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Extended Abstract

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Baculovirus as a new stand-alone prophylactic and therapeutic immunostimulatory agent against malaria

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Introduction: Baculovirus (BV), which is an enveloped insect virus with a circular double-stranded DNA genome, possesses unique characteristics to induce strong innate immune responses in various mammalian cells and in mice.
Aim: Here we show that the innate immune responses induced by BV not only eliminate Plasmodium liverstage parasites but also elicit sterile protection against Plasmodium sporozoite infection through type I IFN signaling pathway. Methodology: Mice had infected with liver-stage parasites before 24h completely prevented blood-stage parasites following a single dose of BV intramuscular (i.m.) administration, which was much superior to primaquine, the only drug approved to eradicate liver-stage parasites.
Findings: This BV-mediated liver-stage parasite elimination was also observed in TLR-9-/-and iNOS- /- mice. In addition to the therapeutic effect, BV i.m. administration sterilely protects mice for at least 7 days from subsequence sporozoite infection, indicating the prophylactic effect. In vivo passive transfer with sera from mice i.m. administered with BV effectively eliminated liver-stage parasites and this effect was canceled by neutralization of IFN-a but not IFN-g in the sera, indicating a killing mechanism downstream of type I IFN signaling pathway. In fact, 6h after BV i.m. administration, both type I and II IFNs were robustly produced in sera and RNA transcripts of interferon-stimulated genes were drastically upregulated in the liver.
Conclusion & Significance: Our results provide a great potential of BV for development of BV-based vaccine and anti-hypnozoite drug as a new stand-alone therapeutic and prophylactic immunostimulatory agent, which is applicable not only for malaria but also for other serious infectious diseases such as viral hepatitis. Baculovirus (BV), an enveloped insect virus with a circular double-stranded DNA genome, possesses unique characteristics that induce strong innate immune responses in mammalian cells. Here, we show that BV administration not only sterilely protects BALB/c mice for at least 7 days from subsequent *Plasmodium berghei* sporozoite infection but also eliminates existing liver-stage parasites completely, effects superior to those of primaquine, and does so in a TLR9-independent manner. Six hours post-BV administration, IFN-α and IFN-γ were robustly produced in serum, and RNA transcripts of interferon-stimulated genes were drastically upregulated in the liver. The *in vivo* passive transfer of post-BV administration serum effectively eliminated liver-stage parasites, and IFN-α neutralization abolished this effect, indicating that the BV liver-stage parasite killing mechanism is downstream of the type I IFN signaling pathway. Our results demonstrate that BV is a potent IFN-inducing prophylactic and therapeutic agent with great potential for further development as a new malaria vaccine and/or anti-hypnozoite drug. Malaria remains a severe public health problem and causes significant economic losses worldwide. There were approximately 216 million malaria cases and an estimated 445,000 malaria deaths, mainly in children under five. Malaria infection is initiated following injection of *Plasmodium* sporozoites into the skin during the taking of a blood meal by *Anopheles* mosquitoes. The sporozoites migrate to the liver and invade hepatocytes. Before clinical symptoms of malaria occur during the blood stage of infection, *Plasmodium falciparum* in the liver develop into exoerythrocytic schizonts for 5 to 6 days. *P. vivax* and *P. ovale* can develop dormant liver-stage forms, known as hypnozoites, which cause relapsing blood-stage infections months or years after the primary infection. Currently, the only licensed drug for the radical cure of *P. vivax* hypnozoites is primaquine (PQ), and artemisinin-based combination therapies are recommended by the World Health Organization (WHO) as the first-line treatment for blood-stage *P. falciparum* malaria. However, PQ has a high associated risk of life-threatening haemolytic anaemia in people with glucose-6-phosphate-dehydrogenase enzyme (G6PD) deficiency. For future malaria eradication strategies, safer radical curative compounds that efficiently kill hypnozoites are required. A series of studies performed by Nussenzweig and colleagues in 1986–1987 revealed that exogenously administered interferon (IFN)-γ effectively inhibits development of liver-stage parasites *in vitro* and *in vivo*. Recently, Boonhok *et al*. reported that IFN-γ-mediated inhibition occurs at least partially in an autophagy-related protein-dependent manner in infected hepatocytes. Additionally, Liehl *et al*. reported that hepatocytes infected with liver-stage parasites induce a type I IFN secretion via the host cells sensing *Plasmodium* RNA, resulting in reduction of the liver-stage burden. These findings suggest that IFN-mediated immunotherapy against liver-stage parasites might be effective. However, new anti-hypnozoite drugs have not been developed yet. *Autographa californica* nucleopolyhedrosis virus (AcNPV), a type of baculovirus (BV), is an enveloped, double-stranded DNA virus that naturally infects insects. BVs possess unique characteristics that activate dendritic cell (DC)-mediated innate immunity through MyD88/Toll-like receptor (TLR9)-dependent and -independent pathways. Takaku and colleagues reported that BV also directly activates murine natural killer (NK) cells through the TLR9 signalling pathway, which leads to induction of NK cell-dependent anti-tumour immunity. Based on the unique adjuvant properties of BV that induce DC maturation and NK cell activation, which are prerequisites for generating robust and long-lasting adaptive immune responses, we have developed BV-based malaria vaccines effective for all three parasite stages, the pre-erythrocytic stage, asexual blood stage, and sexual stage. Here, we investigated BV-mediated innate immunity against the pre-erythrocytic stage parasites.

Our results clearly demonstrate that BV intramuscular administration not only elicits short-term sterile protection against *Plasmodium* sporozoite infection but also eliminates liver-stage parasites completely through the type I IFN signalling pathway. We propose that, due to its potent IFN-inducing function, BV has great potential for development into not only a new malaria vaccine platform capable of protecting vaccinators for a short period before and after malaria infection but also a new non-haemolytic single-dose alternative to PQ. We used BES-GL3 harbouring two gene cassettes consisting of the *luciferase* gene under the control of the *CMV* promoter and the *DAF* gene under the control of the *p10* promoter, which were designed to express luciferase as a transducing marker and to display DAF as protection for BV from complement attack, respectively. BES-GL3 intramuscular administration into the left thigh muscle of mice initially increased the luciferase expression levels robustly, but these levels gradually decreased to 2% on day 28, which is consistent with previous studies. Among various cell types tested *in vitro*, hepatocytes were found to take up BV most effectively, suggesting a potential use for BV as a vector for liver-directed gene transfer. However, direct evidence of *in vivo* liver-directed gene transfer has not been reported because BV-mediated gene transfer into hepatocytes via intravenous injection is severely hampered by serum complement. We next examined the kinetics of proinflammatory cytokines, ALT, and AST in sera following BES-GL3 intravenous administration. IFN-γ and TNF-α levels rapidly reached their peaks at 6 h and decreased to baseline by 24 h. Similarly, the ALT and AST levels rapidly reached their peaks at 12 h and decreased to baseline by 48 h. Compared with intravenous administration, intramuscular administration did not affect the ALT levels; although the AST level trended higher, this difference did not reach statistical significance. ALT is a sensitive indicator of liver damage, so these results suggest that, for BV, intramuscular administration may be less destructive than intravenous administration. BES-GL3 intravenous administration failed to provide protection against challenge with 1,000 parasitized red blood cells (pRBCs) at 6 h post-BV injection, indicating that BV has no residual effect on blood-stage parasites. CpG intramuscular administration at 6 or 24 h prior to challenge conferred protection against sporozoite challenge in 90% or 80% of mice, respectively. This is consistent with previous work showing short-term (2-days) protection induced by CpG intramuscular administration (50 μg) against challenge with 100 *P. yoelii* sporozoites, although only partial protection (50%) was observed when the challenge occurred at 7 days post-CpG intramuscular injection. Thus, the protective efficacy induced by BES-GL3 intramuscular administration is more effective and longer-lasting compared with CpG. All PBS-treated control mice developed blood-stage infection within 6 days following an intravenous injection of 1,000 Pb-conGFP sporozoites.

Bottom Note: *This work is partly presented at* 6th World Congress on NATURAL PRODUCT & SYNTHETIC CHEMISTRY June 24-25, 2019 | New York, USA.