· Original article ·

Immunogenicity of *Plasmodium falciparum* chimeric protein PfCP-2.9 in various strains of mice

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[**ABSTRACT**] **Objective**: To investigate the immunogenicity and characteristics of immune response of the chimeric antigen PfCP-2.9 in various strains of mice. **Methods**: Five strains of mice were immunized with the chimeric antigen formulated with ISA720 adjuvant subcutaneously 3 times at three-week intervals. After immunization, kinetics of antibody responses, isotype of IgG antibody, and antibody responses to the individual components of the chimeric antigen were detected by ELISA. Interactions of the antibodies with native antigens located on surface of merozoite of *Plasmodium falciparum* were analyzed by indirect immunofluorescent assay (IFA). **Results**: Significant immune responses were induced in all strains of mice (detected with ELISA titer $>10^5$) after the third immunization. Moreover, the specific antibodies recognized individual proteins of the chimeric antigen, MSP1-19 and AMA-1 (Ⅲ), as well as native antigens of the cultured *Plasmodium falciparum*. The prevailing isotypes of IgG associated with the immunization were IgG1 and IgG2a. However, there were clear differences in antibody levels and isotypes of IgG among 5 strains of mice. **Conclusion**: The chimeric antigen PfCP-2.9 is highly immunogenic in all strains of mice detected and the anti-PfCP-2.9 antibodies recognize native proteins of the malaria parasite.

[**KEY WORDS**] *Plasmodium falciparum*; chimeric antigen; immunity; mice

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Malaria remains one of the most serious infectious diseases of mankind. During the past several decades, the challenge of anti-malarial treatments is increasing owing to the emergence and spreading of drug-resistant strains of the parasite and insecticide-resistant vector of the mosquito. Thus development of an effective vaccine is an urgent to control malaria. Two antigens of blood-stage, merozoite surface protein 1 (MSP-1) and apical membrane antigen-1 (AMA-1) of *Plasmodium falciparum*, are leading malaria vaccine candidates[1-3]. The 19 000 carboxyl-terminal fragment of MSP1 (MSP1-19) and the C-terminal domain of AMA-1 [AMA-1 (Ⅲ)] are the predominant protective immuno-functional domains of the 2 antigens, respectively[4,5]. A chimeric protein (named PfCP-2.9) comprising MSP1-19 and AMA-1(Ⅲ) of *P. falciparum* was constructed *via* a hinge sequence and expressed in *Pichia pastoris* in secreted form at extremely high level as described before[6]. In this study, we investigated the immunogenicity and characterization of immune responses of the chimeric antigen in the various strains of mice.

1 MATERIALS AND METHODS

1.1 Materials, reagents and animals The recombinant proteins of PfCP-2.9, MSP1-19 and AMA-1 (Ⅲ) were produced in our lab. The hinge peptide of 28 amino acid residues was synthesized by Shanghai Shenyou Biological Technology Co. Ltd.. Montanide ISA720 was purchased from SEPPIC, France. The horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG2b and IgG3 and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG were purchased from Jingmei Biotech Company. 3, 3′, 5, 5′-tetramethylbenzidine (TMB) was purchased from Shanghai Chemical Reagent Co.. BALB/ca, C57BL/6J, C3H/He, DBA/2J and Kunming (KM) mice were purchased from Shanghai Laboratory Animal Center of Chinese Academy Sciences. FCC1/HN and 3D7 isolates of *P. falciparum* were cultured in our lab.

1.2 Vaccine formulation The diluted antigen solution was formulated with ISA720 adjuvant at a ratio of 3 : 7 using homogenizer at speed of 2 500 r/min for 5 min. The quality of the emulsion was controlled by droplet test.

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1.3 Animal immunization Various strains of female mice including BALB/ca, C57BL/6J, C3H/He, DBA/2J and KM, aged 6-8 weeks, were immunized and grouped by 5 mice. Each animal was subcutaneously injected with 0.2 ml of the vaccine emulsion containing 50 μg of PfCP-2.9 antigen. Three vaccinations were given at 3-week intervals. Ten to 14 d after each immunization, approximately 100 μl of blood were taken for analysis of immune responses. All the blood samples were placed at room temperature for 5-10 h and then kept at 4℃ overnight. Sera were isolated from the blood samples by centrifugation at 1 500×g for 20 min and stored at −20 ℃.

1.4 Analysis of antibody responses of immune sera by ELISA 96-well plates were coated with 100 μl of antigen solution (1 μg/ml) diluted in 50 mmol/L carbonate-bicarbonate buffer (pH 9.6) and incubated at 37℃ for 1 h. The sera were diluted before use and 100 μl of serially diluted immune sera were added to each well. The second antibody was 100 μl of the HRP-conjugated goat anti-mouse IgG. The saturated plates were incubated at 37℃ for 1 h. After the color reaction of TMB, 50 μl of 2 mol/L H_2SO_4 per well were added to stop the reaction, and the absorbance of optical density was measured at 450 nm (D_{450}) by using an ELISA microplate reader. The inverse of the highest serum dilution with D_{450} value greater than the cutoff value (twice D_{450} value of control sera) was determined as the titer of the sample.

1.5 IgG isotype analysis The method used for IgG isotype analysis was similar to that described in 1.4 except those anti-IgG isotype antibodies of IgG (IgG1, IgG2a, IgG2b and IgG3) were used as second antibodies.

1.6 Indirect immunofluorescent assay (IFA) Thin blood smears containing the mature schizont of *P. falciparum* were prepared. Serially diluted sera using 0.01 mol/L PBS (pH 7.4) were added to spot on the slide and incubated at 37℃ for 1 h in a moist atmosphere. After washing, the second antibody of FITC-conjugated goat anti-mouse IgG were added and incubated for 1 h under the same conditions. After washing, the slides were examined on a fluorescence microscope.

1.7 Statistical analysis After log transformation of antibody titers, the comparisons of different groups were measured by t test and ANOVA by using SPSS 10.0.

2 RESULTS

2.1 Specific antibody levels in various strains of mice As shown in Fig 1, immune responses to PfCP-2.9 were induced in all 5 strains of mice, and the antibody titers were notably enhanced after each boosting of immunization with the antibody titer more than 10^5. After the third immunization, the specific antibody levels from BALB/ca, C57BL/6J, C3H/He and KM strain of mice were further increased but not from DBA/2J strain in which the peak level was obtained after the second immunization. Comparison of the antibody titers among 5 different strains of mice after primary immunization, there was no significant difference ($F=0.895$, $P=0.485$). However, after the second immunization, remarkable difference of the antibody titers was observed. The levels of specific antibody in BALB/ca mice were statistically higher than those in KM, DBA/2J and C57BL/6J mice ($P=0.009$, 0.026 and <0.001), but no significant difference compared with those in C3H/He mice. Likewise, the levels of the antibody in C57BL/6J strain of mice were significantly lower than those in BALB/ca and C3H/He strain of mice ($P<0.05$), but no significant difference compared with those in KM and DBA/2J strain. Similarly, the antibody titers after the third immunization were distinguished among various groups ($F=10.063$, $P<0.001$). The antibody levels from KM and BALB/ca strain of mice were notably higher than those from C57BL/6J and C3H/He mice, and DBA/2J strain of mice.

2.2 IgG isotype analysis in various strains of mice As shown in Fig 2, the 4 subclasses of IgG against PfCP-2.9 were induced in all immunized mice and both IgG1 and IgG2a isotypes were prevailing subclasses. The high-lower sequence of 4 IgG subclasses was IgG1, IgG2a, IgG2b and IgG3. Similar to total IgG, the levels of both IgG1 and

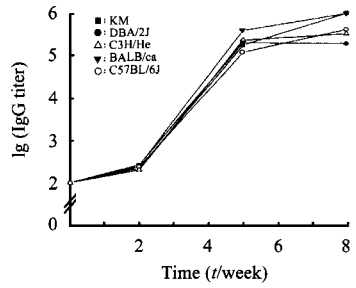

Fig 1 IgG antibody responses of PfCP-2.9 in mice

IgG2a isotypes in KM and BALB/ca strain were significantly higher than those in the rest 3 strains ($P<0.05$). In all 5 strains of mice, KM strain generated the highest IgG2b responses ($P<0.05$) while the lowest level of IgG2b was observed in DBA/2J strain ($P<0.05$), and there was no difference of IgG2b response among BALB/ca, C57BL/6J and C3H/He strains. IgG3 isotype was KM>BALB/ca >C3H/He >C57BL/6J >DBA/2J. The IgG3 titers induced in KM mice were notably higher than those in DBA/2J, C57BL/6J and C3H/He mice ($P<0.05$), but there was no difference compared with those in BALB/ca mice.

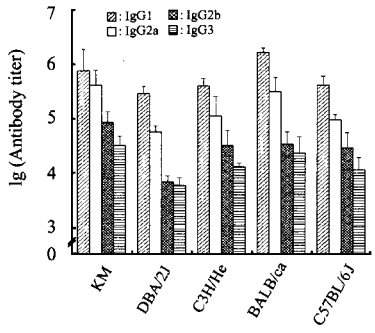

Fig 2 Comparison of IgG isotype titers in different strains of mice

2.3 Interaction of specific antibodies with native antigens on surface of parasite As shown in Fig 3, antibodies to PfCP-2.9 recognized the native antigens of both FCC1/HN and 3D7 strains of *P. falciparum*. There were no significant differences of antibody levels to the native antigens of both strains detected by IFA ($t=0.253$, $P=0.802$). However, significant differences of the antibodies were observed in various strains of mice against 2 strains of *P. falciparum* ($F=3.682$ and 6.520, $P=0.021$ and 0.002). The IFA titers of BALB/ca mice were notably higher than those of DBA/2J and KM mice ($P<0.05$).

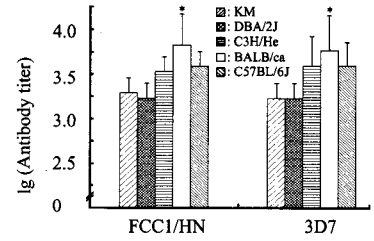

Fig 3 Comparison of IFA antibody titers in different strains of mice

* $P<0.05$ *vs* DBA/2J and KM mice

2.4 Interaction of individual components of PfCP-2.9 with antibodies from various strains of mice As shown in Tab 1, high levels of antibodies against the individual components, MSP1-19 and AMA-1(Ⅲ), were detected in all the 5 strains of mice whereas no antibodies against the hinge was induced. The antibody titer to AMA-1(Ⅲ) was higher than that to MSP1-19 ($t=6.501$, $P=0.003$). However, the antibody titers to the individual components were much lower than those to the entire protein.

Tab 1 Antibody levels in mice immune sera against PfCP-2.9 and its components

Strain	Antibody titers			
	Anti-hinge	Anti-MSP1-19	Anti-AMA-1-(Ⅲ)	Anti-PfCP2.9
KM	<100	13.76×10^4	37.36×10^4	131.4×10^4
DBA/2J	<100	1.54×10^4	2.91×10^4	15.5×10^4
C3H/He	<100	8.74×10^4	18.71×10^4	59.3×10^4
BALB/ca	<100	17.31×10^4	28.74×10^4	106.3×10^4
C57BL/6J	<100	5.67×10^4	8.73×10^4	30.3×10^4

3 DISCUSSION

PfCP-2. 9 chimeric protein is composed of MSP1-19 and AMA-1 (Ⅲ) of *P. falciparum via* a hinge sequence. The main purpose of adding the hinge sequence was to reduce interaction of the 2 cysteine-rich antigens and maintain their disulphide-bond conformations. As shown in this study, immune sera induced by PfCP-2. 9 recognized not only its 2 major components, but also native antigens of the parasite, indicating that the chimeric antigen PfCP-2. 9 resembles the native conformation.

Numerous studies have demonstrated that humoral immunity plays a crucial role in the protection against blood-stage malaria parasite[7]. Specific antibodies to MSP1-19 or AMA-1 can mediate the protective immunity, but it is necessary to induce high level of the antibodies for the protective immunity[4,8]. As shown in this study, the chimeric protein PfCP-2. 9 induced extremely high level of specific antibodies in mice, more than 10^5 after the third immunization. The high level of the antibodies was essential to inhibit growth of the parasite in vitro and associated with the protective immunity. The protective efficacy of humoral immunity in blood-stage malaria was not only correlated to the level of IgG, but also associated with isotypes of IgG. Many studies on serology conducted in malaria endemic regions have demonstrated that cytophilic IgG1 and IgG3 were major isotypes that associated with the protective immunity in human[9]. However, experimental data from mice models indicated that cytophilic IgG2a was major isotype for protective immunity[10]. As shown in this study, the prevailing isotypes of IgG induced by PfCP-2. 9 in different strains of mice were IgG1 and IgG2a, implying that PfCP-2. 9 antigen can induce protective immunity. Genetic background determined by HLA system is highly diversified in human and greatly affects the immune response to vaccination. The SPf66, once of malaria vaccines tested in human widely, had a variable efficacy rates from various trails. The variable results may be due to the diversity of MHC molecule in different populations[11,12]. Similar to human HLA system, H-2 antigen system determines the genetic background of mice. Our data showed that high level of specific antibodies was induced by PfCP-2. 9 vaccine candidate in all 5 strains of mice including the out-bred strain (KM), implying that the vaccine is highly immunogenic in large populations when used in human.

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恶性疟原虫融合抗原 PfCP-2.9 在不同品系小鼠中的免疫特性

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[**摘要**] **目的**: 探讨恶性疟原虫融合抗原 PfCP-2.9 在不同品系小鼠(BALB/ca、C57BL/6J、C3H/He、DBA/2J 及昆明种)中的免疫原性和免疫反应特性。**方法**: 以 ISA720 为佐剂, PfCP-2.9 抗原皮下免疫 5 种品系小鼠 3 次, 间隔 3 周。以 ELISA 检测免疫血清中特异性抗体的动态变化、IgG 抗体亚型及对融合抗原各组分的抗体水平, 以间接荧光抗体实验分析免疫血清对恶性疟原虫天然抗原的识别情况。**结果**: 5 种品系小鼠均能产生针对 PfCP-2.9 的免疫应答, 3 次免疫后血清中特异性抗体滴度达 10^5 以上。PfCP-2.9 所诱生的免疫血清不但能识别 MSP1-19 和 AMA-1(Ⅲ)两个主要片段, 而且能识别恶性疟原虫天然抗原。所诱导的 4 种 IgG 抗体亚型中, IgG1 和 IgG2a 是免疫血清中主要的抗体类型。但特异性抗体水平及抗体亚型分布因遗传背景不同而异。**结论**: 融合蛋白 PfCP-2.9 在 5 种不同品系小鼠中具有强的免疫原性, 其抗体能识别疟原虫天然蛋白。

[**关键词**] 恶性疟原虫; 融合抗原; 免疫性; 小鼠

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· 短篇报道 ·

手部高压喷射伤的临床治疗

Clinical treatment of high-pressure injection injuries of hand

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[**关键词**] 手损伤; 高压喷射伤; 治疗

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手部高压喷射伤(high-pressure injection injuries of the hand, HPIIH)是手外科一种少见的急症创伤, 治疗不当常导致截指或截肢, 所致的截指和(或)截肢率约为 16%～48%[1~3], 其早期症状常不严重, 而致延误正确的治疗。我科在 1998 年 2 月至 2001 年 10 月, 共收治手指高压喷射伤患者 16 例, 采用广泛清创、引流、开放创口、重复扩创及二期闭合创口等方法进行治疗, 术后经 6 个月至 1 年随访, 患者的伤指均得以存活, 功能恢复良好。现总结报告如下。

1 资料和方法

1.1 *一般资料* 16 例患者均为男性, 年龄为 28～36 岁。伤指: 右手示指 2 例、中指 4 例, 左手示指 3 例、中指 6 例、拇指 1 例; 致伤原因: 14 例为喷枪、2 例为油枪; 喷射物: 14 例为液体稀料, 2 例为液体柴油。就诊时间为伤后 1～26 h。主要临床表现为伤指明显肿胀、疼痛, 活动时疼痛加剧。

1.2 *治疗方法* 首先急诊手术清创, 于伤指掌面从掌指关节至远指间关节行"Z"形切口, 广泛显露, 彻底清除注入物及污染组织, 注意保护两侧的神经血管及屈指肌腱腱鞘; 若累及腱鞘, 清创时需保留 A2 及 A4 滑车。1%苯扎溴铵、双氧水及生理盐水反复冲洗, 开放伤口, 湿敷包扎; 根据伤情, 24～72 h 后行第 2 次清创, 术后应用广谱抗生素, 每日湿敷换药。二期手术闭合伤口。术后第 2 天开始物理治疗及康复锻炼。在医生指导下进行主动活动锻炼, 以防肌腱粘连。

2 结 果

11 例伤口缝合后一期愈合; 5 例 2 周拆线时仍有部分伤口未愈, 经换药愈合; 术后随访 6 个月至 1 年, 手指外形理想, 屈伸功能良好, 根据 TAM 功能评定法, 手指功能恢复良者 10 例, 优者 6 例。16 例患者均重返工作岗位。

3 讨 论

1937 年 Rees 首先报道了 1 例手部 HPIIH 的患者, 液体

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