SECOND PROGRESS REPORT FOR THE PERIOD
FEBRUARY 1, 1988 THROUGH JULY 31, 1988

GRANT No. DPE-5542-G-SS-7033-00
PROJECT No. 936-5542

"Analysis of Immunogenic Proteins of Microfilariae, Adult Worms and Excretory/Secretory Products of Onchocerca volvulus"

PROGRAM DESCRIPTION

A.1 Purpose of the Grant:

The purpose of the grant is to develop a new highly sensitive diagnostic test for onchocerciasis.

A.2 Specific Objectives:

A.2.(a) To identify immunogenic proteins associated with microfilariae, adult worms, and excretroy/secretory products of Onchocerca volvulus for use in the development of a more highly sensitive and specific diagnostic test for onchocerciasis.

A.2.(b) To attempt to correlate the presence of individual immunogenic proteins with the severity of onchocercal disease, thereby providing insight into the host-parasite interaction.

A.3 Implementation: Technical Work Plan

A.3.(a) Phase I

A.3.(a).i Measurement of microfilarial skin densities and examination for onchocercal nodules in residents from the study communities; collection of serum samples and nodules.

According to the programmed schedule for visits, Fincas Buena Vista, Las Delicias and Santa Margarita in the onchocerciasis hyperendemic area, was done during February; Fincas La Torre, Mirandilla and Costa Rica, in the mesoendemic area, in March. As it was done in January for the visit to Fincas Panama and Santa Adelaida in the hypoendemic area, informed approval was obtained from each Finca owner before a visit was done.

A total of 1,431 persons have been examined in the three onchocerciasis areas, 424 (29.6%) in the hypoendemic area, 499 (34.9%) in the mesoendemic area, and 508 (35.5%) in the hyperendemic area. Males were 813 (56.8%) and females were 618 (43.2%) (Table I). Children 02-10 years of age were 350 (24.5%), young adults between 11-30 years of age, 591 (41.3%), and adults older than 30 years of age 490 (34.5%) (Table II).

A total of 212 (14.8%) persons examined in the three onchocerciasis areas, 127 (59.9%) males and 85 (40.1%) females,

had onchocerca nodules (onchocercomata). Nodules on the head were present on 165 (62.5%) persons and in 99 (37.5%), on the rest of the body. The nodule rate prevalence according to endemicity was 6.1% (26/424) in the hypoendemic area, 9.4% (47/499) in the mesoendemic area, and 27.4% (139/508) in the hyperendemic area (Table III).

A total of 822 (57.4%) persons examined in the three onchocerciasis areas, 492 (34.4%) males and 330 (23.1%) females, were positive for microfilariae. The microfilarial skin positivity rate prevalence according to endemicity was 33.3% (141/424) in the hypoendemic area, 55.9% (279/499) in the mesoendemic area, and 77.2% (392/508) in the hyperendemic area (Table IV).

The microfilarial skin densities per milligram of skin are being calculated now and we will report them later.

A total of 1,138 (79.5%) of the persons examined during the first visits to the three onchocerciasis areas agreed voluntarily to provide a blood sample for serum. The distribution of serum samples by endemicity is 298 in the hypoendemic area, 432 in the mesoendemic area, and 408 in the hyperendemic area. The serum samples have been aliquoted and stored at -85C until further analysis is required.

Onchocerca nodules for adult filarial worms to prepare whole worm somatic antigens have been obtained from the 139 positive persons in the three endemic areas, after nodulectomies

performed by the paramedic brigade team of the Onchocerciasis Department of the National Malaria Service. The nodules were transported under a liquid nitrogen atmosphere to the laboratory and kept at -85C. The nodules have been digested with collagenase and male and female worms have been maintained at -85C until preparation of antigen for the ELISA tests.

A.3.(a).ii Collection of serum samples in follow-up visits to the study communities.

According to the programmed schedule for visits, Fincas Panama and Santa Adelaida in the onchocerciasis hypoendemic area, was done during May. As in all previous visits, informed approval was obtained from the Finca owner beforehand. A full report was sent to the Finca owner and to the Onchocerciasis Department of the National Malaria Service, informing on the onchocerciasis situation found in the Finca during the first visit.

A total of 336 (79%) of the 424 persons attended the second visit, of which 76 (23%) voluntarily provided blood for serum samples; this represents a total of serum samples from 17.9% the population initially examined.

A.4 Comparisons of actual accomplishments with goals established for the period.

The goals established for this period have been achieved as initially proposed: The first visits to the communities in the three endemic areas have been completed, and serum samples and nodules have been collected. Three problems, however, have been encountered namely, the participation of persons during the follow-up visits, patients treated for onchocerciasis, and collection of nodules for antigen preparation.

On the second visit to Fincas Panama and Santa Adelaida we obtained a poor response for providing additional serum samples by the persons examined initially. The reasons manifested by most of the persons were, for example, that they are born with a given amount of blood and their body will not make any new one no more, others stated that they were made weak after the blood extraction. Although we tried to explain that 10 milliliters of blood will not cause them to feel weak, they still refused. In view of the believes and attitudes that they have we want to propose to increase the time intervals between each follow-up visit, from the initial three to four months, to six month intervals. If we do this change we feel that people will not be reluctant to provide a blood sample at six month intervals. At the end we will still accomplish the overall objectives of the

project, which are to do a longitudinal study of onchocerciasis patients, and four serum samples from each person will be obtained for analysis.

We have not been able to identify patients treated for onchocerciasis at the Rodolfo Robles Hospital since their treatment programs with diethyl carbamazine have been suspended. We are making arrangements with the Onchocerciasis Department of the National Malaria Service and the Hospital Roosevelt to obtain serum samples from patients who are treated with diethyl carbamazine, although this is not routinely done. We have obtained serum samples, however, from onchocerciasis patients treated with Ivermectin, although follow-up from these patients has not been possible.

Due to the population census of the onchocerciasis areas by the Onchocerciasis Department of the National Malaria Service during 1987 and 1988, all nodulectomy activities have been suspended until next year. We have made arrangements to obtain nodules starting in February of next year.

A.5 Other activities.

From June 6 to 9 Dr. Ricardo Lujan attended a PSTC Conference on Biotechnology for Health and Agriculture in Washington, D.C. A presentation entitled "Epidemiology of onchocerciasis in an area of low intensity of infection:

Implications for the development of disease-surveillance methodologies" was presented (enclosed copy of Abstract), as part of the results derived from the project.

TABLE I

Sex distribution of Guatemalan individuals examined for onchocerciasis in three communities [Fincas] of different endemicity, for the study "Analysis of Immunogenic Proteins of Adult Worms, Microfilariae and Excretory/Secretory Products of Onchocerca volvulus," Project No. 6.225, from January to March, 1988

Endemicity / Finca	Males No.	Males %	Females No.	Females %	Total No.	Total %
Low						
Panama	305	71.9	119	18.1	424	100
Medium						
La Torre	132	50.6	129	49.4	261	100
Mirandilla	63	53.4	55	46.6	118	100
Costa Rica	57	47.5	63	52.5	120	100
Total	252	50.5	247	49.5	499	100
High						
Buena Vista	38	44.2	48	55.8	86	100
Las Delicias	104	60.8	67	39.2	171	100
Sta. Margarita	114	45.4	137	54.6	251	100
Total	256	50.4	252	49.6	508	100
TOTAL	813	56.8	618	43.2	1430	100

TABLE II

Age distribution of Guatemalan individuals examined for onchocerciasis in three communities [Fincas] of different endemicity, for the study "Analysis of Immunogenic Proteins of Adult Worms, Microfilariae and Excretory/Secretory Products of Onchocerca volvulus," Project No. 6.225, from January to March, 1988

Endemicity / Finca	Age (years) 02-10	11-30	+ 30	Total
Low				
Panama	94	169	161	424
Medium				
La Torre	75	102	84	261
Mirandilla	37	46	35	118
Costa Rica	36	43	41	120
Total	148	191	160	499
High				
Buena Vista	23	41	22	86
Las Delicias	26	76	69	171
Sta. Margarita	59	114	78	251
Total	108	231	169	508
TOTAL	350	590	480	1430

TABLE III

Sex and anatomical distribution of onchocerca nodules in Guatemalan individuals examined for onchocerciasis in three communities [Fincas] of different endemicity, for the study "Analysis of Immunogenic Proteins of Adult Worms, Microfilariae and Excretory/Secretory Products of Onchocerca volvulus," Project No. 6.225, from January to March, 1988

Endemicity Finca	Number of persons positive (%)	Number of males	females	No. of nodules head	body
Low					
Panama	26 (6.1)	19	7	18	9
Medium					
La Torre	19 (7.3)	13	6	17	8
Mirandilla	8 (6.8)	6	2	7	2
Costa Rica	20 (17.0)	8	12	6	19
Total	47 (9.4)	27	20	30	29
High					
Buena Vista	24 (27.9)	10	14	18	11
Las Delicias	53 (31.0)	36	17	44	22
Sta. Margarita	62 (25.0)	35	27	55	27
Total	139 (27.4)	81	58	117	60
TOTAL	212 (14.8)	127	85	165	98

TABLE IV

Microfilarial skin biopsy results of Guatemalan individuals examined for onchocerciasis in three communities [Fincas] of different endemicity, for the study "Analysis of Immunogenic Proteins of Adult Worms, Microfilariae and Excretory/Secretory Products of Onchocerca volvulus," Project No. 6.225, from January to March, 1988

Endemicity Finca	Microfilarial skin biopsy negative males	negative females	positive males	positive females	Total number positive (%)
Low					
Panama	185	97	119	22	141 (33.3)
Medium					
La Torre	52	74	78	57	135 (51.7)
Mirandilla	28	36	36	18	54 (45.8)
Costa Rica	10	20	47	43	90 (75.0)
Total	90	130	161	118	279 (55.9)
High					
Buena Vista	2	5	38	41	69 (80.2)
Las Delicias	16	14	88	53	141 (82.5)
Sta. Margarita	29	40	86	96	182 (72.5)
Total	47	59	212	190	392 (77.2)
TOTAL	322	286	492	330	812 (56.7)

Epidemiology of onchocerciasis in an area of low intensity of infection: Implications for the development of disease-surveillance methodologies.

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Human onchocerciasis is a disease caused by Onchocerca volvulus, a filarial (nematode) parasite. It occurs across sub Saharan Africa and in six Latin American countries, affecting well over 50 million people and causing blindness in about one million others (WHO Tech Rep Ser 1976, No. 597). For effective diagnosis, treatment, prevention, and control of onchocerciasis, however, more information on the biology of the host-parasite relationship and on its pathogenesis is needed (Henson et al., Bull WHO 1979, 57:667; OCP/OCT/83.1 Geneva: WHO 1983). Diagnostic procedures for onchocerciasis are used for determining the prevalence and intensity of infection, for identifying individuals requiring drug treatment, for evaluating the success of treatment, and for assessing the impact of control efforts (WHO Tech Rep Ser loc cit.; Sasa. Tokyo: Univ Tokyo Press 1978, 166, 687). However, present methods rely on detecting parasites in the skin or the eye, which are crude and insensitive techniques leading to innacurate and inefficient diagnosis. Alternative immunodiagnostic assays that detect specific antibodies to or antigenic fractions of O. volvulus should be developed.

The overall aim of the present study is to identify immunogenic proteins associated with the different life cycle stages of the parasite for use in the development of a more highly sensitive and specific diagnostic test for onchocerciasis. In a recent survey of 498 local Guatemalan residents in Finca Panama (a coffee plantation) in an area of low intensity of onchocerciasis disease (less than 30% of the population positive for skin microfilariae), 16 (3.2%) were positive for both microfilariae (mf) and nodules, 128 (25.7%) for mf only, 11 (2.2%) for nodules only, and 343 (68.9%) negative for both mf and nodules. If the diagnosis for mf in skin snips was based on one to four biopsies (or their combinations), the number of persons with a positive diagnosis varied from 13 to 27.5%. Skin-snip positivity and the density of microfilariae in the skin increased with age, reaching highest levels at 20-24 and 45-49 years, and both were greater in males than in females. Also, this study confirms other observations where up to 39% of persons examined gave a history of nodulectomy, although they were skin-snip negative (Brandling-Bennett et al., Am J Trop Med Hyg 1981, 30:970).

Therefore, prevalence surveys should include a search for nodules and a history of nodulectomy as well as skin-snip examination. However, epidemiologic surveillance of onchocerciasis, specially in areas of low intensity of infection, and assessment of control measurements should be based on more sensitive and specific methods, such as immunodiagnostic assays, which will be discussed.