Unique immunization method provides insights about protective anti-malaria immune response

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In this week's *New England Journal of Medicine*, scientists in Singapore, The Netherlands and France report that they have developed a novel immunization method that will induce fast and effective protection in humans against the life-threatening malaria parasite, *Plasmodium falciparum*, which infects 350 to 500 million people world-wide and kills over one million people each year.

"It is not practical to apply the experimental method used in our study as a means of vaccination," said Laurent Renia, Ph.D., principal investigator at the Singapore Immunology Network (SIgN).

"But, this method of immunization could be applied successfully to similar investigations to find biological markers which would indicate the extent of protection against malaria. It would thus advance the currently limited knowledge of what constitutes protective anti-malaria immunity in humans," added Dr. Renia, who played a pivotal role in the research project by conceptualizing the experimental protocol and designing and conducting the follow-up experiments.

The scientists' experimental approach involved exposing two groups of healthy human subjects to mosquitoes once a month over a three-month period at the Radboud University Nijmegen Medical Centre in The Netherlands. One group (vaccine group) was exposed to mosquitoes infected with the malaria parasite, *P. falciparum*, and the second group

(control group) to uninfected mosquitoes

During the period of exposure, the study participants were treated with chloroquine, an anti-malaria drug that prevented *P. falciparum* from multiplying in the blood. Eight weeks after the last round of immunization and four weeks after the discontinuation of chloroquine administration, the participants in both groups were re-exposed to infected mosquitoes and tested for protection against *P. falciparum*. The four-week period was considered to be sufficient for chloroquine levels to drop below that which might inhibit parasite multiplication and malaria development.

The scientists found that all individuals in the vaccine group had acquired complete protection against the parasite, while those in the control group who did not receive immunization developed parasitemia (parasites in their blood).

This unique method of immunization allowed the human immune system to direct its response to eliminating the *P. falciparum* parasite at the earlier, liver stage of its life cycle. (Chloroquine kills the parasite at the later blood stage.) To induce an immune response, the scientists used malaria parasites that were whole and intact. Other methods have used genetically inactivated parasites or parasites that had been weakened by radiation to induce anti-malaria immunity.

The unique immunization method demonstrated a significant improvement over other experimental malaria vaccines that are currently used in clinical trials and that could induce up to only 50% protection in humans.

Using their novel approach, the scientists examined and gained important insight into the protective anti-malaria immune response in humans, which is difficult to acquire, whether through previous

exposure or vaccination. (Naturally acquired immunity to malaria develops over a period of 10 to 20 years and with repeated exposure to malaria parasites.)

By studying the antibodies, biological substances and cells present in the human subjects from the time of pre- to post-immunization, the scientists identified a specialised group of parasite-specific immune cells that indicated protection against *P. falciparum* in humans.

These immune cells, known as pluripotent effector memory T cells, which can mediate the removal of pathogens from the body, were found in the blood samples of subjects who had been immunized and re-exposed to *P. falciparum*. The control group did not have these specialized cells. These results indicate that these cells could serve as a biological indicator to check for malaria protection in humans during the stages of vaccine development.

"This is an elegant study which uses nature itself to tell us the answer to some basic questions regarding what can induce protective immunity against malaria," said Raymond Lin, M.D., senior consultant and Head of Microbiology at the Department of Laboratory Medicine of Singapore's National University Hospital.

"It shows that exposure to whole unmodified malarial parasites can protect against subsequent infection, while minimizing adverse events through the use of anti-malarial drugs," he added. "This provides hope for future vaccines and offers prospects of alternatives to conventional vaccine approaches. Also, the remarkable experiment studies infection in humans, using real parasites and real mosquitoes yet in a controlled and safe clinical trial setting. Future vaccine researchers will doubtless refer to this paper for guidance. Malaria is a major health threat in this region which Singaporeans are vulnerable to, so having world-class malaria expertise here is important to us."

SIgN Scientific Director Paola Castagnoli, Ph.D., said, "Professsor Renia has made some very significant findings that will contribute to a better understanding of the anti-malaria immune response in humans. His links with important international research centres and hospitals also demonstrate how collaborations that cross national borders can lead to fruitful and meaningful research outcomes. Certainly, such partnerships will help SIgN build up a strong platform in basic human immunology research that will better translate results into medical applications, and advance the search for cures to urgent medical problems."

Before joining SIgN, which is part of Singapore's A*STAR (Agency for Science, Technology and Research), in 2007, Dr. Renia was research director at France's INSERM ,and held appointments as co-director and director of the Department of Immunology at the Institut Cochin in Paris from 2001 to 2006.

At SIgN, Dr. Renia heads the Laboratory of Malaria Immunobiology and works closely with scientists and physicians at hospitals and centres in countries such as Thailand, where malaria is still a burden to public health authorities. Dr. Renia and his lab members travel to the border of Thailand and Myanmar, to conduct follow-up experiments to better understand the molecular basis of the disease.

Of the five malaria parasite species that can cause malaria in humans, Plasmodium falciparum (*P. falciparum*) is the most common cause of infection and is responsible for about 80% of all human malaria cases and about 90% of the deaths from malaria. The other four parasites are *P. vivax, P. malariae, P. ovale* and *P. knowlesi*.

More information: *NEJM* paper, "Protection against a malaria challenge by sporozoite inoculation", published in the 30 July 2009 issue.

Source: Agency for Science, Technology and Research (A*STAR)