Genocea's mark on malaria

By Benjamin Boettner, Senior Writer

Genocea Biosciences Inc. has identified an immunological imprint in malaria based on a cluster of *Plasmodium* epitopes that persists in patients for years after the last clinical episode. The findings suggest immune individuals could be a source of protective T cells, and the biotech will use a recent \$1.2 million grant from the **Bill & Melinda Gates Foundation** to identify biomarkers and create a vaccine.

The notion that malaria leaves a lasting memory on the immune system contradicts the common assumption that immunity to the *Plasmodium* parasites that cause the disease is short lived.

Indeed, most attempts to create malaria vaccines have failed to deliver broad protection and have had no significant impact on the footprint of the disease in sub-Saharan Africa, where it is endemic. The vaccines aim to either prevent people from becoming infected after being bitten by mosquitoes (anti-infection vaccines) or prevent

transmission of *Plasmodium* from people to mosquitos at infectious stages of the parasite's life cycle (transmission-blocking vaccines).

The *Plasmodium* life cycle has three stages: liver and blood stages that occur in the host and a sexual stage that takes place in the gut of the mosquito. Malarial disease—which occurs during the blood stage—is triggered when the parasite is released from the liver into the systemic circulation, where it invades erythrocytes.

Instead of focusing on specific *Plasmodium* antigens and testing their vaccine potential in mice, Genocea used its ATLAS technology to identify T cells that suggest a clinical history of the disease in the blood samples of test subjects.

By finding T cells with memory of disease, Genocea aims to identify antigens that cause the response and highlight which elements of the *Plasmodium* proteome are immunogenic. The idea is that those antigens will be the best candidates for producing a vaccine.

Genocea developed its technology to find antigens for diseases governed by T cell immunity that normally do not involve a notable humoral response and designed it around a high throughput screening process that mimics the immune response to antigens. The system uses an unbiased approach in which libraries of antigens for a given pathogen are expressed in *E. coli*. The antigens are fed to patient-derived antigen-presenting cells and then mixed with T cells from the same patient. If the patient has been previously exposed to the antigen and the T cell retains a memory of that exposure, the T cell responds by secreting interferon- γ (IFNG; IFN- γ) (see **Figure 1, "Genocea's ATLAS technology"**).

Figure 1. Genocea's ATLAS technology. Full-length proteins of the pre-erythrocytic *Plasmodium falciparum* proteome are generated in *Escherichia coli* that expresses cytosolic *Listeria monocytogenes* listeriolysin O (cLLO). Individual *E. coli* clones are then mixed with autologous antigen-presenting cells obtained from individuals who had resided in endemic malaria areas. The dendritic cells intracellularly process *P. falciparum* proteins into fragments by degradation in the proteasome, and transporter associated with antigen processing (TAP) mediates loading of a portion of the fragments onto molecules of major histocompatibility complex class I (MHC). Following translocation of the loaded MHC complexes to the cell surface, antigens are presented to CD8⁺ T cells derived from the same individual (autologous CD8⁺ T cell). If the T cell has encountered the same antigen before in the individual's history, it will secrete interferon- γ (IFNG; IFN- γ), which can be measured in a high throughput format.

Figure reproduced from ref. 1 with permission from **Genocea Biosciences Inc.**

Genocoea believes the strength of its strategy is that it can isolate antigens from thousands of possible candidates for each pathogen and select antigens that could provide protection across genetically diverse populations.

Platform for *Plasmodium*

To apply its platform to *Plasmodium falciparum*, Genocoea researchers prepared a bacterial expression library in *Escherichia coli* encompassing 735 of the 1,193 pre-erythrocytic-stage proteins. According to Jessica Baker Flechtner, Genocoea's SVP of research, those 735 were the proteins that the company could successfully express in *E. coli*.

Next, the company obtained monocytes and CD8⁺ T cells from blood samples donated by 100 individuals who immigrated to the U.S. from sub-Saharan areas with endemic *P. falciparum*.

The researchers differentiated the monocytes into dendritic cells capable of presenting antigens and, separately, expanded the CD8⁺ T cells nonspecifically. They then mixed *E. coli* clones expressing *P. falciparum* proteins with dendritic cells that processed and presented the cells for T cell recognition.

In the last step, the cells were exposed to the expanded CD8⁺ T cells using a multiwell plate format and IFN- γ levels were measured.

Computational analysis of the results revealed a discrete set of 15 antigens that were 14 times more likely to appear in individuals who previously had clinical malaria than in individuals who had remained free of the infection.

"There are experimental vaccines that use liver immunity by direct injection of immunogenic proteins into the liver and give rise to protective T cells," said Jean-Luc Bodmer, director of vaccine research at Genocoea. "Our system to some extent mimics the presentation of *Plasmodium* antigens by infected hepatocytes and the ensuing T cell response. It came as a big surprise to us that this was indeed measurable."

The findings were presented at the **American Society of Tropical Medicine and Hygiene** meeting in November.¹

Since presenting the findings at the meeting, Genocoea has constructed a bacterial expression library of over 1,600 pre-erythrocytic-stage proteins that it will screen in 2015, Baker Flechtner said.

But she told *SciBX* that it is not yet clear whether the reactive T cells provide protection. "We are therefore now looking at the converse scenario and are trying to identify *P. falciparum* signatures in individuals who do not get sick but have been naturally exposed to the agent," she said. "The data coming out of these studies can be superimposed with data from additional control settings."

Bodmer told *SciBX* that in these controlled settings, healthy subjects are infected with a fully infectious pathogen, which enables responses to be determined accurately.

Because malaria infection is fully curable if caught early, researchers have been able to use naïve human subjects as a safe "challenge model" to evaluate responses and potential vaccines. If a volunteer is not protected, the infection can be treated.²

"All of those samples and studies will be absolutely crucial to defining generally protective antigens," Bodmer said. "Each individual presented with the same protein will process it in a specific way. As a result, certain epitopes will come up within one subset but not in others. Thus, finding the right mix could provide considerable advantages to generating a vaccine."

Baker Flechtner told *SciBX* that the strategy has worked for Genocoea in other programs. "We have applied the same metrics

in our herpes simplex program, and a resulting vaccine was taken into people—the first vaccine ever that was shown to provide protection."

GEN-003, a T cell vaccine for treating patients infected with herpes simplex virus-2 (HSV-2), is in Phase II testing. The vaccine consists of two HSV-2 antigens, infected cell polypeptide 4 (ICP4) and GD2, combined with the adjuvant matrix M that the company obtained through its 2009 deal with Isconova AB, a subsidiary of **Novavax Inc.**

Genocoea has received funding and engaged in a collaboration with the Bill & Melinda Gates Foundation since 2012 for its malaria vaccine program. In September, it received \$1.2 million for the T cell malaria vaccine program to advance the findings presented at the ASTMH meeting.

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2. Talley, A. et al. *PLoS One* 9, e109654; published online Nov. 18, 2014; doi:10.1371/journal.pone.0109654

COMPANIES AND INSTITUTIONS MENTIONED

American Society of Tropical Medicine and Hygiene,
Deerfield, Ill.

Bill & Melinda Gates Foundation, Seattle, Wash.

Genocoea Biosciences Inc. (NASDAQ:GNCA), Cambridge, Mass.

Novavax Inc. (NASDAQ:NVAX), Gaithersburg, Md.

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