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STUDIES ON THE CHARACTERIZATION OF *PLASMODIUM VIVAX* STRAINS FROM CENTRAL AMERICA

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During the past several decades, characterization of American strains of malaria has been almost totally neglected except for a few strains which were characterized in the 1940's.[1] Recently, the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, has been collaborative with the Central America Malaria Research Station in El Salvador on characterization of strains of human malaria from the Central and South American region.

This report deals only with studies on isolates of vivax malaria from three Central American countries. Complete characterization of strains of malaria includes such features as prepatent period, course of infection, relapse activity, sporozoite cycle, transmissibility, antigenic composition, drug response, and related phenomena. The features which will be discussed here relate only to prepatent periods, relapse activity, and drug response.

The strains were isolated in clean, intact *Aotus trivirgatus* (owl or night monkeys) by the intravenous and or intraperitoneal inoculation of parasitized blood from human donors. The monkeys were inoculated in El Salvador and shipped to the Chamblee, Georgia facilities. Once patent infections were obtained in the owl monkeys, the strains were maintained either by passage of parasitized blood or by inoculation of sporozoites. The strains of vivax malaria characterized to date were acquired from Panama (Panama strain), El Salvador (Sal I and Sal II strains), and Nicaragua (NICA strain).

Volunteers at the Unit on Human Malaria at the U.S. Penitentiary in Atlanta, Georgia were exposed to infection by the bites of *Anopheles freeborni* or *A. maculatus* mosquitoes which had been allowed to feed on owl monkeys whose infections showed gametocytemia. Volunteers were followed daily or 3 times a week by blood smears. At each attack, the infections were treated after a minimum of 3 days patent parasitemia with one of the standard antimalarial drugs at the recommended dosage regimen.

Table 1 shows the results of our studies with the Panama strain. It can be seen that the prepatent periods for the ten infections ranged from 12 to 17 days (median of 13.5 days). The first relapse activity in 8 of 10 volunteers occurred at from 94 to 226 days (median of 165.5 days) after exposure to infection. Of the 2 remaining volunteers, 1 did not experience relapse through the observation period of 167 days before he received radical curative therapy of chloroquine (1.5 g base) and primaquine (15 mg base for 14 consecutive days). The other received radical cure at day 15.* Five of the 8 volunteers who exhibited one relapse experienced a second relapse at day 17 to 56 or a median of 51 days after the first relapse. The three volunteers who did not receive radical curative therapy after the second relapse experienced a third relapse at days 251 to 298 (median 271) or 52 to 57 days after their second relapse. Of the 8 infections which showed any relapse activity, 7 did so after a minimum latent interval of 143 days or 20 weeks after exposure to infection.

The response of the Panama strain to chloroquine and to quinine was normal, with parasite clearance occurring the 2nd to the 4th day. The response of this strain was also found to be normal (clearance in less than 72 hours) in three of four treatments with pyrimethamine. Parasite clearance in one of the pyrimethamine treatments required between 168 and 192 hours. One infection was treated at second relapse with a chlo-

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* In this connection, whenever a volunteer decides that he no longer wants to participate in our malaria studies, we honor his decision and administer radically curative therapy at that time. Thus, a number of volunteers in this study received radically curative antimalarial therapy before their infections could have been expected to relapse.

TABLE 1

Relapse activity of a Panama strain of Plasmodium vivax *in volunteers exposed to infection by the bites of infected* Anopheles *mosquitoes*

Volunteer	Primary attack	Infection day*		
		1st relapse	2nd relapse	3rd relapse
K. A.	13 (C&P at 15)			
P. I.	13 (C) (C&P at 167)			
P. E.	11 (C)	143 (C) (C&P at 179)		
P. A.	14 (C)	164 (C) (C&P at 265)		
H. A.†	17 (Py)	205 (C&P at 250)		
Mc. C.	12 (Py)	94 (Py)	149 (C&P at 163)	
W. E.	15 (C)	167 (C)	218 (CLE) (C&P at 258)	
T. O.	14 (Q)	154 (C)	244 (C)	271 (Q) (C&P at 363)
C. L.	13 (Q)	174 (C)	197 (C)	251 (C&P at 253)
D. E.	12 (Q)	226 (Q)	246 (Q)	298 (C&P at 301)

* (C), chloroquine; (CG), chloroguanide; (Py), pyrimethamine; (Q), quinine; (CLE), Cleocin®; (C&P), chloroquine and primaquine radical curative therapy initiated on the given infection day.
† Negro.

rinated lincomycin (semi-synthetic antibiotic) marketed as Cleocin®; the parasite clearance was somewhat prolonged (up to 132 hours). The radical curative rate for the 14-day regimen of 15 mg primaquine daily observed for this strain was 100% irrespective of whether it was given for treatment of the primary attack or the first, second, or third relapse.

Table 2 summarizes our observations on the Salvador I strain. The prepatent periods for eleven infections ranged from 12 to 16 days (median of 15 days). No relapse activity was seen in five infections before radical curative therapy was initiated at 98 to 240 days after exposure to infection. In the five volunteers whose infections showed relapse activity, relapses occurred at 192 to 229 days (median 205) after exposure to infection. A second relapse was observed in only one volunteer, at day 229. The other four volunteers received radical curative therapy at day 195, 200, 227, and 272 (8 to 60 days after relapse). No additional relapse activity occurred.

Table 3 shows the relapse activity of the Salvador II strain of vivax malaria. The prepatent periods for the eleven infections ranged from 10 to 16 days (median of 13 days). In six infections, no relapse activity was seen before

TABLE 2

Relapse activity of the Salvador I strain of Plasmodium vivax *in volunteers exposed to infection by the bites of infected* Anopheles *mosquitoes*

Volunteer	Primary attack	Infection day*	
		1st relapse	2nd relapse
Mc. C.	12 (C & P at 19)		
R. I.	12 (Q) (C & P at 98)		
M. A.	15 (C) (C & P at 239)		
C. A.	16 (Py) (C & P at 141)		
D. E.	16 (CG) (C & P at 171)		
L. I.	16 (Q) (C & P at 240)		
H. U.	13 (Q)	192 (Q) (C & P at 195)	
W. I.	16 (C)	200 (CLE) (C & P at 200)	
Mc. K.	12 (Q)	205 (Q) (C & P at 227)	
Mc. N.†	15 (C)	229 (C) (C & P at 272)	
G. O.	14 (Q)	205 (Q)	229 (C & P at 235)

* (C), chloroquine; (CG), chloroguanide; (Py), pyrimethamine; (Q), quinine; (CLE), Cleocin®; (C&P), chloroquine and primaquine radical curative therapy initiated on the given infection day.
† Negro.

TABLE 3

Relapse activity of the Salvado: II strain of Plasmodium vivax *in volunteers exposed to infection by the bites of infected* Anopheles *mosquitoes*

Volunteer	Primary attack	Infection day*		
		1st relapse	2nd relapse	3rd relapse
W. I.	10 (C) (C&P at 169)			
G. A.	11 (C&P at 14)			
L. A.	11 (Q) (C&P at 170)			
H. I.	13 (Q) (C&P at 88)			
M. A.	14 (Q) (C&P at 170)			
J. O.	15 (Q) (C&P at 221)			
S. T.	15 (Py) (C&P at 256)			
R. O.†	13 (C)	235 (C&P at 246)		
R. A.	16 (CG)	262 (C)	323 (C&P at 326)	
R. E.	10 (Q)	172 (Q)	203 (Q)	231 (Q) (C&P at 255)
L. E.†	13 (C)	205 (CLE)	284 (CLE)	342 (C&P at 343)

* (C), chloroquine; (CG), chlorguanide; (Py), pyrimethamine; (Q), quinine; (CLE), Cleocin®; (C&P), chloroquine and primaquine radical curative therapy initiated on the given infection day.
† Negro.

radical curative therapy at 88 to 256 days. Four infections showed first relapses at 172 to 262 days (median 221.5) after exposure to infection. Three infections showed a second relapse at 203 to 323 days (median 284) or 31 to 79 days after the first relapse. There were two infections which exhibited a third relapse at 231 and 342 days.

The response of the Salvador strains (Sal I and Sal II) to chloroquine and to quinine was normal, with the parasite clearance time ranging from 1 to 4 days. The response of these strains to the recommended dosage regimens of either pyrimethamine or chlorguanide was slow, although within normal limits (parasite clearance time ranging from 4½ to 6 days). The response to the new semi-synthetic antibiotic Cleocin® was as slow as with pyrimethamine or chlorguanide (4½ to 6 days). The radical curative rate of primaquine (14-day regimen) for the two strains from El Salvador was also 100%. The 7-day course of Cleocin (total of 12.6 g) had no significant radical curative activity against the Sal II strain, since relapses occurred after treatment with this drug.

Table 4 shows the results of studies with the NICA strain. The prepatent periods for the six infections ranged from 10 to 12 days (median 10.0 days). The infections in two of the volunteers (W. I. and T. R.) did not relapse before they received radical curative therapy at days 64 and 71. The four remaining infections (L. O., M. A., P. A., G. A.) showed first relapse activity at 85 to 194 days after exposure to infection (median of 120.5). One of these volunteers (L. O.) received radical curative therapy at day 201.

The three remaining volunteers experienced a

TABLE 4

Relapse activity of the NICA strain of Plasmodium vivax *in volunteers exposed to infection by the bites of infected* Anopheles *mosquitoes*

Volunteer	Primary attack	Infection day*				
		1st relapse	2nd relapse	3rd relapse	4th relapse	5th relapse
W. I.	10 (Q) (C&P at 71)					
T. R.	12 (C) (C&P at 64)					
L. O.	12 (C)	194 (C&P at 201)				
M. A.	10 (Q)	146 (Q)	164 (C&P at 169)			
P. A.	10 (Q)	94 (Q)	115 (Q)	135 (Q)	154 (C&P at 155)	
G. A.	10 (Q)	85 (Q)	107 (Q)	128 (Q)	149 (Q)	171 (C&P at 171)

* (C), chloroquine; (CG), chlorguanide; (Py), pyrimethamine; (Q), quinine; (CLE), Cleocin®; (C&P), chloroquine and primaquine radical curative therapy initiated on the given infection day.

second relapse at days 107 and 164 (median 115) or 21 to 22 days after the first relapse. Volunteer M. A. received radical curative therapy at day 169. The other two volunteers (G. A. and P. A.) experienced a third relapse at days 126 and 135 (median of 131.5) and a fourth relapse at days 149 and 154 (median of 151.5), respectively. Volunteer P. A. received radical curative therapy at day 155. G. A. experienced a fifth relapse at day 171 and received radical curative therapy beginning on that day. The drug sensitivity of the NICA strain has been tested only for chloroquine and quinine. Normal parasite clearance (<96 hours) was observed for each. The six infections have been treated with 14 days of primaquine with an apparent 100% radical cure. The observations on this strain suggest that it differs a little from the other three strains in that infections in 2 of 4 volunteers not receiving radical curative therapy exhibited relapse activity earlier than 150 days.

Strains of vivax malaria have generally been classified into two major types, based on their life pattern characteristics. The life pattern of the *temperate zone type* is characterized by an early primary attack (short prepatent period) followed by a long latent period before appearance of frequent relapse activity. The *tropical zone type* of life pattern also is characterized by an early primary attack, but with a short latent period following the initial attack before appearance of frequent relapse activity. The classical examples of temperate zone and tropical zone types are the St. Elizabeth (USA) and the Chesson (New Guinea-South Pacific) strains of *Plasmodium vivax*, respectively. Coatney et al. observed relatively short prepatent periods of 11 to 18 days with the St. Elizabeth strain followed by latent periods of a minimum of 5 months after treatment of the primary attack before first relapse activity was observed.[3] The same investigators reported that, like the St. Elizabeth strain, infections with the Chesson strain exhibited early primary attacks of 9 to 16 days but differed radically from it in that there was no long latent period between the primary attack and the first relapse. Rather, when a Chesson vivax attack was interrupted by effective blood schizonticidal therapy, relapse usually occurred promptly, sometimes as early as 6 days. Further relapses continued at frequent and/or regular intervals; later, they gradually became more widely spaced.

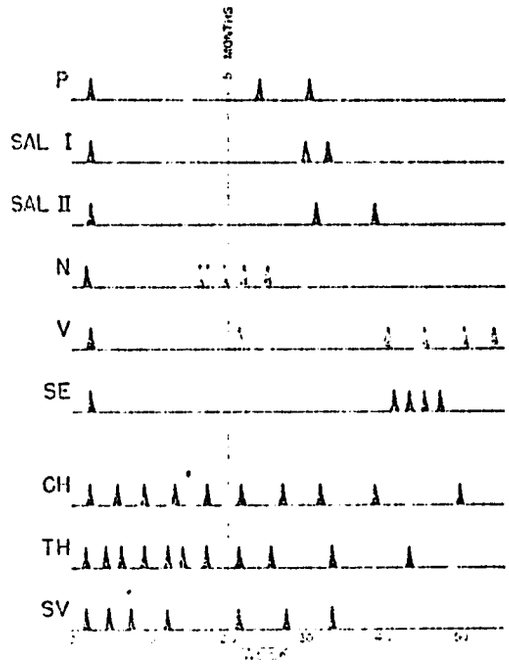

FIGURE 1. Patterns of relapse activity of infections with various strains of *Plasmodium vivax* in volunteers exposed to infection by the bites of infected *Anopheles* mosquitoes. P, Panama strain; Sal I and Sal II, Salvador strains; N, Nicaraguan strain; V, Venezuelan strain; SE, St. Elizabeth strain; CH, Chesson strain; TH, Thailand strain; SV, South Vietnam strain.

Figure 1 presents a summary of the patterns of relapse activity for strains of vivax malaria studied by our group during the past 10 years with the exception of the SE and CH strains. The data for these two patterns were taken from Coatney et al.[3,5] The patterns for each strain represent median values. As mentioned earlier, the CH (Chesson strain) pattern is characteristic for the tropical zone type of relapsing vivax malaria with no long period of latency between primary attack and first relapse. The TH (Thailand) and SV (South Vietnam) patterns fall into this tropical zone relapse pattern. The SE (St. Elizabeth) pattern is representative of the temperate zone type, which has a long period of latency between primary attack and first relapse. The V pattern represents a strain from Venezuela. The other four patterns represent the strains reported herein. It can be seen that the median patterns for all four Central American strains are generally similar, with a suggestion that the strain

from Nicaragua is a "fence-rider" with a few relapses occurring before 5 months.

On the basis of their life patterns, it could be concluded that the four Central American vivax strains fall into the "temperate zone" category. However, because the Central American region is tropical, this classification, which appears to be based primarily on temperature, might be considered somewhat inconsistent. This classification in all probability evolved from the explanations of many investigators, including Hackett,[6] who thought that the characteristic of latency provided an explanation for the overwintering of the parasites and, consequently, for the spring wave of malaria. He further suggested that strains with this characteristic would have greatly enhanced chances for survival in temperate zones. This phenomenon of delayed relapse activity could also be explained as a more efficient means in tropical areas for malaria parasites to survive through periods of prolonged dry season with low anophelism.

Covell stated that "the theory seems to have grown up on the assumption that Nature had provided this pattern to enable the Plasmodium to survive in places where the transmission period is a short one."[7] However, he did not consider this implication to be justified since non-relapsing falciparum malaria survives without much difficulty in areas where the transmission season is short.

In a monograph by Coatney et al.[8] it is held that malaria spread from Southeast Asia to Africa and then to Europe; and only recently (16th Century) was it introduced into the New World. It is interesting to note that vivax malaria from Southeast Asia and the South Pacific all have been of the Chesson (tropical zone) type (Fairley;[9] Coatney et al.;[8] Contacos et al.[10]) It is as interesting to note that, in contrast, the vivax malaria of the Americas appears to be of the St. Elizabeth (temperate zone) type. Obviously, something happened to the vivax parasite on its long and circuitous journey from Southeast Asia through Africa and Europe and finally to the New World.

If strains of vivax malaria with this late relapse mechanism have evolved in areas with short transmission seasons, then one would not expect to commonly find such strains in areas where transmission extends over a great portion of the year. The antithesis of this should also obtain. It will be of interest to see if areas in the Americas with an almost continuous transmission season do support or sustain strains of the tropical zone type of vivax malaria.

The responses of the four Central American vivax strains to the usually recommended dosage regimens of chloroquine pyrimethamine, chlorguanide, or quinine are considered to be within normal limits. Therefore, any one of these drugs should cure an attack of this malaria; and, if given along with the 14-day course of primaquine, a 100% radical cure rate should be obtained. The treatment of choice, however, continues to be chloroquine, 1.5 g base, followed by primaquine, 15 mg base, for 14 consecutive days.

Knowledge of the relapse characteristics of local or regional strains of vivax malaria is important for several obvious reasons, foremost among them being (1) the interpretation of malariometric data in endemic areas and (2) the development of antimalarial chemotherapeutic programs as well as the interpretation of the results of such drug programs.

SUMMARY

Four strains of vivax malaria from three Central American countries (Panama, El Salvador, Nicaragua) were studied in volunteers for characteristics of their relapse patterns and for their response to recommended dosage regimens of some standard antimalarial drugs. The relapse patterns of the four Central American vivax strains fell into the "temperate zone" category of relapse pattern; namely, an early primary attack (short prepatent period) followed by a long period of latency before appearance of frequent relapse activity. The characteristics and classification of relapse patterns are discussed.

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