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研究課題名（和文）免疫制御細胞の原虫破壊機構の解明と抗原虫ペプチドの開発

研究課題名（英文）Elucidation of mechanisms by which host immune cells kill protozoan parasites and development of antiparasitic peptides

研究代表者

テルカウィ　アラー（Terkawi, Alaa）

北海道大学・医学研究科・助教

研究者番号：００７２３０７４

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研究成果の概要（和文）：宿主の分子防御機構を解明し、マラリア原虫を除去するエフェクター分子を同定することは、新たな抗マラリア治療に重要である。マクロファージや好中球は微生物に対して迅速に認識・殺傷する能力があり、免疫応答で重要な役割を担う。我々は、DNAマイクロアレイ技術を用いた転写解析を行い、マラリア原虫を殺傷するマクロファージ由来の宿主エフェクター分子を識別することに注目した。その結果、マクロファージ由来の-defensin-130と好中球由来のカテプシンＬがマラリア原虫の殺傷作用に関与することが判明した。本研究の結果より、マラリア原虫に対する新たな治療標的を明らかにした。

研究成果の概要（英文）：Understanding the molecular defense mechanism of phagocytes and identifying their effector molecules against malarial parasites may provide important clues for the discovery of new therapies. Macrophages and neutrophils are professional phagocytes that play key role in the innate immune response against infection through their ability to rapidly recognize and kill microorganisms. An attempt was made to identify the host effector molecules derived from macrophages and neutrophils that kill malaria parasites using DNA microarray technology.
Results suggested that DEFB130 from macrophages and CTSL from neutrophils are involved in the clearance mechanism of malarial parasites. The data obtained from this project broaden our knowledge on the immunological response of macrophages and neutrophils to malaria parasites and shed light on a new target for therapeutic intervention.

研究分野：　Immunology

キーワード：　Macrophages　Neutrophils　Phagocytes　Effector molecules

１．研究開始当初の背景

Malaria, caused by apicomplexan protozoa of the *Plasmodium falciparum*, remains one of the top public health concerns worldwide, killing nearly 0.5 million people every year. Resistance to the current antimalarial drugs in many endemic regions of the world exaggerates this health threat and proposes the urgent need for a new frontline antimalarial therapy. Macrophages and neutrophils are professional phagocytic cells that play key roles in the innate immune response against infection through their ability to rapidly recognize, phagocytize, and kill microorganisms. Upon recognition by these cells, foreign agents are internalized and engulfed into phagosomes to be exposed to various antimicrobial effectors, including proteases, hydrolytic enzymes, and antimicrobial peptides. Understanding the molecular defense mechanism of macrophages and neutrophils, and identifying their effector molecules against malarial parasites may provide important clues for the discovery of new therapies.

２．研究の目的

In the current study, an attempt was made to identify the host effector molecules derived from macrophages and neutrophils that kill malaria parasites using DNA microarray technology.

３．研究の方法

Human macrophages and neutrophils were cultured with *Plasmodium falciparum* parasitized erythrocytes (iRBCs) to obtain high number of iRBCs-phagocytizing cells. Phagocytes were harvested for RNA extraction at the peak of phagocytic activity after 2 hrs for macrophages and 1 hr co-culture for neutrophils. Transcriptional analyses by DNA microarray technology were performed to identify genes profile of human macrophage specifically induced by phagocytosis of iRBCs. Gene knockdown was carried out using esiRNA in human primary macrophages and THP1 cell line. Gene overexpression was carried out in HL human cell line for mostly regulated genes in the DNA microarray data. P3XFLAG-CMV14 mammalian expression vector was used as carrier vector and electroporation was performed in a nucleofector device. The toxic activity of cells was examined *in vitro* against iRBCs.

４．研究成果

Transcriptional analyses by DNA microarray technology indicated that macrophages generated a broad and vigorous set of gene expression in response to malarial parasites represented in 216 upregulated genes and 168 downregulated genes. Importantly, the upregulated genes were involved in multiple cellular functions including growth, proteolysis, coagulation and adhesion. Interestingly, microarray results identified β-defensin 130 (DEFB130) as one of the top three upregulated genes in macrophages phagocytizing iRBCs. These results led to identify DEFB130 as an antimicrobial host-defense peptide against malarial parasites. Confocal laser scanning microscopic examination showed that macrophages phagocytizing iRBC had higher levels of DEFB130, which appeared within the cytosol of the macrophages and accumulated around the engulfed iRBC or malaria pigment (Fig. 1). Transfection of esiRNA-mediated knockdown of DEFB130 into macrophages resulted in a remarkable reduction in their antiplasmodial activity *in vitro* (Fig. 2).

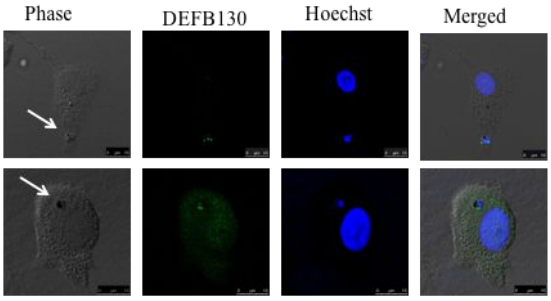

Fig.1. Intracellular localization of DEFB130 in macrophages phagocytizing iRBCs examined by confocal microscopy.

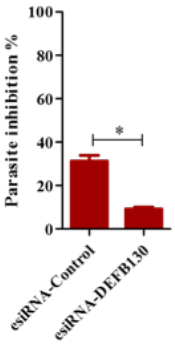

Fig. 2. Parasite inhibition after culturing transfected macrophages with enriched-iRBCs.

Furthermore, DEFB130 synthetic peptide exhibited a modest toxic effect on *P. falciparum in vitro* and *P. yoelii in vivo*, unlike scrambled DEFB130 peptide, which showed no antiplasmodial activity (Fig. 3).

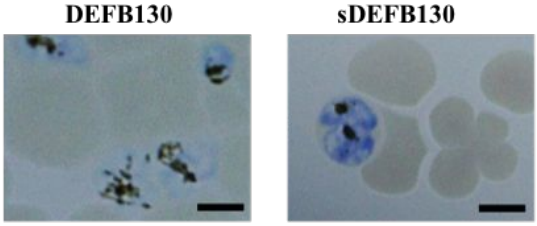

Fig. 3. Morphological changes in the parasites after 3 h of culture with 50 μM peptide.

To further define the effective region of DEFB130, we synthesized analog peptides for the N-terminal (32 amino acids) and C-terminal

(15 amino acids) domains of DEFB130 and examined their inhibitory effects against *P. falciparum* parasites *in vitro*. Strikingly, the analog peptide of the N-terminal but not the C-terminal domain was effective against the growth of parasites (Table 1). Together, these results suggest that DEFB130 might be one of the macrophage effector molecules for eliminating malarial parasites and its maximal cytolytic activity likely depends on the full cationic charge, hydrophobicity, and amphipathicity of the peptide.

	Parasite strains (IC_{50} μM)		
	3D7	**Dd2**	**HB3**
DEFB130	47.12 ± 2.22	43.53 ± 3.81	49.22 ± 3.16
sDEFB130	>200	>200	>200
Nt-DEFB130	93.02 ± 0.88	91.31 ± 2.09	90.55 ± 1.63
Ct-DEFB130	>200	>200	>200

Table 1. Antimalarial activity of DEFB130. The IC50 values of synthetic DEFB130, scrambled peptide, and N-terminal and C-terminal domain peptides against different strains of *P. falciparum* parasites *in vitro*.

Furthermore, the same strategy was applied on human neutrophils phagocytizing infected erythrocytes to identify their effective molecules. Notably, microarray data demonstrated that neutrophils induced a broad and vigorous set of changes in gene expression in response to malarial parasites, represented by 384 downregulated and 148 upregulated genes. The transcriptional response was characterized by regulation of numerous genes encoding multiple surface receptors, proteins involved in signal transduction pathways, and defense response proteins. Several genes were targeted for *in vitro* studies and the antiplasmodial activities of gene manipulated HL-60 human cell line were examined. Results revealed the involvement of cathepsin L (CTSL) in mechanism by which neutrophils kill malarial parasites (Fig. 4).

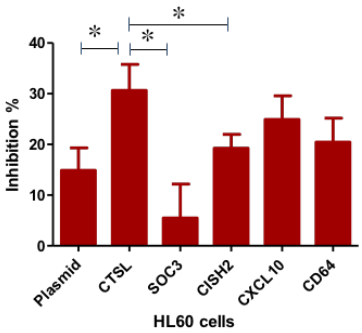

Fig.4. Percentage of growth inhibition by transfected HL cells. Top regulated genes were overexpressed in neutrophil-like cells and then cultured with iRBCs.

In conclusion, these data obtained from this project broaden our knowledge of the immunological response of macrophages to malaria parasites and shed light on a new target for therapeutic intervention.

５．主な発表論文等

（〔雑誌論文〕（計 5 件）

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〔学会発表〕（計 4 件）meeting

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〔図書〕（計　0　件）

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権利者：
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名称：抗原虫薬
発明者：加藤健太郎、アラー テルカウィ、高野量
権利者：国立大学法人帯広畜産大学
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番号：特願 2015-233632
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〔その他〕
ホームページ等
とくになし

６．研究組織
(1)研究代表者
Mohamad Alaa Terkawi（アラー テルカウィ）
北海道大学・大学院医学研究科・特任研究助教
研究者番号：00723074

(2)研究分担者
なし

研究者番号：

(3)連携研究者
なし

研究者番号：

(4)研究協力者
加藤健太郎（Kato Kentaro）
原虫病研究センター帯広畜産大学

高野量（Ryo Takano）
原虫病研究センター帯広畜産大学