Antigenic Diversity and the Transmission Dynamics of *Plasmodium falciparum*

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The average age of humans at their first infection with *Plasmodium falciparum* is typically less than 1 year in most endemic areas. This has been interpreted as evidence of the high transmissibility of the parasite, with the implication that control of malaria will require high levels of coverage with a potential vaccine. This interpretation is challenged by mathematical models that demonstrate that the long period required to develop immunity to malaria permits a high risk (or low average age) of infection even when parasite transmissibility is low. Patterns of seroconversion to five antigenically distinct isolates of *P. falciparum* in a highly malarious area of Papua New Guinea indicate that each is only mildly transmissible and that malaria, as a construct of several such independently transmitted strains, has a basic reproductive rate (or transmissibility) that is an order of magnitude lower than other estimates.

The transmission success of a parasite may be measured by reference to the basic reproductive rate of infection, R_0, which records the average number of secondary cases of infection generated by one primary case in a susceptible population. To block transmission by mass vaccination soon after birth, the fraction, p, that must be successfully immunized is related to the magnitude of R_0 by the simple expression (*1*)

$$p > 1 - 1/R_0 \quad (1)$$

Prevailing estimates of the R_0 of *P. falciparum* malaria, which kills around 2 million children every year, are of the order 80 to 100, suggesting that an immunization level of 99% is required to interrupt *P. falciparum* transmission (*1*). Such high values of R_0 typically derive from the analysis of the early increase with age in the proportion of individuals that have experienced *P. falciparum* infection. If infection induces lifelong immunity, R_0 is inversely related to the average age at infection, A, by the simple relation

$$R_0 = L/A \quad (2)$$

where L is the average life-span of the host (*1*). The value of A is thus low for highly transmissible diseases such as measles [1 to 2 years in large urban areas in developing countries before mass vaccination (*2*)] and higher for less transmissible diseases such as rubella [9 to 10 years in developed countries before mass vaccination (*3*)]. For *P. falciparum* malaria in hyperendemic areas, serological data that record the proportion of infants, relative to the total number of infants, positive for antibodies to total parasite extracts indicate that A is less than 1 year (*4*), suggesting that the R_0 of malaria lies between 50 to 100.

However, exposure to malaria does not induce lifelong immunity to further infection. Young children in hyperendemic malaria zones may experience anywhere between one to five clinical attacks of malaria per year (*5, 6*). Although older children do develop a functional but nonsterilizing immunity that manifests itself in a reduction of clinical episodes, a substantial reduction in parasite rates (the proportion of individuals with asexual malaria parasites) is observed only in adults (*7–16*). Thus, in contrast with measles, a large proportion of exposed individuals are not immune to reinfection. This observation may be expressed formally in the following terms: the mean duration of immunity across all age classes, H, is effectively equal in measles to the average life-span of an individual, L, whereas for malaria $H << L$.

The predicted relation between age and fraction of exposed individuals for various values of H obtained by an age-structured mathematical model is illustrated in Fig. 1A. A rapid rise with age in the proportion exposed may result even with low transmissibility (low R_0 values), provided H is short. For comparison, similar age-exposure profiles are shown for a range of R_0 values with the assumption that immunity is lifelong (Fig. 1B). The relation between the magnitude of R_0; average duration of immunity, H; average duration of infectiousness, D; and average age at first infection, A_H, is given by

$$A_H = (H + D\gamma)/(R_0 - 1) \quad (3)$$

where γ is the average number of blood meals a mosquito takes during its lifetime. Equation 3 shows that an early A_H may occur even when R_0 is small, provided H and D are small. Reliable estimates of D are difficult to obtain because it is not clear how long an individual is infectious in the course of a particular infection. Whereas the prevalence of infection, recorded by the presence of asexual parasite stages, may be high in hyperendemic situations, the prevalence of persons with gametocytes (the transmissible stages) is typically much lower (*4, 6, 8–16*). This observation suggests that D is much shorter than the duration of infection and is likely to be of the order of a few weeks. However, even if infectiousness lasted as long as infection, D would only be of the order of a few months. Thus, A_H is principally determined by H ($A = H/R_0$, to a good approximation). We therefore conclude that early A_H's with *P. falciparum* are compatible with low parasite transmissibility (*17*).

The delay in the development of immunity may be a consequence of the antigenic diversity of the parasite in which immunity develops only after exposure to many different antigenic types or "strains" circulating in a given locality (*18*). If this is the case, we expect to see a slower rise in exposure to a particular strain, but a much faster rise in exposure to "malaria," where the latter is defined as the experience of any one of several strains. When a number (n) of strains of the parasite are independently circulating in a defined region, the average age at first exposure to malaria, A_n (under the assumption that strain-specific immunity is lifelong), is given by

$$A_n = \frac{L}{\Sigma R_{0i}} \quad (4)$$

where R_{0i} is the basic reproductive rate of strain i. This equation makes clear that a

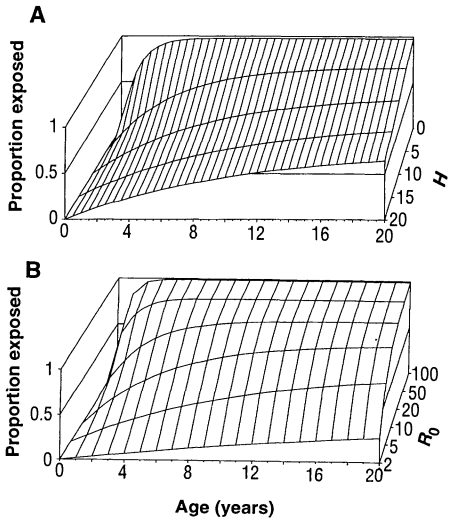

Fig. 1. (**A**) The rise with age in the proportion of each age class exposed to an infectious agent for different H's ($R_0 = 2$) (*30*). (**B**) For comparison, similar profiles are shown for different values of R_0 when immunity is lifelong.

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low A_n can arise with malaria (defined as a composite of a variety of antigenic types) when the transmissibilities of all strains, defined by the values of their R_0's, are low. Comparing Eqs. 3 and 4, we see that a low A is compatible with low transmissibility either when immunity to malaria is short-lived (Eq. 3) or when immunity is lifelong only to each of the different antigenic types or strains circulating in the community (Eq. 4). The low value of A_n arises from the summation of the low transmissibilities of the strains. In contrast, R_0 for malaria is the weighted average of the R_0's of the constituent strains. These conclusions regarding the relation between age-exposure profiles and parasite transmissibility have a general application in that any infection that does not induce lifelong immunity, such as gonorrhea, and which exists as a construct of strains may conform to this paradigm.

To test whether the R_0 of malaria may be calculated by classifying the parasite population into antigenically distinct strains, we applied the mathematical methods described here to a set of epidemiological data collected in Madang, Papua New Guinea (PNG). Infection and disease patterns in this region of year-round transmission are similar to most areas of stable malaria transmission (*6, 12*), and entomological inoculation rates (*19*) are comparable to, if not higher than, those in many areas of sub-Saharan Africa (*4, 10, 11, 20, 21*). In Fig. 2A, we record by age the rise in exposure to five *P. falciparum* isolates that differ in parasite-induced erythrocyte surface antigens (PIESAs). Isolates 1935, 1776, 1934, and 1917 were collected in PNG in 1987 (*22*). The clone HB3 derives from a Honduran isolate (*23*). Each of the PNG isolates has a distinct PIESA serotype, as defined by patterns of agglutination reactivity with acute and convalescent sera from the donors of these isolates (*22*). Researchers have shown that a single exposure to a particular PIESA serotype results in a specific agglutination antibody response 21 to 28 days after acute infection (*22, 24*), which is predominantly isolate-specific (*25*). The proportion exposed to any one particular PIESA serotype rises only slowly with age, so that typically less than 25% of children will have seen the corresponding strain by the age of 4 years. In contrast, the proportion in each age class with experience of any one of the five strains rises rapidly with age. The observed patterns are consistent with the idea that serotype-specific immunity may endure for long periods (*22, 24, 26*), but they do not preclude the hypothesis that repeated exposure to one serotype is necessary to maintain immunity (in which case, we would be overestimating R_0). Exposure to the surface antigen associated with a single wild isolate of *P. falciparum* in rural Gambia (*27*) shows a similar age profile to those recorded in Fig. 2A. In the Gambia study, evidence of exposure to the surface antigen was the only immune factor that showed a consistent protective effect against clinical malaria, out of a variety of host immune responses examined for protective effects against asexual blood stages.

The PIESAs described here have been shown to undergo antigenic variation (*28*), which may appear to complicate the interpretation of our data (Fig. 2A). It seems unlikely, however, that seroconversion to any of the five antigenic types in our study occurs as a consequence of clonal antigenic variation because in vitro studies (*28*) reveal rapid rates of emergence of antigenic variants that are inconsistent with the slow accumulation of exposure of the five PIESA serotypes as recorded in Fig. 2A. That this response is isolate-specific also indicates that there is limited overlap between the variant repertoires of the isolates. It seems likely that antigenic diversity, as reflected by the multitude of strains constituting malaria, is of significance in ensuring parasite persistence in the host population. Antigenic variation through the switching of agglutination phenotypes during clonal expansion in the host, however, is of significance in extending the duration of a single infection (and hence the infectious period) and increases the likelihood of transmission from a single host.

The proportions of each age class that have experienced exactly n serotypes are shown in Fig. 2B. The predicted proportion of individuals of a given age a that will have seen exactly n strains out of five may be calculated as

$$C_n^5 x(a)^n [1 - x(a)]^{5-n} \quad (5)$$

where x is the number of exposed hosts and C is the probability of transmission from host to vector, given complete independence of strain transmission and assuming that R_0's are essentially similar between strains. If infection confers lifelong immunity, then $x(a) = 1 - e^{-(L/R_0)a}$. The matrix of proportions in age class that have seen exactly n strains may thus be calculated for a given value of R_0. The expected distribution of individuals in each age class by number of strains experienced for an average R_0 value of 7 is shown in Fig. 3A, revealing a correspondence with the pattern recorded in Fig. 2B. A comprehensive analysis with various values of R_0 reveals that the best fit to the observed pattern is provided by R_0 values in the range of 6 to 7 (Fig. 3B). This suggests that the R_0 of *P. falciparum* malaria in a highly endemic area like PNG is lower than other estimates (*1*).

In conclusion, we have shown that a low A with *P. falciparum* does not reflect high transmissibility of the parasite but may instead be an indication of the circulation of many distinct antigenic types or strains of the parasite, each with moderate to low transmissibility. In such circumstances, immunity to malaria (where the latter is defined as the experience of one or more strains) may appear short-lived, when in reality immunity to a specific strain is lifelong and reinfection occurs as a result of exposure to different strains.

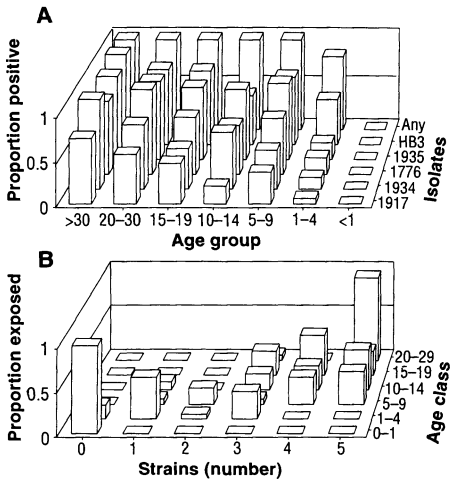

Fig. 2. The rise by age in exposure to five *P. falciparum* isolates (*31*), shown as the proportion in each age class with agglutinating antibodies to each strain (**A**) and alternatively as the proportion in each age class exposed to exactly n strains (**B**).

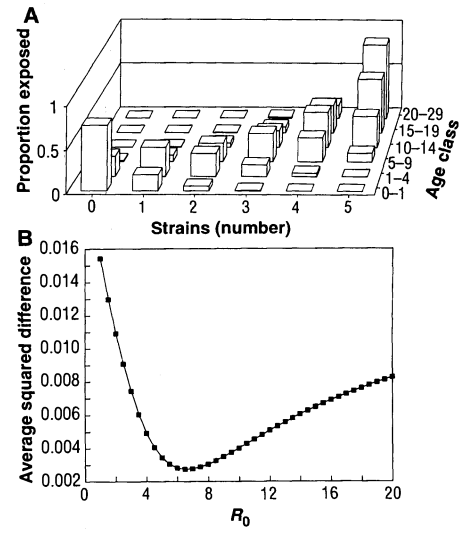

Fig. 3. (**A**) The predicted distribution of the proportions of each age class exposed to exactly n strains given $R_0 = 7$. (**B**) The goodness of fit (*32*) of the average squared difference, S, across a range of values of R_0, showing a best fit around 6 to 7.

Eradication of malaria may be achieved in such a system with a vaccine directed against conserved determinants, which may be naturally poorly immunogenic but may be rendered more effective by artificial methods. Vaccination strategies involving conserved antigens must be based on the R_0 of the strain with the highest transmissibility, as is the case for the measles-mumps-rubella vaccine (*1*), where the R_0 of measles determines the requisite vaccine coverage. At the low values of R_0 indicated by our analysis of epidemiological data, malaria may be easier to control by mass vaccination (*29*) than expected.

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30. We use an age-structured mathematical model for malaria transmission with overlapping categories of exposed, x, and immune, z, hosts. Nonimmunes acquire infection from contact with infectious mosquitoes, present in proportion y in the vector population, and become immune. Immunity is lost at rate h. Once exposed, however, an individual remains in the exposed category for life; hence, the rate of loss of exposed individuals is identical to the community death rate, μ. If infection induces lifelong immunity, then $h = \mu$. The transmission system can be described by the following set of partial differential equations

$$\frac{\delta x}{\delta a} + \frac{\delta x}{\delta t} = m\alpha b(1 - x)y - \mu x$$

$$\frac{\delta z}{\delta a} + \frac{\delta z}{\delta t} = m\alpha b(1 - z)y - hz$$

$$\frac{dy}{dt} = m\alpha^2 bD(1 - y)y[1 - \int \mu z(a)e^{-\mu a}\partial a] - \frac{1}{L_M}y$$

Here, m is the number of mosquitoes per human host, α is the mosquito biting rate, b is the probability of transmission from vector to host, L_M is the average life-span of the mosquito, and D is measured in the human host. Within this system, $R_0 = m\alpha^2 bDL_M$ [J. L. Aron and R. M. May, in *Population Dynamics of Infectious Diseases: Theory and Applications*, R. M. Anderson, Ed. (Chapman and Hall, London, 1982), pp. 139–179].
31. We performed agglutination assays on plasma samples using lines selected for the expression of agglutination phenotypes (by binding to C32 amelanotic melanoma cells) using methods as described [K. P. Day *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8292 (1993)]. Trophozoite-infected cells of the above lines were reacted with sera as described (*22*) from a cross-sectional survey involving the following numbers of individuals (n) in each age class: for 1 year of age with isolates 1776, HB3, 1934, and 1935 (n = 3) and with isolate 1917 (n = 2); for age 1 to 4 years with isolates 1776, HB3, and 1917 (n = 18) and with isolates 1934 and 1935 (n = 17); for 5 to 9 years with isolates 1776, HB3, and 1934 (n = 16), with isolate 1917 (n = 14), and with isolate 1935 (n = 13); for 10 to 14 years with isolates 1776, HB3, and 1934 (n = 11), with isolate 1917 (n = 10), and with isolate 1935 (n = 9); for 15 to 19 years with isolates 1776, HB3, 1934, and 1917 (n = 11) and with isolate 1935 (n = 10); for 20 to 29 years with isolates 1776, HB3, 1934, and 1917 (n = 18) and with isolate 1935 (n = 15); and for ≥30 years with isolates 1776, HB3, and 1934 (n = 16), with isolate 1917 (n = 15), and with isolate 1935 (n = 13).
32. To determine whether there was a correlation with R_0 in the correspondence of the distribution generated by the model with the observed pattern, we used a simple inverse measure: $S = \Sigma$(observed pattern − expected pattern)^2, of the "goodness of fit," where S is the average squared difference. In Fig. 3B, this measure is plotted with respect to R_0, showing a best fit around values between 6 and 7. Maximum likelihood estimates of R_0 for each strain (assuming that each confers lifelong immunity) were in the range of 2 to 10. Note that there may be some bias in these values because of host heterogeneity.

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Diverse Essential Functions Revealed by Complementing Yeast Calmodulin Mutants

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Calmodulin, a cytoplasmic calcium-binding protein, is indispensable for eukaryotic cell growth. Examination of 14 temperature-sensitive yeast mutants bearing one or more phenylalanine to alanine substitutions in the single essential calmodulin gene of yeast (*CMD1*) revealed diverse essential functions. Mutations could be classified into four intragenic complementation groups. Each group showed different characteristic functional defects in actin organization, calmodulin localization, nuclear division, or bud emergence. Phenylalanine residues implicated in calmodulin localization and nuclear division are located in the amino-terminal half of the protein, whereas those implicated in actin organization and bud emergence are located in the carboxyl-terminal half.

Calmodulin is a Ca^{2+}-binding protein implicated in many functions in eukaryotic cells (*1*). It interacts with more than 20 different proteins including several metabolic enzymes, protein kinases, a protein phosphatase, ion transporters, receptors, motor proteins, and cytoskeletal components (*1*, *2*). Budding yeast (*Saccharomyces cerevisiae*) contains a single gene encoding calmodulin (*CMD1*), which is essential for cell growth (*3*). Calmodulins from yeast and vertebrates have structural, biochemical, and biophysical similarity (*4*) and are functionally conserved (*5*). Extensive analysis of two temperature-sensitive mutations (*cmd1-1* and *cmd1-101*) indicated that a defect in mitosis (*6*, *7*)—specifically, impaired spindle pole body (SPB) function (*6*)—is responsible for the temperature-sensitive lethality. However, the finding of many functions unrelated to mitosis in vitro indicated that nuclear division might not be the only essential function requiring calmodulin (*8*).

If calmodulin indeed has diverse essential roles, it might be possible to find additional conditional-lethal mutations in *CMD1* that impair each of these essential functions. Conversely, identification of several distinct defects specified by different *cmd1* mutations would serve to demonstrate the multiple essential roles of calmodulin in cell growth. Mutations were obtained by specific alteration of the phenylalanine residues in calmodulin, which were predicted

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