AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

AUTHOR REPLY

Reply to "Parasite Strain, Host Immunity, and Circulating Blood Cells with Dead Parasites: Why Predicting Malaria Parasite Clearance Is Not a Simple Task"

Ian M. Hastings, Katherine Kay, Eva Maria Hodel

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

We welcome the comments of Simpson et al. (1). There is clearly a need to understand the basic properties of parasite clearance rates and their potential role as policy decision tools. Simpson et al. state that our models are "limited with respect to two key parameters, the infecting parasite strain and host immunity." We address each point in turn, noting that our nomenclature (2) recognized that it is infected red blood cells (iRBC), not parasites directly, which are cleared from the circulation.

Simpson et al. (1) note that "immunity is complex" (and that we acknowledged this in our paper) and make the specific point of immunity reducing the parasite multiplication factor at the end of the 48-h cycle. We were aware of this factor and addressed it on page 6432 in the text starting "The dynamics can be understood as the interactions among the three factors that determine iRBC clearance dynamics, i.e., spleen clearance rates, sequestration rates, and new merozoite release rates" (2). We argued that spleen clearance rates completely dominate any decrease in the rate of successful RBC invasions of merozoites (equivalent to merozoite release rates) so took this potential immune effect into account in our modeling.

Simpson et al. (1) also noted the effect of "infecting parasite strain." Importantly, *Plasmodium falciparum* is sexual, with frequent genetic recombination, so resistant and sensitive "strains" are defined only by short regions of DNA surrounding the resistance locus. The remainder of the strain genetic background (which includes antigen-encoding loci) reflects that of the local population. Interestingly, we did address this issue of immunity acting against the resistance/sensitivity genotype (or strains) in our original submission in a paragraph that read as follows (with citation numbers updated):

> There is another, less obvious, way in which iRBC clearance rates may reflect parasite drug resistance levels. This occurs if a resistance mutation(s) simultaneously affects both artemisinin sensitivity and the iRBC immune profile (i.e., the extent to which iRBC are recognized by host clearance mechanisms). Interestingly, this hypothesis occurs fairly regularly in the literature, most explicitly in the work of Koella (3), who also cites earlier work by Clyde in 1958 (4) and by Peters in 1987 (5). Koella discusses three ways in which this phenomenon may occur: (i) if separate genes affecting resistance and immune profile are physically closely linked on the same chromosome, (ii) if a single gene affects both resistance and immune profile, and/or (iii) if the mutation alters drug accumulation within the iRBC and the degree of drug accumulation affects the immune properties of the iRBC. [We also conjecture that changes in drug accumulation might affect the physical properties of iRBC, such as deformability, that might affect splenic clearance rates]. Koella's arguments were explicitly applied to chloroquine resistance but appear equally applicable to artemisinin resistance, although, in both drugs, the first explanation seems less than compelling because current genetic analyses did not identify immune loci within the resistance linkage regions. Koella's second hypothesis seems more plausible, as changes in the levels of molecules, such as membrane-bound pumps, may be associated with resistance levels and also affect the iRBC immune profile. Note also that these immune effects may produce a counterintuitive result: if resistance enhances the immune profile, for example by overproduction of a membrane-bound pump recognized by immunity, then resistance may actually become associated with higher clearance rates.

This paragraph was removed on the robust advice of an anonymous reviewer. We are delighted to resurrect it because it is highly pertinent to the current correspondence and to the previous paper (6), which did not cite this literature. Hastings has longstanding interest in this area (see, e.g., references 7 and 8), which made us reluctant to explicitly model "strain" effects, particularly because relating immunity directly to the resistance genotype would allow any resistance/clearance relationship to be predicted.

The title of the letter of Simpson et al. (1) states that "predicting malaria parasite clearance is not a simple task"; we agree entirely, as this requires details of immunity and parasite biology of which we remain profoundly ignorant. We argue strongly that even if we cannot predict them, it is straightforward to interpret them; they reflect primarily the level of patients' acquired immunity unless and until drug effectiveness becomes so low that parasites are not actually cleared. This makes clearance rates extremely insensitive and nonspecific metrics of drug effectiveness, which was the key point that we made in our paper.

REFERENCES

1. **Simpson J, McCaw JM, Fowkes FJ.** 2016. Parasite strain, host immunity, and circulating blood cells with dead parasites: why predicting malaria parasite clearance is not a simple task. Antimicrob Agents Chemother **60:**1172. http://dx.doi.org/10.1128/AAC.02528-15.
2. **Hastings IM, Kay K, Hodel EM.** 2015. How robust are malaria parasite clearance rates as indicators of drug effectiveness and resistance? Antimi-

Citation Hastings IM, Kay K, Hodel EM. 2016. Reply to "Parasite strain, host immunity, and circulating blood cells with dead parasites: why predicting malaria parasite clearance is not a simple task." Antimicrob Agents Chemother 60:1173–1174. doi:10.1128/AAC.02570-15.

Address correspondence to Ian M. Hastings, Ian.Hastings@LSTMed.ac.uk.

This is a response to a letter by Simpson et al. (doi:10.1128/AAC.02528-15).

crob Agents Chemother **59:**6428–6436. http://dx.doi.org/10.1128/AAC.00481-15.
3. **Koella JC.** 1993. Epidemiological evidence for an association between chloroquine resistance of *Plasmodium falciparum* and its immunological properties. Parasitol Today **9:**105–108. http://dx.doi.org/10.1016/0169-4758(93)90218-5.
4. **Clyde DF.** 1958. Multiple infections of *Plasmodium falciparum* susceptible and resistant to pyrimethamine. Riv Malariol **37:**263–276.
5. **Peters W.** 1987. Chemotherapy and drug resistance in malaria. Academic Press, London, United Kingdom.
6. **Dogovski C, Xie SC, Burgio G, Bridgford J, Mok S, McCaw JM, Chotivanich K, Kenny S, Gnädig N, Straimer J, Bozdech Z, Fidock DA, Simpson JA, Dondorp AM, Foote S, Klonis N, Tilley L.** 2015. Targeting the cell stress response of *Plasmodium falciparum* to overcome artemisinin resistance. PLoS Biol **13:**e1002132. http://dx.doi.org/10.1371/journal.pbio.1002132.
7. **Hastings IM, Wedgwood-Oppenheim BA.** 1997. Sex, strains and virulence. Parasitol Today **13:**375–383. http://dx.doi.org/10.1016/S0169-4758(97)01110-1.
8. **Mzilahowa T, McCall PJ, Hastings IM.** 2007. "Sexual" population structure and genetics of the malaria agent *P. falciparum*. PLoS One **2:**e613. http://dx.doi.org/10.1371/journal.pone.0000613.