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学位論文名	Characterization of proteases involved in the processing of *Plasmodium falciparum* serine repeat antigen (SERA) （熱帯熱マラリア原虫のSERA抗原のプロセッシングに関与するプロテアーゼの解析）
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論文内容の要旨

Aim

The serine repeat antigen (SERA) of *Plasmodium falciparum* is a blood stage malaria vaccine candidate. The function of this protein, however, is unknown. The protein is synthesized at trophozoite- and schizont-stages in the erythrocytic cycle of the parasite and secreted as a 120 kDa protein into the parasitophorous vacuole. Shortly before schizont rupturing, SERA (120 kDa) is proteolytically processed into a N-terminal 47 kDa fragment (P47) and a 73 kDa fragment (P73). P73 is then processed into a 56 kDa fragment (P56) and a C-terminal 18 kDa fragment (P18). P56 is further converted to 50 kDa fragment (P50), while P47 is further processed into two 25 kDa fragments depending on the allelic type of SERA gene. P50 has s significant homology to papain-family proteases but has a serine instead of cysteine at the predicted active site ; whether or not SERA contains a protease activity is unknown. In order to get a clue to the unknown function of SERA, the mechanism behind the processing of this protein was investigated in the present study.

Methods and Results

1. To establish an *in vitro* cell-free system for the analysis of SERA processing, a recombinant SERA was expressed and purified from baculovirus-expression system. When the recombinant SERA was incubated with an extract of parasitized erythrocytes, it was converted to fragments corresponding to P47, P73, P56, P50 and P18 that are observed in parasite cultures, as demonstrated by immunoblotting with antibodies specific for different domains of SERA. The processing activities apprared at late schizont stage of the parasite.

2. On examining the extracts from different fractions of schizont-infected erythrocytes, it was found that the fraction containing components of the parasitophorous vacuole has processing activities. The proteases appeared to be membrane associated. This is consistent with the secretion and accumulation of SERA within the parasitophorous vacuole before processing.

3. When inhibitors specific for individual classes of proteases were tested for inhibibion of SERA processing in the *in vitro* system, it was found that the activity responsible for the processing of SERA into P47 and P73 was inhibited by serine protease inhibitor DFP. In contrast, the activity responsible for the conversion of P56 into P50 was inhibited by each of the cysteine protease inhibitors E-64, leupeptin and iodoacetamide. The conversion of P73 to P56 and P18 wes only partially inhibited by the same cysteine protease inhibitors. When added to cultures of middle-stage schizonts, DFP significantly caused accumulation of the unprocessed SERA, while both leupeptin and E-64 markedly caused accumulation of P56 but not P73. These observations suggest that SERA processing is mediated by at least three distinct proteases.

4. To examine the effects of DFP, leupeptin, and E-64 on the intraerythrocytic proliferation of the parasite, cultures of middle- stage schizonts were treated with individual inhibitors. DFP, leupeptin and E-64 individually blocked 35-46% of the schizonts from rupturing. Together with the observation that SERA processing is initiated most likely at late schizont stage and completed within the paresitized erythrocytes just prior to schizont rupture, these results suggest that SERA processing correlates to schizont rupture.

Conclusion

An *in vitro* cell-free system has been established that mimics the SERA processing that occurs in parasitized erythrocytes. The proteases involved in the processing of SERA have been partially characterized. The results obtained from the present study also suggest that SERA processing correlates to schizont rupture. This provides the basis for further investigation of the physiological role of SERA.

論文審査の結果の要旨

熱帯熱マラリア原虫のSERA抗原（serine repeat antigen）は流行地において自然獲得されるマラリア免疫の最も重要な標的抗原であり、組換えSERA蛋白質はマラリアワクチン候補として臨床試験が予定されている。SERA（120 kD）は寄生体胞内に浮遊し、分裂体が破裂してメロゾイトを感染赤血球から放出する際に47 kD、50 kD、18 kDの断片に切断される。しかしながら、マラリア原虫の増殖におけるSERA蛋白質の生理的機能は未だに不明である。本研究では、SERAの機能解明を目指して、SERAプロセッシング機構の解析を行った。先ず、バキュロウイルスを用いて発現させたレコビナントSERAをマラリア原虫の粗抽出液と反応させ、in vitro系でSERAプロセッシングを行なわせることに成功した。次にこの系を利用し、プロテアーゼ阻害剤の効果を解析した結果、少なくとも3種類のプロテアーゼがSERAのプロセッシングに関与していることを明らかにした。さらに、培養中の原虫分裂体にこれらプロテアーゼ阻害剤を加えることよりSERAプロセッシングを阻害したところ、寄生体胞膜の壊裂が阻害された。これらの結果は、SERAプロセッシングはマラリア原虫分裂体の壊裂と強く相関することを示唆する。本研究はSERAのプロセッシング機構、さらにSERA抗原の機能の解明の分子基盤となるものである。よって、博士学位の授与に値するものと認める。