Developing new malaria vaccine

Professor Moriya Tsuji tells International Innovation about the efforts to 'wipe malaria off the face of the earth'

Can you explain about the background to your research, your aims and where the concept came from?

The most advanced malaria vaccine candidate so far is called RTS,S vaccine, which consists of a fusion protein of a portion of the circumsporozoite (CS) protein with the hepatitis B surface antigen, incorporated in a mixture of three adjuvants. This vaccine is now in Phase III trial. However, the efficacy of this vaccine as of Phase II trials has been around 50-60 per cent against disease, and the protective immunity seems to be primarily mediated by humoral response. Therefore, for the purpose of improving the efficacy of malaria vaccine, we are aiming to develop a new malaria vaccine that can induce; 1) cell-mediated immunity (CMI) against 2) novel malarial antigen(s).

PROFESSOR VICTOR NUSSENZWEIG

How did you first become involved with the programme and what has your input been so far?

I studied T cell biology in graduate school almost 22-26 years ago. After my graduation, my mentor, Prof. Tomio Tada at the University of Tokyo in Japan, sent me to the Department of Medical & Molecular Parasitology at New York University School of Medicine headed by Prof. Ruth Nussenzweig, the wife of Prof. Victor Nussenzweig, where I studied cell-mediated immunity (CMI) against malaria, as well as the development of T cell-based malaria vaccine.

Since then, I determined the roles of various T cell subsets, including CD4+ T cells, gamma-delta T cells and natural killer T (NKT) cells, in protective immunity against malaria in a mouse model. I have also determined that adenovirus is an excellent vector for inducing protective immunity against malaria.

What is the wider impact of your research?

Once we identify a new malarial antigen that can elicit protective CMI, then the antigen should play a pivotal role in complementing the existing RTS, S vaccine for a SUCCESSFUL malaria vaccine.

Are there any 'partners' involved in your research? If so, can you explain their expertise, what they contribute and what they will gain from their involvement?

Apart from the NIH, we have been collaborating with GlaxoSmithKline (GSK) for the identification of a new malarial antigen. Since GSK has been the leading force for testing the efficacy of RTS,S vaccine in children, it is logical for us to team up with them to identify new antigenic targets for a malaria vaccine.

Furthermore, has your organisation collaborated with international partners – has the collaborative approach worked?

In the past, my group had collaborated with the Crucell Holland B.V, and together, we found that adenovirus serotype 35 (Ad35) can be a potent vaccine vector as a malaria vaccine. This study became a basis for the Crucell to move onto some of the currently ongoing clinical trials using Ad35 vector for a malaria vaccine.

What are the expectations and objectives of the project?

Malaria is a scourge of humanity, and, hence, this project is vital in order to save millions of lives of innocent newborn children every single year. A successful malaria vaccine developed by this project may not only save these children's lives, but also ultimately eradicate this globally devastating infectious disease.

How it has progressed thus far?

We have identified a few candidate malarial antigens that may facilitate the development of successful malaria vaccines.

Can you tell us if you faced any major challenges?

Malaria parasite posses more than 5000 genes and thousands of potential antigens for a vaccine, and it has been very difficult for us to narrow down and identify the "mighty" antigen that can mount a long lasting protective immunity by way of a vaccine.

What is the expected output of the project?

With more funding and cutting edge technologies, we are determined to identify "novel" and "protective" malarial antigens that will lead to wiping malaria off the face of the earth.

Improving malaria pre-erythrocytic vaccines

When the World Health Organisation (WHO) estimates that 2-3 million children die of malaria each year, the search to find an effective vaccine is increasingly important. Moriya Tsuji & Victor Nussenzweig describe the process of their research in this vital field of expertise

MALARIA IS A severe disease that ranks among the most prevalent infections in tropical areas throughout the world. Approximately 300-500 million people become infected yearly, with relatively high rates of morbidity and mortality. Severe morbidity and mortality occur particularly in young children and in adults migrating to a malaria endemic area without having undergone prior malaria exposure. The WHO estimates that between two and three million children die of malaria every year in Africa alone.

Malaria infection starts when mosquitoes inject sporozoites into the skin. The parasites enter the bloodstream, and after reaching the liver, develop into exoerythrocytic forms (EEFs) inside hepatocytes. The EEFs mature and then divide rapidly to form thousands of merozoites that re-enter the blood and infect erythrocytes, causing the disease we recognise as malaria.

The widespread occurrence and the increasing incidence of malaria in many countries are caused by drug-resistant parasites (Plasmodium falciparum, recently also P. vivax), insecticide-resistant vectors (Anopheles mosquitoes), and economic/political deterioration in many African countries. These underscore the need for developing new methods for the control of this disease. However, one complication is that during its life cycle the parasite undergoes many changes, each associated with a diverse set of stage-specific protective antigens. Most vaccine efforts are directed against the pre-erythocytic stages (sporozoites and EEFs), and blood stages. These vaccines aim at preventing the progression of the life cycle of the parasites or decreasing the severity of the disease. Other vaccines target the mosquito stages of the parasite and aim at the interruption of malaria transmission.

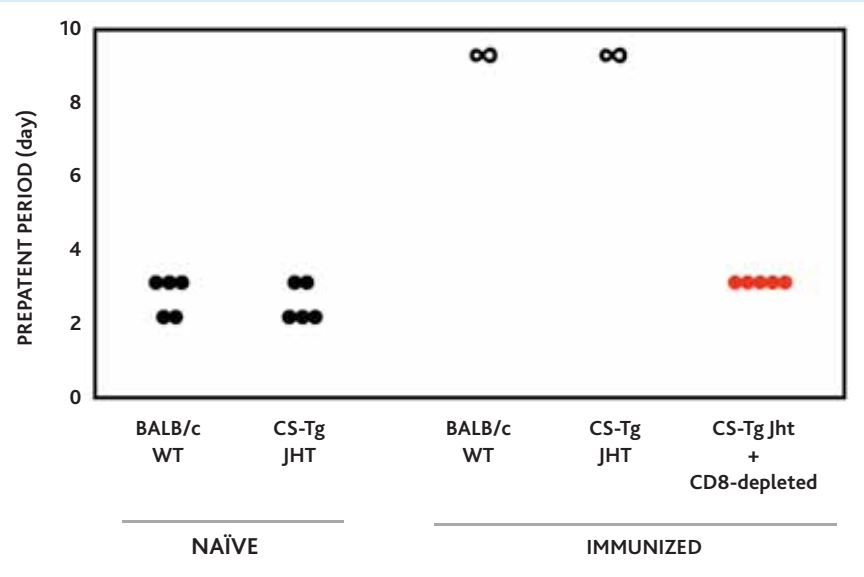

FIGURE ONE. MULTIPLE IMMUNISATION OF CS-TG JHT MICE WITH IRRADIATED SPOROZOITES INDUCED PROTECTIVE ANTI-MALARIAL IMMUNITY THAT IS MEDIATED BY CD8+ T CELLS.

The concept that a malaria vaccine is feasible was shown in the late 1960s by the demonstration that the repeated immunisation of rodents with irradiated sporozoites (IrSp) led to complete protection against sporozoite challenge. This was followed by immunisation of human volunteers with the bite of P. falciparum infected and radiation-attenuated Anopheles mosquitoes. Protection was stage-specific, i.e. vaccination did not protect against challenge with blood stages of the parasites, and was species-specific. Importantly, IrSp vaccination was effective not only against the P. falciparum parasite isolate used for vaccination, but also against parasite isolates from different geographical isolates.

Protection mediated by IrSp is multifactorial: it involves sporozoite neutralisation by antibodies and various T cell subsets that inhibit EEF development. The best characterised neutralising antibodies are directed against the immunodominant, species specific, repeat domains of the circumsporozoite (CS) protein. In P. falciparum, the repeats (NANP)n are conserved among isolates from all areas of the world. Monoclonal antibodies or Fab fragments that recognise the CS repeats inhibit sporozoite motility and invasion. Although studies in rodents, monkeys and humans demonstrate that antibodies alone can protect against malaria infection, complete protection is only achieved if the serum concentration of antibodies is sufficiently high to prevent the entry of all sporozoites in hepatocytes. The parasites that escape neutralisation and enter hepatocytes can only be the eliminated by effector T cells. The CD4+ and CD8+ T cell epitopes of the CS protein of P. falciparum have been extensively studied both in naïve individuals immunised with IrSp, and in individuals living in malaria endemic areas.

The CS-based vaccine currently undergoing human trials is named RTS,S, which is a fusion protein of a portion of the CS with the hepatitis B surface antigen, incorporated in a

INTELLIGENCE

CONTACT

Professor Moriya Tsuji
Associate Professor/Staff Investigator

455 First Avenue Room 747
New York, NY 10016 USA

SPECIALTIES

Academic, Research Scientist
Allergy and Immunology

T (001) 212-448-5021
E mtsuji@adarc.org

www.adarc.org/research/moriya/

AFFILIATION

Rockefeller University/Aaron Diamond AIDS Research Center

Identification of novel synthetic analogues of alpha-GalCer, as an immuno-enhancing molecule for vaccines against HIV and malaria

Although many attempts have been made to develop HIV and malaria vaccines, they have been unsuccessful to date. The main reason for this failure is that the vaccines have not been immunologically strong, that is immunogenic enough to prevent the infection and/or to prolongue anti-HIV/malaria immunity. Therefore, it is essential to develop a compound that can significantly enhance the immunogenicity of HIV/malaria vaccines. Our previous studies have demonstrated that a CD1d molecule-binding, natural killer T (NKT) cell ligand, alpha-galactosylceramide (alpha-GalCer), can enhance the immunogenicity of vaccines against HIV and malaria in a mouse model. Most recently, we have discovered that modification of alpha-GalCer can results in compounds with stronger biological activity (NKT cell stimulating activity) than alpha-GalCer. Therefore, our main goal is to determine whether some of the alpha-GalCer analogues could display a far superior immuno-enhancing activity, thereby greatly improving the efficacy of vaccines against HIV and malaria.

mixture of three adjuvants. In the latest trial in Mozambique, three doses of RTS,S vaccine were given to 214 babies, and the trial resulted in a 65 per cent reduced risk of contracting new malarial infections. This latest trial, however, involved a relatively small number of babies, and statistical analysis shows that the efficacy of RTS.S in babies is not significantly different from that of previous trials in infants. Protection appears to be dependent on the serum levels of antibodies against the CS repeats, but there is some evidence for a role of T cells. Importantly, there was no evidence in the RTS,S trials that the lack of protection in some volunteers was due to infection with P. falciparum isolates whose T cell epitopes differed from those in the vaccine. The importance of the CS protein in protective immunity is highlighted by studies showing that the response to a universal CD4+ T cell epitope of the CS in non-immunised Gambian individuals correlates with protection against both infection and disease. It is conceivable that the efficacy and duration of protection of RTS,S can be increased by enhancing its immunogenicity, either by using more powerful adjuvants or by using prime/boosting systems where RTS,S is boosted with recombinant virus or other vectors expressing CS. Another approach would be to search for additional, powerful non-CS sporozoite/liver stage T cell antigens that could be used in combination with a CS-based vaccine (see below) or alone.

Using CS-transgenic JHT mice, in which immune response to the CS antigen is tolerated and, in addition, generation of antibodies is impaired, we have shown that CS antigen alone accounts for close to 90 per cent of the protection elicited by immunisation with IrSp of a rodent malaria parasite, Plasmodium yoelii. However, we have also found that hyper-immunising these mice with IrSp could induce a very strong protective anti-malaria immunity, which is mainly mediated by CD8+ T cells (see figure one). This finding underscores the presence of sub-dominant "minor" protective antigens in IrSp. Therefore, in this project, we seek to identify CD8+ T cell responses against some of the non-CS "minor" protective antigens generated by immunisation with IrSp.

We hypothesise that a "minor" protective antigen in the context of whole IrSp will turn out to be very immunogenic and protective if delivered in isolation. To test this hypothesis, we will first construct recombinant adenoviruses expressing new P. yoelii antigens that are orthologous to P. falciparum proteins. These antigens will be selected based on their highly transcribed profile in sporozoites, and their possession of a signal sequence and/or functional Pexel/VTS motifs. We will then determine the protective capacity of the new non-CS antigens by immunising mice with recombinant adenoviral vaccines, followed by a malaria challenge. We will also characterise the nature of CD8+ T cells induced by the non-CS antigens, using potential CD8+ T cell epitopes identified by the presence of the MHC class I-binding motifs within the amino acid sequence of the candidate antigens through NIH computer algorithm and murine malaria genome sequence databank.

The identification of novel malaria antigen(s) should facilitate the development of pre-erythrocytic malaria vaccines.

Reference

1. **Nussenzweig RS, Vanderberg J, Most H, Orton C:** Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. *Nature* 1967, 216:160-162
2. **Moorthy VS, Good MF, Hill AV**: Malaria vaccine developments. *Lancet* 2004, 363:150-156.
3. **Tsuji M, Zavala F**: T cells as mediators of protective immunity against liver stages of Plasmodium. *Trends Parasitol.* 2003, 19:88-93
4. **Aponte JJ, Aide P, Renom M**, *et al*. Safety of the RTS,S/AS02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomised controlled phase I/IIb trial. *Lancet* 2007 370:1543-1551.
5. **Reece WH, Pinder M, Gothard PK,** *et al*. A CD4(+) T-cell immune response to a conserved epitope in the circumsporozoite protein correlates with protection from natural Plasmodium falciparum infection and disease. *Nat. Med.* 2004, 10:406-410
6. **Kumar AK, Sano G, Boscardin S, Nussenzweig RS, Nussenzweig MC, Zavala F, Nussenzweig V**: The circumsporozoite protein is an immunodominant protective antigen in irradiated sporozoites. *Nature* 2006, 444:937-940
7. **Ophorst OJAE, Radosevic K, Havenga MJE, Pau MG, Holterman L, Berkhout B, Goudsmit J, Tsuji M**: Recombinant human adenovirus serotype 35-based malaria vaccine: Immunogenicity and protection against Plasmodium yoelii in mice. *Infect. Immun.* 2006, 74:313-320.