Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 20 - Molecular Mechanisms of Uninfected Red Cell Phagocytosis in Severe Malarial Anemia

7. **Start and End Date of Research Project:** 5/1/2009 - 6/30/2010

8. **Name of Principal Investigator for the Research Project:** José A. Stoute, M.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 61,927

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Torres	Research Assistant	50%	$9,752

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Stoute	Principal Investigator	25
Mbugua	Post-doctoral Fellow	100%
Byrnes	Res Ass't	50%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___x_____ No_____

If yes, please describe your plans:

At the present time we are preparing an NIH application to continue this work.

12. Future of Research Project. What are the future plans for this research project?

We have developed an animal model of severe malarial anemia which shows significant erythrophagocytosis in the liver. We are proposing to study this model in vivo and in vitro to dissect the mechanisms of uninfected red cell destruction during malaria.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____x_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes____x_____ No_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

    Amos Mbugua, Ph.D., post-doctoral fellow was hired to work on this project. He was previously at the University Medicine and Dentistry of New Jersey.

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___x_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    Dr. Amos Mbugua, although not directly supported by the grant, was brought in from another institution to work on this project. Dr. Mbugua developed techniques for tracking red blood cells. These techniques were new to our laboratory and thus expanded the quality of our work. In addition, because of this we had the opportunity to establish new relationships with investigators in the Cancer Institute.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Overview

The broad research objective was to develop interventions to prevent the destruction of uninfected red cells by the host innate immune system during malaria infection.

Specific Aims:
1. Identify the red cell ligands and macrophage receptors involved in phagocytosis of red cells in a rodent SMA model developed in our laboratory.
2. Determine the roles of malaria pigment (hemozoin) and glycosylphosphatidylinositol (GPI) in stimulating in vitro erythrophagocytosis and identify reagents to block this effect.

To achieve the above aims we studied the role of red cell ligands and macrophage receptors on the in vitro phagocytosis of red cells from mice infected with rodent malaria using a new model of SMA developed in our laboratory. We planned to block specific ligands and receptors using lectins, receptors, and antibodies that have been shown to be effective in blocking the phagocytosis of aged or C3b-coated red cells. In addition, we planned to study the role of GPI and hemozoin in the stimulation of macrophage-dependent phagocytosis as well as the effect of toll-like receptor (TLR) inhibitors and inhibitors of the transcription factor NF-κB on the erythrophagocytosis of GPI and hemozoin-stimulated macrophages. We hoped to identify compounds that potentially could be used for the treatment of SMA and that could be tested in mice in vivo in subsequent studies.

Summary of Research Completed
The focus of our investigation on erythrophagocytosis in our rodent SMA model involved the use of labeled red cells whose florescence signal increased as a result of internalization by macrophages. The outline of our approach was as follows:
a) Testing of three florescent dyes (cypHer5E, pHrodo and CFSE) for red cell staining
b) Phagocytosis experiments using the *P.chabaudi*, *P.berghei* and SMA (*P.chabaudi* and *P.berghei*) models
c) Assessing the effect of potential phagocytosis modulators such as, pigment (hemozoin) and glycosylphosphatidylinositol (GPI)

Methods
RBC staining for monitoring phagocytosis
We optimized the staining of red cell cells using two pH-sensitive (cypHer5E and pHrodo) and one pH-independent dye (carboxyfluorescein diacetate succinimidyl ester, CFSE). Both cypHer5E and pHrodo floresce minimally in neutral environments but exhibit increased red florescence in acidic environment such as in the endosomes formed after particle internalization.

CFSE on the other hand passively diffuses into cells and remains colorless and non-fluorescent until its acetate groups are cleaved by intracellular esterases. The green florescent conjugates that bind strongly to intracellular amines are retained by the cells following cell divisions and are ideal for tracking cells for weeks.

The procedure for labeling red cells for monitoring phagocytosis began with harvesting blood from a mouse by cardiac puncture using citrate-phosphate-dextrose (CPD) as the anti-coagulant. The blood was centrifuged, plasma removed and a 5% (v/v) hematocrit suspension prepared using phosphate buffered saline (PBS), pH 7.4. Dye was added to the 5% blood suspension to a final dilution of 1:250 (cypHer5E and pHrodo) or 1:600 (CFSE). This was mixed, protected from light by covering the tube(s) with aluminium foil and incubated for 30 min on a rocking platform. For CFSE stained red cells the reaction was stopped using an equal volume of fetal bovine serum (FBS). The stained red cells were then centrifuged and washed three times with PBS and then analyzed by flow cytometry (Figure 1). After testing the three dyes we proceeded to use pHrodo in our erythrophagocytosis assays.

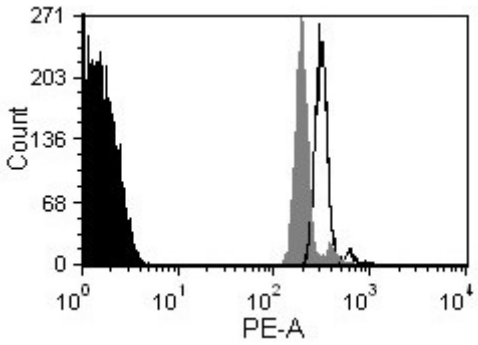

Figure 1. pHrodo stained red cells. Histograms from flow cytometry analysis of unstained (black) and pHrodo stained red cells at pH 7 (gray) and pH 4 (white).

In vitro red cell phagocytosis assay

The protocol for determining *in vitro* erythrophagocytosis was as follows. First, macrophages were obtained by cell culture (J774) or isolated from C57BL/6 mice (splenic, peritoneal, PBMCs and bone marrow) using standard procedures. Murine macrophages were harvested from both uninfected and *P. chabaudi*, *P.berghei* or *P. chabaudi* -*P.berghei*-infected (Anemia model) mice. Red cells were also collected from these mice as needed. Three populations of red blood cells were used in our studies: uninfected RBCs from uninfected mice (uiRBC/uiM), uninfected RBCs from infected mice (uiRBC/iM) and infected RBCs from infected mice (iRBC/iM). Separation of infected and uninfected red cells from infected animals was achieved using Percoll/sorbitol density gradients with Percoll densities of 80%, 70%, 60%, 40% and 20% (Figure 2).

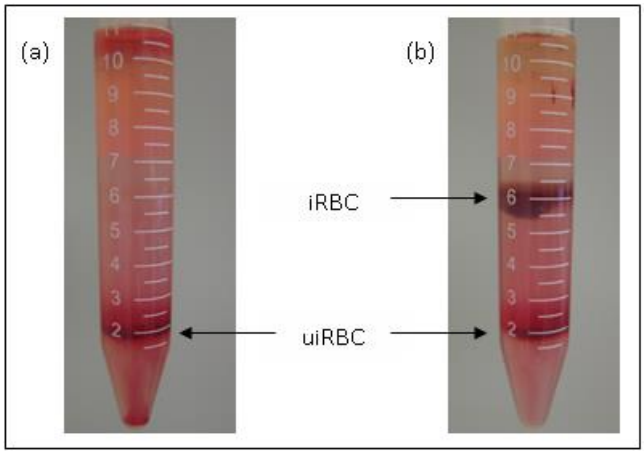

Figure 2. Percoll gradient separation of infected and unifected red cells. Uninfected red cells from uninfected mouse (a) and infected alongside uninfected red cells from an infected mouse (b).

The positive control for red cell phagocytosis were uninfected red cells from an uninfected mouse (uiRBC/uiM) coated with a monoclonal against mouse red cell glycophorin (anti-TER 119) while anti-TER

119-coated RBCs co-incubated with cytochalasin D (a potent inhibitor of phagocytosis) were used as the negative control. A typical assay was set up as follows: on the day before the phagocytosis assay, macrophages cultured in DMEM/10%HI-FBS were plated at a concentration of 1 x 10^6 per well on each of six wells on a 24-well plate to be analyzed by flow cytometry. For comparison between 4 x 10^4 and 1 x 10^5 of the same macrophages were added to each of six wells on an 8-well Labtek chamber slide to be analyzed my light microscopy. The next day the old medium was replaced with fresh medium and in selected wells medium containing 50 uM cytochalasin D added 30 minutes prior to addition of pHrodo-labeled red cells. Red blood cells were then added to the macrophage at a ratio of 10:1 to both the 24-well plate and the 8-well chamber slide. These were then placed on a rotating shaker and incubated at 37ºC, 5%CO_2 for 2 hours. Nonadherent red cells were then washed off by replacing the media with RPMI. After removal of the RPMI the macrophages on the 24-well plates were detached using cold PBS/10mM EDTA/1%BSA. To prepare them for analysis by flow cytometry an equal volume of 2% paraformaldehyde was added and the suspension transferred to 5 ml polystyrene round bottom test tubes. The detachable chambers on the 8-well slides were removed and the macrophages fixed with methanol before staining with Giemsa for viewing by light microscopy.

Results

We investigated whether there was an increased susceptibility to phagocytosis of uninfected red cells obtained from infected mice as compared to those from uninfected mice. This was tested in three mouse models: *P.chabaudi*, *P.berghei* and the *P. chabaudi-P. berghei* SMA model The dynamics of these infections differed with the peak parasitaemias of 40% noted in both the *P.chabaudi*, and SMA models (Fig.3a and 4a) compared to 15% in the *P.berghei* model (Fig 5a). Anemia was most pronounced in the *P.chabaudi* (60% reduction in RBC count, Fig.3b), and SMA models (50% reduction in RBC count, Fig 4b) while it was less (20% reduction in RBC count, Fig. 5b) in the *P.berghei* model. Most of the experiments were done using J774 macrophages as the effector cells while a number of experiments were performed with macrophages (splenic, peritoneal or PBMCs) harvested from our anemia model while one investigation was conducted on macrophages from the *P.berghei* model. Similarly, the majority of the red cells

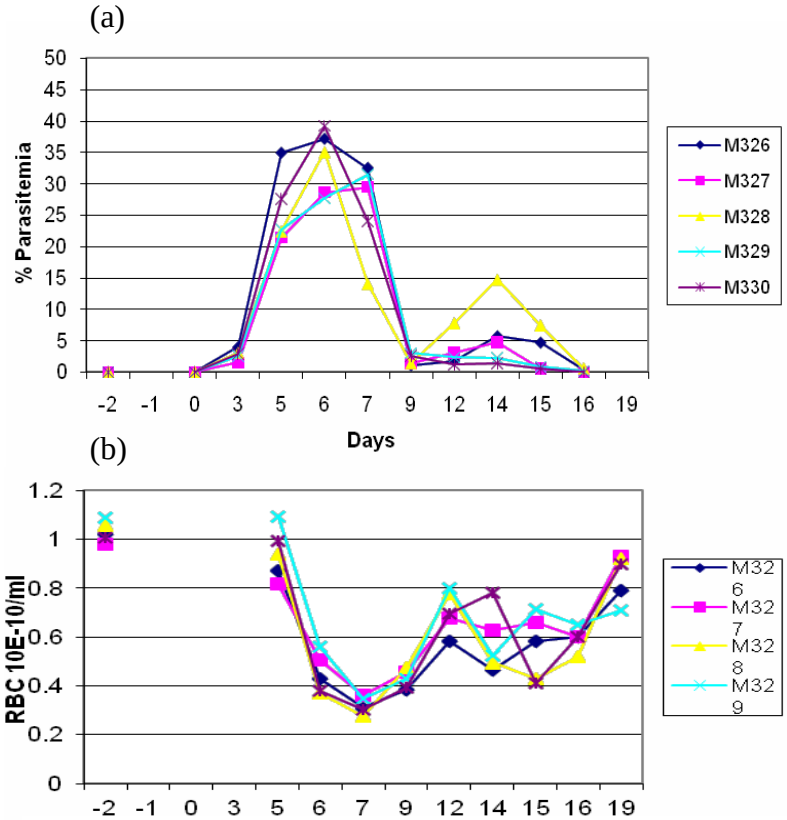

Figure 3. *P.chabaudi* mouse model infection dynamics. Following infection of C57 Bl/6 mice with *P.chabaudi* the parasitaemia peaked between 30 – 40% on Day 6 and then declined to baseline levels on Day 9 (a). Red cell counts dropped by over 60% by Day 7 and gradually returned to normal levels by Day 19

used for the assays were obtained from our SMA model given that of the three models we interrogated it is most representative of malaria anemia in humans.

In the *P.chabaudi* model the higher level of phagocytosis of uiRBC/iM compared to uiRBC/uiM was consistent across the three time points tested with the greatest difference observed pre-peak parasitemia (Day 5) with phagocytosis of uiRBC/iM at 38% and that of uiRBC/uiM at 12% (Figure 6). No such difference in susceptibility to phagocytosis was observed in either the *P.berghei* or SMA (*P.chabaudi* and *P.berghei*) models (Figures 7 and 8). Overall the extent of erythrophagocytosis was much lower in macrophages isolated from the infected mice in comparison to the J774 cell line.

We also tested potential modulators of phagocytosis. The assumption was that such modulators may be essential in onset and sustenance of phagocytosis *in vivo*. These included malaria pigment (hemozoin), human TNF and spleen cytokines from a Day 5 post-infection anemia model mouse. None of these however decisively inhibited or increased the extent of phagocytosis in our positive control (J774 macrophages ingesting anti-TER 119-coated RBCs) and neither did they enhance phagocytosis on our SMA model. Given that we did not detect any significant phagocytosis in our SMA model we could not achieve one of our aims namely, to identify red cell ligands and macrophage receptors involved in this process.

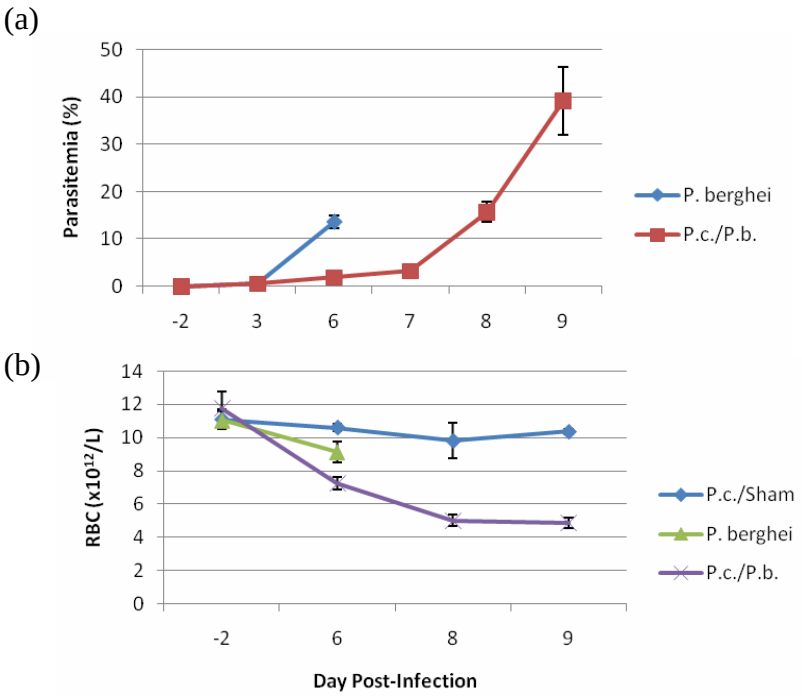

Figure 4. Anemia mouse model infection dynamics. Sequential infection of C57 Bl/6 mice with *P.chabaudi* a nd then later with *P.berghei* resulted in a peak parasitaemia of 40% on Day 9 (a). Red cell counts dropped by almost 50% by Day 8 (b).

Discussion and Conclusions:

Our results suggest that uninfected red cells *P. chabaudi* –infected mice are more susceptible to erythrophagocytosis. This is contrary to our expectations since red cell destruction due to hyperparasitemia appears to be the main mechanism in the pathogenesis of anemia in the *P. chabaudi* model (Figure 3). Furthermore, our findings do not provide any evidence for increased susceptibility of uninfected red cells to erythrophagocytosis in either the *P.berghei* or SMA (*P.chabaudi* and *P.berghei*) models. One potential explanation is that the target cells may not be optimal. We have demonstrated that in the *P. chabaudi-P. berghei* model a large part, if not most, of the erythrophagocytosis takes place in the liver by Kupffer cells. J774 cells are of monocyte origin. Kupffer cells may have a set of receptors distinct from other macrophages and therefore, we may have missed this interaction. Therefore, further studies will concentrate on the use of primary hepatic cell Kupffer cells.

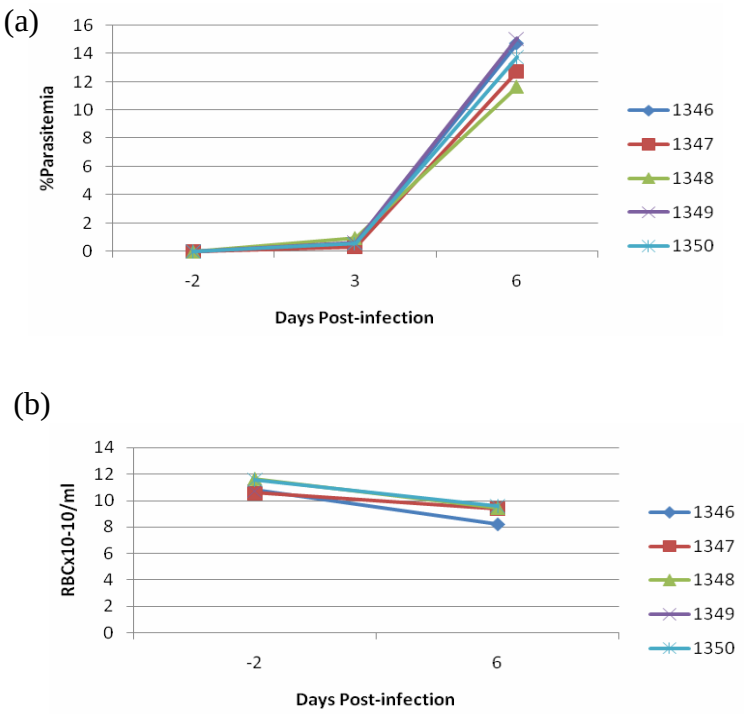

Figure 5. *P.berghei* mouse model infection dynamics. Following infection of C57 Bl/6 mice with *P.berghei* the parasitaemia peaked at 15% on Day 6 and the mice did not recover from this lethal infection (a). Red cell counts dropped by almost 20% on Day 6 with no recovery to normal levels (b).

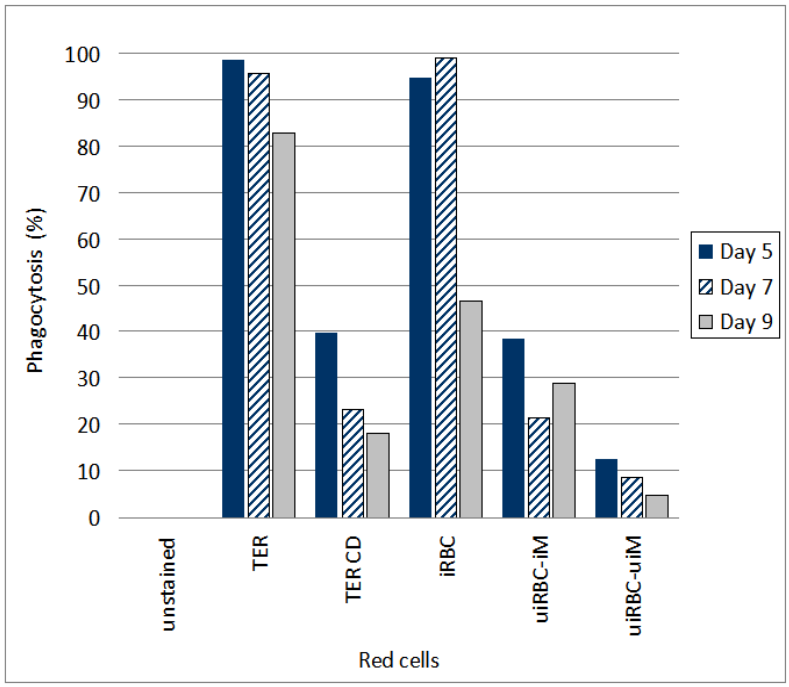

Figure 6. Erythrophagocytosis in *P.chabaudi* mouse model. The percent phagocytosis by J774 macrophages using red cells from *P.chabaudi*-infected mice (iRBC and uiRBC-iM), from uninfected mice (uiRBC-uiM) and anti-TER 119-coated RBCs co-incubated with (TER CD) and without cytochalasin D (TER)

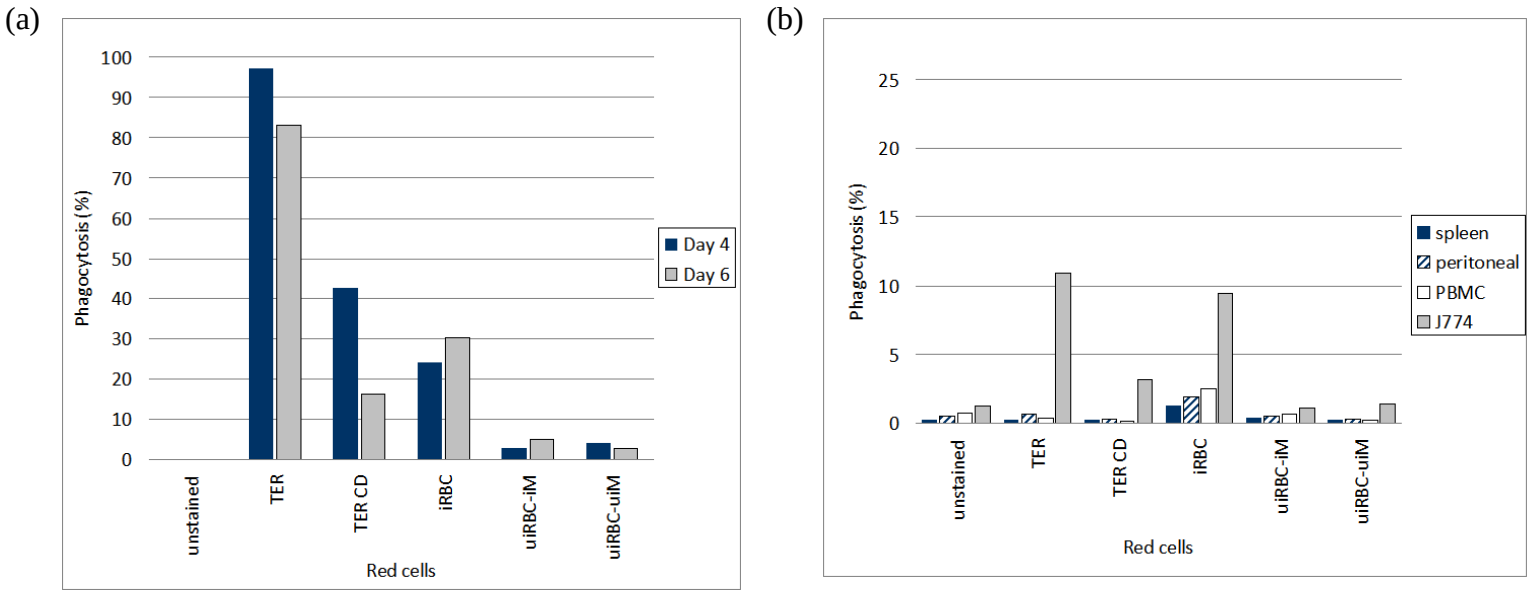

Figure 7. Erythrophagocytosis in *P.berghei* mouse model. The percent phagocytosis by J774 macrophages (a) and by splenic, peritoneal macrophages, or PBMCs from *P.berghei*-infected mice (b) using red cells from *P.berghei*-infected mice (iRBC and uiRBC-iM), from uninfected mice (uiRBC-uiM) and anti-TER 119-coated RBCs co-incubated with (TER CD) and without cytochalasin D (TER)

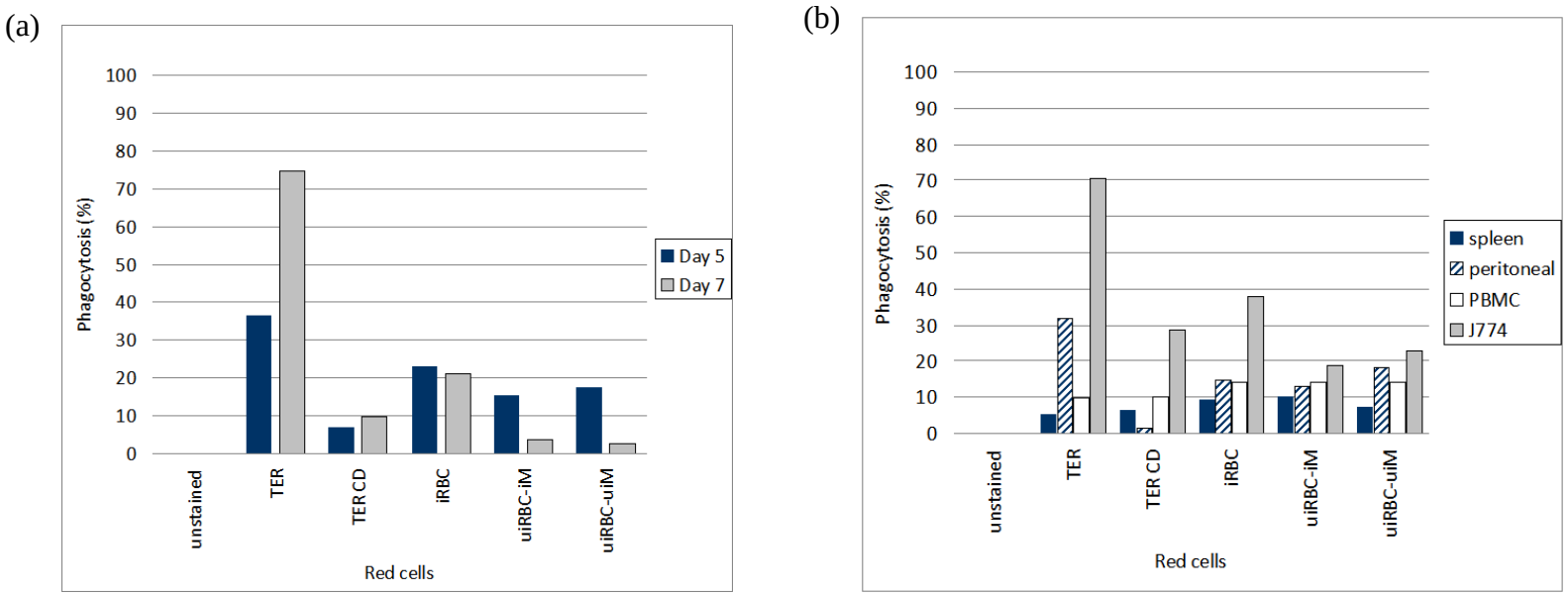

Figure 8. Erythrophagocytosis in Anemia mouse model. The percent phagocytosis by J774 macrophages (a) and by splenic and peritoneal macrophages and PBMCs from *P.chabaudi/P.berghei*-infected mice (b) using red cells from *P.chabaudi/P.berghei*-infected mice (iRBC and uiRBC-iM), from uninfected mice (uiRBC-uiM) and anti-TER 119-coated RBCs co-incubated with (TER CD) and without cytochalasin D (TER)

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__x____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two

publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Sequential Plasmodium chabaudi and Plasmodium berghei Infections Provide a Novel Model of Severe Malarial Anemia	J Harris, TM Bohr, C Stracener, ME Landmesser, V. Torres, A Mbugua, C Moratz, JA Stoute	Infection and Immunity	Nov. 2011	☐Submitted ☐Accepted ☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No____x_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention,

diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
José A. Stoute	Associate Professor Medicine, Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login) jstoute	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of South Florida, Tampa, FL	B.A.	1978-1981	Biology
University of Miami School of Medicine, Miami, FL	M.D.	1981-1985	Medicine
Emory University, Atlanta, GA	Residency	1985-1988	Internal Medicine
Walter Reed Army Medical Center, Washington, DC	Fellowship	1991-1994	Infectious Diseases

A. Personal Statement

I am a physician scientist. My clinical training is internal medicine and infectious diseases. I received further training in basic science and laboratory research throughout my career to include my infectious diseases fellowship at the Walter Reed Army Institute of Research. Following my infectious diseases fellowship in 1994 I led the first successful trial of the RTS,S malaria vaccine. Following this success, I relocated to Kenya with the US Army where I became interested in the problem of severe malarial anemia and began to study the role of complement and complement receptors in the pathogenesis of malaria. In particular, I focused on the role of the complement receptor 1 (CR1). My laboratory has made a number of important and novel observations about the role of this receptor and complement in severe malaria and is now recognized as one of the leading laboratories in the field. Our experience working with CR1 in the area of pathogenesis led us to be the first laboratory to identify this receptor's role in invasion of red cells. We recently develop an animal model of severe malarial anemia and we are investigating this model to learn more about the pathogenesis of this illness in particular the mechanisms of erythrophagocytosis.

B. Positions and Honors

Professional Experience:

1988-1991 Staff, General Internal Medicine, Darnall Army Community Hospital, Ft. Hood, TX. Chairman, Critical Care Committee and Member, Infection Control Committee.
1990-1991 Battalion Surgeon, Task Force 1-8, 1st Cavalry Division, Persian Gulf War.
1994-1997 Infectious Disease Officer, Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.
1994-1997 Staff, Infectious Disease Service, Walter Reed Army Medical Center, Washington, DC.
1994-2005 Assistant Professor, Dept. of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland.
1997-2003 Chief, Malaria Immunology, US Army Medical Research Unit, Kenya.
2003-Present Principal Investigator, Dept. of Cellular Injury, Division of Military Casualty, Walter Reed Army Institute of Research.
2005- Present Associate Professor, Uniformed Services University of the Health Sciences, Bethesda, Maryland.

2007-2008 Chief, Malaria Pathogenesis Section, Division of Malaria Vaccine Development, Walter Reed Army Institute of Research
2008-Present Associate Professor, Department of Medicine, and Microbiology and Immunology, Pennsylvania State College of Medicine, Hershey, PA.

Honors:

1980 - Received Dr. Sylvan Myer's Award for Outstanding Achievement in Physics and Mathematics, University of South Florida.
1981 - Finished undergraduate studies as Magna Cum Laude.
1985 - Received M.D. degree with an additional distinction for scientific research performed.
1985-1988 Residency training in Internal, Medicine Emory University Affiliated Hospitals, Atlanta, Georgia.
1994 - Bailey K. Ashford Clinical Research Award, Walter Reed Army Medical Center
1998 - WRAIR Association, Edward L. Buescher Young Scientist Award.
2006 Order of Military Medical Merit

Professional Certifications:

1988 - Diplomate of the American Board of Internal Medicine.
1994- Diplomate of the American Board of Internal Medicine in the Subspecialty of Infectious Diseases.
2005- Recertification in the Subspecialty of Infectious Diseases

Medical Licensures

Georgia 029798 Expiration 4/30/2012
Pennsylvania MD434601 Expiration 12/31/2012

Professional Societies:

Member of the American College of Physicians.
Member of the American Society of Tropical Medicine and Hygiene.
American Association for the Advancement of Science.
Member of the Infectious Disease Society of America.

C. Selected Peer-reviewed Publications (out of 33 total)

1. Waitumbi JN, Opollo M, Misore A, Muga R, **Stoute JA.** Red Cell Surface Changes and Erythrophagocytosis in Children with Severe *Plasmodium falciparum* Anemia. Blood 95(4):1481-1486. 2000.
2. **Stoute JA,** Odindo AO, Owuor BO, Mibei EK, Opollo MO, and Waitumbi JN**.** Loss of red blood cell complement regulatory proteins and increased levels of circulating immune complexes are associated with severe malarial anemia. Jour Infect Dis 187:522-525, 2003.
3. Schwenk R**,** Ahser LV, Chalom I, Lanar D, Sun P, White K, Keil D, Kester KE, **Stoute J**, Heppner DG, and Krzych U. Opsonization by antigen-specific antibodies as mechanism of protective immunity induced by *Plasmodium falciparum* sporozoite protein-based vaccine. Parasite Immunol 25:17-25, 2003.
4. Ohas EA, Adams JH, Waitumbi JN, Orago ASS, Barbosa A,[3] Lanar DE, **Stoute JA**. Measurement of Antibody Levels Against Region II of the Erythrocyte Binding Antigen 175 (EBA-175) of *Plasmodium falciparum* in a Malaria Holoendemic Area of Western Kenya. Infect Immunity. 72:735-741,2004.
5. Waitumbi JN, Donvito B, Kisserli A, Cohen JH, **Stoute JA**. Age-related Changes in Red Blood Cell Complement Regulatory Proteins and the Susceptibility to Severe Malaria. J Infect Dis. 190:1183-1190, 2004.
6. Mibei EK, Orago AS, and **Stoute JA**. Immune complex levels in children with severe Plasmodium

falciparum malaria. Am J Trop Med Hyg 2005; 72:593-9.
7. **Stoute JA**. Complement-regulatory proteins in severe malaria: too little or too much of a good thing? Trends Parasitol 2005; 21:218-23.
8. Thathy V, Moulds JM, Guyah B, Otieno W, **Stoute JA**. Complement receptor 1 polymorphisms associated with resistance to severe malaria in Kenya. Malar.J 2005;4:54.
9. Ryan JR, **Stoute JA**, Amon J, Dunton RF, Mtalib R, Koros J et al. Evidence for transmission of Plasmodium vivax among a duffy antigen negative population in western Kenya. Am J Trop Med Hyg 2006; 75(4):575-581.
10. **Stoute JA**, Heppner DG, Jr., Mason CJ, Siangla J, Opollo MO, Kester KE, Vigneron L, Voss G, Walter MJ, Tornieporth N, Cohen JD, Ballou WR. Phase 1 safety and immunogenicity trial of malaria vaccine RTS,S/AS02A in adults in a hyperendemic region of western Kenya. Am.J.Trop.Med.Hyg. 2006;166-70.
11. **Stoute,J.A.**, Gombe,J., Withers,M.R., Siangla,J., McKinney,D., Onyango,M., Cummings,J.F., Milman,J., Tucker,K., Soisson,L. *et al.* (2007) Phase 1 randomized double-blind safety and immunogenicity trial of Plasmodium falciparum malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya. *Vaccine*, **25**, 176-184.
12. Owuor BO, Odhiambo CO, Otieno WO, Adhiambo C, Makawiti DW, **Stoute JA**. Reduced immune complex binding capacity and increased complement susceptibility of red cells from children with severe malaria-associated anemia. Mol Med 2008 Mar;14(3-4):89-97.
13. Mibei E, Otieno WO, Orago ASS, **Stoute JA**. Distinct Pattern of Class and Subclass Antibodies in Immune Complexes of Children with Cerebral Malaria and Severe Malarial Anemia. Parasite Immunol In Press.
14. Bull PC, Buckee CO, Kyes S, Kortok MM, Thathy V, Guyah B, **Stoute JA**, Newbold CI, Marsh K. Plasmodium falciparum antigenic variation. Mapping mosaic var gene sequences onto a network of shared, highly polymorphic sequence blocks. Mol Microbiol. 2008 Apr 28.
15. Odhiambo CO, Otieno W, Adhiambo C, Odera MM, **Stoute JA**. Increased deposition of C3b on red cells with low CR1 and CD55 in a malaria endemic region of western Kenya: Implications for the development of severe anemia. BMC Med 2008 6:23.
16. Ogonda LA, Orago AS, Otieno MF, Adhiambo C, Otieno W, **Stoute JA**. The levels of CD16/Fc gamma receptor IIIA on CD14+ CD16+ monocytes are higher in children with severe Plasmodium falciparum anemia than in children with cerebral or uncomplicated malaria. Infect Immun 2010; (78): 2173-2181.
17. Spadafora C, Awandare GA, Kopydlowski KM, Czege J, Moch JK, Finberg RW, Tsokos GC, **Stoute JA**. Complement Receptor 1 Is a Sialic Acid-Independent Erythrocyte Receptor of *Plasmodium falciparum*. PLoS Pathog 2010 Jun 17;6(6):e1000968.
18. Awandare GA, Spadafora C, Moch JK, Dutta S, Haynes JD, **Stoute JA**. Plasmodium falciparum field isolates use complement receptor 1 (CR1) as a receptor for invasion of erythrocytes. Mol.Biochem.Parasitol. 2011; 177:57-60.
19. **Stoute JA**. Complement Receptor 1 and Malaria. Cell. Microbiol. 2011; 13(10):1441-1450.
20. Odera M, Otieno W, Adhiambo C, **Stoute JA**. Dual role of complement receptor type 1 in immune complex-mediated macrophage stimulation: Implications for the pathogenesis of Plasmodium falciparum malaria. Clin Exp Immunol. 2011;166(2):201-207.