

## SEASONAL VARIATION OF ANTI-RESA/Pf155 *PLASMODIUM FALCIPARUM* ANTIBODIES IN THREE LOCALITIES FROM THE STATE OF AMAPÁ, BRAZIL

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### SUMMARY

Anti-RESA/Pf155 antibodies were assayed in sera of individuals from three localities (Laranjal do Jari, Vila Padaria and Vila Paraíso) in the State of Amapá, Brazil, during the long-rains and short-rains seasons. All of these had negative blood smears for malaria. Most of the sera collected were positive in Indirect Fluorescent Antibody (IFA) with *P. falciparum* parasites, with no seasonal variation. A high percentage of these sera (62% to 100%) was RESA positive by Modified Indirect Fluorescent Antibody (MIFA), with a significant ( $p < 0.05$ ) increase of geometric mean titers during the short-rains season, when the transmission of the disease is highest. ELISA with three repetitive RESA peptides (EENV)<sub>3</sub>(4x3), (EENVEHDA)<sub>2</sub>(8x2) and (DDEHVVEPTVA)<sub>2</sub>(11x2) did not reveal statistically significant seasonal variations, although a small enhancement of positivity was observed in V. Padaria (15.3 to 38.8%) in the short-rains season with the 8x2 peptides, and with 4x3 and 8x2 peptides in V. Paraíso, with a decrease in 11x2. MIFA titers appeared to be correlated mainly to the peptide 4x3 and it was the immunodominant in the three localities.

**KEYWORDS:** RESA/Pf155; *P. falciparum*; Seasonal variation.

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### INTRODUCTION

Malaria is the endemic disease of highest incidence in the world. W.H.O. estimates that there are 270 million new cases every year and 2.1 billion people live in malarious areas (W.H.O., 1990)<sup>28</sup>.

According to the report of Fundação Nacional da Saúde, in 1990 there were 560396 malaria cases in Brazil and the majority came from the Amazon Region. Approximately 50% of these were by *P. falciparum*.

PERLMANN et al. (1984)<sup>13</sup> reported that RESA (Ring-infected Erythrocyte Surface Antigen) may elicit an immune response conferring partial protection against reinfection of subjects in endemic areas. This

resistance was demonstrated both "in vitro" (WAHLIN et al., 1984)<sup>25</sup> and "in vivo" (COLLINS et al., 1988)<sup>6</sup> by several authors.

There are several sero-epidemiological studies mainly in Africa, correlating high or low titers of anti-RESA antibodies to age, time of exposure to the disease, clinical signs, parasitemia levels and seasonal variation (WAHLGREN et al., 1986<sup>24</sup>; DELORON et al. 1987<sup>7</sup>, CHIZZOLINI et al., 1988<sup>4</sup>; DELORON et al., 1989<sup>8</sup>).

In South America, there is little information about the profile of anti-RESA antibodies in the endemic areas. In Brazil, our group made a study of those antibod-

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ies in order to correlate levels of anti-RESA antibodies detected by Modified Indirect Fluorescence Antibody (MIFA) with the occupation of the individuals from agricultural and mining areas in the State of Pará and Rondônia (KLOETZEL et al., 1990) <sup>11</sup>.

This paper tries to follow the seasonal variation of anti-RESA antibody titers evaluated by MIFA and ELISA against the three synthetic peptides representing repeat aminoacid sequences of *P. falciparum*: the C-terminus sequences (EENV and EENVEHDA) and the N-terminus sequence (DDEHVEEPTVA) in the same adult individuals living in three localities of Companhia Florestal de Monte Dourado (C. F. M. D.)

(State of Amapá) during the season of long-rains (March to May) and the short-rains season (October and November).

## MATERIALS AND METHODS

### Study Areas

The areas we chose were Laranjal do Jari (L. Jari), Vila Padaria (V. Padaria) and Vila Paraíso (V. Paraíso) (Fig. 1).

The first area's population (about 12554 inhabitants) is dedicated to local commerce and many family members stay periods of time at mining projects

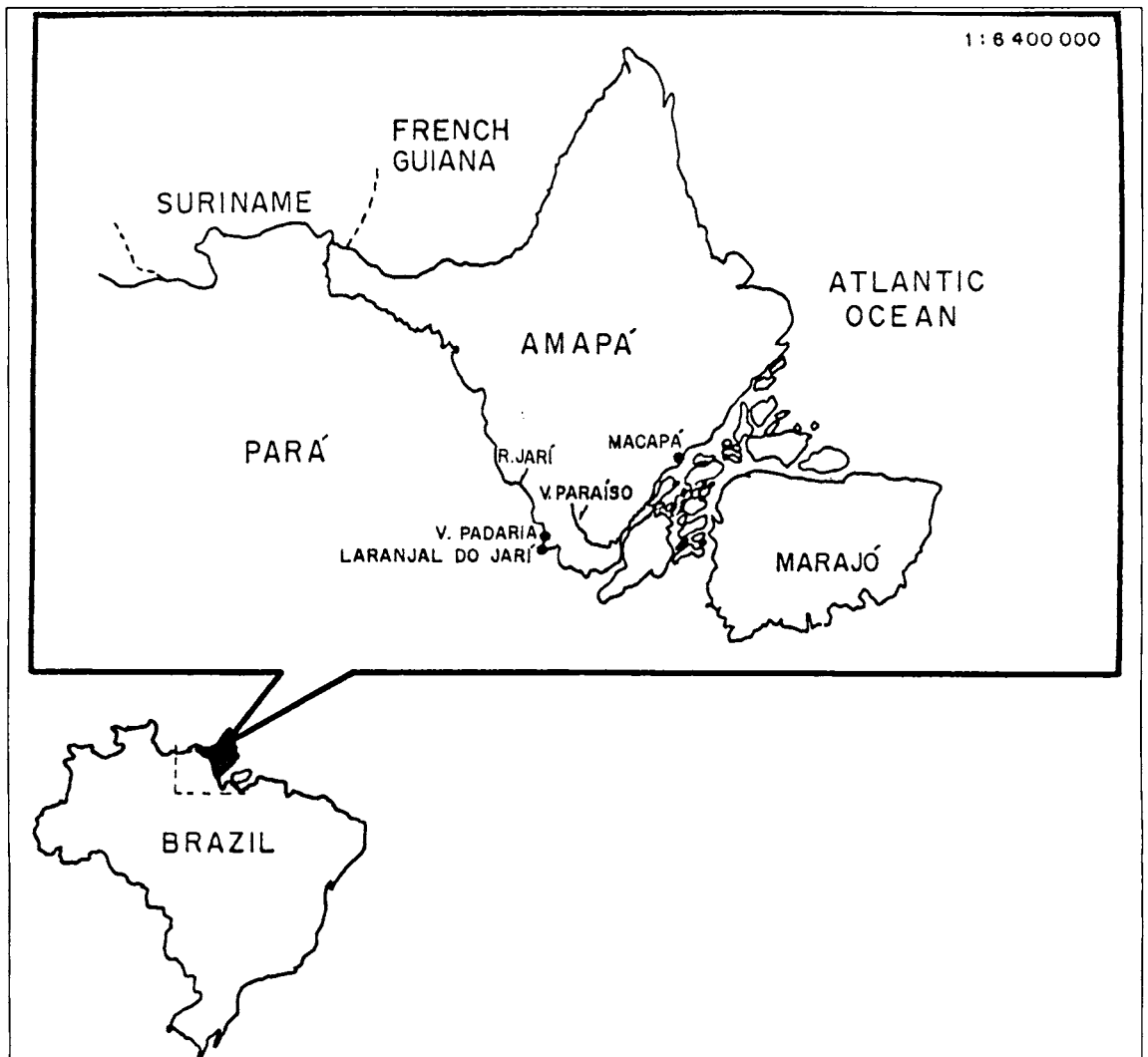


Figure 1. - Geographic localization of the endemic areas.

("garimpos") being responsible for local outbreaks of malaria on their return.

Vila Padaria (about 190 inhabitants) has reasonable access to medical facilities and the population is dedicated to agricultural work.

Vila Paraíso, interior of the State (about 190 inhabitants) is dedicated to cattle raising and access to medical facilities is very difficult. At our second visit, there was a serious outbreak of both *P. vivax* and *P. falciparum* malaria in children, but very few adults were affected.

Samples were collected during the season of long-rains (March to May) and during short-rains season (October and November).

According to data from C.F.M.D. the peak of *P. falciparum* and *P. vivax* malaria occurs in October and November.

### Subjects

Venous blood samples were obtained with informed consent by all individuals.

We collected 37 samples of sera from the same individuals from L. Jari, 19 from V. Padaria and 16 from V. Paraíso, aged above 13 years. A questionnaire was applied at the time of collection and this included: number of malaria attacks, time of residence in the area, specific anti malaria treatment and kind of malaria parasites.

Thick blood smears were made at the time of bleeding. All of these were negative both for *P. vivax* and *P. falciparum*, but previous malaria attacks were reported.

### Antibody Assays

Sera were tested by Indirect Fluorescent Antibody (IFA) and those who were IFA positive were evaluated by MIFA and ELISA against the three synthetic peptides representing RESA repeats: (EENV)<sub>3</sub>, (EENVEHDA)<sub>2</sub> and (DDEHVVEPTVA)<sub>2</sub>. These peptides will be referred to as 4x3, 8x2 and 11x2 respectively.

IFA was carried out using as antigen unfixed air-dried human erythrocytes infected with later forms of *P. falciparum* (FERREIRA & SANCHEZ, 1988)<sup>10</sup> while MIFA was carried out with human erythrocytes with early forms (ring-forms) flash-fixed with glutaraldehyde

and air dried, using anti-human IgG and biotin-avidin immunofluorescence technique (PERLMANN et al., 1984)<sup>13</sup>. Cut off values of both tests were those reported in the literature and checked in our laboratory with fifty sera from a local blood bank. In both reactions parasites were obtained from "in vitro" cultures (TRAGER & JENSEN, 1976)<sup>22</sup> of strain Pf67 isolated from a patient of Amazonas State.

### Peptides

RESA peptides were prepared by the solid method (STEWART & YOUNG, 1984)<sup>21</sup> using the tert-butyloxycarbonyl protection strategy for the alpha amino group. The following side-chain protecting groups were employed: benzyl for Thr, cyclohexyl for Asp and Glu and p-toluene sulphonyl for His. Peptide chains were assembled in 4-methylbenzhydrylamineresin and the carboxamide-peptides obtained by HF cleavage were purified by gel permeation and preparative HPLC. They were conjugated to BSA at molecular rate of 20: 1 (peptide: BSA) with glutaraldehyde (BERZINS et al., 1986)<sup>1</sup>.

All positive MIFA sera were assayed by ELISA at a dilution of 1:100, with peptides at concentration of 66µg/ml. Serum dilutions and peptide concentration were standardized previously, to obtain maximum sensitivity and specificity. Reactions were considered positive to peptides 4x3, 8x2 and 11x2 at absorbances > .053, .083 and .088 O. D. (492nm) respectively. Cut-off values had been obtained by ELISA reactions of fifty blood donors from a blood bank outside the endemic area adding 2 standard deviations to the mean O. D.

### Statistical Analysis

All percentages were tested by confidence interval (95%). Differences in means were tested by Student's t-test. P values < 0.05 were considered significant.

## RESULTS

As can be seen in Table 1, the majority of the subjects had previous contact with malaria and the positivity of these sera detected by IFA varied between 92% to 100%. The positivity of these sera for MIFA was also high. We observed that this positivity tends to increase during the short-rains season in the three micro-regions despite of these difference not being statistically significant. Only three individuals from L. Jari reported having contracted malaria between the collections and

TABLE 1

Indirect Fluorescent Antibody (IFA) and Modified Indirect Fluorescent Antibody (MIFA) positivity rate (%) of sequential samples\*\* collected during the long-rains (1<sup>st</sup>) and short-rains (2<sup>nd</sup>) seasons

Localities	IFA		MIFA	
	1st	2nd	1st	2nd
Laranjal do Jari n	92 (34/37)*	92 (34/37)*	62 (21/34)+	68 (23/34)+
Vila Padaria n	100 (19)	100 (19)	68.4 (13/19)+	94.7 (18/19)+
Vila Paraiso n	100 (16)	100 (16)	87.5 (14/16)+	100 (16)

\* = positive/total  
 + = positive/total of IFA positive sera.  
 \*\* = all individuals with negative smears for both *falciparum* and *vivax* malaria.  
 IFA cut-off value = >1/40.  
 MIFA cut-off value > 1/5.

one of them was infected by *P. falciparum*. This infection, however, did not change the antibody titer detected by MIFA during the long-rains season. The other two individuals remained negative for MIFA.

There was no correlation between geometric mean titers (GMT) and seasonal variation when the sera were assayed by IFA (Table 2). However, we observed higher GMT of MIFA during the short-rains season ( $p > 0.05$ ) in the three micro-regions.

Despite of the increase of MIFA positive sera in the short-rains season (Table 3), the individuals of the three

TABLE 2

Indirect Fluorescent Antibody (IFA) and Modified Indirect Fluorescent Antibody (MIFA) of sequential samples ++ collected during the long-rains (1st) and short-rains (2nd) seasons.

Localities	Geometric Mean Titers			
	IFA		MIFA	
	1st	2nd	1st	2nd**
Laranjal do Jari n	366 (34)*	443 (34)*	27 (21)*	65 (23)*
Vila Padaria n	1713 (19)*	796 (19)*	38 (13)*	194 (18)*
Vila Paraiso n	3620 (16)*	3319 (16)*	444 (14)*	1457 (16)*

\*\* =  $p < 0.05$ .  
 \* = total of IFA positive sera.  
 + = total of MIFA positive sera.  
 ++ = all individuals with negative smears for both *falciparum* and *vivax* malaria.

TABLE 3

Number of malaria episodes reported by the same MIFA positive individuals\* (%) at the first (long-rains season) and second (short-rains season) collections in the three localities.

Episodes/ Localities	L. Jari		V. Padaria		V. Paraiso	
	1st	2nd	1st	2nd	1st	2nd
N. R. n	9.5 2/21	17.3 4/23	53.8 7/13	55.5 10/18	7.1 1/14	18.7 3/16
1-3 n	57.1 12/21	52.1 12/23	46.1 6/13	44.4** 8/18	78.5 11/14	68.7 11/16
> 4 n	33.3 7/21	30.4 7/23			14.2 2/14	12.5 2/16

N. R. = no malaria infections reported.  
 \* = individuals with negative blood smears.  
 \*\* = Two individuals reported infection by *P. falciparum* before the first collection, by MIFA turned positive at the second collection.

localities did not report malaria episodes during this period. In V. Padaria, two individuals contracted malaria before the first collection and were negative by MIFA. Their sera became positive only on the second collection (short-rains season).

Table 4 represents ELISA with the three synthetic peptides (4x3, 8x2 and 11x2) of sera positive in MIFA. All sera from L. Jari reacted with peptide 4x3 during the short-rains season but we did not observe any statistically significant differences of seasonal variation in positivity by ELISA with the three peptides.

TABLE 4

ELISA with RESA peptides. Sera from Modified Indirect Fluorescent Antibody (MIFA) positive individuals\* (%) of the three localities detected during the long-rains (1<sup>st</sup>) and short-rains (2<sup>nd</sup>) seasons

Localities	Peptides					
	4x3		8x2		11x2	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Laranjal do Jari n	71.4 (15/21)	100 (23)	33.3 (7/21)	47.8 (11/23)	52.4 (11/21)	43.4 (10/23)
Vila Padaria n	76.9 (10/13)	66.6 (12/18)	15.3 (2/13)	38.8 (7/18)	69.2 (9/13)	61.1 (11/18)
Vila Paraiso n	78.5 (11/14)	81.2 (13/16)	35.7 (5/14)	50.0 (8/16)	85.7 (12/14)	62.5 (10/16)

\* = all individuals with negative smears for both *falciparum* and *vivax* malaria.  
 4x3 = (EENV)<sub>2</sub>.  
 8x2 = (EENVEHDA)<sub>2</sub>.  
 11x2 = (DDHIVEPTVA)<sub>2</sub>.  
 sera tested at 1:100 dilution.  
 peptide concentration: 66µg/ml.

Enhancement of positivity during the short-rains season to 8x2 peptide in V. Padaria (15.3% to 38.8%) was not significant (confidence interval 95%). Practically there was no seasonal variation of positivity with the other peptides.

There was a small enhancement (not significant) of the positivity during the short-rains season with 4x3 and 8x2 peptides in V. Padaria and the decrease of the positivity to the 11x2 peptide was not significant.

Table 5 represents the arithmetic means of absorbances from the same sera analyzed in Table 4. There was no significant seasonal difference in these arithmetic means in the three localities. The apparent decrease of these means in the short-rains season with the 4x3 and 8x2 peptides in L. Jari and with the 11x2 peptide in V. Paraíso was not statistically significant.

TABLE 5

Arithmetic means of absorbances in ELISA from individuals\* positive by MIFA from the three localities during the long-rains (1<sup>st</sup>) and short-rains (2<sup>nd</sup>) seasons.

Localities	Peptides					
	4x3		8x2		11x2	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Laranjal do Jari	502	347	352	147	176	276
n	(15)	(23)	(7)	(11)	(11)	(10)
Vila Padaria	340	276	114	136	080	151
n	(10)	(12)	(2)	(7)	(9)	(11)
Vila Paraíso	293	264	141	149	611	235
n	(11)	(13)	(5)	(8)	(12)	(10)

\* = all individuals with negative smears for both *falciparum* and *vivax* malaria.

4x3 = (EENV)<sub>3</sub>.

8x2 = (EENVHDA)<sub>2</sub>.

11x2 = (DDHVEEPTVA)<sub>2</sub>.

sera tested at 1:100 dilution.

peptide concentration: 66µg/ml

## DISCUSSION

CHOUGNET et al. (1990)<sup>5</sup> and DELORON & COT (1990)<sup>9</sup> correlated positivity and GMT of anti-RESA antibodies detected by MIFA to seasonal variation. However, in our study, we did not observe a correlation between positivity of these antibodies detected by MIFA to seasonal variations in the three micro-regions.

We only observed a significant increase of the GMT of anti-RESA antibodies detected by MIFA in the three microregions during the short-rains season, when

transmission is reported to be highest. Among the total of 37 subjects from L. Jari, three related having contracted malaria between the two collections and one of these was *falciparum*. That new infection did not modify RESA antibody titers. In the case of a new *vivax* infection this is a consistent finding (PERLMANN et al., 1984)<sup>13</sup>. The remaining individuals with higher anti-RESA antibodies may have contracted a new infection, which worked as a booster, aborting clinical manifestations of the disease. Thick smears are known to have low sensitivity and we were not able to detect any parasites in all those subjects that had no clinical malaria manifestations.

However, these findings do not explain the absence of a correlation between seasonal variation of positivity or arithmetic mean of absorbances detected by ELISA to the three peptides. These findings are in agreement with those reported by CHOUGNET et al. (1990)<sup>5</sup>.

There was a high percentage of people with anti-whole parasite *P. falciparum* antibodies (IFA) and anti-RESA antibodies detected by MIFA and ELISA, who claimed they never had a malarial attack. The majority had been living in L. Jari for several years (about 4 years or more) and those at V. Padaria and V. Paraíso were living there since their birth. When they were asked about previous malaria attacks they reported only "Flu" or episodes of low fever but these symptoms had never been ascribed to malaria and they claimed never having been submitted to anti-malarial treatment.

Asymptomatic malaria is known to be frequent in Africa and has been reported previously in Brazil by PRATA et al. (1988)<sup>16</sup>, although it is considered to be an exception here. Our findings seem to indicate that malaria without classical symptoms may exist in Amapá.

Like the results reported by RZEPczyk et al. (1989)<sup>19</sup> in our study the 4x3 peptide seems to be immunodominant over the 8x2 peptide.

There appeared to be a direct correlation between MIFA and (EENV)<sub>3</sub> peptide. These correlations were 77.2%, 87% and 82% for L. Jari, V. Padaria and V. Paraíso respectively. Other authors (BJORKMAN et al., 1990<sup>2</sup>, 1991<sup>3</sup>, PETERSEN et al., 1990<sup>15</sup>) observed a correlation between the 4 amino-acid peptide from the Pf155/RESA C' terminus and MIFA.

Some sera with anti-RESA antibodies detected by

MIFA did not react with any of the three peptides from RESA.

RESA is deposited in the infected erythrocyte membrane being the dominant but not the only membrane antigen (MATTEI et al., 1989<sup>12</sup>; WAHLIN et al., 1992<sup>26</sup>). Therefore RESA as well as other antigens may be detected by MIFA. ELISA with the peptides is more specific in detecting anti-RESA antibodies (PETERSEN et al., 1989)<sup>14</sup>.

Independently of the absence of a correlation between anti-RESA antibodies detected by MIFA and ELISA with seasonal variation, some authors try to study the correlation between RESA and genetic factors or the cooperation between T and B cell epitopes.

RILEY et al. (1991)<sup>17</sup> observed differences in the prevalence of anti-RESA peptide antibodies between ethnic groups from a riverine population from Gambia. SJOBERG et al. (1992)<sup>20</sup> did not find any correlation between anti-RESA antibodies and the differences in class II haplotypes for the 8 and 4 amino-acid peptides. The authors suggested that the regulation of the Pf155/RESA antibody may reflect the impact of factors encoded by genes outside the HLA class II region. Thus, TROYE-BOMBERG & PERLMANN (1993)<sup>23</sup> related that it is important to define the non-MHC-encoded factors regulating the human immune responses to *P. falciparum*. These authors also reported that it is unknown whether the genetic regulation at the antigen-presenting cells level reflects differences in antigen processing and presentation or release of factors such as interleukin 1.

Some authors have been discussing the discrepancy of results obtained in several studies and the different epidemiologic conditions of the areas studied. The seasonal differences and the diverse transmission in each region or country could be responsible for variation in results.

We emphasize that our study only tries to discuss the profile of anti-RESA antibodies in one endemic area of Brazil and the profile in these three micro-regions does not necessarily represent the epidemiologic reality of the State of Amapá as a whole or the rest of endemic areas from Brazil.

#### ACKNOWLEDGEMENTS

This work was financed by Companhia Florestal de

Monte Dourado (C. F. M. D.), CNPq-Conselho Nacional de Desenvolvimento Científico e Tecnológico and by Laboratórios de Investigação Médica do Hospital das Clínicas. We thank Fundação Nacional da Saúde (Pará) for local logistic support and Dr. C. E. Tosta - Universidade de Brasília for the original strain of *P. falciparum*. Skillful technical assistance of Angela M. Lourenço, Regina A. de Paula and Mauro T. Marrelli is acknowledged, as well as line drawing of Brazil's map by Almir Robson Ferreira.

#### RESUMO

##### Variação sazonal de anticorpos anti-RESA/Pf155 de *Plasmodium falciparum* em três localidades do Estado do Amapá, Brasil.

Anticorpos anti-RESA/Pf155 foram testados em soros de indivíduos de três localidades (Laranjal do Jari, Vila Padaria e Vila Paraíso) localizadas no Estado do Amapá, Brasil, durante as estações de chuvas "longas" e "curtas". Todos os esfregaços apresentaram-se negativos para malária.

A maioria dos soros coletados foram positivos na reação de Imunofluorescência Indireta (IFI) para *P. falciparum*, não apresentando variação sazonal. Uma alta porcentagem destes soros (62% a 100%) foram positivos para RESA na reação de Imunofluorescência Indireta Modificada (IFIM), com aumento na média geométrica dos títulos significante ( $p < 0.05$ ) durante a estação das chuvas "curtas", época de maior transmissão da doença. O teste de ELISA com os três peptídeos repetitivos do RESA: (EENV)<sub>3</sub>(4x3), (EENVEHDA)<sub>2</sub>(8x2) e (DDEHVEEPTVA)<sub>2</sub>(11x2), não revelou variações sazonais estatisticamente significantes, embora um pequeno aumento na positividade tenha sido observado, na época de chuvas "curtas", em V. Padaria (15,3 para 38,8%) com o peptídeo 8x2 e em V. Paraíso com os peptídeos 4x3 e 8x2, decrescendo com o peptídeo 11x2. Os títulos de IFIM pareceram se correlacionar principalmente com o peptídeo 4x3 e este mostrou-se imunodominante nas três localidades.

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- Recebido para publicação em 08/10/1993.  
Aceito para publicação em 10/02/1994.