

considered is the duration of protective antibodies. Recent findings relevant to the present work indicated that when *P. vivax* transmission-blocking vaccine candidate Pvs25 was injected s.c. with incomplete Freund's adjuvant into BALB/c mice, it induced a strong antigen-specific serum IgG response that was maintained for more than 6 months (our unpublished data). i.n. vaccination with Pvs25 plus CT induced a level of serum IgG comparable to that induced by s.c. vaccination formulated with incomplete Freund's adjuvant, but the level gradually decreased over 6 months. However, we found that i.n. vaccination with Pvs25 plus CT was generally more potent based on the magnitude and duration of the specific serum IgG response than s.c. vaccination with Pvs25 formulated with aluminum hydroxide (unpublished data).

In this study we used CT as a mucosal adjuvant; however, the use of CT for humans is hampered by the toxicity of this compound. Also, as mentioned above, issues related to the potential allergic response and the duration of antibodies need special consideration. Fortunately, however, nontoxic and thus safer adjuvants, but adjuvants that are as effective as CT, are being developed, making a mucosal malaria vaccine a feasible goal (1, 15). For example, we recently found that when a nontoxic subunit of CT, CTB, was fused to malaria OSP, it was efficacious by both the mucosal and s.c. routes for blocking parasite transmission (unpublished data). Thus, if the mucosal transmission-blocking vaccine efficacy data obtained with this rodent infection model can be reproduced in human clinical trials with guaranteed safety, OSP antigens formulated as non-invasive vaccines may become a powerful tool for use against human malaria.

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#### REFERENCES

- Agren, L. C., L. Ekman, B. Löwenadler, and N. Y. Lycke. 1997. Genetically engineered nontoxic vaccine adjuvant that combines B cell targeting with immunomodulation by cholera toxin A1 subunit. *J. Immunol.* 158:3936-3946.
- Arakawa, T., A. Komesu, H. Otsuki, J. Sattabongkot, R. Udomsangpetch, Y. Matsumoto, N. Tsuji, Y. Wu, M. Torii, and T. Tsuboi. 2005. Nasal immunization with a malaria transmission-blocking vaccine candidate, Pfs25, induces complete protective immunity in mice against field isolates of *Plasmodium falciparum*. *Infect. Immun.* 73:7375-7380.
- Arakawa, T., T. Tsuboi, A. Kishimoto, J. Sattabongkot, N. Suwanabun, T. Rungruang, Y. Matsumoto, N. Tsuji, H. Hisaeda, A. Stowers, I. Shimabukuro, Y. Sato, and M. Torii. 2003. Serum antibodies induced by intranasal immunization of mice with *Plasmodium vivax* Pvs25 co-administered with cholera toxin completely block parasite transmission to mosquitoes. *Vaccine* 21:3143-3148.
- Carter, R. 2001. Transmission blocking malaria vaccines. *Vaccine* 19:2309-2314.
- del Carmen Rodriguez, M., P. Gerold, J. Dessens, K. Kurtenbach, R. T. Schwartz, R. E. Sinden, and G. Margos. 2000. Characterisation and expression of pbs25, a sexual and sporogonic stage specific protein of *Plasmodium berghei*. *Mol. Biochem. Parasitol.* 110:147-159.
- Escalante, A. A., A. A. Lal, and F. J. Ayala. 1998. Genetic polymorphism and natural selection in the malaria parasite *Plasmodium falciparum*. *Genetics* 149:189-202.
- Genton, B. 2008. Malaria vaccines: a toy for travelers or a tool for eradication? *Expert Rev. Vaccines* 7:597-611.
- Gozar, M. M., O. Muratova, D. B. Keister, C. R. Kensil, V. L. Price, and D. C. Kaslow. 2001. *Plasmodium falciparum*: immunogenicity of alum-adsorbed clinical-grade TBV25-28, a yeast-secreted malaria transmission-blocking vaccine candidate. *Exp. Parasitol.* 97:61-69.
- Gozar, M. M., V. L. Price, and D. C. Kaslow. 1998. *Saccharomyces cerevisiae*-secreted fusion proteins Pfs25 and Pfs28 elicit potent *Plasmodium falciparum* transmission-blocking antibodies in mice. *Infect. Immun.* 66:59-64.
- Greenwood, B. 2008. Can malaria be eliminated? *Trans. R. Soc. Trop. Med. Hyg.* 103S:S2-S5.
- Greenwood, B. M., D. A. Fidock, D. E. Kyle, S. H. Kappe, P. L. Alonso, F. H. Collins, and P. E. Duffy. 2008. Malaria: progress, perils, and prospects for eradication. *J. Clin. Investig.* 118:1266-1276.
- Hafalla, J. C., M. L. Santiago, M. C. Pasay, B. L. Ramirez, M. M. Gozar, A. Saul, and D. C. Kaslow. 1997. Minimal variation in the Pfs28 ookinete antigen from Philippine field isolates of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 87:97-99.
- Harakuni, T., H. Kohama, M. Tadano, G. Uechi, N. Tsuji, Y. Matsumoto, T. Miyata, T. Tsuboi, H. Oku, and T. Arakawa. 2009. Mucosal vaccination approach against mosquito-borne Japanese encephalitis virus. *Jpn. J. Infect. Dis.* 62:37-45.
- Hisaeda, H., A. W. Stowers, T. Tsuboi, W. E. Collins, J. S. Sattabongkot, N. Suwanabun, M. Torii, and D. C. Kaslow. 2000. Antibodies to malaria vaccine candidates Pvs25 and Pvs28 completely block the ability of *Plasmodium vivax* to infect mosquitoes. *Infect. Immun.* 68:6618-6623.
- Holmgren, J., J. Adamsson, F. Anjuere, J. Clemens, C. Czerkinsky, K. Eriksson, C. F. Flach, A. George-Chaudy, A. M. Harandi, M. Lebens, T. Lehner, M. Lindblad, E. Nygren, S. Raghavan, J. Sanchez, M. Stanford, J. B. Sun, A. M. Svennerholm, and S. Tengvall. 2005. Mucosal adjuvants and anti-infection and anti-immunopathology vaccines based on cholera toxin, cholera toxin B subunit and CpG DNA. *Immunol. Lett.* 97:181-188.
- Holmgren, J., and C. Czerkinsky. 2005. Mucosal immunity and vaccines. *Nat. Med.* 11:S45-53.
- Kaslow, D. C. 1997. Transmission-blocking vaccines: uses and current status of development. *Int. J. Parasitol.* 27:183-189.
- Kaslow, D. C., C. Bathurst, T. Lensen, T. Ponnudurai, P. J. Barr, and D. B. Keister. 1994. *Saccharomyces cerevisiae* recombinant Pfs25 adsorbed to alum elicits antibodies that block transmission of *Plasmodium falciparum*. *Infect. Immun.* 62:5576-5580.
- Kaslow, D. C., I. A. Quakyi, and D. B. Keister. 1989. Minimal variation in a vaccine candidate from the sexual stage of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 32:101-103.
- Richie, T. L., and A. Saul. 2002. Progress and challenges for malaria vaccines. *Nature* 415:694-701.
- Saul, A. 2008. Efficacy model for mosquito stage transmission blocking vaccines for malaria. *Parasitology* 135:1497-1506.
- Seder, R. A., and A. V. Hill. 2000. Vaccines against intracellular infections requiring cellular immunity. *Nature* 406:793-798.
- Shi, Y. P., M. P. Alpers, M. M. Pova, and A. A. Lal. 1992. Single amino acid variation in the ookinete vaccine antigen from field isolates of *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 50:179-180.
- Stowers, A. W., D. B. Keister, O. Muratova, and D. C. Kaslow. 2000. A region of *Plasmodium falciparum* antigen Pfs25 that is the target of highly potent transmission-blocking antibodies. *Infect. Immun.* 68:5530-5538.
- Tsuboi, T., Y. M. Cao, Y. Hitsumoto, T. Yanagi, H. Kanbara, and M. Torii. 1997. Two antigens on zygotes and ookinetes of *Plasmodium yoelii* and *Plasmodium berghei* that are distinct targets of transmission-blocking immunity. *Infect. Immun.* 65:2260-2264.
- Tsuboi, T., Y. M. Cao, M. Torii, Y. Hitsumoto, and H. Kanbara. 1995. Murine complement reduces infectivity of *Plasmodium yoelii* to mosquitoes. *Infect. Immun.* 63:3702-3704.
- Tsuboi, T., O. Kaneko, Y. M. Cao, M. Tachibana, Y. Yoshihiro, T. Nagao, H. Kanbara, and M. Torii. 2004. A rapid genotyping method for the vivax malaria transmission-blocking vaccine candidates, Pvs25 and Pvs28. *Parasitol. Int.* 53:211-216.
- Tsuboi, T., D. C. Kaslow, M. M. Gozar, M. Tachibana, Y. M. Cao, and M. Torii. 1998. Sequence polymorphism in two novel *Plasmodium vivax* ookinete surface proteins, Pvs25 and Pvs28, that are malaria transmission-blocking vaccine candidates. *Mol. Med.* 4:772-782.

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