Preclinical Studies on a New Vaccine Formulation of BK–SE36, 
a Malaria Vaccine Candidate

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An effective blood-stage vaccine remains elusive but necessary if we are to reduce malaria morbidity and mortality. The SE36 antigen derived from serine repeat antigen 5 (SERA5) of Plasmodium falciparum remains a promising blood-stage malaria vaccine candidate. The protective efficacy of BK–SE36, SE36 recombinant protein and aluminum hydroxide gel, is continuously being studied in clinical trials. However, the efficacy of BK–SE36 may still be improved with the use of DNA sequences containing CpG motifs that can selectively promote cellular and/or humoral immune responses. Preclinical studies in non-human primates show promising results. A clinical trial of the vaccine candidate BK–SE36/CpG in a malaria-exposed population is awaited as this can give valuable clues on the immune responses towards K3 CpG formulation in malaria-endemic populations.

Key words: BK–SE36, malaria vaccine; Plasmodium falciparum; TLR9 ligand adjuvant, K3 CpG

Malaria is an Anopheles–mosquito–borne infectious disease. Of the five species of malaria parasites that can infect humans, P. falciparum is the most virulent, causing pandemics mainly in tropical and subtropical regions. Recently, with increasing support for preventive, diagnostic and treatment measures, progress has been achieved in reducing malaria burden (World Malaria Report 2014: http://apps.who.int/iris/bitstream/10665/144852/2/9789241564830_eng.pdf?ua = 1). However, still an estimated 198 million cases (uncertainty range 124–283 million) occurred globally in 2013 with 584,000 deaths (367,000–755,000) of which children under 5 years old in Africa account for 78% of all deaths. Because of country level limitations in implementation of control strategies, uncertainty in international funding1, spread of drug and insecticide–resistant malaria2–4 as well as concerns in global warming5, there has been great expectations for developing a vaccine as an additional intervention for global malaria control and eradication. In the latter part of 2014, RTS,S, the most advance vaccine candidate in development, was applied for licensure. This pre-erythrocytic vaccine (based on the circumsporozoite protein and hepatitis B surface antigen fusion protein) induces approximately 30–50% reduction in the risk of clinical malaria. Thus, a vaccine with higher levels of protection is still sought6. A blood–stage vaccine, either alone or as a component of a multi–stage vaccine, is needed to protect against clinical disease or epidemic malaria.

Serine repeat antigen 5 (SERA5) protein (120 kDa) is an antigen abundantly secreted in the parasitophorous vacuole by the malaria parasite7–9. It is a member of a multigene family that encodes proteins with a putative papain–like cysteine protease motif10. Recently, SERA5 has been demonstrated to have no enzymatic role, yet indispensable, during blood–stage growth and is suggested to be involved in substrate–like interactions or in regulation of active enzyme counterparts that leads to parasite egress11. The N-terminall part of the protein has been identified as a critical domain that
correlates with reduced parasite density\textsuperscript{12} and absence of clinical symptoms in infected children/adults\textsuperscript{13–15}. Moreover, the N-terminal repetitive sequence regions were identified as immunodominant IgG epitopes in Ugandan malaria-immune volunteers and the physicochemical properties of these protective epitopes suggest that they are intrinsically unstructured and, thus, a strict tertiary structure of SE36 epitopes is not required to elicit protective antibodies\textsuperscript{16}.

A recombinant form of SERA5 N-terminal domain (based from \textit{P. falciparum} Honduras I strain), SE36, together with aluminum hydroxide gel (AHG) as adjuvant is referred to as the malaria vaccine candidate BK–SE36 (SE36/AHG). BK–SE36 is produced under Good Manufacturing Practice (GMP) conditions at the Research Foundation for Microbial Diseases of Osaka University (BIKEN).

**Pre-clinical studies in primates**

Good Laboratory Practice (GLP) tests for BK–SE36 were conducted in 2003 with no safety issues identified. In addition, immunogenicity of the vaccine product was assessed in chimpanzees, which have a similar immune system to humans. The study showed significant increase in anti-SE36 antibody titer with no safety concerns at doses of 10/100, 50/500 or 450/4500 SE36/AHG (μg/μg)\textsuperscript{15}. Antibodies induced after administration of 50/500 or 450/4500 SE36/AHG were maintained for 2 years and two administrations were sufficient for serum anti-SE36 antibody levels to reach a plateau level.

Experimental infection studies in squirrel monkeys also demonstrated that monkeys who received two subcutaneous administrations of 50/500 SE36/AHG, with two-week vaccination interval, and then challenged with heterologous parasites (\textit{P. falciparum} IPC/Ray strain) have markedly lower parasitemia than control monkeys that received phosphate buffered saline alone. Another critical observation was that the antibody titer induced by the vaccine was further boosted by the challenge infection. Thus, BK–SE36 can prime the immune response to the SERA5 protein of the infecting \textit{Plasmodium} strain, suggesting that vaccine efficacy in endemic areas can be further enhanced by malaria infection.

**Phase 1a in malaria naive Japanese adults**

After nonclinical studies, in 2005 a phase 1a clinical trial was conducted to evaluate safety and immunogenicity in humans. Based on data from preclinical studies, two doses of BK–SE36 were selected for testing: 50 μg SE36 (half-dose or 0.5

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**Figure 1** Anti-SE36 antibody responses in non-immune Japanese adult volunteers that participated in the phase 1a trial

A. Geometric mean of the antibody titers in two arms (half- and full-dose) before each vaccination at Days 0, 21 and 42.

B. Sero-conversion rates during phase 1a. Number of volunteers who have anti-SE36 antibodies/total number of subjects vaccinated.
ml of BK-SE36) and 100 μg SE36 (full-dose or 1.0 ml of BK-SE36). Each dose was subcutaneously administered on Days 0, 21 and 42.

The first-in-human clinical trial had no safety concerns after three administrations of either the half- or full-dose of BK-SE36 as shown in Table-1. Anti-SE36 protein sero-positivity rate as measured by ELISA was 93.3% (14/15 subjects) and 100% (14/14 subjects) after second vaccination for half- and full-dose of BK-SE36, respectively. Sero-conversion was 100% in all vaccinees after three vaccinations (Figure-1). Notably, even if 100% sero-conversion rates were achieved, the mean anti-SE36 antibody titer was several fold lower than that in African high-titer pooled sera.

Phase 1b in malaria immune Ugandan adults and young children

From 2010 through 2011, a phase 1b clinical trial was conducted in Uganda, followed by a 1-year follow-up study. Local and systemic adverse events were more or less similar to the phase 1a clinical trial, with no volunteers withdrawn due to adverse events (Table-2); although transient elevation in AST and ALT were noted in one volunteer right after treatment for lower respiratory tract infection. There was one serious adverse event of acute gastritis.

Table 1: Local and systemic reactogenicity in Japanese non-immune adults

<table>
<thead>
<tr>
<th></th>
<th>Half-dose (0.5 ml) BK-SE36</th>
<th>Full-dose (1.0 ml) BK-SE36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local reactogenicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induration</td>
<td>15/15</td>
<td>10/15</td>
</tr>
<tr>
<td>Pain</td>
<td>0/15</td>
<td>1/15</td>
</tr>
<tr>
<td>Erythema</td>
<td>14/15</td>
<td>10/15</td>
</tr>
<tr>
<td>Swelling</td>
<td>2/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Itchiness</td>
<td>0/15</td>
<td>4/15</td>
</tr>
<tr>
<td><strong>Systemic reactogenicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>2/15</td>
<td>4/15</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2/15</td>
<td>2/15</td>
</tr>
</tbody>
</table>

Table 2: Local, systemic reactogenicity and other adverse events in Ugandan adults and young children

<table>
<thead>
<tr>
<th></th>
<th>21-32 years old</th>
<th>6-20 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK-SE36 (1 ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local reactogenicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induration</td>
<td>33/36</td>
<td>32/33</td>
</tr>
<tr>
<td>Pain</td>
<td>14/36</td>
<td>11/33</td>
</tr>
<tr>
<td>Erythema</td>
<td>1/36</td>
<td>4/33</td>
</tr>
<tr>
<td>Swelling</td>
<td>3/36</td>
<td>0</td>
</tr>
<tr>
<td>Tenderness</td>
<td>23/36</td>
<td>17/33</td>
</tr>
<tr>
<td>Redness</td>
<td>1/36</td>
<td>0</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>2/36</td>
<td>1/33</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>1/33</td>
<td>0</td>
</tr>
<tr>
<td><strong>Systemic reactogenicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>5/36</td>
<td>1/33</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2/36</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure decrease</td>
<td>1/36</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure increase</td>
<td>5/36</td>
<td>0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1/36</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>2/36</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory adverse events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated AST(^1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Elevated ALT(^2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serious Adverse Event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute gastritis</td>
<td>1/36</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\)AST, aspartate aminotransferase
\(^2\)ALT, alanine aminotransferase
event due to acute bacterial gastritis.

In this first clinical trial of BK-SE36 in malaria endemic areas, the vaccine was demonstrated to have acceptable immunogenicity (Figure-2). The follow-up study generated data on malaria incidences covering two malaria peak seasons (from 130 to 365 days post-second vaccination). Hazard ratio to first episodes of $\geq 5,000$ parasites/$\mu$l in BK-SE36 was 0.50 (95% CI, 0.28–0.92, p = 0.02) and for first episodes of high parasitemia + fever, 0.28 (95% CI, 0.12–0.66, p < 0.01). Against all/multiple malaria episodes the hazard ratio for high parasitemia was 0.57 (95% CI, 0.33–0.99, p = 0.05) and for high parasitemia + fever, 0.34 (95% CI, 0.15–0.76, p = 0.01). From these, there was substantial difference in time-to-first high parasitemia and multiple malaria episodes of BK-SE36 vaccinees in a malaria endemic area where 84% coverage of bednets and minimal practice of indoor insecticide spraying was reported.

**Immunostimulatory DNA sequences**

The phase 1b trial in Uganda demonstrated low sero-conversion in malaria exposed adults (Figure-2) which might be interpreted as due to immunotolerance\textsuperscript{18, 19}. Also, AHG is generally regarded as a weak adjuvant for antibody induction to recombinant antigens. We expect that the utility of BK-SE36 can be further enhanced with an adjuvant that can stimulate innate immunity. Likewise, current effective adjuvant formulations consist of several molecules that act synergistically by activating both innate and acquired immune mechanisms\textsuperscript{20}. Innate immunity is induced by exposure to conserved pathogen-associated molecular patterns that are expressed in a variety of infectious microorganisms, the recognition of which is mediated by several receptors including the most extensively studied Toll-like receptors (TLRs)\textsuperscript{20, 21}. There are now four licensed vaccines that contain multiple TLR agonists: BCG vaccine which contains both TLR2 and TLR4; Cervarix and Fendrix which contains alum and monophosphoryl lipid A; and the *Mycobacterium indicuspranii* adjuvant (Immuvac/Cadi-05) for leprosy\textsuperscript{20}. These developments attest to the success in multi-adjuvancing, and consequently, agents that support the efficient induction of innate immunity.

Synthetic oligonucleotides (ODN) containing unmethylated cytosine guanine dinucleotide (CpG) motifs have been shown to be strong adjuvants for DNA or protein-based immunogens and have been used for human trials\textsuperscript{22–26}. Because CpGs are only present at

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**Figure-2** Fold increase in anti-SE36 antibody titers in malaria-exposed Ugandan adults and young children that participated in the phase 1b trial

A. Geometric mean of the fold-increase in antibody titers in two arms (half- and full-dose) at each age group.

B. Proportion of subjects in whom antibody titer increased by > 1.9-fold after two vaccinations in comparison with pre-vaccination titer/total subjects vaccinated per cohort.
about 25% in mammalian DNA and are highly methylated, once recognized, these DNA sequences stimulate the immune system through a specific receptor, TLR9. TLR9 activation, restricted to B cells and plasmacytoid dendritic cells (pDC) in man, enhance the immune system biased towards a Th1 type response. B cells are activated to secrete interleukins (IL-6 and IL-10), immunoglobulin (IgG), and to express increased levels of co-stimulatory molecules. Monocytes and macrophages secrete IL-12, leading to natural killer (NK) cell activation and interferon (IFN-γ) secretion. Moreover, expression of co-stimulatory molecules can also lead to enhanced antigen presentation. Thus, these immunostimulatory properties have been tapped for a range of non-antigen specific therapy of allergens and cancers; as well as antigen-specific generation of B and T cell immune responses to synthetic peptide- and recombinant protein–based vaccines. Besides antibodies which are crucial for parasite clearance in the latter stages of infection, several studies have shown that the control of malaria is partially dependent on the generation of Th1-type immunity.

CpG 7909 (Coley Pharmaceutical Group, Wellesley, MA) has been studied extensively in humans in combination with hepatitis B vaccine, inactivated influenza vaccine and anthrax vaccine. In the malaria field, a number of studies also demonstrated that CpG7909 boosted the immunogenicity of alum-based vaccines (Table–3). With regards to the safety aspect, so far, there were transient fluctuations in white blood cell counts and platelet count as expected with CpG 7909 and other TLR9 agonists. No clinical events associated with these fluctuations have been reported by several authors and given the rapid return to baseline, their clinical significance has been judged to be minimal. So far, there were no reports of clinically relevant changes in liver or renal function. Systemic symptoms

| Table–3 Some studies using CpG ODN for candidate malaria vaccines |
|-------------------------------------------------|------------------|------------------|------------------|------------------|
| CpG ODN in vaccine formulation | Vaccine candidate | Phase | Outcome | References |
| CpG 2006 | | | | |
| CpG 1826 | \(Plasmodium vivax\) MSP1α in Montanide ISA 51 | Pre-clinical and sporozoite challenge | Protective efficacy in murine model | Kumar, et al., 2004 |
| CpG10105 | (250μg) P625/Pv25 in Alhydrogel or Montanide ISA720 | Pre-clinical (Rhesus monkey and mosquito feeding assay) | Evaluation of transmission–blocking activity | Miura, et al., 2007 |
| CpG 1826 | Killed parasites (whole-parasite vaccine) and aluminium hydroxide | Pre-clinical (rodent) | Vaccine with CpG adjuvant elicited strong, cross-reactive T cell responses | Pinzon-Charry, et al., 2010 |
| CpG7909 | \(P. falciparum\) AMA1-C1/Alhydrogel | Phase 1a (non-immune adults) | Safety, immunogenicity and in vitro growth inhibition (GIA) studies | Mullen, et al., 2008 |
| | | Phase 1a (non-immune adults) | Safety, immunogenicity and GIA studies | Ellis, et al., 2009 |
| | | Phase 1b (malaria exposed adults) | Safety and immunogenicity; Acquisition of memory B cells | Sagara, et al., 2009 |
| | | Phase 1/2a (non-immune adults) | Human experimental malaria infection studies | Duncan, et al., 2011 |
| | | Phase 1 (non-immune adults) | Safety, immunogenicity and GIA studies | Ellis, et al., 2012 |
| | | Phase 1 (non-immune adults) | Safety, immunogenicity and GIA studies | Ellis, et al., 2010 |

1TCGTCGCTGTTGTCGTTPCTTTTCTT  
2TCGTCGCTGTTGTCPTTTTGTGTT  
3TCGTCGCTGTTGTCGTCTTTGTTTTCTT  
4TCGTCGCTGTTGTCGTCTTTTTCGA  
5TCGTCGCTGTTGTCGTCTTGTGTT
short-lived flu-like symptoms, fatigue, rigor, myalgia, pyrexia) were most commonly described ranging from mild to moderate in severity; with frequency increasing with higher CpG doses (refer to Table-3 studies). These reactogenicities were deemed generally manageable and acceptable given that volunteers mounted significantly higher levels of antibodies against antigenic proteins (from 2- to 49-fold)\(^{33, 34}\). Disappointingly, the increase in antibody titers achieved by the vaccinees, did not protect volunteers against \textit{in vivo} malaria infection or reduce parasite multiplication rate in the CpG7909 + apical membrane antigen 1 trial\(^{35}\).

Further assessments are ongoing to identify additional CpGs; establish their safety and activity profiles; and identify populations and conditions where addition of CpG ODNs exert substantial advantage\(^ {36-37}\).

**Studies of BK–SE36 with K3 CpG**

A study to optimize the immunogenicity of BK–SE36 was done in combination with K3 CpG (ATCGACTCTCGAGCGTTCTC), D35 CpG (GCTgctagtagagggGG) (GeneDesign, Inc), or synthetic hemozoin (sHZ) as adjuvant\(^ {38}\). K3 CpG activate memory B cells to proliferate and differentiate into IgG producing cells, secrete IgM, stimulate pDC precursors to mature and secrete IL-8, and indirectly activate monocytes to produce IL-6. D35 CpG, on the other hand, induces plasmacytoid DCs to produce IFN-\(\alpha\) and IFN-\(\gamma\), either directly or indirectly trigger IFN-\(\alpha\) production by NK, and enhance maturation of myeloid DC\(^ {39} \textit{ et al.}\) sHZ was

**Figure-3** Kinetics of the antibody response in cynomolgus monkeys

A. Anti SE36-specific IgG antibody titers against the day the animals were immunized and/or bled. Cynomolgus monkeys were administered subcutaneously on days 0, 22, 101, and 365 (arrows) with a mixture of 10 \(\mu\)g SE36 and 125 \(\mu\)g AHG with or without 500 \(\mu\)g K3 CpG, or D35 CpG; or 1.5 mM sHZ in 1.0 ml. Closed circles, triangles, squares, and diamonds show the median titers (n = 3/group) of BK–SE36, BK–SE36 + K3 CpG, BK–SE36 + D35 CpG, and BK–SE36 + synthetic hemozoin (sHZ), respectively. Bars represent standard error of the mean.

B. Comparison of antibody titers on days 36 (i), 112 (ii), and 379 (iii). Results from individual monkeys are shown. Non-parametric ANOVA (Kruskal–Wallis) with Dunn’s post-hoc test was used for statistical analyses between groups; * indicates significant difference at \(p<0.05\).
included in the study as it was reported to be a potent adjuvant for malaria antigens\(^\text{41}\). The original formulation of BK–SE36 was used as control. Although earlier reported to give primarily a Th2 response in mice, it is now known that in humans, proteins with alum adjuvants activate a mixture of Th2 and Th1 cells\(^\text{28,42}\). These different formulations were compared and preclinical studies were done in cynomolgus monkeys, an animal model used as a surrogate species for humans. Twelve cynomolgus monkeys were divided into four groups (BK–SE36, BK–SE36 with K3 CpG, BK–SE36 with D35 CpG, and BK–SE36 with sHZ). Monkeys were immunized subcutaneously four times (Day 0, 22, 101 and 365), followed by approximately 600-day follow-up with periodic blood-sample collection for serum anti-SE36 antibody titer measurements (Figure-3). TLR9 ligand adjuvants were demonstrated to significantly enhance antibody titers compared to BK–SE36 alone. Antibody titer in the K3 CpG adjuvant group was approximately 10 times greater than BK–SE36 (Figure-3A). K3 CpG also has dose sparing effect, as no statistical difference was observed in post-immunization antibody titers between the third and fourth immunization, indicating that three administrations are sufficient to induce maximal antibody titers (Figure-3A and B [ii]–[iii]).

To investigate the involvement of SE36 antigen–specific helper T cells, cytokine secretion levels were measured under \textit{ex–vivo} SE36 stimulation that included IFN–\(\gamma\) as a Th1 response marker and IL–5 and IL–13 as Th2 response markers. Significant inductions of IFN–\(\gamma\), IL–5, and IL–13 were observed in the K3 CpG adjuvant group indicating that K3 can induce both Th1/Th2 responses, while sHZ adjuvant induces only the Th2 response (Figure-4). As in earlier studies, injection of crude \textit{P. falciparum} 3D7 extract resulted to an increase in anti-SE36 antibody titer suggesting that the immune response can be boosted by malaria infection.

Further studies on K3 CpG were conducted using squirrel monkeys as an animal model for human malaria. Three groups were evaluated: BK–SE36 with K3, BK–SE36 alone and AHG with K3 CpG only. The monkeys were immunized subcutaneously twice within a 3–week interval and at 9-weeks after the first immunization received a challenge infection of \(5 \times 10^8\) parasites. Monkeys immunized with BK–SE36 and K3 CpG effectively suppressed parasitemia\(^\text{38}\). Anti–SE36 antibody titer was also subsequently boosted by malaria infection. From these promising results, a combination of TLR9 ligand adjuvant with BK–SE36 can induce higher humoral and cellular immune

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Levels of IFN–\(\gamma\), IL–5, and IL–13 in PBMCs of cynomolgus monkeys stimulated \textit{in vitro} with SE36 antigen. Formulation of BK–SE36 with K3 CpG adjuvant promotes both Th1 and Th2 responses, in contrast to BK–SE36 + D35 CpG or BK–SE36 + sHz. PBMCs of cynomolgus monkeys on Day 36 were purified by Ficoll and isolated cells stimulated with SE36 (‘36’) (closed symbols) antigen or PBS (‘N’) (open symbols) at 37°C for 24h. * indicates significant difference from PBS at \(p < 0.05\). Statistical analysis was performed for pre– and post–immunization sera using a Mann–Whitney U test.}
\end{figure}
Concerning safety, toxicity studies using single-dose/repeated intramuscular administration to rats, foreign body-related granuloma at the vaccination site was observed, but there was no systemic toxicity (Horii et al., unpublished). In a safety pharmacology study using rats, there were no influence on the central nervous or respiratory systems. Using dogs, there was likewise no influence on the cardiovascular system. In a topical irritation study involving intramuscular administration to rabbits, Grade 2 topical skin-irritating properties were noted. Histopathologically, fibrosis was advanced, suggesting recovery, although topical skin reactions macroscopically persisted to 35 days after vaccination (Horii et al., unpublished). Further safety and GLP tests are underway. In parallel, supported by results from vaccine trials in non-human primates, a first-in-human investigator-initiated clinical trial was done for BK-SE36/CpG. The trial has just been completed (Horii et al., in preparation) and follow-up of vaccinees is on-going.

**Conclusion**

There are a number of challenges in malaria vaccine development: (a) the parasite’s complex life cycle and diverse protein repertoire; (b) antigenic variation and polymorphisms across parasite strains and species; and (c) the parasites’ developed strategies for host immunity evasion. SERA5 may likely overcome challenges with regards to extensive polymorphism and strict structural requirement for protective epitopes. Further studies on the phase Ib clinical trial in Uganda suggest that BK-SE36 does not show allele-specific efficacy (Arisue et al., in preparation) and protective efficacy may not be influenced by African HLA II haplotype (Tougan et al., unpublished). Although early indicators of vaccine efficacy for BK-SE36 is promising, use of K3 CpG as an adjuvant to enhance immunogenicity and possibly efficacy may be one approach to broaden immune responses. A robust immune response may overcome immune tolerance or help immunocompromised individuals. Indeed, immunity against malaria involves timely and coordinated interplay of both innate and adaptive immunity involving multiple cell types like dendritic cells, NK cells; B cells, CD4+ and CD8+ T cells. Adjuvant formulations have been described taking into account not only the amount of antibody induced by the vaccine but also the quality of the antibody (different fine specificities or the maturation of the antibody response). K3 CpG in our studies was identified as an effective TLR9 ligand that can induce both humoral and cellular immunity when compared to BK-SE36 alone. The advantage in the activation of multiple innate receptors that could target redundant pathways of innate responsiveness rather than a single pathway was observed with RTS, S. RTS, S administered with alum did not confer protection, whereas, RTS, S mixed with TLR-4 ligand resulted to increased

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**Figure 5** Parasitemia in squirrel monkeys at different time points during a vaccine trial. Parasite densities among the different treatment groups (BK-SE36, BK-SE36 with K3 CpG and AHG with K3 CpG) are compared up to 14 days post challenge infection. The monkeys were immunized with the different formulations nine weeks before challenged with $5 \times 10^8$ infected IPC/Ray red blood cells. "dt" stands for a monkey that was euthanized, or succumbed to infection. Closed circles for BK-SE36 group, triangles for BK-SE36 with K3 CpG and squares for AHG with K3 CpG. Results for individual monkeys are shown.
antibody production and induction of Th1 cell immunity with 30–50% protection\(^{20}\).

A public health concern, is the ability of CpG adjuvants to cause autoimmune disease. These are in part based on observations that infections can trigger some autoimmune diseases and pattern recognition receptors are capable of breaking tolerance in animal models\(^{20}\). The occurrence of Wegeners Granulomatosis (an autoimmune vasculitis affecting the lungs and kidneys) was reported in a volunteer who received a recombinant hepatitis B vaccine adjuvanted with the oligonucleotide ISS (TGACTGTGACGTTCGAGATGA)\(^{20}\). However, it is also noted that specific antigen–adjuvant combinations can influence both safety and immune responses. What we know now is that TLR9 binding is influenced partly by specific base sequences, as well as the sugar phosphate backbone, conjugation to the antigen and dose of the antigen, as well as administration route and schedule\(^{25}26\)–\(^{42}\)–\(^{47}\). These highlight considerations on careful formulation and evaluation of the antigen with CpG ODN. Further clinical studies would assess these concerns, especially with the advent of some in vitro assays that can be predictors of in vivo toxicity or, whenever possible, assay for extensive T–cell profiling\(^{42}\). Research and clinical trials to answer whether the CpG adjuvants cause autoimmune diseases or not are underway.

Acknowledgments

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