clinical symptoms. It is also interesting to do a follow-up study whether asymptomatic individuals with low IgG levels develop clinical symptoms in near future.

In conclusion, impaired antibody response to MSP1 of *P. falciparum* could be a risk factor for developing clinical symptoms in the holo-endemic Guadalcanal. Regulatory system involved in the present phenomenon is malaria-specific, however, there might be regulatory system specific for selective epitopes in case of antibody response to MSP1. Target epitopes of MSP1 for this immunoregulation could be involved in immunopathogenesis as well as protective immunity during falciparum malaria.

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Table 1.

Heterogeneous IgG responses to M3 and M6 blocks of MSP1 in symptomatic patients with falciparum malaria in Guadalcanal.

	Positive response to			Responses to M3/M6			
Subjects	M1/6	M3	M6	+/+	+/-	-/+	-/-
Symptomatic (N=23)	15 (65.2%)	13 (56.5%)	11 (47.8%)	9	4	2	8
Asymptomatic (N=18)	18 (100%)	15 (83.3%)	17 (94.4%)				
p value	<0.01	ns*	<0.01		<0.05		

* not significant

Table 2.

Comparison of MSP1-specific IgG antibodies in asymptomatic donors in plasma samples collected in two different years in Guadalcanal.

			OD values in ELISA	
Subject ID	Age	Sex	in 1994	in 1995
205	23	female	0.611	0.643
206	60	male	1.24	1.12
235	44	female	0.852	1.16
248	50	female	1.19	1.18
402	7	male	0.112	0.161
403	18	male	1.03	1.23
417	11	female	0.47	0.111
418	11	male	1.18	1.27
424	35	male	1.18	0.991

Figure 1.

MSP1-specific IgG antibodies in symptomatic and asymptomatic individuals infected with *P. falciparum*. MSP1-specific IgG level in symptomatic group was significantly low compared with asymptomatic donors ($p<0.001$). Cutoff line for positive IgG level was tentatively determined as the mean OD value + 3 SD of the Japanese negative controls. More than 1/3 of symptomatic cases were negative for MSP1, and the frequency of negative cases significantly elevated in the symptomatic group ($p<0.01$).

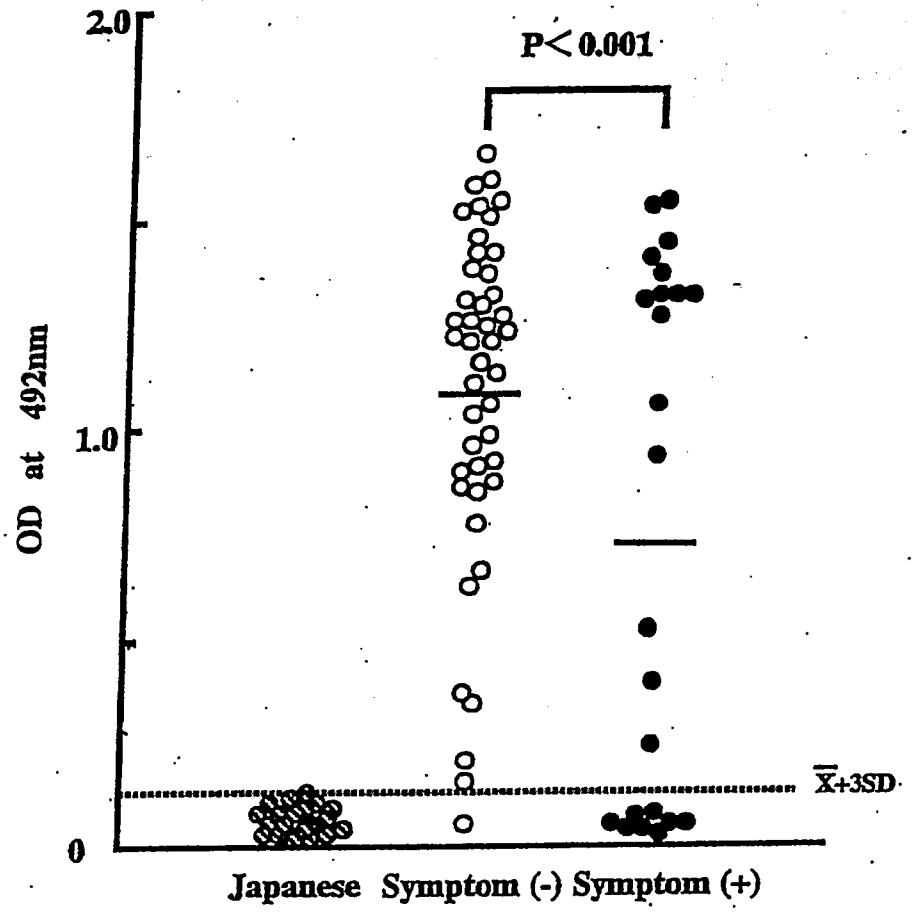

Figure 2.

Antibody titers of anti-polio virus antibody in individuals in Guadalcanal. There was no difference in antibody titers to polio virus between the two groups. Titers higher than x4 are positive, and the cutoff line is shown in a dotted line.