Bilateral Project of JSPS with Royal Society

"Evaluation of a novel human transmission-blocking vaccine using rodent parasites bearing Plasmodium vivax Pvs25 protein"

Description of project:

We have recently developed a new experimental vaccine vector system based on Autographa californica nucleopolyhedrosis virus (AcNPV) termed the "Baculovirus Dual Expression System", which drives expression of vaccine candidate antigens by a dual promoter that consists of tandemly arranged baculovirus-derived polyhedrin and mammalian-derived CMV promoters. This study utilised this system to express a *Plasmodium vivax* transmission-blocking immunogen (AcNPV-Dual-Pvs25). AcNPV-Dual-Pvs25 not only displayed Pvs25 on the AcNPV envelope, exhibiting aspects of its native three-dimensional structure, but also expressed appropriately immunogenic protein upon transduction of mammalian cells. Both intranasal and intramuscular immunization of mice with AcNPV-Dual-Pvs25 induced high Pvs25-specific antibody titres, notably of IgG1, IgG2a and IgG2b isotypes, indicating a mixed Th1/Th2 response. Importantly, sera obtained from subcutaneously immunized rabbits exhibited a significant transmission-blocking effect (96% reduction in infection intensity, 24% reduction in prevalence) when challenged with human blood infected with *P. vivax* gametocytes using the standard membrane feeding assay. Additionally, active immunization (both intranasal and intramuscular routes) of mice followed by challenge using a transgenic *P. berghei* line expressing Pvs25 in place of native Pbs25 and Pbs28 (clone Pvs25DR3) demonstrates a strong transmission-blocking response, with a 92.1% (intranasal) and 83.8% (intramuscular) reduction in oocyst intensity. Corresponding reductions in prevalence of infection were observed (88.4% and 75.5% respectively). This project therefore offers a novel tool for the development of anti-malarial transmission-blocking vaccines against the sexual stages of the parasite.

Departments and institutions involved:

Division of Cell and Molecular Biology, Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, Imperial College Road, London SW7 2AZ, UK.

Laboratory of Vaccinology and Applied Immunology, Kanazawa University School of Pharmacy Kakuma-machi, Kanazawa, 920-1192, Japan

Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok 10400, Thailand.

Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Ehime 790-8577, Japan.

How collaboration started:

Our relationship started in 2008 with an exchange of scientific information relating to transmission-blocking in *Plasmodium*. At Imperial College, we had developed novel chimeric rodent malaria parasites expressing human vaccine targets, allowing quick, easy and cheap screening of anti-malarial transmission blocking vaccines. Correspondingly, Prof. Yoshida's lab had characterised the use of the baculovirus dual expression system for the delivery of immunogens. An obvious synergy

between our laboratories quickly developed. Crucially, Prof. Yoshida and a student visited London under the funding of JSPS to further facilitate our collaboration.

Amount of money awarded:

4480,000 JPY (2009.4.1-2011.3.11)

How participants benefitted from the scheme:

During the project, both labs obtained knowledge regarding each other's traditional field of interest (developing chimeric parasites and baculoviral-based expression of vaccine targets). The work we carried out was successful, and the results offer a novel tool for the development of anti-malarial transmission-blocking vaccines. Results were successfully published (Intranasal and intramuscular immunization with Baculovirus Dual Expression System-based Pvs25 vaccine substantially blocks Plasmodium vivax transmission, Andrew M. Blagborough,, Shigeto Yoshida,, Jetsumon Sattabongkot, Takafumi Tsuboi, Robert E. Sinden, Vaccine 28 (2010) 6014–6020). Young post-doc researchers of both labs presented their data at International Conferences (Yamamoto et al., The 12th International Congress of Parasitology 2011, Melbourne, Australia; Blagborough et al., The 58th American Society of Tropical Medicine and Hygiene 2011, Washington DC, USA). Thus, our collaboration has encouraged and supported career buildings of young researchers.

What have been the collaborative developments since the project finished:

Our relationship is still continuing. Our initial research involved assessing the transmission blocking potential of a single-recombinant baculovirus expressing a single immunogen (Pvs25). Following the success of our first studies, we are using the baculovirus dual expression system to examine and characterise novel transmission-blocking antigens. We are additionally producing additional chimeric rodent malaria parasites to assess transmission-blocking vaccines in a quick, accurate and ethically acceptable manner. We feel that our research is a unique and highly complementary mix of parasitology and vaccinology, and are actively perusing additional projects and avenues of funding.

Has there been further applications to JSPS for funding:

To extend our relationship, we have applied a new Bilateral Project of JSPS with Royal Society, which aims to develop improved malaria transmission-blocking vaccines.

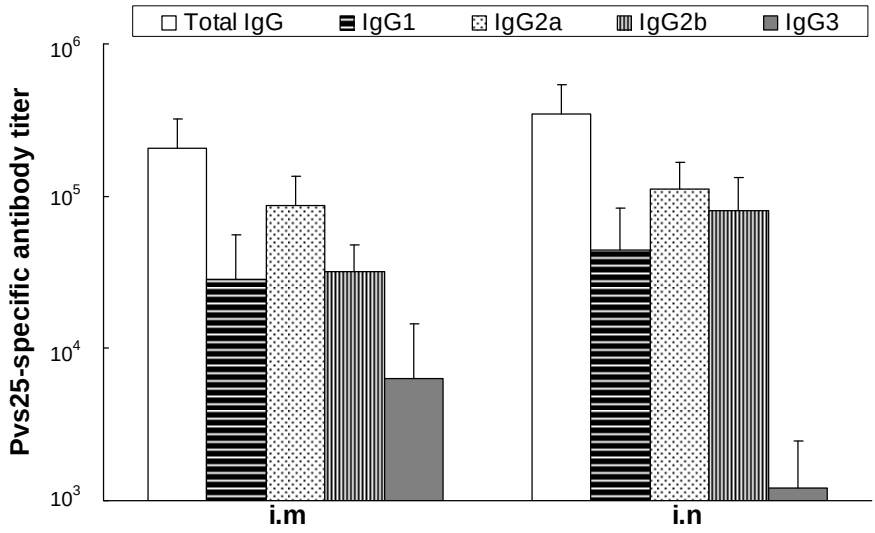

Fig 1: Pvs25-specific antibody responses. Sera were collected from individual mice (6 mice/group) three weeks after the final immunization. The individual sera were tested for total IgG, IgG1, IgG2a, IgG2b and IgG3 specific for Pvs25 by ELISA. The data represent one of two experiments, which had similar results. Data are the mean±S.E.M. of groups. Significant differences of total IgG titres between different groups were evaluated using the two-tailed Fisher's exact probability test. *, $p < 0.01$.

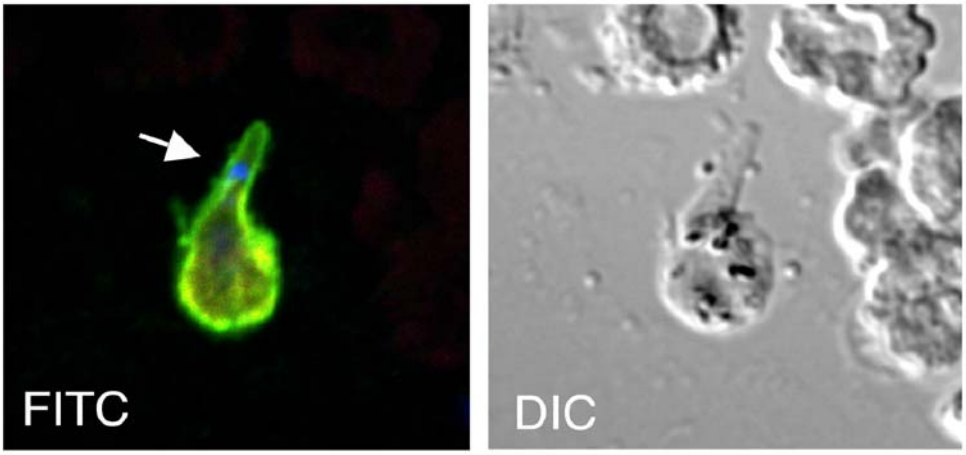

Fig. 2: Confocal fluorescence microscopy of sera obtained from mice immunized with AcNPV-Dual-Pvs25. The entire surface of cultured retorts/zygotes was clearly stained (green) by serum (1:500 dilution) obtained from a mouse immunized i.n. with AcNPV-Dual-Pvs25. Cell nuclei were visualized by blue DAPI staining (arrow) (immunofluorescence assay [IFA]). Right panel represents image obtained by differential interference contrast (DIC) microscopy. Scale bar, 10µm.