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TITLE: DEVELOPMENT OF SEROLOGIC ASSAYS FOR THE DIAGNOSIS OF NEW WORLD LEISHMANIASIS

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Murine monoclonal antibodies have been used to recover the genus-specific antigens of the New World Leishmania. These antigens have been used for the development of species-specific serodiagnostic assays and to study the immunopathogenesis of the disease. Seven manuscripts and 10 abstracts have resulted from these efforts.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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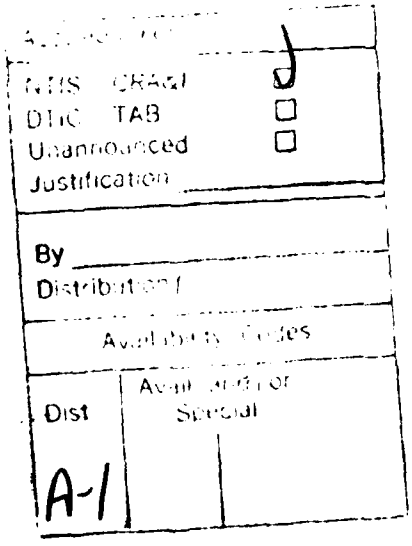

Narrative Summary:

1. Cultivation and maintenance of parasites:

The laboratory has become proficient in the maintenance and cultivation of both promastigotes and amastigotes of the leishmania species as well as epimastigotes of the trypanosomes. An inventory of parasite isolates which are now held in our cryobank can be found in our Annual Report No. 2 (1983-1984).

2. Monoclonal Antibody Production.

The laboratory has produced and characterized numerous panels of murine monoclonal antibodies to the surface and subcellular antigens of the major species and subspecies of New World Leishmania, Trypanosoma cruzi and Trypanosoma rangeli . Immunogens have included extracts of whole culutred forms (promastigotes or epimastigotes), purified pellicular membranes, insect forms and amastigotes.

3. Use of monoclonal antibodies for the speciation of isolates:

The primary objective of our contract was to develop monoclonal antibodies for the speciation of New World Leishmania isolates. Although were were not successful in differentiation of some of the sub-species we are now confident in the genus-specificity of some antibodies and most importantly, our ability to differentiate members of the mexicana versus the braziliensis complex. However, the differences in surface antigen expression are often quantitative and not, as reported by others, commonly qualitative. The antigenic profiles of isolates from mucocutaneous disease are also very different from members of the same species from cutaneous disease. At least one monoclonal antibody is specific for an epitope which, thus far, is peculiar to a subpopulation of promastigotes of an Leishmania braziliensis panamensis isolate. Many of these monoclonals are now in use at WRAIR for the speciation of trypanosomatids recovered from sandflies collected in The Republic of Panama. Other monoclonals are being used at WRAIR for the speciation of leishmania recovered from human lesions.

4. Recovery of species- and strain-specific antigens:

The second major goal of the contract was to use the monoclonal antibodies as ligands, in immunoaffinity chromatography, for the recovery of strain-specific and species-specific antigens. A complete list of the antigens recovered and assessed is presented in the Annual Report No. 2 (1983-1984).The leishmania display four dominant genus-specific antigen on their surface membrane with molecular sizes of 72, 55, 42 and 15 kd respectively. The low molecular weight protein is aslo excreted into the medium which supports growth of the promastigotes. The 72 and 55 kd moieties have no external exposure, as determined by flow cytometry, and thus probably play little role, if any, in the immune response. Minor membrane antigens consited of 4 additional polypeptides with kd values of 63, 58, 56 and 25. The three larger molecules share a common antigenic epitope. All of the antigens have been characterized and quantitated on the surface

membrane of all leishmania isolates, by using techniques of immunoblotting and flow microfluorometry. Several have also been visualized by immunoelectronmicroscopy.

5. Development of enzyme linked immunosorbent assays to demonstrate serum antibodies against strain-specific and species-specific antigens of leishmania:

Although we were not successful in developing an ELISA for detecting species-specific antibody responses (the third objective of the contract), we have recovered and purified a genus-specific antigen which is highly reactive with sera from human leishmaniasis but not reactive with sera from individuals with Chagas' disease. This antigen has now been used in several serosurveys in The Republic of Panama.

6. Use of monoclonal antidodies to detect parasites in infected tissues:

Another objective was to ascertain whether moncolonal antibodies could be used to enhance assays designed to detect intracellular amastigotes in biopsy specimens. Using a Balb/c mouse model, we were able to confirm that several of our monoclonal antibodies did facilitate identification of amastigotes in infected tissues. These monoclonal antibodies are now be used at WRAIR for the identification of amastigotes in human tissues.

7. Use of monoclonal antibodies to identify parasite antigens which may contribute to the pathology of the disease.

Throughout the course of these studies, we also discovered that several monoclonal antibodies can act as opsonins and actually enhance parastie binding and uptake by mouse peritoneal macrophages. The antigens recognized by these monoclonals have been characterized and purified. Conversely, several other monoclonals inhibit the binding and uptake of the parasites by the macrophages. We suspect that these antibodies are recognizing the parasite receptor which is required for attachment.

One monoclonal antibody recognized an parasite antigen which was expressed on the surface membrane of the parasitized macrophage. Flow cytometric analyses of the kinetics of expression of this antigen suggested that it has an intracellular origin and is processed within the phagolysosome. Results have also been confirmed by immunoelectronmicroscopy. This antigen has been purified and we now have evidence that it stimulates lymphocyte transformation in vitro .Its capacity to induce lymphokines involved in the activation of macrophages is currenty under investigation.

Many other mohoclonal antibodies recognized epitopes which have apparently been highly conserved throughout phylogeny and thus are expressed in vertebrate cells. These antibodies are reactive with an array of cytoskeletal elements, nuclear components and cytoplasmic organelles. The role which these cross-reactive antigens play in the immunopathogenesis of disease remains unclear.

Papers:

1. Anthony, R. L., K. M. Williams, J. B. Sacci and D. C. Rubin. 1985. Subcellular and taxonomic specificity of monoclonal antibodies to New World Leishmania . Am. J. Trop. Med. Hyg., 34: 1085-1094.

2. Williams, K. M., J. B. Sacci, and R. L. Anthony. 1986. Characterization and quantitation on membrane antigens of New World Leishmania species using monoclonal antibodies in western blot and flow microfluorometric assays. J. Protozool. (in press).

3. Williams, K. M., J. B. Sacci, and R. L. Anthony. 1986. Flow cytometric analysis of the effects exerted by monoclonal antibodies on binding and uptake of Leishmania mexicana mexicana promastigotes by murine peritoneal macrophages. Infect. Immun. (in press).

4. Williams, K. M., J. B. Sacci, and R. L. Anthony. 1986. Identification and recovery of Leishmania antigen displayed on the surface membrane of mouse peritoneal macrophages infected in vitro . J. Immunol. (in press).

5. Anthony, R. L., K. M. Williams and J. B. Sacci. 1986. Identification, recovery and immunogenecity of an antigen common to Trypanosoma cruzi and the vertebrate cell matrix. Infect. Immun. (submitted for publication).

6. Williams, K. M., J. B. Sacci, and R. L. Anthony. 1986. Rapid identification of Leishmania amastigotes using strain- and species-specific monoclonal antibodies. Am. J. Trop. Med. (submitted for publication).

7. Sacci, J. B., H. A. Christensen, A. Vasquez, and R. L. Anthony. 1986. Serodiagnosis of New World Leishmania by using a genus-specific antigen in enzyme linked immunosorbent assays. (in preparation).

Abstracts:

Anthony, R.L., and Constantine, N.T.: Identification and characterization of surface and subcellular antigens of Trypanosoma cruzi by means of monoclonal antibodies. Fed. Proc. 41: 585, 1982.

Anthony, R.L., Phelps, P.C., and Williams, K.M.: Serologic cross-reactivity between flagellar antigens on the Trypanosomatidae and cytoskeletal components of mammalian cells. Am. Soc. Trop. Med. Hyg., Cleveland, Ohio, November 11, 1982.

Constantine, N.T., Williams, K.M., and Anthony, R.L.: Visualization of leishmania in parasitized macrophages using monoclonal antibodies of four specificities. Am. Soc. Trop. Med. Hyg., Cleveland, Ohio, November 9, 1982.

Williams, K.M., Constantine, N.T., and Anthony, R.L.: Differentiation of New World Leishmania species by means of their reactivity with monoclonal antibodies in immunofluorescent assays. Fed. Proc. 42: 854, 1983.

Anthony, R.L., Phelps, P.C., and Williams, K.M.: Monoclonal antibodies to rat brain tubulin: Reactivity with cultured mammalian cells. Fed. Proc. 42: 1301, 1983.

Sacci, J.B., Williams, K.M., and Anthony, R.L.: Specificity of monoclonal antibodies to New World Leishmania: Assessment by enzyme linked immunosorbent assays. Am. Soc. Trop. Med. Hyg., Baltimore, MD. 1984.

Williams, K.M., Sacci, J.B., and Anthony, R.L.: Monoclonal antibodies recognizing stage-specific antigens of Leishmania mexicana mexicana and components of infected and uninfected peritoneal macrophages. Am. Soc. Trop. Med. Hyg., Baltimore, MD. 1984.

Anthony, R.L., Williams, K.M., and Sacci, J.B.: Specificity of monoclonal antibodies to parasitic tyrpanosomatids. Fourth European Congress of Parasitology, Izmir, Turkey, 1984.

Anthony, R.L., Sacci, J.B., and Williams, K.M.: Trypomastigotes of Trypanosoma Cruzi possess a 58 KD flagellar antigen which elicits antibodies to cytoskeletal polypeptides in human disease. Am. Soc. Trop. Med. Hyg., Miami, FL. 1985.

Sacci, J.B., Williams, K.M., and Anthony, R.L.: Promastigotes or Leishmania braziliensis panamensis have a genus - specific surface antigen which can be purified and used for the serodiagnosis of human disease. Am. Soc. Trop. Med. Hyg., Miami, FL. 1985.

SPECIAL LECTURES, INVITED SEMINARS

Tropical Medicine Association of Washington, D.C. Guest speaker. NIAID, Bethesda, Maryland, February 25, 1982.

Tropical Medicine Course. Invited speaker. Walter Reed Army Institute of Research, Washington, D.C., July 23, 1982.

Thomas W. Holbrook Memorial Lectureship, Medical University of South Carolina, Charleston,SC, April 16, 1984.

Invited Lecture. Monoclonal antibodies and the antigenic dissection of New World Leishmania. Walter Reed Army Institute of Research. Washington, D.C. November 13, 1985.

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Dissertation title:
"Immunogeneic and Immunopathologic Properties of Leishmanial Antigens Identified by Murine Monoclonal Antibodies"

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