

Conserved anti-plasmodial defenses and signaling in *Anopheles stephensi* and *Anopheles gambiae*

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Anopheles stephensi and *Anopheles gambiae* respond to malaria parasite infection with inducible expression of nitric oxide synthase (NOS; 1, 2). The midgut response in *A. stephensi* is biphasic with induction at 6h and at 36-48h following infection (3), indicating that parasite presence in the blood mass is sufficient for NOS induction. Catalytic activity of AsNOS results in the synthesis of inflammatory levels of nitrogen oxides (NO_x; 3) that limit parasite development. Several reactive nitrogen intermediates (RNIs) with significant potential for toxic effects on both host and parasite are formed in the midgut; these RNIs indicate that significant nitrosative and oxidative stresses exist in the *A. stephensi* midgut following infection. Induction and activity of AsNOS are dependent on ingested midgut blood components including mammalian growth factors/cytokines that crosstalk with mosquito cells and blood proteins that function as substrates for RNI chemistry.

We have focused on how NO synthesis is induced and controlled as a basis for identifying novel targets to regulate parasite development. *Plasmodium falciparum* glycosylphosphatidylinositols (PfGPIs), the “malaria toxin” in mammals (4), drive biphasic induction of midgut NOS in both *A. stephensi* (5) and *A. gambiae*, indicating that PfGPIs are critical inflammatory mediators in both mosquitoes and mammals. In addition to signaling through pathways associated with inflammation (e.g., NF-κB-dependent; 6), PfGPIs mimic the effects of insulin on mammalian cells (4). Although PfGPIs are not insulin-mimetic to *A. stephensi* cells, they do activate insulin-signaling associated Akt and ERK in the mosquito midgut and this activation is necessary for AsNOS induction (5). In mammalian cells, Akt phosphorylates more than 50 gene products associated with cell growth, defense, survival, and metabolism and has been identified as a major therapeutic target for a number of human diseases (7), indicating that study of Akt signaling in *A. stephensi* is likely to reveal new gene targets for engineering of anti-parasite resistance. In addition to MEK/ERK and Akt, we are continuing studies of other signaling pathways and effector genes that are activated by PfGPIs.

In mammals, transforming growth factor (TGF)-β1 maintains immunological balance during malaria parasite infection (8), primarily through an effect on inducible NOS (9). We have demonstrated that ingested mammalian TGF-β1 dose-dependently regulates malaria parasite development in *A. stephensi* (3), in part through an effect on AsNOS catalytic activity. Our observations indicated for the first time that TGF-β-dependent Smad signaling regulates an important anti-parasite defense in the insect host. The signaling architecture for TGF-β/activin and BMP/GDF signaling is in place in the *A. stephensi* midgut (10), indicating that this tissue can respond to endogenous and exogenous ligands in this large protein superfamily. The success of cytokine therapy for management of infectious disease in humans (11) suggests that a similar strategy may be feasible in mosquitoes. In mammals, however, TGF-β1 regulates a myriad of genes that are associated with cell growth and death, differentiation, morphology,

extracellular matrix synthesis and immunity which, in turn, influence the development and physiology of nearly every major tissue and organ system (12). As such, we are using genomics and proteomics approaches to understand the global effects of TGF- β 1 as well as parasite signals and other immunomodulatory factors on mosquito cells.

Our observations indicate that the midgut is a complex environment of radical chemistry and immunomodulatory signals derived from the parasite, from ingested blood, and from the mosquito response to parasite infection that do not act in isolation from one another. As such, we suggest that evaluation of transgenes in this milieu should account for the diversity and balance of crosstalking factors likely to be encountered in human hosts in the field to better predict the outcome of genetic manipulation on parasite development.

References

- (1) Luckhart S, Vodovotz Y, Cui L, Rosenberg R. 1998. The mosquito *Anopheles stephensi* limits malaria parasite development with inducible synthesis of nitric oxide. *Proc. Natl. Acad. Sci. USA* 95:5700-5.
- (2) Dimopoulos G, Seeley D, Wolf A, Kafatos FC. 1998. Malaria infection of the mosquito *Anopheles gambiae* activates immune-responsive genes during critical transition stages of the parasite life cycle. *EMBO J.* 17:6115-23.
- (3) Luckhart S, Crampton AL, Zamora R, Lieber MJ, Dos Santos PC, Peterson TM, Emmith N, Lim J, Wink DA, Vodovotz Y. 2003. Mammalian transforming growth factor- β 1 activated after ingestion by *Anopheles stephensi* modulates mosquito immunity. *Infect Immun.* 71:3000-9.
- (4) Schofield L, Hackett F. 1993. Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. *J Exp Med.* 177:145-53.
- (5) Lim J, Gowda DC, Krishnegowda G, Luckhart S. 2005. Induction of nitric oxide synthase in *Anopheles stephensi* by *Plasmodium falciparum*: mechanism of signaling and the role of parasite glycosylphosphatidylinositols. *Infect Immun.* 73:2778-89.
- (6) Zhu J, Krishnegowda G, Gowda DC. 2005. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: the requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-kappaB pathways for the expression of proinflammatory cytokines and nitric oxide. *J. Biol Chem.* 280:8617-27.
- (7) Hanada M, Feng J, Hemmings BA. 2004. Structure, regulation and function of PKB/AKT – a major therapeutic target. *Biochim Biophys Acta.* 1697:3-16.
- (8) Omer FM, Kurtzhals JA, Riley EM. 2000. Maintaining the immunological balance in parasitic infections: a role for TGF- β ? *Parasitol Today.* 16:18-23.
- (9) Vodovotz Y. 1997. Control of nitric oxide production by transforming growth factor-beta1: mechanistic insights and potential relevance to human disease. *Nitric Oxide* 1:3-17.
- (10) Lieber MJ, Luckhart S. 2004. Transforming growth factor- β s and related gene products in mosquito vectors of human malaria parasites: signaling architecture for immunological crosstalk. *Mol. Immunol.* 41:965-77.
- (11) Aoki N, Xing Z. 2004. Use of cytokines in infection. *Expert Opin. Emerg. Drugs.* 9:223-36.
- (12) Madsen J. 2000. How cells read TGF- β signals. *Nat. Rev. Mol. Cell Biol.* 1:169-78.