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جامعة اليرموك
YARMOUK UNIVERSITY

Irbid - Jordan

Tel. 271100 - 271115. Telex 51533 YARMOUK JO. Cable: YARMOUK

Ref.

Date

FACULTY OF SCIENCE
DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. John A. Daly,
Acting Director,
Office of Research,
AID,
Bureau for Research and Development,
Room 320 SA-18,
Washington D.C. 20523-1818,
U.S.A.

Reference: PSTC Project No. 6.132.

Dear Dr. Daly,

Enclosed please find a final report on PSTC Project No. 6.132 "Development and Characterization of monoclonal antibodies to hydatid disease antigens", which has been completed December 1989.

Thank you.

Yours sincerely,

Sami K. Abdel-Hafez, Ph.D.
Professor

c.c. Dean of Research and Graduate Studies, Yarmouk University.
Health and Population Officer, USAID Office, Amman, Jordan.

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FINAL REPORT

Project Title: Development and Characterization of Monoclonal Antibodies to Hydatid Disease Antigens.

Principal Investigator: Professor Sami K. Abdel-Hafez.

Co-investigator: Dr. Fadwa M. Al-Yaman.

I. Background:

The project started December 1986 and was completed December 1989. The following were the objectives of the project:

1. Prepare hydatid antigenic material from human and domestic animal sources as well as from in vitro cultured stages (excretory/secretory antigens).
2. Produce monoclonal antibodies (MCA) against some of these antigens.
3. Screen the MCA produced against a battery of antigens, and select 'relevant' ones for potential of utilization in serodiagnosis and isolation of appropriate antigens.
4. Use of selected MCA in seroepidemiological situations and for the detection of E/S antigens in sera of humans and domestic animals infected with the hydatid cysts.

II. Work Carried Out:

To fulfil these objectives we have carried out the following research work:

1. Preparation of antigenic material from hydatid cyst fluid, protoscolices, and protoscolices tegument as well as in vitro cultured stages at various phases of cultures.

2. Establishment of in vitro culture of Echinococcus up to the adult sexual stage. These cultures were initiated from sheep, donkey and human hydatid protoscoleces. Differences in the behaviour and success of in vitro cultures from the different sources were noted. These studies helped in the strain identification of E. granulosus in Jordan and in the preparation of in vitro derived antigens.
3. Excretory/secretory antigens were prepared from protoscoleces of sheep hydatid cysts which were in vitro cultured up to 35 days. Characterization of these antigens was carried out using SDS-PAGE and immunoblot techniques and was found to contain many protein and glycoprotein fractions.
4. Monoclonal antibody production against protoscoleces tegumental antigenic extract was carried out using immunized Balb/c mice as spleen cell donors. Three cell lines were expanded and characterized. The specificity of these MCA's was studied using a battery of antigenic preparations from different parasites. Under non-reducing conditions immunoblot analysis showed that one of the prepared MCA's identified a 31 kDa single band while the other two MCA identified 2 or 3 bands simultaneously. Under reducing conditions, the 1st MCA identified a <14.4 kDa single band while the other 2 MCA identified many small Mr bands. The respective antigens for each of these 3 MCA's were affinity purified but were not found to be E. granulosus specific. Affinity purified antigens with these MCA were tested for possible use in serodiagnosis. The greatest sensitivity (81.3% of positive cases) was obtained using affinity purified antigens by 5B4G7 MCA. The epitopes recognized by one of these 3 MCA were of protein nature (in 2 MCA) and glycoprotein nature (1 MCA).
5. Monoclonal antibody production against a purified antigen from crude sheep hydatid fluid. The antigen used was a 50-100 kDa fraction. Immunized Balb/c mice were used as spleen cell donors. Five MCA's whose isotypes were IgG₁(2), IgG_{2b}(1) and IgM(2) were characterized. Two of these MCA's were E. granulosus specific, one was tainiid specific and two were crossreactive with Fasciola hepatica, and Dictyocaulus floria antigens. The epitopes recognized by these MCA's appeared to be non-carbohydrate in nature.

III. Publications Supported by Grant USAID 6.132:

1. Al-Yaman, F.M., Abdel-Hafez, S.K. and Saliba, E.K. 1988. Hydatidosis: Global and local importance part II: Hydatidosis in Jordan. *Jordan Med. J.* 22: 7-12.
2. Al-Yaman, F.M., Abdel-Hafez, S.K., Assaf, L.M. and Malkawi, F.K. 1988. Evaluation of various serodiagnostic tests for human hydatidosis. *Jap. J. Parasitol.* 37: 133-138.
3. Said, I., Abdel-Hafez, S.K. and Al-Yaman, F.M. 1988. Morphological variation of Echinococcus granulosus protoscoleces from hydatid cysts of human and various domestic animals in Jordan. *Intern. J. Parasitol.* 18: 1111-1114.
4. Abdel-Hafez, S.K. and Al-Yaman, F.M. 1989. Spleen hydatidosis in sheep from North Jordan. *Vet. Parasitol.* 30: 191-196.
5. Hijjawi, N.S., Abdel-Hafez, S.K. and Al-Yaman, F.M. 1992. In vitro culture of the strobilar stage of Echinococcus granulosus of sheep and donkey origin from Jordan. *Parasitol. Res.* 78: 607-616.
6. Hijjawi, N.S., Abdel-Hafez, S.K. and Kamhawi, S.A. 1992. Echinococcus granulosus: possible formation of a shelled egg in vitro. *Intern. J. Parasitol.* 22: 117-118.
7. Hussein, A., Abdel-Hafez, S.K. and Khalil, A. Isolation and characterization of excretory/secretory products from in vitro developmental stages of Echinococcus granulosus. *Jap. J. Parasitol.* "In press".

IV. Publications Submitted or in Preparation:

1. Al-Melhim, W., Al-Yaman, F. and Abdel-Hafez, S.K. Production of monoclonal antibodies against partially purified hydatid fluid of Echinococcus granulosus.
2. Abbas, M., Al-Yaman, F.M. and Abdel-Hafez, S.K. Characterization of monoclonal antibodies against Echinococcus granulosus protoscoleces tegumental antigenic extract.
3. Moosa, R.A., Abdel-Hafez, S.K. and Al-Yaman, F.M. Human unilocular hydatidosis in Jordan: Serodiagnosis and seroepidemiological studies.
4. Abbasi, I. and Abdel-Hafez, S.K. Strain and immunological characterization of unilocular hydatidosis in Jordan by in vivo methods.

V. Titles of Thesis Work Supported by the Grant:

These include thesis work which utilized equipment and facilities made possible through this grant.

| Title | Graduate Student | Date |
|---|-------------------|------------|
| 1 Production and characterization of monoclonal antibodies against <u>Echinococcus granulosus</u> partially purified sheep hydatid fluid. | Walid Al-Melhim | Aug. 1989 |
| 2 Production and characterization of monoclonal antibodies against <u>Echinococcus granulosus</u> protoscoleces tegumental antigen extract. | Manal Abbas | Aug. 1989 |
| 3 Isolation and characterization of excretory/secretory antigens from various <u>in vitro</u> developmental stages of <u>Echinococcus granulosus</u> . | Ayman S. Hussein | Sept. 1990 |
| 4 Unilocular hydatidosis in Jordan: Strain and immunological characterization by <u>in vivo</u> methods. | Ibrahim Abbasi | Aug. 1990 |
| 5 Studies on the <u>in vitro</u> culturing of <u>Echinococcus granulosus</u> from sheep and donkey cysts in Jordan. | Nawal S. Hijjawi | May, 1989 |
| 6 Human unilocular hydatidosis in Jordan: Serodiagnosis and sero-epidemiological studies. | Riyad A. Moosa | Jan. 1989 |
| 7 Histopathological, cellular and immunological changes in the spleen of sheep and mice infected with hydatid cysts of <u>Echinococcus granulosus</u> . | Siddieg A. Rahoud | Dec. 1987 |