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Parasite virulence and disease patterns in *Plasmodium falciparum* malaria

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ABSTRACT **Heterogeneity in parasite virulence is one of several factors that have been proposed to contribute to the wide spectrum of disease severity in *Plasmodium falciparum* malaria. We use observed age-structured patterns of disease to define a population structure of *P. falciparum*, where the latter contains several independently transmitted antigenic types or "strains" that each induce some degree of strain-specific antidisease immunity upon infection. Patterns of incidence of severe and mild disease may be explained by assuming that a majority of these strains are associated with mild disease and that although severe malarial anemia is a complication occurring in a certain proportion of early infections with "mild" parasites, cerebral malaria is caused by a few distinct highly virulent strains. Considerable variation in parasite virulence, as a major factor of disease severity in malaria, is made possible by the absence of competition between the various parasite strains, arising from weak shared immune responses. The theoretical framework presented in this paper can explain other epidemiological observations, such as the results of interventions with insecticide-impregnated bednets.**

The relationship between virulence and transmissibility is an important theme in analysis of host–parasite interactions in natural populations (1). However, there are few studies of the effects of parasite virulence on the population dynamics of major infectious diseases of humans. Data from the era of malaria therapy (2, 3) and recent molecular studies (4) indicate that there may be considerable variation in virulence of the *Plasmodium falciparum* parasite, which causes >1 million malaria deaths each year. In this paper we explore the population dynamic and population genetic implications of such proposed parasite diversity to ask whether they may explain some of the now well-defined epidemiological features of malarial disease.

In African children, among whom the great majority of malaria deaths occur, *P. falciparum* malaria can be clinically resolved into "mild" and "severe" types. This distinction describes a clear, and readily recognizable, clinical differentiation of malaria into a majority (≈99%) of uncomplicated cases with a very low mortality (<<1%) and a small number of severe cases with a mortality of 10–20% under treatment (5). Furthermore, severe malarial disease manifests as either severe malarial anemia (SMA) or cerebral malaria (CM), both pathologically distinct from mild malaria. Hence, this classification is not just an arbitrary division of a continuum of disease severity but reflects a clear bimodality in the severity of malarial disease.

Although data from animal models (6) and induced malaria experiments (2, 3) indicate that isolates can vary in the severity of disease caused, differences in *P. falciparum* virulence have yet to be conclusively demonstrated in the field. Recently, however, a number of parasite phenotypes have been identified as possible virulence factors; these include cytoadherence (adherence of infected cells to epithelial surfaces), rosetting (of uninfected erythrocytes by infected erythrocytes), cytokine production, and less specifically, immune evasion mechanisms and variation in the intrinsic growth rate of the parasite (4). The variability in the clinical outcome of *P. falciparum* infection may thus be a consequence of heterogeneity in parasite phenotypes associated with the pathology of severe disease. There is strong evidence that host genetic susceptibility influences the clinical outcome of malarial infection; the immunological, nutritional, and sociological status of the host may also be of various degrees of importance (7). Disease severity may also depend on the size of initial parasite inoculum (8). It is apparent that whether an individual develops mild or severe malaria must depend on a complex combination of host and parasite factors. However, the epidemiological patterns of mild and severe disease observed within the population at large may be predominantly influenced by a few key variables. In this paper, we propose that certain characteristic epidemiological features of mild and severe disease may be explained by heterogeneity in parasite virulence.

The Effect of Virulence Factors on Transmissibility

Parasite transmissibility may be expressed as a combination of parameters, known as the basic reproductive rate (R_0), which is a measure of the average number of secondary infections generated by one primary infection in a totally susceptible population (1, 9). Parasite phenotypes associated with virulence are likely to have both positive and negative effects on the transmissibility (R_0) of malaria. High or persistent parasitemia, precipitated by certain virulence factors such as cytoadherence, may enhance the production of infectious stages (gametocytes) (10, 11) and, therefore, increase transmissibility. Splenic evasion through cytoadherence may play a more direct role in increasing infectiousness as developing gametocytes of *P. falciparum* also sequester until mature and infectious (12). A negative effect on transmission of all virulence factors occurs as a consequence of host mortality (thus reducing the average duration of infectiousness). However, the adverse effects may be more subtle. For instance, high parasitemia could induce a severe nonspecific immune response that could act to reduce the infectiousness of gametocytes. Fever/paroxysms are associated with high plasma levels of the pyrogenic cytokine tumor necrosis factor α (13, 14) that, along with other cytokines such as interferon, appears to affect circulating gametocytes, as demonstrated by membrane feeding experiments on *Plasmodium vivax* infections in Sri Lanka (15). As a result of these

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Abbreviations: CM, cerebral malaria; SMA, severe malarial anemia.

tensions between the different effects of parasite phenotypes associated with virulence, the relationship between virulence and transmissibility (R_0) is nonlinear, with the latter likely to peak at some intermediate value of virulence. Though in contrast to the classical coevolutionary paradigm where host and parasite are expected to attain some state of mutual harmlessness, selection for intermediate levels of virulence has been widely demonstrated, both experimentally and through theoretical exercises (16–18). Parasite types with the intermediate degree of virulence that maximizes transmission (R_0) will have an evolutionary advantage over other types, provided there is competition between the different parasite types. Under these circumstances, the system may be expected to evolve toward the intermediate degree of virulence that maximizes transmissibility.

The question then arises of how polymorphisms are maintained at genetic loci governing the expression of virulence factors, given that selection will occur for alleles that maximize transmissibility. In a situation where there is strong competition between different parasite types (as a result of shared immune responses, for instance), coexistence is only possible if the strains have very similar basic reproductive rates (19). Thus a more virulent strain must compensate for higher host mortality (which reduces the duration of infectiousness, and hence transmissibility) through higher infectiousness. However, selection for optimum virulence (i.e., maximum transmissibility) will only occur if the different strains are in competition. If there is no competition between the strains, the constraint that the basic reproductive rates must be similar can be relaxed (19). Within each antigenic system, there will be selection for a level of virulence to achieve maximum transmissibility. Thus R_0 will peak independently at some higher level of virulence for a "severe" strain than for a "mild" strain. This scenario is favored by the observed persistence of high prevalence of parasitemia in older children, which suggests that infection-blocking immunity is mainly strain-specific or develops only after a long history of exposure to shared determinants (20). The typically abrupt decline in parasite rates between the ages of 10 and 15 years in endemic areas suggests that immune responses to shared determinants must accumulate to a certain threshold to block further infection (21). This delayed development of "cross-immunity" may serve as a weak source of competition and limit the range of variation in R_0 between the parasite types (19).

We thus propose that the maintenance of heterogeneity in parasite virulence is made possible by the virtual lack of effective cross-immunity between strains of differing virulence, given that they are unlikely to be of similar transmissibility. These variously virulent strains thus essentially constitute separate transmission systems. In the following section, we show that this argument can offer a satisfactory basis for the hitherto unexplained age-structured patterns of severe and mild disease.

Age-Structured Patterns of Disease

Fig. 1 documents age-structured patterns of mild and severe malaria in The Gambia, West Africa. Fig. 1*A* records the age distribution of cases of mild malaria in terms of average number of clinical episodes per year. Similar patterns have been recorded in other malaria endemic areas (23, 24). Fig. 1*B* shows the age distribution of cases of CM and SMA observed in a large case-control hospital-based study in The Gambia (22); a similar study in coastal Kenya reports the same disjunction between the two age profiles (4). The average age of children with SMA has been reported as 27 months and that of children with CM has been reported as 45 months in The Gambia (5). The corresponding ages in coastal Kenya are 22 months and 40 months (4).

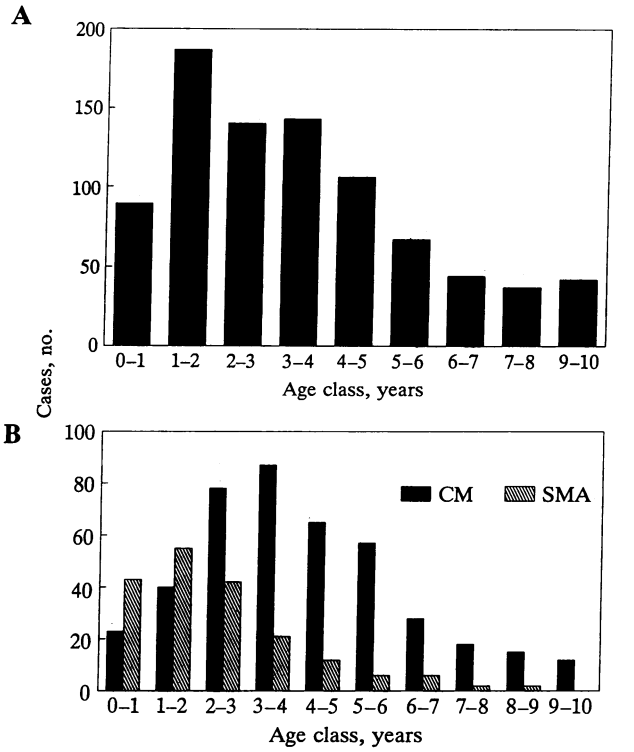

FIG. 1. Age-structured patterns of mild and severe malaria in The Gambia, West Africa. (*A*) Age distribution of cases of mild malaria presenting at the out-patient department of the Medical Research Council clinic in Fajara, The Gambia, from October 23 to December 31, 1992. (*B*) Age distribution of cases of CM and SMA observed in a large case-control hospital-based study in the same area (for details, see ref. 22).

The steep increase and slow decline with age in the incidence of mild malaria (Fig. 1*A*) can be explained by the existence of a number of independently transmitted antigenic types or strains that each induce some degree of antidisease or protective immunity upon exposure (i.e., first infection).

The average age of exposure to mild malaria, defined as the experience of any one of n different strains is given by:

$$A_n = \frac{H}{\sum R_{0i}}, \qquad [1]$$

where R_{0i} is the basic reproductive rate of strain i and H is the average duration of immunity against reinfection by the same strain (25). The latter is determined by the relative proportions of the population who are immune (mostly adults) and nonimmune (mostly children) to a given strain, rather than by the rate of decay of immunity within an individual. In the limit, where each strain induces lifelong strain-specific immunity against reinfection, H is equal to the average lifespan of the human host (25).

Eq. 1 implies that a large bulk of children will have experienced mild malaria at a very young age (<1 year), as a consequence of the large number of strains circulating within a given region, even if the basic reproductive rate of each strain (and hence of mild malaria as a whole) is very low. The circulation of a large number of strains that each induce strain-specific antidisease (protective) immunity upon exposure can explain the slow uniform decline in the incidence of mild disease. As the experience of different strains accumulates, the probability of infection with a previously unencountered strain diminishes. Thus, the incidence of disease will decline slowly with age, as shown in Fig. 1*A*. The broken lines in Fig. 2 record the output of a mathematical model

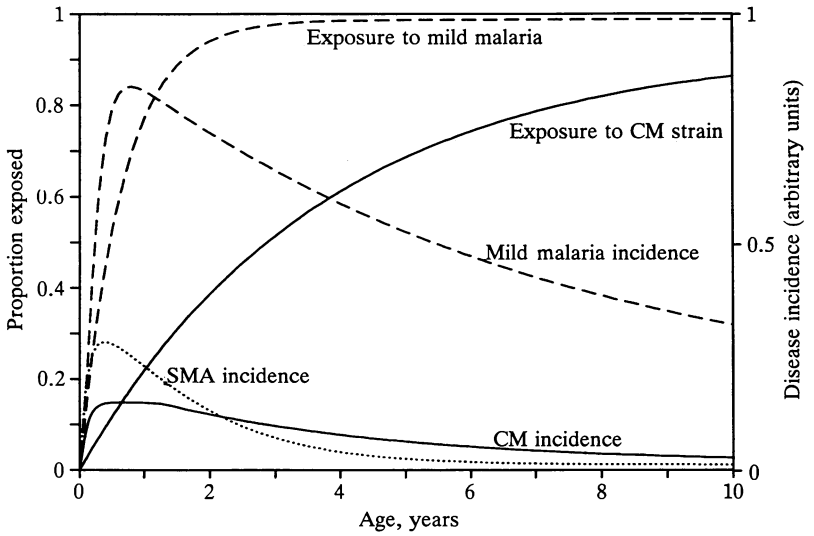

FIG. 2. Simulations of a mathematical model with 50 strains of mild malaria and a single strain of CM, as described in *Appendix*, in terms of the patterns of exposure to mild malaria and CM parasites, and the associated patterns of mild and severe disease. The incidence of SMA and CM is magnified 10 times to bring their respective patterns into view.

based on the above assumptions, where the number of strains of mild malaria is 50 (for mathematical details, see *Appendix*). Exposure to mild malaria, defined as the experience of any one of these strains, rises rapidly, as does the incidence of mild disease. The incidence of mild disease subsequently declines, as the number of new strains encountered diminishes.

Both SMA and CM appear to decline more rapidly with age than mild malaria. For SMA, this may be an effect of protection from past exposure to mild malaria, as the sharp decline with age in the incidence of SMA correlates with the steep rise with age in the proportion exposed to mild malaria. Fig. 2 shows the pattern of incidence of SMA generated by the assumption that the latter is a rare consequence of first infection (or exposure) with mild malaria parasites (any of the 50 strains). Thus, the age-specific pattern of incidence of SMA may be explained by assuming that the latter is a rare complication of infection with any mild malaria strain, occurring mainly among those who have little previous experience of mild malaria.

The age-structured pattern of CM cannot, however, be explained in this way by an association with lack of exposure to mild malaria. Furthermore, a recent study in The Gambia indicates that lack of previous exposure is not a risk factor for the development of CM (26). Children with CM, in this study, were shown to recognize as wide a diversity of parasite isolates as children of similar age with mild malaria. This result suggests that CM may be caused by a single or a few independently transmitted parasite types. The observed time–space clustering of the incidence of severe malaria in coastal Kenya (27) lends further support to this hypothesis. The higher average age of CM as recorded in Fig. 1*B* can be thus explained as a consequence of a slower rise with age in exposure to parasites causing CM, in comparison with parasites causing mild malaria (and SMA). Eq. 1 indicates that exposure to mild malaria may rise more rapidly than to CM because the former may be caused by a larger number of strains. Within this framework, the more rapid decline in the incidence of CM with age, in comparison with mild malaria, can be explained as characteristic of a disease caused by a single or a few strains.

The rise in exposure to mild malaria, defined as the experience of any one of a large number strains, will occur more rapidly than for the few strains capable of causing CM, even if the transmissibility of mild malaria (a weighted average of the R_0 values of the constituent strains) is lower than the R_0 of CM (25). As the proportion unexposed to each type diminishes, the incidence of the associated severe disease will decline. Fig. 2 contrasts the respective patterns of CM and SMA, generated by a mathematical model in which CM is caused by a single parasite strain, while mild malaria may be caused by any of 50 strains. There is a slow increase in exposure to the CM strain and a corresponding pattern of decline in the incidence of CM.

In both mild and severe cases, a significant degree of protection against disease at birth, decaying rapidly with age, must be invoked to account for the low numbers in the first few years of life. In the absence of such a mechanism, the highest incidence of disease is likely to occur in the youngest age class, as it contains the highest concentration of susceptibles. Clearly, this is not the case for either mild or severe malaria. The nature of this well-recognized resistance of very young children to clinical malaria remains poorly understood. As a result of this resistance, the incidence of disease will, in all cases, appear to peak at a given age, rather than exhibiting a monotonic decline. The age at which incidence peaks will be determined by the rate of decline of disease incidence in the absence of this protective mechanism. The later peak for CM thus essentially reflects a slower decline in disease incidence in comparison with SMA. We assume that the degree of protection decays exponentially at a rate of 80%, such that a significant proportion of children in the 0- to 1-year and 1- to 2-year age classes and a small fraction of the 2–3 year olds are protected against disease. With the incidences in the first three age classes accordingly scaled down, the age distributions of SMA and CM peak, respectively, in the 1- to 2-year and 3- to 4-year age classes. The rate of decline required in this model to produce the observed age profiles may be of value in defining this protective mechanism.

Effects of Heterogeneity in Parasite Virulence on Vector Control

The stratification of the parasite population into strains of differing virulence and transmissibility, where most such strains induce mild disease, can be used to explain the observation that the use of bednets can reduce the incidence of mild disease without inducing any significant change in prevalence of infection (28, 29). The latter has previously been explained by assuming that the development of malarial disease is a function of the size and frequency of innoculation. Within the strain framework, the effect of bednets may be very simply explained as a reduction in the number of new strains encountered (i.e., those capable of causing disease). Although a similar proportionate reduction will have oc-

curred in the total number of strains (i.e., those capable of causing infection) encountered, the effects of this will be lost due to saturation in the prevalence of parasitemia. Disease incidence, however, will not have saturated, since not every infection leads to disease and because the duration of disease is short in comparison with duration of parasitemia. Therefore, a reduction in the number of new strains encountered may manifest itself in a reduction in disease.

Alternatively, the effect of bednet use may be interpreted as the consequence of the differential impact of reduction in transmission (by bednet use) upon parasite types associated with disease. Fig. 3 demonstrates the effect of a reduction in biting rate (a) on the population prevalence of two different parasite strains, where the basic reproductive rate of strain A is 5 times that of strain B, in the absence of bednets ($a = 0.4$ bites per day). It is clear that the differential impact of reducing access to hosts will be much greater in the case of strain B. The reduction in mild disease may thus be explained by a subspectrum of virulence among mild strains, where the more virulent strains are less transmissible than those that lead to asymptomatic infections.

Discussion

The spectrum of malarial disease may be explained by the existence of several different antigenic types that each induce some degree of strain-specific antidisease immunity upon infection but may or may not elicit adequate infection-blocking responses. Age-specific patterns of incidence of severe and mild disease suggest that the majority of these antigenic types are associated with mild disease. Epidemiological studies, viewed within this framework, suggest that while SMA may be a complication occurring in a certain proportion of early infections with mild parasites, CM is caused by a few distinct antigenic types. Thus, exposure to mild malaria rises more rapidly than to CM (as manifest in the higher average age of CM cases), simply because the former can be caused by any of a large number of strains. Conversely, the incidence of mild malaria declines at a much slower (and more uniform) manner than the sharp nonlinear

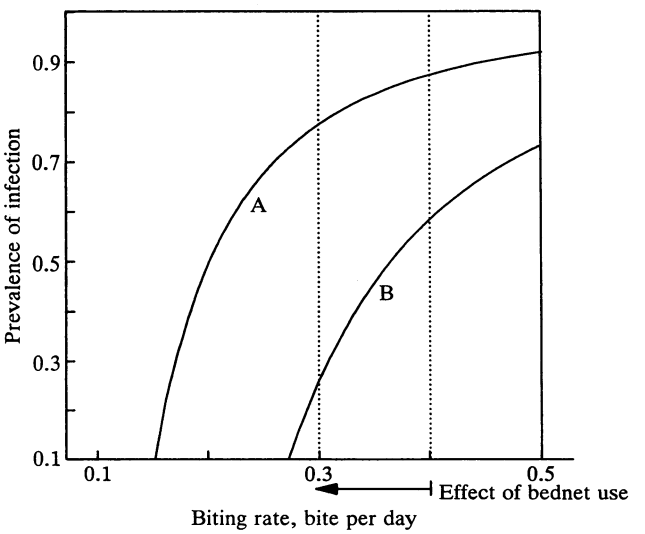

FIG. 3. Effect of a reduction in biting rate on the population prevalence of two different parasite strains where the basic reproductive rate of strain A is 5 times that of strain B, in the absence of bednets ($a = 0.4$ bite per day). The effect of bednet use is shown as a shift in biting rate from 0.4 bite per day to 0.3 bite per day; the impact of this change on prevalence of infection is minimal for strain A and significant for strain B. Thus if strain B is the more virulent strain, there will be a reduction in disease that is not accompanied by a reduction in prevalence of parasitemia in the population.

decline observed in CM cases that is more typical of a disease associated with a single strain. The extremely rapid decline in incidence of SMA with age can be explained by assuming that the probability of developing the syndrome drops rapidly with number of past infections with mild malaria parasites. Any mild malaria strain is assumed to be capable of precipitating SMA, provided the host has not been infected with malaria more than a few times.

Certain molecular observations support the stratification of the parasite population into strains of differing virulence and transmissibility, where those causing CM constitute a distinct subset. Although a multiplicity of parasite factors is associated with the diverse pathological consequences of malaria, we assume that the major determinant of CM is the blockage of brain vessels by cytoadherence of infected erythrocytes. A high molecular weight antigen designated Pf EMP1 has been associated with the expression of the cytoadherence phenotype (30). Polymorphism in this molecule, leading to variation in cytoadherence, has been suggested as a possible explanation for heterogeneity in parasite virulence (31–33). The putative parasite cytoadherence ligand Pf EMP1 is believed to be the target of agglutinating antibodies that are isolate- and variant-specific (34). These antibodies have been shown to correlate with protection against mild disease (35). Epidemiological patterns of seroconversion to these antigenic types (as defined by the agglutinating antibodies), recorded in the Madang region of Papua, New Guinea, suggest that they are independently transmitted (25). Recent molecular data (36) suggest that the Pf EMP1 genotype may be directly associated with the transmissibility of a strain and thus support the stratification of the parasite population into strains of differing virulence and transmissibility with respect to this phenotype.

The putative cytoadherence ligand Pf EMP1 and the associated agglutination phenotype have both been shown to undergo clonal antigenic variation (37, 38). This does not, however, complicate the definition of a malaria strain, since *in vitro* studies (38) reveal very rapid rates of emergence of new antigenic variants that are inconsistent with the slow accumulation of experience of agglutination phenotypes of field isolates (25). It seems likely that antigenic variation through switching of agglutination phenotypes during clonal expansion within the host is of significance in extending the duration of a single infection but not in protecting against a range of strains, possibly because there is little overlap between the variants associated with each strain. Thus, a cytoadherence genotype, composed of a series of variants, may function in the same manner as trypanosome serodemes (distinct repertoires of variable antigen types), which have been shown to cocirculate within a transmission system (39).

If more than one locus determines virulence (and hence transmissibility), then genetic recombination may interfere with the stratification of the parasite population into independently transmitted antigenic types or strains. However, rates of recombination will depend on the intensity of transmission, and recombination may be a relatively rare event in areas of low transmission, given the short duration of infectiousness. Evidence is gradually emerging that a large degree of clonality may prevail in endemic situations (K.P.D., unpublished data), thus isolating strains differing in virulence and transmissibility.

The maintenance of considerable variation in parasite virulence, which acts as an important determinant of malarial disease severity, is made possible by the absence of competition between the various parasite strains, arising from weak shared immune responses associated with limited overlap in antigenic variants of the different strains, and by the low probabilities of recombination occurring as a result of short infectious periods. Heterogeneity in host resistance is a further factor in the maintenance of parasite polymorphisms associated with variable virulence (21). The theoretical framework

presented in this paper serves to explain several epidemiological observations and should stimulate further research on the population genetics and transmission dynamics of parasite virulence.

Appendix

Let $y_i(a, t)$ denote the proportion of the population of age a, at time t, with experience of i strains of mild malaria. The dynamics of the system may be represented by the following set of equations: $(\delta y_1/\delta t) + (\delta y_1/\delta a) = n\lambda(1 - \Sigma y_1) - [(n - 1)\lambda + \mu]y_1$ and $(\delta y_i/\delta t) + (\delta y_i/\delta a) = (n - i + 1)\lambda y_{i-1} - [(n - i)\lambda + \mu]y_i$, where the total number of strains circulating within the system is n and λ is the per capita force of infection per strain (R_0/H). Thus, individuals who have experienced i strains are at risk of disease from $n - i$ strains, each with force of infection, λ. Upon infection, these individuals move into the category y_{i+1}. Individuals in the compartment y_n are immune to disease. We seek solutions to these equations at equilibrium and thus set the time derivatives to zero.

The proportion as yet unexposed to mild malaria (defined as not having experienced any one of the n strains) at age a is given by $X(a) = 1 - \Sigma y_i(a)$. The incidence of mild disease between the ages a_1 and a_2 is given by $Z(a_2) - Z(a_1)$, where $dZ(a)/da = \lambda[\Sigma (n - i)y_i(a) + nX(a)]$, where mild disease is assumed to be a consequence of infection with a new strain.

The incidence of SMA between the ages a_1 and a_2 is given by $Z'(a_2) - Z'(a_1)$, where $dZ'(a)/da = n\lambda X(a)$, in the extreme where SMA can be precipitated by any malaria strain among those without past experience of mild malaria. The same set of equations can be adapted to calculate exposure to and incidence of CM, by reducing the number of strains n.

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