6. 225

THIRD PROGRESS REPORT FOR THE PERIOD AUGUST 1, 1988 THROUGH JANUARY 31, 1989

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 excretory/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.

Rec'd in SCI NOV 29 1989

A.3 Implementation: Technical Work Plan

A.3.(a) Phase I

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

According to the programmed schedule for follow-up visits, the second visit to Fincas Buena Vista, Las Delicias, and Santa Margarita, in the onchocerciasis hyperendemic area, were done during July/August, 1988; and Fincas La Torre, Mirandilla, and Costa Rica, in the mesoendemic area, in October, 1988. As it was done previously, informed approval was obtained from each Finca owner before a visit was done.

A total of 636 (44.4%) of the 1,431 persons examined during the first visits to the three onchocerciasis areas agreed voluntarily to provide a second blood sample for serum. The distribution of serum samples by onchocerciasis endemicity was 77 in the hypoendemic area (results of Fincas Panamá and Santa Adelaida provided in the Second Progress Report), 273 in the mesoendemic area, and 286 in the hyperendemic area. The serum samples have been aliquoted and stored at -85C until further analysis is required.

A third visit to Fincas Panamá and Santa Adelaida in the hypoendemic area, was done in November, 1988. A total of 113 (26.6%) of the persons examined during the first visit agreed voluntarily to provide a third blood sample for serum.

An estimated number of at least 84 (5.9%) persons, from which information could be obtained, had either left the Fincas or have died during the interval between the first and second visits (a period of four to seven months).

A.3.(a).ii Collection of serum samples from control patients.

Serum samples from control patients have been obtained, as indicated in the Project: (a) 100 residents of Guatemala City who have no known exposure to *O. volvulus* but are infected with various intestinal helminths (*Ascaris*, hookworms, *Strongyloides*, *Trichuris*, etc.); (b) 100 residents from Guatemala City who have no known exposure to *O. volvulus* and have no evidence of intestinal helminth infections by stool examination; (c) 100 persons from the United States without evidence of intestinal helminth infections by stool examination, obtained from the serum bank of the Centers for Disease Control (CDC) in Atlanta; and (d) 4 persons from West Africa with other filarial infections (*Loa loa*) but without evidence of *O. volvulus* infection.

A.3.(a).iii Collection of nodules.

Onchocerca nodules to obtain adult filarial worms and prepare whole worm somatic antigens have been obtained from the 187 positive persons in endemic areas, after nodulectomies performed by the paramedic brigade teams of the Onchocerciasis Department of the National Malaria Service. The nodules were transported under a liquid nitrogen atmosphere to the laboratory and kept at -85 C. The nodules have been digested with collagenase and male and female worms are maintained at -85 C until preparation of antigens for the ELISA and EITB tests.

A.3.(a).iv Microfilarial skin densities.

The geometric mean of the microfilarial skin densities per milligram of skin (MFD) were calculated for all persons examined during the first visit to the Fincas in the three endemicity areas. As outlined in the Grant, patients in the study communities were divided into six different groups based on MFD and the frequency of onchocerca nodules: (a) <1-10 MFD, (b) 11-50 MFD, (c) 51-100 MFD, (d) >100 MFD, (e) nodules only, and (f) no microfilariae and nodules. The distribution of individuals according to MFD in a total of 1,376 permanent residents from Fincas located at three endemicity

areas was: 530 (38.5%) with no microfilariae, 551 (40.0%) with <1-10 MFD, 213 (15.5%) with 11-50 MFD, 53 (3.9%) with 51-100 MFD, and 29 (2.1%) with >100 MFD. The results are shown in Table I.

A.3.(b) Phase II:

A.3.(b).i <u>Preparation of Onchocerca whole worm somatic antigens for trans-blot ELISA</u>.

<u>Onchocerca</u> whole worm somatic antigens for trans-blot ELISA (or enzyme-linked immuno-electro transfer blot, EITB) were prepared as described in the Grant according to the procedures of Boto et al., 1984. J. Immunol., 133:975. Briefly, 4.33 gm (wet weight) of adult <u>O. volvulus</u> males and females were macerated in extraction buffer (Tris 50 mM) containing protease inhibitors (EDTA 5 mM, PMSF 1 mM, TPCK 50 ug/mL), Triton X-100 (1%) and sodium deoxycholate (0.25%). After six cycles of sonication at 4 C, the insoluble material was sedimented at 100,000 g at 4 C for 1 hour. The sediment was processed as described before, the supernatants were combined, and dialyzed at 4 C for 24 h against eight changes of 1 L each of 10 mM Tris buffer, pH 7.39. Protein concentration (4.69 mg/mL) was determined by the Bio-Rad method and the antigen solution was stored in small aliquots at -80 C until used.

A.3.(b).ii Learn the trans-blot ELISA (EITB) procedure in Atlanta and adapt to O. volvulus.

As indicated in the Grant, a training component with Dr. Victor Tsang (the collaborator in this Project) at the Centers for Disease Control in Atlanta, GA to learn and apply the EITB procedure to O. volvulus, was included for laboratory personnel in the Project. This training was provided to two persons from the Universidad del Valle during January, 1989. The EITB procedure was performed according to the procedures of Tsang et al. (1986. Enzyme-Linked Immunoelectrotransfer Blot Technique (Western Blot) for Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus (HTLV-III/LAV) Antibodies. Immunology Series No. 15, U.S. Department of Health and Human Services, 25 pp.). Results of the antigen prepared for EITB [described in section A.3.(b).i] and applied to Guatemalan onchocerciasis sera derived from patients with different MFD and nodules, respectively, gave the following results. The optimal antigen concentration to load the PAGE-SDS gels was 0.6 ug/well. In the EITB, no apparent cross-reactivity was observed with normal human sera. Sera derived from African patients with Wuchereria bancrofti infections, however, demonstrated some cross reactivity with an antigen of a molecular weight between 14.44 Kd and 20.10 Kd. Preliminary analysis of the molecular weight bands recognized by

the different onchocerciasis sera indicated that bands of 200 Kd and 17 Kd are not recognized by patients without onchocerciasis; antigens of 66 - 97 Kd are recognized by patients with onchocerciasis. One band of <14 Kd was recognized by all sera, including normal human sera (Figures 1 and 2).

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 second follow-up visits to the communities in the three endemic areas have been completed, serum samples and nodules have been collected, antigen for EITB has been prepared, and the training component for EITB at CDC has been accomplished.

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. These same problems were outlined already in the Second Progress Report of Activities.

Patient compliance has been very poor in follow-up visits. Out of 1,431 persons initially examined and called again for re-examination, only 980 (68%) attended the second visit of which only 636 (56%) provided a blood sample. 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 have increased the time intervals between each follow-up visit, from the initial three to four months, to six month intervals. In spite of this change, people are still reluctant to provide a blood sample even at six month intervals. We have tried to gain their confidence by providing free physical medical examinations, vitamins, and some food supplements. Most people recognize and appreciate our efforts, but their cultural believes are a handicap and a barrier when it comes to providing blood samples. We would like to propose that in future studies, an educational campaign and a promotion by a social worker will be extremely important and helpful to bridge over these barriers. Nevertheless, 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 sequential serum samples from specified patient groups will be obtained for analysis.

We still 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. As indicated in the Second Progress Report of Activities, we have obtained serum samples, however, from onchocerciasis patients treated with Ivermectin, although follow-up from these patients has not been possible.

Due to an ongoing Ivermectin pilot study in five Fincas in the onchocerciasis areas by the Onchocerciasis Department of the National Malaria Service, all nodulectomy activities have been reduced or suspended until next year. We have made arrangements, however, to obtain nodules starting in February or March of 1989.

A.5 Other activities.

From December 5 to 10, Dr. Ricardo Luján and Licenciada Magda Chávez attended the VIII Central American Congress for Microbiology and I National Congress for Microbiology in San Salvador, El Salvador. Three presentations were given (enclosed a copy of each Abstract), as part of the results derived from the project: "Epidemiology of onchocerciasis in areas of different endemicity in Guatemala:

Implementation of "Sentinel Fincas" for the evaluation of control programs," "Analysis of the quantitative skin biopsy to determine onchocerciasis prevalence," and "Onchocerciasis in migratory population ("cuadrilla") of Guatemala: Implications for the implementation of a national program for control of the disease"

TABLE I

Geometric mean microfilarial densities for onchocerciasis in permanent residents of communities [Finca] from three different endemicity levels

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	Geometric Mean Microfilarial Densities (MFD)*				
	0	<1-10	11-50	51-100	>100
Low					
Panama	239**	138	26	3	0
Medium					
La Torre	115	98	28	2	3
Mirandilla	63	38	16	1	0
Costa Rica	30	57	27	3	3
Total	208	193	71	7	6
High					
Buena Vista	7	37	27	9	6
Las Delicias	20	60	40	29	10
Sta. Margarita	56	123	49	5	7
Total	83	220	116	43	23
TOTAL	530	551	213	53	29

* MFD = geometric mean of number of microfilariae/mg of wet weight of skin taken from four skin biopsies/individual.
** Number of individuals.

SUEROS O N C H O

Sueros: 1:50, 1 hora
Conjugado: Cabra Anti-IgG hum/HRPOD
1:1,000 1 hora
Sustrato: DAB, 5 min

MUESTRAS: # 1

62-0005
62-0123
63-0014
63-0028
63-0033

61-0011
61-0012
61-0021
61-0042
61-0073

61-0020
61-0049
61-0060
61-0066
61-0083

61-0044
61-0064
62-0083
62-0145
62-0159

Pool ON
SHN

Western Blot
8-9, Enero, 1989
CDC, Atlanta, Georgia

VIII CONGRESO CENTROAMERICANO DE MICROBIOLOGIA
I CONGRESO NACIONAL DE MICROBIOLOGIA
San Salvador, El Salvador, 5 - 10 Diciembre 1988

Instrucciones para la Preparación del resumen

1-Escriba el resumen con maquina eléctrica, a 1 espacio, en el interior del cuadro adjunto, sin salirse de los márgenes.

2-Para correcciones utilice liquido corrector. No borre.

3-El título debe ir escrito con letras mayúsculas. Escriba los apellidos y las iniciales de los autores según las reglas habituales, y subraye el nombre del autor que hará la presentación.

Necesitaré:

Proyector de diapositivas (X)

Retroproyector ( )

Otros ( )

-Especifique.

Fecha límite de Inscripción de trabajos:

31 de octubre de 1988.

EPIDEMIOLOGIA DE ONCOCERCOSIS EN AREAS DE DIFERENTE ENDEMICIDAD EN GUATEMALA: IMPLEMENTACION DE "FINCAS CENTINELA" PARA EVALUACION DE PROGRAMAS DE CONTROL.

Chávez Escandón, M.J., Luján, R., Luján Trangay, A., y Galindo Rodríguez, D.D.

Centro de Investigaciones en Enfermedades Tropicales, Universidad del Valle de Guatemala y Departamento de Oncocercosis, Servicio Nacional de la Malaria y Programas Anexos, Guatemala.

Los métodos tradicionales para el control de la oncocercosis se han basado en campañas extensas de lucha antilarvaria, nodulectomía o por tratamiento con dietilcarbamazina. A pesar de ello, la enfermedad de Robles aún continúa siendo un problema de salud en varios países de Latinoamérica y Africa. La Ivermectina, una droga microfilaricida, ofrece una alternativa nueva y efectiva para el tratamiento de la enfermedad. Sin embargo, antes de distribuir esta droga a nivel comunitario deberían establecerse "Fincas centinela", en áreas de diferente endemicidad, que sirvieran como indicadores sensibles para medir y evaluar el impacto que puedan tener las campañas nacionales de control de la oncocercosis con Ivermectina.

El objetivo del presente trabajo fue describir la epidemiología de oncocercosis en poblaciones de áreas con diferente endemicidad y definir los factores que contribuyen al grado y severidad de la enfermedad, para obtener información basal como "Fincas centinela". Se describen los resultados de la prevalencia de oncocercosis por biopsias cutáneas, nódulos y examen oftalmológico en relación a sexo, grupo etáreo y ocupación de 1,364 colonos en siete fincas de áreas meso e hiperendémicas de oncocercosis en Guatemala.

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Otros ( )

-Especifique.

Fecha límite de Inscripción de trabajos:

31 de octubre de 1988.

ANALISIS DE LA BIOPSIA DE PIEL CUANTITATIVA PARA DETERMINAR LA PREVALENCIA DE ONCOCERCOSIS.

Luján, R., Chávez Escandón, M.J., Luján Trangay, A., y Galindo Rodríguez, D.D.

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La biopsia de piel (bx), determinando la presencia de microfilarias (mf), permanece aún como el método parasitológico más sensible y específico para el diagnóstico de la oncocercosis. Kawabata et al. (1983, Trans Roy Soc Trop Med Hyg, 77:215) demostraron que en Guatemala la densidad de mf (dmf) es mayor sobre la espina de la escápula (E) y la cresta del hueso ilíaco (I); y que en los hombres es mayor en I mientras que en mujeres es en E. Por ello, se ha recomendado tomar dos bx (una E y una I) de hombres y dos E de mujeres.

El objetivo del presente trabajo fue determinar cuál es el número y la combinación de bx que debería utilizarse para para determinar la prevalencia de oncocercosis. Para esto se estudiaron 1,364 individuos (771 hombres y 593 mujeres) provenientes de áreas de mediana y alta endemicidad de oncocercosis. De cada uno de ellos se tomaron cuatro bx con un esclerodermótomo tipo Holth (2.0 mm), dos del lado derecho (D) y dos del izquierdo (I), una E y una I. Las bx se incubaron en medio RPMI 1640 y a las 24 h se contaron las mf.

Con una bx se encontró un 12-16% de falsos negativos tanto en hombres como en mujeres, y la combinación de dos bx, ED/EI ó ID/II subestimó un 5-12% de individuos positivos dependiendo del área de endemicidad, mientras que la de ED/II ó EI/II, sólo en un 2-4%.

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Otros ( )

-Especifique.

Fecha límite de Inscripción de trabajos:

31 de octubre de 1988.

ONCOCERCOSIS EN POBLACION MIGRATORIA ("CUADRILLA") DE GUATEMALA: IMPLICACIONES PARA LA IMPLEMENTACION DE UN PROGRAMA NACIONAL PARA EL CONTROL DE LA ENFERMEDAD.

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Los aspectos descriptivos de la epidemiología de oncocercosis en Guatemala están bien documentados, basados en exámenes periódicos de la población local o colonos. En 1983 se estimó 233,975 personas en riesgo y 28,451 ya infectadas (Zea Flores, 1983. Memorias VI Congr. C.A. de Microbiol.). Durante el año, especialmente en los meses de octubre a enero, la población local aumenta hasta un 50% por población migratoria proveniente de áreas no endémicas. Sin embargo, la "cuadrilla" nunca ha sido examinada y se desconoce la importancia que pueda tener en el mantenimiento de la transmisión de oncocercosis.

El objetivo del presente trabajo fue determinar la prevalencia de oncocercosis en "cuadrilleros". Para ésto se estudiaron 57 individuos, 42 hombres y 15 mujeres comprendidos entre los 4 y 58 años.

Once (26.2%) hombres y 4 (26.7%) mujeres fueron positivos para microfilarias (mf) en piel, comparado con 77.9% de los hombres y 63.3% de mujeres colonos. La densidad de mf/mg de piel en los positivos fue de 0.52-3.92, comparado con 0.33-448.09 en colonos. Dos (3.5%) personas presentaron nodulos en la cabeza y el cuerpo, comparado con 20.5% de los colonos. Siete (12.3%) indicaron historia previa de 1-4 nodulectomías en los últimos 2-25 años, comparado con el 64% de los colonos. A pesar de la baja prevalencia e intensidad de infección oncocercósica, la "cuadrilla" debería incluirse en los programas nacionales de control de la oncocercosis con Ivermectina.

Financiado en parte por una donación de la AID/SCI/PSTC (Grant DPE-5542-G-SS-7033-00)

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