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9. ABSTRACT  
 Malaria can be transmitted by blood transfusions. Thus it is important to have a simple and effective test for determining whether blood donors are infected with malaria. Ten cases of transfusion-induced malaria involving 110 donors in the U.S. were investigated. All of the available donors were contacted and case histories obtained. Serum samples and blood slides were collected from the 47 donors most suspect from their histories. Malaria antibody was detected by the indirect fluorescent antibody test in one donor from each of the nine cases. In eight cases, the donor's highest antibody titer was with the infecting species of malaria. In three cases, the positive donor was proven to be infected. This was established by positive blood films, bone marrow smears, or sub-inoculation of blood into a human volunteer. In the other six cases, the presence of malarial antibody was the only evidence of malaria infection found in the blood donors involved. One case was not resolved by serology, because two highly suspect donors were not available for testing. To decrease the threat of transfusion-induced malaria, either high-risk groups must be eliminated from donor pools or some dependable means of malaria detection must be employed at the time of blood donation. The IFA test, although it is not rapid, can be used to screen the donor pool by eliminating only those individuals with a positive malaria antibody response. It may also be useful in malaria eradication programs to detect occult infections which constantly threaten to restore an active malaria transmission cycle.

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THE INDIRECT FLUORESCENT ANTIBODY TEST  
FOR DETECTION OF OCCULT MALARIA IN BLOOD DONORS\*

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#### SYNOPSIS

Occult malaria presents a threat of blood transfusion-induced infections and, possibly, the restoration of active mosquito transmission in areas currently considered nonendemic for Plasmodia. The diagnosis by routine blood films of asymptomatic infections with extremely low parasitemias is very difficult and time-consuming. Malaria serology, particularly the indirect fluorescent antibody test (IFA), can be of great value in these cases.

This paper presents a report of 10 cases of transfusion-induced malaria. Malaria antibody was detected in one donor from each of 9 cases. The usefulness of the IFA test for detection of occult malaria is discussed.

Malaria induced by blood transfusion from asymptomatic carriers is a problem for blood banks (Brooks and Barry, 1969). Transfusion-induced malaria is recognized in many countries where malaria transmission has been eliminated (Lepes, 1965; Duhanina and Zukova, 1965; Lupascu et al., 1967; Ambroise-Thomas, 1969; Fisher, 1969). The standards for blood donation established by the American Association of Blood Banks regarding malaria have been recently revised in an attempt to prevent transfusion malaria in the United States (Standards, 1970). Although these standards have been helpful, at least five cases of transfusion-induced malaria occurred in the first eight months of 1970.

Detection by the indirect fluorescent antibody (IFA) test of donors responsible for transfusion malaria has been reported (Lupascu et al., 1967; Lupascu et al., 1969; Ambroise-Thomas, 1969; Brooks and Barry, 1969; Fisher and Schultz, 1969). The sera from the last two reports mentioned above were tested in this laboratory. The value of the IFA test in detecting occult malaria and the use of the test in decreasing transfusion-induced malaria are the subject of this report.

#### MATERIALS AND METHODS

Ten cases of transfusion-induced malaria were reported to the Center for Disease Control (CDC); an extensive epidemiologic investigation was performed on each case by members of the Parasitic Diseases Section, Epidemiology Program. Serum samples and blood smears were obtained from both the recipient and all donors involved in 6 of the 10 cases. In the other 4 cases, not all of the donors could be contacted. All blood slides were examined at the

National Malaria Repository, CDC, for the presence of parasites. When a particular donor's serum was found to contain malarial antibody, his blood smears were intensively studied.

The IFA test was performed as described by Sulzer et al. (1969). Species determination was based on the method outlined by Gleason et al. (1971). All tests were coded and randomly assorted to reduce bias. To serologically determine the infecting Plasmodium species, we used all available species of human malarial parasites as antigen. All antigens contained schizonts, and the comparison of results was based on the titer of the schizont reaction.

#### RESULTS

Of the 10 cases of transfusion-induced malaria reported, malarial antibody was detected in one donor in each of 9 cases (Table 1). The donor's highest antibody titer was with the infecting species in 8 cases, but in one case (Case 5), antibody titers to two antigens were equal. One case (Case 10) was not resolved by serology; two of the 7 donors were not available for testing, and one of these missing donors was a Vietnam veteran.

All donors with positive IFA titers were investigated thoroughly (Table 2). In Case 1, degenerate parasites and pigment were seen in bone marrow from the positive donor. Two parasites were found in multiple blood films from one donor taken after a 500 ml phlebotomy (Case 3). Blood from the positive donor in Case 6 was inoculated into a volunteer who subsequently developed P. malariae. The other 6 donors were examined only by blood films and serologic (IFA) tests; the presence of malarial antibody was the only evidence of malaria infection found.

## DISCUSSION

We have previously reported that a positive antibody reaction can be expected in the IFA test in at least 95% of sera from cases that had positive blood films (Sulzer et al., 1969). We also reported that, in U.S. military returnees from South East Asia, an antibody titer of 1:256 or greater is highly suggestive of current malaria infection if there has been no chemotherapy in the preceeding 6 months (Wilson et al., 1970). Basing our interpretation on these and other studies (Coudert et al., 1966; Ambroise-Thomas, 1969), we considered a positive antibody reaction in one donor from each of 9 transfusion cases as strong presumptive evidence that the responsible donor was detected. Demonstration of infection in three donors with positive IFA reactions further supports this conclusion.

Raghaven (1966) reported that, when circulating parasitemias fall below a certain level, malaria parasites, though present, will probably not be detected by examination of blood films. The number of parasites in the blood drawn for the purpose of transfusion from such an individual may be sufficient to produce infection in the nonimmune debilitated recipient.

It is, of course, possible that donors with negative antibody reactions might also have been infected. We believe, however, that circulating parasitemias which pose a threat of infection in blood transfusion will stimulate a detectable antibody response in essentially all cases.

Antibody titers to more than one species of malaria were found in 8 positive reactors. This could be due to either production of cross-reacting antibodies or infection with more than one Plasmodium species. The significant factor is that we could identify the infecting species in 8 of 9 positive reactors by comparing titers obtained with the different species antigens.

Five of the positive donors in the 10 cases described were Vietnam veterans who donated blood 6 to 13 months after their return to the United States. According to the previous standards for blood donors, these men satisfied the criteria for travelers in endemic areas who took antimalarial prophylaxis. However, they are not acceptable now according to the revised standards for blood banks which call for deferring potential donors for 2 years after cessation of suppressive therapy (Standards, 1970). Four of the responsible donors were immigrants from countries currently considered endemic for malaria; they would now be permanently rejected for whole blood donation.

Cases 9 and 10 (Tables 1 and 2) occurred in 1970. Five of the 8 donors involved were Vietnam veterans who, at the time of donation, denied a history of malaria (Dr. D. S. Stern, personal communication\*). Denial of past infection or travel in endemic areas effectively negates the purpose of the new blood bank standards concerning malaria. To overcome this obstacle, either the donor pool must be decreased by elimination of high risk groups or some dependable means of malaria detection must be employed at the time of blood donation.

Todorovic et al. (1968) reported the development of a tube latex agglutination test for malaria using a soluble antigen isolated from the blood of chickens acutely infected with Plasmodium gallinaceum. This initial study showed promising results of a rapid and easily performed test but it has not been completely evaluated. The indirect hemagglutination test has been well evaluated (Rogers et al., 1968) but preservation of the antigens has been a problem.

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In the absence of an evaluated rapid serologic test for malaria antibody, the IFA test might be used as a method of detecting occult malaria. In the U.S., blood donors in the age group selected for military service and immigrants from past or present endemic areas might be tested for malaria antibody and the serologic positives eliminated as donors. This procedure would be helpful in two ways: 1) by eliminating those with positive antibody responses who might be a source of transfusion-induced malaria, and 2) at the same time, by increasing the donor pool. The expense of performing the test would have to be weighed against the risk of transfusion-induced malaria or significant reduction of the donor pool. Screening at one dilution, perhaps 1:64, would reduce test cost in terms of time and reagents.

Ambroise-Thomas (1969) reported the use of the IFA test for screening blood donors in Lyon, France. Of 225 people who would not have been eligible for blood donation because of travel in malarious areas within the past 5 years, 57.6% were accepted on the basis of negative malaria antibody response. Conversely, 28.7% of 345 people who had returned more than 5 years before were still positive, and thus eliminated from the donor pool.

If further work confirms the suggestion by several authors (Ambroise-Thomas, 1969; Wilson *et al.*, 1970) that high malarial IFA titers in untreated persons, otherwise completely normal, is evidence that occult infection exists, the implication for malaria control programs is apparent. Although the gametocytemias in such cases may be well below the threshold needed to infect mosquitoes, an increase in parasitemia may occur at any time. In such an instance, mosquitoes may become infected and thus restore the transmission cycle. The presence of cases of occult malaria in areas where it is thought

to have been eliminated is a continuing threat of restoration of transmission. If malaria is to be totally eradicated, such individuals must, of necessity, be sought out and their parasitemias terminated by curative chemotherapy. The use of the IFA test, in such a program, would be an essential technique in accomplishing this end.

## SUMMARY

Ten cases of transfusion-induced malaria involving 110 donors in the United States were investigated by the Center for Disease Control, Atlanta, Georgia. All of the available donors were contacted and case histories obtained. Serum samples and blood slides were collected from the 47 donors most suspect from their histories. Malaria antibody was detected by the indirect fluorescent antibody test in one donor from each of 9 cases. The donor's highest antibody titer was with the infecting species in 8 cases. In 3 cases, the positive donor was proven to be infected by positive blood films, bone marrow smears, or sub-inoculation of blood into a human volunteer. The presence of malarial antibody was the only evidence of malaria infection found in the blood donors involved in the other 6 cases. One case was not resolved by serology: two highly suspect donors were not available for testing.

To decrease the threat of transfusion-induced malaria, either high risk groups must be eliminated from donor pools or some dependable means of malaria detection must be employed at the time of blood donation. The IFA test, although not a rapid test, can be used to increase the donor pool by eliminating only those individuals with a positive malaria antibody response. It may also be useful in malaria eradication programs to detect occult infections which constantly threaten to restore an active malaria transmission cycle.

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TABLE 1

IFA REACTIONS OF SERUM SAMPLES FROM BLOOD DONORS INVOLVED IN 10 CASES OF TRANSFUSION-INDUCED MALARIA

Case No.	Infecting species	No. of donors	No. tested by IFA	No. positive by IFA	IFA Titers		
					vivax	falciparum	malariae
1	<i>P. falciparum</i>	2	2	1	1:16	1:256	Neg.
2	<i>P. malariae</i>	2	2	1	1:16	1:16	1:256
3	<i>P. falciparum</i>	4	4	1	1:64	1:1024	1:256
4	<i>P. malariae</i>	12	2	1	1:64	1:16	1:1024
5	<i>P. falciparum</i>	7	3	1	1:64	1:256	1:256
6	<i>P. malariae</i>	56	9	1	1:64	1:256	1:4096
7	<i>P. falciparum</i>	6	6	1	1:64	1:256	1:64
8	<i>P. falciparum</i>	13	13	1	1:16	1:256	1:16
9	<i>P. falciparum</i>	1	1	1	Neg.	1:256	Neg.
10	<i>P. vivax</i>	7	5	0			

TABLE 2  
HISTORY OF IFA POSITIVE DONORS

CASE NO.	HISTORY
1	Vietnam veteran donated blood 8 months after return to U.S. Experienced chills, fever, and sweating 1 month before donation. Blood slides negative, but bone marrow contained degenerated schizonts and malarial pigment. (NCDC Annual Summary, 1967)
2	Immigrated to U. S. from Oaxaca, Mexico, in 1956, returned for brief visit to Mexico City 4 years before blood donation. Denied history of malaria, and blood films negative. NCDC Annual Summary, 1967)
3	Student from Nigeria in U. S. for 32 months. Denied history of malaria, but multiple blood films taken after 500 ml phlebotomy contained 2 <i>P. falciparum</i> -like trophozoites. (Brooks and Barry, 1969)
4	Immigrated to Hawaii from the Philippines in 1930, returned for 2 weeks in 1967, and donated blood 10 months later. Denied history of malaria, and blood films not available. (NCDC Annual Summary, 1968)
5	Vietnam veteran donated blood 13 months after return to U. S. Denied history of malaria and blood films negative. (Fisher and Schultz, 1969)
6	Student from Nigeria in U. S. for 24 months. Denied history of malaria. Blood films negative, but blood inoculation into volunteer produced infection with <i>P. malariae</i> . (NCDC Annual Summary, 1968)
7	Vietnam veteran donated blood 6 months after return to U. S. Denied history of malaria, and blood films negative. (Fisher and Schultz, 1969)
8	Vietnam veteran, denied overseas military service and history of malaria at time of donation. Blood drawn 6 months after return to U. S. (NCDC Annual Report, 1969)
9	Vietnam veteran donated blood 7 months after return to U. S. Originally denied overseas military service; blood films negative. (NCDC, Morbidity and Mortality Weekly Report, April 11, 1970)
10	One donor not tested by serology had been in Vietnam 5 months before blood donation. The other not tested had been in Greece, Turkey, and Egypt 4 years before donation. (Stern, personal communication)