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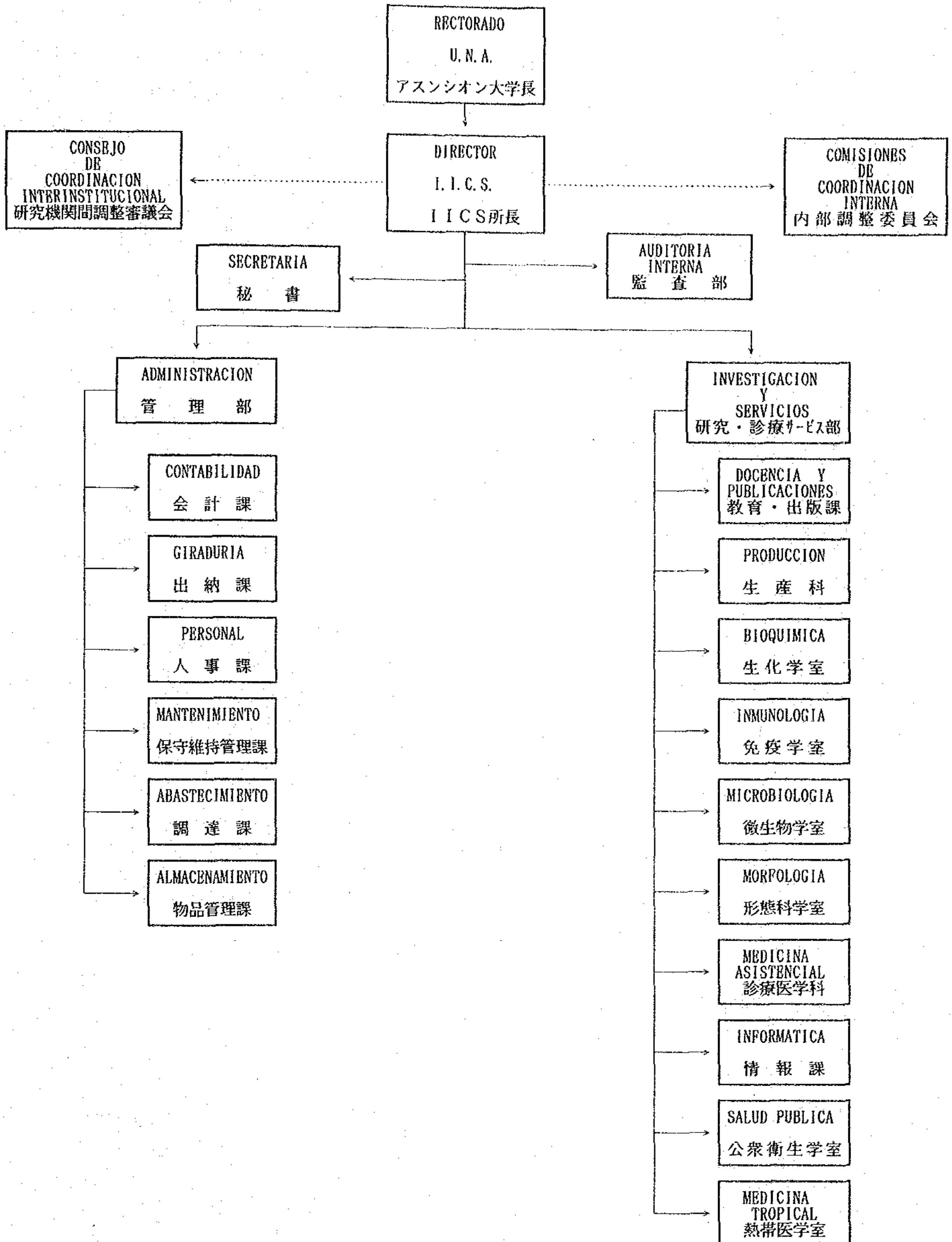

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MEASUREMENT OF PLASMA FIBRONECTIN IN PATIENTS CHAGAS' DISEASE WITH

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INTRODUCTION :

Attachment of Trypanosoma cruzi, the causative agent of Chagas' disease, to mammalian cells involves the interaction of the plasma membranes of the pathogen and the host cell.

It is generally accepted that many ligand - cell and cell - cell interactions are mediated by cell surface glycoproteins. Fibronectin, a 500,000 dalton glycoprotein present in blood, connective tissue, and on the cell surface, participates in a number of cell surface interactions with the extracellular microenviroment. It has been proven that fibronectin mediates the attachment of pathogens such as Treponema pallidum and Leishmania species. Ouaissi et al. demonstrated that fibronectin interacts th a specific receptor which is presumably on the plasma membrane of T.cruzi trypomastigotes. In fact, they have isolated and characterized this membrane receptor for fibronectin. Involvement of this fibronectin receptor in the interaction between T.cruzi and vertebrate cells has been reported.

Studies of T.cruzi provide strong evidence in support of the concept that this parasite uses host fibronectin as a bridge for its association with the host cell it infects.

Using hemoagglutination inhibition assay or an ELISA assay we will measure plasma fibronectin in patients with Chagas' disease in order to find out if there is a variation in plasma fibronectin levels in these patients.

MATERIAL AND METHODS

Purification of human plasma fibronection: 100 ml of human plasma was applied to a cyanogen bromide activated Sepharose 4B column to remove non-specific binding materials in plasma. Then, it was applied to a cyanogen bromide activated Sepharose 4B column coupled with gelatin. The application was repeated more than 3 times for a period 2 hours at room temperature. The column, previously equilibrated with tris-EDTA buffer, was washed with 100 ml of the same buffer.

Elution was done using Tris-HCl buffer containing 4M urea and 5 ml fractions were collected. Measurements of the OD were done at 280 nm for each fraction.

Peak fraction were collected and concetrated with 40% saturated ammonium sulfate. Finally, the fractions containing the fibronectin were dialyzed against PBS.

Determination of the optimal concentration of rabbit antihuman fibronectin antiserum titer.

a) Conjugation of the fibronectin to sheep red blood cells (SRBC). A 50% suspension of SRBC was mixed with 0.01 mg/ml of fibronectin solution at the same volume. Then, a 1% solution of $CrCl_3.H_2O$ in saline was added at the same volume and agglutination was observed within 2-5 minutes. The SRBC conjugated with fibronectin were washed with saline 3 times and the pellet was suspended at a concentration of 2-4%.

b) Hemoagglutination assay. Rabbit antihuman fibronectin antiserum. Was diluted floo-fold in soft type microplates at a volume of 25 ul. Then, 25 ul.Then, of fibronectin conjugated SRBC were added. After 2-4 hours at room temperature hemoagglutination inhibition titer was checked.

Hemoagglutination inhibition assay for the measurement of plasma fibronectin in Chagas' disease. Dilution of Test samples which were expected to contain fibronectin were senally diluted at a volume of 25 ul. 25 ul of fibronectin conjugated SRBC were added. Then, 25 ul of rabbit antifibronection antiserum with a two fold higher concentration of hemoagglutination point was added.
After 2-4 hours at room temperature hemoagglutination inhibition titer was checked.
ELISA assay (inhibition assay) for the measurement of plasma fibronectin in Chagas' disease. Hard type microplates were coated with a 1,000 - 2,000 fold dilution of rabbit antihuman fibronectin antiserum. The plates were the washed with PBS 3 times, and saturated with 1% BSA in PBS a room temperature for 30 minutes. 20 ul of test samples or a known concentration of fibronectin were added and Incubated at room temperature for 2 hours followed by 6 washes with PBS. Substrate solution, 1, mg/ml p-Nitrophenyl phosphate disodium in diethanolamine, was added and was allowed to react for 30 minutes at room temperature. Measurements were taken in a ELISA reader at 405 nm.

FUTURE PLANS

First of all, upon obtaining plasma fibronectin levels in Chagas' disease patients it would be interesting to compare these levels with the clinical aspect of each patient. If a variation in plasma fibronectin levels is found, it would be possible to establish a relationship between both, clinical aspects and plasma fibronectin levels.

Secondly, the rabbit antihuman fibronectin antiserum can be used to confirm the fibronectin role in **T.cruzi** binding to host cells. Addition of this antiserum to **T.cruzi** - monocyte co-cultures will allow us to check any variation in the attachment of the parasites to the cells.

RHEUMATOID FACTOR AND CHAGAS' DISEASE

Cabello, A.; Maldonado, M.; Nishinarita, s.

INTRODUCTION:

Rheumatoid factors (RF) are immunoglobulins with specificity for the Fc fragment of IgG. They can belong to the IgG, IgM, IgA, IgD, or TgE classes of antibody.
Most of the laboratory methods, however, detect RF IgM 19 S.
In rheumatoid arthritis, the most characteristic serum finding is an elevated RF, present in more than 75 % of patients. However, this elevation is not exclusive to rheumatoid arthritis patients, since it is also found in SLE patients (30 %), in sjogren syndrome patients (90 %), and less frequently in acleroderma or polymyositis patients. It has also been observed in patients with hypergammaglobulinemia associated with hepatic disease, kala-azar, sarcoidosis and syphilis, as well as in chronic illnesses such as leprosy and tuberculosis.
Epidemiological studies have shown that a small number of normal people are positive for RF.
The search for this factor in Chagas' disease patients has yielded evidence that these patients produce RF-like antibodies, mainly of the IgM class; high titers of this antibody were detected in the sera from 95 % of patients in the acute stage of the disease up to approximately 1 year from the onset of symptoms, and in 25 % of patients in the chronic stage, with or without lesions. These findings suggest that there is no correlation between the presence of RF and the course of the infection by T.cruzi, but they do appcar to give another evidence for a possible autoimmune disorder in Chagas' disease.

OBJECTIVES:

1. Search for RF in Chagas' disease patients, in different stages of disease.
2. Determine the classes of RF found in these patients.
3. Determine a role for the presence of this factor in the pathogenesis of the disease.

MATERIALS AND METHODS

1. Patient selection: Patients with different stages (acute, intermediary and chronic) of Chagas' disease were selected.

2. RF detection assay: the method used was ELISA:

a) Rabbit IgG was fixed on a plate, and incubated overnight. Thereafter it was washed with PBS and saturated with 1% BSA-PBS for 30 min.
b) 100 ul of different dilutions of sera from Chagas' patients was added, and the mixture was incubated for 2 hours in a moisture chamber; then washed 5 times with PBS.
c) 100 ul of anti-human IgG, IgA, IgM and IgE, conjugated with alkaline phosphatase was added, and incubated for 2 h in a moisture chamber. Thereafter it was washed 5 times with PBS.
d) The substrate (Sigma 104 indiethanolamine buffer, 1 mg/ml) was added, and the mixture was incubated for 30 min at room temperature, then readings were performed with the spectrophotometer.

PLANS FOR THE FUTURE

1. Determine the type of Chagas' patient with positive RF determination .
2. Assay for the class of Rf (IgG, IgA, IgM, IgE) present in these patiennts.
3. Study RF-positive patients for other markers of autoimmune disease (ANA, LE cells, anti-DNA antibodies, etc.).

DETECTION OF R.F. IN CHAGAS' DISEASE PATIENTS

TIENT N°

			R. F.	
DX.	IgG	IgA	IgM	IgE
INDETER.	NEG.1:50	neg.1:50	POS.1:6250	neg.1:50
INDETER.	pos.1:6250	neg.1:50	neg.1:50	neg.1:50
INDETER.	neg.1:50	neg.1:50	neg.1.50	neg.1:50
INDETER.	pos.1:6250	neg.1:50	neg.1:50	neg.1:50
INDETER.	pos.1:250	neg.1:50	pos.1:250	neg.1:50
MIOCAR.	NEG.1:50	neg.1:50	neg.1:50	neg.1:50
MIOCAR.	neg.1:50	neg.1:50	neg.1:50	IOS.131250
MEG.	neg.1:50	neg.1:50	neg.1:50	neg.1:50
MEG.	neg.1:50	neg.1:50	neg.1:50	NOT tested
MEGAES.	pos.1:50	neg.1:50	neg.1:50	NOT tested
INDETER.	pos.1.1250	neg.1:50	POS.1:1250	NOT tested
INDETER.	neg.1:50	neg.1:50	neg.1:50	
MIOCAR.	neg.1:150	neg.1:50	neg.1:50	
MIOCAR.	neg.1:50	neg.1:50	POS.1:250	

TRYPOMASTIGOTE PRODUCTION USING AN IN VITRO CELL CULTURE SYSTEM

Graciela Russomando and Tom Chiller.

INTRODUCTION:
The parasitic protozoan **Trypanosoma cruzi**, the causative agent of Chagas' disease in humans, has four distinct morphological forms throughout its life cycle(1).The epimastigote and metacyclic trypomastigote forms are found in its insect vector. Trypomastigotes are found in the bloodstrean of the vertebrate hosts, whereas the amastigotes forms live as intracellular parasites. Surface proteins of these different stages have been defined in terms of their molecular weights and their recognition by serum antibodies from infected hosts(2,3). A major goal of these studies is to indentify surface antigens, as they are good candidates for the development of diagnostic tools and vaccines.The isolation of **T. cruzi** surface proteins is an obligatory step in assessing their effectiveness as immunogens for protection against infection(4).Since the first step in parasite interiorization into host cells is the mutual contact between their respective plasma membranes, it is likely that surface components of the trypomastigote form are involved in the mechanism of interiorization.Trypomastigote surface antigens may thus be potential targets for antibody mediated immunity.The purpose of this work is the production of parasite antigens specific to the infective trypomastigote stage suitable for use as diagnostic and vaccine reagents, and the use of these proteins to detect hybridoma cell lines producing monoclonal antibodies directed aginst trypomastigote surface proteins.In the present study,a tissue culture system was adopted which allowed the production of large numbers of infectious **T. cruzi** trypomastigotes (TM) in vitro by infection of BHK LLC-MK2 (Kidney, Rhesus monkey,macaca mulata).Initially, BHK cells were used in order to obtain the best qualitative and quantitative antigens of the trypomastigote forms of T.cruzi. Since no reference was available on the use of this cell line in T. cruzi infection, it was necessary to perform a comparative and systematic analysis of the different experimental conditions necessary for the T.cruzi-BHK cells interaction assays.Later, the BHK infection results were compared with another cell line, LLC,MK2. A method for obtaining **T. cruzi** trypomastigotes (Y strain) with the LLC-MK2 cells has been previously described(5),and since that time it has been widely used as a trypomastigote source for antigen preparation according to several reports.

MATERIALS AND METHODS.

PARASITES; T. cruzi, Y strain, isolated by Silva and Nussenzweig (1953) from a human host was obtained from Belo Horizonte, Brasil, and has been maintained in our laboratory by intraperitoneal passage in mice every seven days.
PARASITES AND HOST CELLS; The method for obtaining T. cruzi trypomastigotes (Y strain) in cultured monkey epithelial cells (LLC-MK2) has been previously described (5). LLC-MK2 cell monolayers were grown in Dulbecco's modified Eagle's medium (DME) supplemented with 10 % fetal calf serum (FCS) and antibiotics at 37°c in a 5 % Co_2 incubator. FCS concentration was reduced to 2% in infected cultures.This cell line is propagated every 7 days by subculture at a ratio of 1:10. Each subculture is performed once confluency has been reached.
Parasites used for infection of LLC-MK2 cells were allowed to be in contact with the recipient cells for 48 hours, with DME medium, supplemented with 2% FCS. After this time of incubation, the cells were washed out with fresh medium and finally DME, 2% FCS was added.
The trypomastigotes released from the cultured cells around the 5-6th day through the 8th day after inoculation, were harvested by centrifugation at 3,500 rpm for 30 min followed by washing two times with PBS before experimetal use, or otherwise cryopreserved in liquid N_2.
The BHK cells were maintened in Minimum Essential Media (MEM) supplemented with 5% (FCS) and antibiotics at 37°c in a 5% CO_2 incubator. This cell line is propagated every 48 hrs. Twenty thousand cells were seeded per cm^2 in each subculture.
During the time of infection performed for 2 to 18 hours, the cells were left in contact with the parasites in a serum-free MEM meidum. After this time, they were maintained in MEM, supplemented with 5% FCS. Parasites were harvested daily by removing the supernatant starting from the fifth day post-infection.
Blood-stream trypomastigotes were partially purified from blood taken from a 7-day infected mouse. Blood was taken by intracardiac puncture in sterile conditions using sodium citrate 3.8%. Erythrocytes were removed by low-speed centrifugation at 1,000 rpm for 15 min, followed by an incubation at 37°c for 30 min. Parasites were recovered from plasma by a 3,500-rpm centrifugation for 30 min. The pellet was resuspended in medium with 20 % FCS, and incubated at 37°c for no longer than 30 min in order to remove plasma proteins. The clump formed is released and centrifuged at 3,500 rpm for 30 min, and the pellet was resuspended in the cell culture medium.
Trypomastigotes in the harvested supernatant fluids were counted by means of a haemocytometer. The morphology of parasites was assessed by examination of a Giemsa- stained preparation.

GROWTH KINETICS OF LLC-MK2 CELLS: To determine the growth kinetics of LLC_MK2 cells, individual groups of 9.6 cm^2 Petri dishes were seeded at the following cell densities: $3x10^4$ cells/cm^2 and $0.5x10^4$ cells/cm^2.Samples from Petri dishes from each group were removed daily from days 1 through 8 following inoculation,and the adherent cells were removed by trypsinization.Cells were counted with the aid of a haemocytometer and the viability was established by trypan blue exclusion.

PARASITE INFECTIVITY IN MICE: Cell culture- derived parasite infectivity was established by tritation in Swiss mice.With the BHK-derived trypomastigotes, this was performed using 10^4, 10^5 and 106 parasites per mouse; whereas using LLC MK2-derived trypomastigotes,three mice were inoculated with 10^7 parasites each.

RESULTS:

TRYPOMATIGOTES FROM BHK CELLS: The infection was done when the cells presented a monolayer aspect.It was found that the optimum time of parasite - cell interaction that allowed a good infection of the cells was approximately 18 hours in a serum-free medium.It was considered that the fourth day following infection was the end of the first cell cycle, accompanied by a destruction of a high number of cells, and the finding of a large number of parasites in the supernatant. The fact that the initial infection was made in an unsynchronized way, and cell division was supposed to continue, it was difficult to determine by this experiment the multiplicity of infection.However, the fact that after the fourth day following infection a higher number of parasites were collected,would indicate that at the first time of infection, there was more than one parasite per cell, and that the continuous cell division has a considerable influence on these results.
The inoculations were done at an infectivity ratio of 1:1,1:5,1:10 parasite - cell, respectively. Independent of these ratios, between the 8th and 9th day of infection, the total number of parasites collected did not show any significant difference.
Dvorak (1976) pointed out that all dividing unifected cells will produce two uninfected daugther cells and some infected cells will produce one infected and one uninfected daugther cell.Thus, at cell division the parasites segregate independent of the daughter cells, with an overall reduction occurring in the percentage of synchronization of parasite growth in tissue culture. Two major factors affect variability; the inherent variation in dividing time between parasites in different cells, and the unequal distribution of parasites after cell division. Monolayer cell lines eliminate the second variable and thus facilitate a relatively synchronous infection of stationary host cells.

GROWTH KINETICS OF LLC-MK2 : The cell proliferation which resulted from different levels of inocula is shown in fig. (2). Analyses showed a linear relationship between log. of transformed cell count and time (exponential growth at each of the levels studied).
These individual slopes did not represent significant differences; so an overall estimate of doubling time of 24 hrs. was determined.

TRYPOMASTIGOTES FROM LLC-MK2: In regard to the parasite - cell ratio, it was found that increasing levels of initial infection resulted in a better production of parasites, released between days five and seven. When the parasite - cell ratio is less than one, the harvest of parasites during these days is very poor and increases during the eighth through the fourteenth day after infection. However, the longer one waits to remove the parasites from the supernatant, the more contamination with amastigote forms are found.
It was found that when bloodstream trypomatigotes used for initial infection were partially purified from blood of infected mice, by low speed centrifugation, which only removes erythrocytes there is a loss of 50% of the total initial parasites used for the infection atfer the 48 hrs.
It was possible to increase the multiplicity of infection parasite - cell, by removing plasma proteins from the treated blood.
All the mice used to test the infectivity of parasites derived from cell cultures showed parasitemia, and in some cases they died 10 days after inoculation.
It is concluded that because of the low doubling time of the BHK cells, there is an overgrowth before the parasite production reaches its full potential. This causes monolayer to peel off and individual cells die, releasing intracellular parasites of questionable quality.
Finally, the LLc-MK2 cells are highly susceptible to infection by T.cruzi, and are capable of supporting parasite growth. Furthermore, as a monolayer cell line, the LLC-MK2 cells do not overgrow, which allows the harvest of organisms from adhered, viable cells.

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IN VITRO ASSAY FOR A CYTOTOXIC EFFECT OF SPLEEN AND PERITONEAL CELLS OF PREIMMUNIZED MICE ON TRYPOMASTIGOTE FORMAS OF TRYOANOSOMA CRUZI

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INTRODUCTION:

Chagas' disease is endemic all over Latin America affecting more than 20 million people, according to the most recent WHO report (1). Although a great deal of effort has been invested in attempts to develop a safe preventative method to control the spread of the disease, little progress in this regard has been achieved thus far.

It is apparent, from previous reseach, that both humoral and cellular immune mechanisms concur in trying to control the parasite aggression in the infected organism.Several reports sustain the participation of antibody-mediated lysis (2), whereas others strongly favor the supremacy of cellular immunity (3) in the defense against this infection.In the past few years,most researchers tend to concentrate in the study of cell-mediated immunity against **T. cruzi**. This bias can be attributed to the accumulation of evidence favoring the higher effectiveness of this mechanism during the course of infection. A recent publication reports the success of an in vitro assay for parasite killing by mast cells isolated from previously immunized mice (4). This work was based on the observation of considerable mast cell infiltrate in the sites of inflammation in infected organisms. This would suggest a putative active role of these cells in the immune response to this infection.The present work investigates the presence of a cytotoxic T cell activity against **T. cruzi** trypomastigotes in vitro, when incubated with peritoneal and spleen cells, respectively, from mice previously immunized with both UV-irradiated whole trypomastigotes and insoluble fractions thereof.

MATERIALS AND METHODS

Parasites: **T. cruzi** Y strain was used in all the experiments. Epimastigote forms of the parasite were obtained from culture in LIT medium.Trypomastigotes were produced at a fairly high efficiency by infection of LLC-MK2 cells(5) in an in vitro culture with boodstream trypomastigotes obtained from infected mice.

Mice: Swiss albino mice were employed as a source of both peritoneal and spleen cells for the cytotoxicityd assay.

UV- irradiation of parasites: Parasites were irradiated by placing 10 ml of the parasite suspensions in 6-cm diameter Petri dish and exposing them with the lids removed- to UV light in a UV chamber. Epimastigotes were irradiated for various lengths of time in order to determine the dose of UV light required to block parasite replication without affecting its viability.

Trypomastigotes were irradiated with the suitable UV dose capable of stopping parasite proliferation for inoculation into the mouse host.

Assessment of suitable UV light dose for replication blockage: To determine the UV dose necessary for blocking parasite replication without affecting seriously its viability, epimastigotes were irradiated for 0.5, 1, 2, 6, 16 and 25 min. Viability and parasite replication were assessed by [3H]- uridine uptake and [3H]-thymidine uptake, respectively [3H]- uridine and [3H]-thymidine uptake were determined by incubating the irradiated parasites, suspended in LIT medium with 2 uCi of the radiolabeled nucleosides, respectively, in LIT medium in a micro-well plate.Following a 72- hour incubation period, parasites were harvested using a semiautomatic multiple cell harvester (Labco Science Co.,Ltd.) onto glasswool filter paper and washed.Their radioactivities were counted in a Beckman LS 5000 TA Liquid Scintillation Counter, using the Beckman Ready Safe TM liquid Scintillation cocktail.

Disruption of parasites and fractionation: Approximately 3 x 10^8 trypomastigote-form parasites were collected and disrupted by intermitent sonication with a Branson Sonifier 250, at the maximum energy output, with 1- min intervals, for 3 min. The disrupted parasites were examined using an inverted microscope.Continuous and stepwise 5%-60% sucrose gradient solutions were prepared in order to test the ability of both types of gradients to separate the sonicated parasite fractions. The stepwise gradients consisted of fractions of 5% ,10%, 20%,40% and 60%, on one hand, and 5% , 10%, 15%, 20% and 60% on the other hand.Portions of 0.5 ml of the sonicated parasite suspension were layered on top of the gradient solutions and centrifuged at 100,000 x g for 4 hours.

Immunization of mice with UV- irradiated and intact trypomastigotes: Parasites frozen with 10% dimethylsulfoxide(DMSO) were recovered by thawing at 37° C, pelleted by centrifugation at 3,000 rpm for 30 min, and washed twice with phosphate buffered saline (PBS).They were resuspended in 0.6 ml PBS and the concentration of parasites in the suspension was determined by counting in a Neubauer chamber. Three mice were inoculated intraperitoneally with 0.1 ml of suspension containing ca.10^7 resucitated intact parasites and three mice were likewise inoculated with the same number of parasites after a 6- min UV irradiation.One week after, the immunized mice received a booster following the same procedure as in the first immunization.

Immunization of mice with fractions of trypomastigote components: Three milliliters of solutions withdrawn from the sucrose gradients from the zones corresponding to the initial positions of the 5%, 10%, 20%, and 40% fractions, respectively, and 0.5 ml of the solution corresponding to the top layer, were each inoculated intraperitoneally into different mice. After one week, the immunization was boostered following the same procedure.

Parasite labeling: Trypomastigotes were labeled by incubating 12

with 250 uCi of [3H]- uridine per 1 x 10^7 parasites in 5 ml Dulbecco Modified Eagle Mediun (DMEM).The parasites were harvested by centrifugation at 3,000 rpm for 10 min, and washed three times with DMEM. The pellet was resuspended in 5 ml RPMI 1640 medium, containing 10% fetal calf serum (FCS), 5 x 10^{-5} M 2-mercaptoethanol (2-ME), non-essential amino acids (NEAA), and sodium pyruvate. An aliquot (0.1 ml) of the suspension was taken and counted in the scintillation cocktail. The number of cells were also counted in a Neubauer chamber and the parasite concentration was adjusted to 10^5 parasites per milliliter.

Isolation of effector cells: Immunized and non-immunized mice were sacrificed and peritoneal cells were removed by washing the peritoneal cavity with 10 ml Hank's solution.

Spleens were removed from the same animals and cells were extruded through a 100-mesh screen of a stainless steel tissue homogenizer. The extruded cells were suspended in Hank's solution. Spleen cells were washed three times with Hank's solution and finally resuspended in 2 ml RPMI 1640 medium, containing 10% FCS, 5 x 10^{-5} M 2-ME, NEAA, and sodium pyruvate.Twenty milliliters of tris-HCL buffer containing ammonium chloride was added in order to destroy unnucleated cells. After 60 sec, the volume was brought up to 30 ml with RPMI medium with supplements, and centrifuged at 2,000 rpm for 7 min, at 4°C. The supernatant was decanted and the pellet resuspended in 10 ml of RPMI medium with suplements. Peritoneal cell suspensions were treated similarly, except that no tris-HCL/NH_4Cl solution was added to them. The pellet was also resuspended in 10 ml RPMI medium with supplements. Both spleen and peritoneal cells were counted in a Neubauer chamber, using Turk's solution, and subsequently the cell concentrations were adjusted to 10^7 and 10^6,respectively

Cytotoxic T cell assay: One milliliter of spleen cell suspensions containing 10^7 and 10^6 cells, respectively, were mixed with 1 ml of parasite suspension each, containing 0.5 x 10^5 parasites. On the other hand 1 ml of peritoneal cell suspencion, containing 10^6 cells, was mixed with 1 ml of parasite suspension (0.5 x 10^5) parasites/ml).A tube containing only parasites was also prepared in the same manner. The tubes were incubated overnight 37°C. They were centrifuged at 3.500 rpm for 15 min, 15 ml of the supernatants were placed in scintillation vials with Beckman Ready SafeTM cocktail and counted. All assays were done in duplicate.

RESULTS

Assessment of UV- irradiation time required for blocking parasite replication: Since it is well known that intact organisms are the best inducers of cell-mediated immune response, whole parasites were necessary for mouse immunization. In addition, the greater the amount of antigen used for immunization the better cellular immune response is obtained. However, because the T. cruzi strain is known to be extremely virulent, the inoculation of 10^7 trypomastigotes would lead to such a high parasitemia in the mice,which would kill them even before an appropriate level of cellular immune response is reached. Blockage of parasite replication was thus needed before inoculation. Therefore, it was necessary to determine a suitable UV dose which would wipe out the ability of the parasites to replicate without affecting seriously their viability. For this purpose, epimastigotes were used, as they are able to replicate in vitro. Epimastigotes suspended in LIT medium were irradiated with UV light for 0.5, 1.0, 2.0, 6.0, 16.0, and 25 min. This test was done by thymidine and uridine uptake. Ten microliters of parasite suspensions were placed in microwells together with 2 uCi of both [^{3}H]-uridine and [^{3}H]-thymidine and incubated in a total volume of 200 ul for 72 hours. Non irradiated intact parasites were included in the assay as well as parasites killed by submerging the tube in boiling waterfor 10 min. Both before and after incubation, the microwells werechecked for morphological appearance of the parasites with the aid of an inverted microscope.

The non-irradiated parasites looked normal with constant movement. However, several , of the irradiated ones looked round-shaped, arranged clusters in and non-motile. These features increased with increasing irradiation time.

The heat-treated samples, on the other hand, looked completely shrunk. Once incubation time was over, the parasites were harvested from the microwell plate, onto glasswool filter paper and washed for 30 sec with PBS. The blots were cut out and placed in scintillation vials containing scintillation cocktail and counted in a Beckman scintillation counter. The average values, in CPM,are presented in table 1, and the graph in Fig. 1 shows that both thymidine and uridine uptakes fall quickly and nearly in paralell during the 6-min irradiation. Further increase in irradiation time appears to have little affect on the nucleosides uptake. In order to dismiss the counts which might be due to an unspecific adsorption of radiolabel onto the surface of the parasites, the heat-killed parasites incubated with radiolabels were also counted. The counts obtained were of a value around those corresponding to the background counts. Therefore,radiolabel surface adsorption has no affect on the results for thymidine and uridine uptake by these parasites.

Parasite disruption and fractionation: The source of trypomastigotes was a frozen stock, which was thawed and washed with PBS three times. Approximately 3 x 10^8 parasites were resuspended in 3 ml of PBS and sonicated for 3 min, with 1-min. intervals, holding the container in an iced water bath to avoid overheating. Six tubes with 9 ml of a 5, 10, 20, 40, and 60% sucrose gradient were prepared and 0.5 ml of sonificated parasites were layered on the top of the density gradient solutions.

Centrifugation was performed at 29,000 rpm for 4 hours, at 4°C, in a SW41Ti rotor. When fractionation was performed prior to the first immunization, no bands were observed, probably because of the low amount of parasites used for sonication. However, the second fractionation, used as a booster, resulted in a well visible band in the zone corresponding to a region between the initial positions of the 40% and the 60% sucrose solutions of the gradient, and two more bands in the regions between the initial 40% and 20% and 20% and 10%, respectively. Fractionation performed in a continous sucrose gradient only gave two diffuse bands.

Immunization: Mice were injected intraperitoneally with the top layer of the sucrese gradient (o.5 ml), and with 3 ml of the fractions corresponding to the initial positions of the 5%, 10%, 20%, and 40% solutions of the gradient in order to ensure that most (if not all) of parasite antigens are used for immunization. Mice inoculated with the 40% and 20% sucrose solutions were found dead the day following inoculation . On the other hand, mice immunized with whole irradiated and non-irradiated parasites were followed up in terms of their parasitemia level.Table 2 and Fig. 2 show the parasitemia values at different times following inoculation.Mice which received UV- irradiated parasites were negative for parasitemia, whereas those injected with nontreated parasites showed a normal parasitemia curve. The mouse which received the higher dose (1×10^7 parasites) died at the 11th following infection.

Cytotoxicity assay: The in vitro cytotoxicity assay consisted incubating [^{3}]-uridine-labeled trypomastigotes (0.5×10^5 parasites) with different numbers of spleen and peritoneal cells isolated from immunized mice for 12 hours. If cytotoxic cells are present in the cultures, trypomastigotes are expected to be destroyed, releasing their labeled RNA content into the medium, which could be detected by counting the radioactivity of the supernatant after centrifugation. The results are shown in Table 3. No significant difference was observed between the counts of the different samples including those containing only labeled parasites, and those incubated with spleen and peritoneal cells taken from normal mice, therefore, no conclusion can be drawn from these results.

DISCUSSION

It is well known that acute phase of T.cruzi infection is characterized by a high parasitemia, which persists for a long period of time. This suggests the poor effectiveness of the immune system of the infected organism to cope with this aggression. Therefore, if any cytotoxic activity is involved in fighting the disease in vivo, it is apparently not sufficient in eliminating the parasites under normal conditions.

However, the possibility still remains that the cytotoxic cells are not activated enough to be able to clear the organism out of the parasite. For this reason, it is worthowhile to perform in vitro assays to test the cytotoxic effect of T lymphocytes isolated from immunized animals on the bloodstrean form of T.cruzi. This would give us an idea about the presence of such an affect in the defense mechanism against this parasite.

In the case that cellular immunity plays a key role in the immune response against T. cruzi, and should the key effector cells and the key parasite antigen be identified, a new hope for the development of a vaccine would exist.

No meaningful results have arisen from our first experiments. However, it is very likely that a poor washing of the parasite cells from the excess radiolabel was the major point of error, rather than a spontaneous lysis of all the parasites during the overnight incubation. A second experiment will be performed where care will be taken to make sure the parasite suspension is free from surface- adhered radiolabel before incubation with the effector cells. For this purpose supernatants will be checked for radioactivity after each round of washing. Should the spleen and peritoneal cells from immunized mice fail to provoke parasite lysis, the same cells will be incubated in vitro with UV- irradiated trypomastigotes with the purpose of activating the putative effector cells. This plan is based on the hypothesis that the cytotoxic cells have not been activated enough in vivo, and therefore need a further activation in vitro.

A clonal selection and specifie cell proliferation are expected to result from this incubation, triggered by certain T. cruzi antigens.If successful, once the importan antigen is identified, plans should be worked out for a mass production of this antigen.

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TABLE I

[3H]-TdR AND [3H]-UR UPTAKE BY UV- IRRADIATED TRYPOMASTIGOTE FORMS OF TRYPANOSOMA CRUZI

Irrad. time (min)	[3H]-TdR (cpm)	[3H]-UR Uptake (cpm)	BKg (cpm)
0,0	3,920	10,169	32
0,5	4,495	5,777	35
1,0	4,092	5,095	24
2,0	2,837	4,279	22
6,0	1,598	2,531	23
16,0	1,895	2,765	23
25,0	1,423	1,976	29
Heat-Killed	207	100	24

TABLE II

Course of parasitemia for UV- irradiated and non- irradiated trypomastigote- inoculated mice

Mouse	UV- Irrad.	Parasites inoculated	Parasitemia(Paras./ml) Day 5	Day 7	Day 10
1	no	1.0x107	3.1x106	6.6x105	1.4x106
2	no	1.0x107	4.0x104	8.9x104	4.1x104
3	no	0.5x107	8.1x103	1.6x104	1.9x105
4	yes	1.0x107	------	----	----
5	yes	1.0x107	-----	----	----

Note. Mice were inoculated intraperitoneally with parasites suspended in PBS. Parasitemia level was followed by counting the parasites in blood obtained by pricking the mouse's tails. Counting was performed by the Method of Arias and Ferro (6).

TABLE III

In vitro cytotoxic assay for trypomastigote Killing by spleen and peritoneal cells from T. cruzi-immunized mice

Effector cell	Counts(cpm)
a) Spleen cells	
Normal	
1 x 107	107,573
1 x 106	107,060
UV-irradiated	
1 x 107	107,616
1 x 106	110,493
b) Peritoneal cells	
Normal	
1 x 106	111,903
UV_ irradiated	
1 x 106	111,360
c) Parasites alone	
1 x 105	114,426

Note: The cytotoxicity assay was performed according to the technique described by Numata et al.

ISOLATION OF TRYPANOSOMA CRUZI STRAINS FROM PATIENTS

Antonieta de Arias.

INTRODUCTION

Three main groups ofT.cruzi strains have been described so far in Latin America. These these groups, representatives of which infect man, have:

- Different geographic distribution.
- They are assosiated with different transmission cycles, and
- They seem to be related to different forms of chronic Chagas' disease.The chronic digestive pathology is common in Brazil, and in contrast, the chronic cardiac pathology caused by T.cruzi infection is the predominant manifestation in Argentina.

This has led to the isolation and characterization of different T.cruzi strains, which have helped establish a relationship between the geographic distribution of the strain and the predominant pathology.

The collection and analysis of isoenzymes, the use of DNA probes, and data obtained by immunoblotting from parasites in different geographic areas could useful in assessing whether different genotypes are associated with different disease forms in man. Thus establishing some relationship between the genetic heterogeneity of T.cruzi and the prognosis of infected patients. Comparative studies on strains from different geographic areas under standardized conditions would be desirable, and therefore the mounting of a research program for mapping T.cruzi zymodemes as well as other strain markers would be necessary for detecting recombinations and a relationship between the biochemical, biologic,and clinical characteristics of infection.

In our country, the cardiac and digestive forms of the chronic disease coexist,and it is necessary to study the isolation and characterization of T.cruzi strains in order to establish the morphologic and biochemical characteristics of their infection.

MATERIALS AND METHODS

The technique used for isolation of T.cruzi is hemoculture. LIT is used as a culture medium. 4-6 ml of blood are taken and distributed in 2 tubes containing 5-7 ml of LIT medium. The tubes are vigorously shaken and then incubatd at 28° two weeks later the cultures ave observed using an inverted microscope, and thereafter observations are made weekly. Once the hemoculture is positive, it is concentrated and cryopreserved at -199° for later use.

RESULTS:

Hemocultures were performed on blood from 15 patients with positive serology for CHAGAS' disease, two of which yielded parasites. In both cases, the patients were in the undetermined stage of the disease.

15 patients with hemoculture-------- 2 (+) hemocultures = 13.3 %

Patient	Date of hemoculturing	Date of parasite detection	Days elapsed
1 IF: 1/320	13/05/88	22/07/88	83
2 ELISA: 1/320	13/03/89	03/07/89	122

Blood was also drawn from 12 monkeys experimentally infected with T.cruzi, with an approximate infection time of 2.5 years, and hemocultures were performed. Of these hemocultures, 6 resulted positive.

12 monkeys with hemoculture---------6 (+) hemocultures = 50

Monkey No.	Date of hemoculturing	Date of parasite detection	Days elapsed
74 IF: 1/32	14/10/88	15/11/88	32
68 IF: 1/8	14/10/88	02/12/88	49
82 IF	11/10/88	15/12/88	65
84 IF: 1/32	14/10/88	15/12/88	62
41 IF: 1/64	11/10/88	15/02/89	127
14 IF: 1/16	14/10/88	27/03/89	164

PLAN FOR THE FUTURE

IN 1989 the following procedures will be performed:

- Hemoculture in patients with positive serology for CHAGAS' disease and lesions compatible with T.cruzi infection.
- Cryopreservation of the isolated strains.
  Starting from 1990 onwards the work Schedule is presented in appendices 1 and 2.

APPENDIX 1

CHAGAS PROJECT — PROCEDURE FOR ISOLATION OF T.CRUZI STRAINS

PASSAGE	1. PROCEDURE
a.Vector to LIT	a.1.Minced intestines from positive vectors are cultured in LIT according to xenoculture technique
b.Culture to mouse through inocul	b.1. Old cultures are inoculated into mice.
c.Mouse to	c.1 Xenodiagnosis is applied on infected mouse using 10 3rd.- stage triatome.
d.Mouse to LIT	d.1 Hemoculture with blood from infected mouse, estracted by cardiac puncture, the cultured in LIT.
e.Blood+culture to vector	e.1 Artificial feeding through a membrane.

2. ADVANTAGES	3. DISADVANTAGES
a.2.1. High sensitivity a.2.2. Fast parasite growth	a.3.1. Expensive a.3.2. Easy
b.2.1. Trypomastigote b.2.2. Establishment of parasitemia curves on middle range	b.3.1. Low parasitemia in mice b.3.2. Very laborious work
c.2.1. Recovery of low-parasitemia strains	c.3.1. Immobilization may cause death in mice
c.2.2. Increase in parasite Nº in "broad" strains	c.3.2. Maintenence of triatome
d.2.1. High sensitivity d.2.2. Less risk of contamination	d.3.1. long follow-up for results (120 days)
e.2.1. High sensitivity e.2.2. Animals are not needed for triatome feeding	e.3.1. There is species-selectivity e.3.2. Maintenance of triatome

OBS: One disadvantage common to all procedure is the selection of the most appropriate strains, depending on the host utilized.

APPENDIX 2

STANDARDIZATION OF STRAIN ISOLATION FROM PATIENTS

Patient

Hemoculture (2 tubes)
+

Cryopreservation   Passages   LIT-LIT _ maintenence

4-6 times v-v-

cloning and amplification

Aged culture

Inoculum to mouse---------curve

(broad)   (slender)

MOUSE- MOUSE Trypomastigote extraction	MOUSE - VECTOR passages for strain recovery	MOUSE-LIT cyclic passages

ASSESSMENT OF LEISHMANIASIS VECTORS IN PARAGUAY

Inchausti, A.

INTRODUCTION:

Leishmaniasis is a disease which gives rise to important socioeconomic and public health problems in rural areas. It presents itself as endemic in certain areas of neotropical America. Although this represents a large geographic area we can, only observe the disease in zones favorable to the vectors. In our country, the endemic area consists of a large part of the Región Oriental (Eastern Region), including the departaments of San Pedro, Alto Parana, Canindeyú, Amambay, Caaguazú, Caazapá and Guairá. Little is known about the species of vectors prevalent in each endemic site. The first work performed on the systematics of Phlebotomus Sp. Was carried out in 1948 by Barreto, and 3 species were described: Lutzomia migonei, L.intermedia, and L.longipalpis. In 1955 Hertihg and Wharton described 2 additional species: L.Whitmani and L. monticulosa. In 1984, a seroepidemiologic study was carried out in Tavapy 2, Alto Parana, by A. Rojas de Arias, who collected 3 already mentioned species. Knowledge of the demographis, dynamics, and taxonomy of the Phlebotomus vector is essential for understanding epidemiologic cycles and preparing strategies to fight the disease.

OBJECTIVES:

General: Determine the vectors of leishmaniasis in Paraguay.

Specific:
- collect phlebotomus specimens in field from 3 endemic areas (Alto Parana, Caaguazu and Caazapa).
- Prepare the insects for indentification in the laboratory.
- Construct a map showing distribution of the collected species.

MATERIALS AND METHODS

a) Insect collection:

1. Aspirator (Castro collector): Consists of a 30 cm-long, 1 cm-wide glass tube. In one of its ends, a rubber tube of variable length (40-60 cm) is inserted and in the boundary between both tubes, a gauze sheet is placed, wich acts as a filter to avoud swallowing of the insects on aspiration.

Technique for collection: the glass end of the tube thus constructed is applied on the resting insect, and the collector aspirates through the end. The insect so captured is then sent to a special flask with a blow.

2. Collecting flasks: 400-ml plastic flasks, with an opening for introducing the insects and another one for respiration, were used. Inside the flasks, wet sand or leaves can be placed.

3.Traps (shannon traps): consist of a central compartment and two lateral ones. The central one has a rectangular shape, and is open in its inferior facet. It is suspended
in the air by means of 4 ropes tied on the corners. A light source is placed inside the trap in order to attract the insects.
4. Baits (human bait): The skin of a volunteer is uncovered in a way such that the hematophagous insects (Phlebotomus) can bite the subject. The subject should stay immobile in a dark place and with the help of a flashlight. Should light up the site of the bite. Then the collector can be used for capturing the vector.

B) Specimen conservation and transport:
The collected specimens are sacrificed using chloroform vapors or ethyl acetate, and then kept in 70 % alcohol.
C) Clearing and dehydration prodcedures
The specimens kept in alcohol are treated as follows:
Phenol.......................... 24 h
Potas 10 %...................... 12 h

Washed twice in distilled water
Acetic acid 10 %................. 10 min
Acid Fucsne...................... 10 min
Acetic acid 10 %................. 10 min
Alcohol 70 %..................... 10 min
Alcohol 95 %..................... 10 min
Adsolute alcohol................. 10 min
Vegetal creosote
Mounting on balsam of Canada.

D) Classification:
Members of Phlebotomus Sp. present a group of structures, the identification of which is necessary in order to correctly classify the specimen. For example:
- Head: Maxillary palps; antennae; wings
- Characteristics of the male
- Characteristics of the female, sperm reservoir.
Observing all these characteristics and others more, the findings are passed through the identification table in order to determine the species.

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PLANS FOR THE FUTURE

1. Epidemiologic census of Leishmaniasis cases in endemic areas in Paraguay.
   1.1. cutaneous tests
   1.2. Isolation of Leishmaniasis strains from patients

2. Determination of Leishmaniasis vectors
   2.1. Collection of _Phlebotomus_
   2.2. Dissection of flies in order to find natural infections

3. Search for reservoirs of leishmania
   3.1. Examination of wild mammals
4. Identification of Leishmania strains in Paraguay.
5. Experimental infections by Leishmania in _phlebotomus_

TUMOR NECROSIS FACTOR (TNF) PRODUCTION IN CHAGAS DISEASE

Maria Elisa Vera Antola; Watanabe, T.

Tumor Necrosis Factor (TNF) is one type of cytokine which regulates the immune response.
It is known that the immune system in the host is affected by **Trypanosoma cruzi** infection.
We want to investigate whether the production of TNF is affected or not by the infection.

Materials and methods:

1-Mice:

Mice were used at 6 to 8 weeks of age.

Macrophages-Lymphocytes -Polymorphonuclear leukocytes:

Cells were prepared in the following way. Briefly, ca. 4 ml of 3 % PP (Proteose- Peptone) was injected into the peritoneal cavity of mice, and the peritoneal exudate cell (PEC) was harvested 3 days later. The cells were centrifuged at 1200 r.p.m. for 5 min. at 4°C. The pellet was washed twice with RPMI 1640 medium (GIBCO Laboratories) suplemented with Penicillin, Streptomycin,Hepes(10 mMol) and NaHCO3 (2g/l). The PEC were resuspended in RPMI 1640 containing 5% of heat-inactivated Foetal Bovine Serum (Whittaker M.a. Bioproducts). The cells were cultured for 4 hs. In 5% CO_2 and 95% air incubator, with LPS (10 ug/ml) (SIGMA CHEMICALS).

Cytolytic assay:

Cytolysis of L 929 cells (Mouse fibroblast cells) was measured by the method of Ruff and Glifford. Briefly, 50 ul of culture supernatant of PEC (Macorphages, lymphocytes and polymorphonuclears) and 100 ul of L 929 cells ($5 * 10^5$/ml) were mixed in the wells (7 mm diameter) of flat-bottomed microplates. 50 ul Actinomycin D (20 ug/ml) was added to this mixture and then incubated for 18 hs at 37°C incubator.
Then , the medium was removed and remaining cells were stained for 15 min with Crystal violet. The staining solution was removed after that time and the wells were washed two times with running water. The cytolytic affect was studied after the plate was dried.
Observation: The last step of this method, which consist of the addition of 0.1 ml of Sodium Dodecyl Sulfate (0.5 %) and the measure, ment of Absorbance at 590 nm of the supernatnt was not performed, because of the lack of the adecuated filter.

Results:

In mice:

1- The ratio of Macrophages and Lymphocytes in the PEC decreased after infection with T.cruzi.

Normal mice	Macrophages	58 %
1 Week after infection		2 %
2 Weeks after infection		30 %
3 Weeks after infection		17 %

2- TNF activity in culture sepernatants was enhanced with the infection of T.cruzi, when PEC were stimulated with LPS.
3- In this experiment, there was no difference between the activity of culture supernatant from normal and one week after infection on L-929, but in another experiment we found slight difference between the effect of normal and one week after infection supernatants on L-929 cells.

Conclusion
In infected mice the number of macrophages was smaller than in normal one, however the TNF activity in the culture supernatant was higher.

Future:
Retry. Check the results.
1. The ratio between M/L.
2. If there are differences between TNF activity with supernatants from infected and normal mice.
3. Check whether TNF is produced mainly by Macrophages or Lymphocytes.

MATERIALS AND METHODS:
2. In human:
Lymphocytes and Monocytes:
About 6 ml of peripheral blood was takes with heparine as anticoagulant from: a- One person with chronic Chagas' disease.
b- One normal patient as negative control.

The blood was diluted two times with MEM and then it was layered on Ficoll - paque (PHARMACIA) and centrifiged at 1500 r.p.m. for 30 min at room temperature.
The layer containing the cells was washed three times with RPMI supplemented with 1 % FBS. The cell count was performed and the cells were brought up to the desired volume with medium.
1 ml of cell suspension ($5 * 10^6$cells) was incubated in 6 well plate for about 4 hs with or without LPS (10 ug/ml).
The supernatant was removed and centrifuged for 30 min at 3500 r.p.m. at 4°c.

CYTOLYTIC ASSAY:
Cytolysis of L 929 cells (Mouse fibroblast celss) was measured by the method of Ruff and Glifford. Briefly, 50 ul of culture supernatant from human monocytes and lymphocytes and 100 ul of L 929 cells ($5 * 10^5$ /ml) were mixed in the wells (7mm diameter) of flat-bottomed microplates. 50 ul Actinomycin D (20 ug/ml) was added to this mixture and then incubated for about 18 hs at 37°c. Then, the medium was removed and the remaining cells were stained for 15 min. With Crystal violet. The staining solution was removed after that time ane the wells were washed two times with running water.The cytolytic affect was studied after the plate was dried.
Results: We tried this experiment just one time and found that TNF activity was higher in a normal person than in an inffected one.

Future: We must repeat the same experiment with more samples.

BLASTOGENESIS IN CHAGAS' DESEASE

Dras. M.Agueda Cabello S., N.Ohta and C.M. de Cabral.

INTRODUCTION:The Chagas' disease involves many changes in the immune cellulor response of the host, the parasites may immunosupres or stimulate the response, for that reason it is important to study the in vitro action.
The aim of this study was to find out the optimal antigen dose that can stimulate immunoresponse and also to determine if this response is specific or not.

MATERIALS AND METHODS
Antigen of epimastigotes ag. were obtained from soluble extracts.
PHA was used as an ag. control at a final concentration of 4%.
Patients were in the non-acute phase of Chagas's disease.
As normal control subjets (Chagas disease free).
Cell suspensions: Mononuclear cells were obtained from heparinized venous blood and then were centrifugated with Ficoll(SIGMA).The buffy coat was washed with RPMI 1640 mediun twice and they was brought up to the desired concentration with RPMI 1640 supplemented with 10% FCS.(1.106 /ml).
Blastogenesis assay: the cells were incubated with PHA and different concentrations of epimastigote ag. in microwells during 6-7 days at 37°C with CO_2 and humidity.
The blastogenesis was determined by microscope.
The control of PHA stimulation was given the score of 10.

RESULTS:

Patient	19	control	23	cont.	25	cont.	32	cont.
medium	1,0	0,3	0,3		0,7	0,0	0,3	0,0
PHA-M 1%	10	10	10		10	10	10	8
EPI 1,5625ug/ml	4,3	0,3	1,0		1,0	0,0	3,7	0,0
3,125	4,0	0,0	1,7		2,0	0,0	4,0	0,0
6,25	4,3	0,0	1,7		1,7	0,0	3,3	0,0
12,5	3,3	0,0	1,3		1,3	0,0	2,3	0,0
25,0	1,0	0,0	0,3		0,0	0,0	1,7	0,0
50,0	0	0	0					

CONCLUSIONS:
1- antigen doses between 5-10 ug/ml gave optimal conditions.
2- 6-7 days incubation was optimal.
3- diversity in blastogenesis to epimastigote antigen may be divided in to two groups; high responder and low responder.
4- specific blastogenesis response was only seen in patients.

Future projects: to try again blastogenesis but determine the score by a beta counter, which is much more accurate than observation by microscope.
Also the next assay will use diferent fractions of antigens from the parasites to determine which one would stimulate a high respon

PLAQUE FORMING CELLS ASSAY

INTRODUCTION:

This assay is used for testing the humoral immune response when using sheep red blood cell (SRBC) as antigen to stimulate the production of antibody by B cells.
We improved the method by first using SRBC with Alsever solution that contained sodium azide to avoid contamination, but our results were not satisfactory and for that reason we tried to work without sodium azide.
The aim of this study is to investigate the changes that have been found by other authors during the study of humoral immune response in Chagas' disease.
The IMMUNE RESPONSE changes according with the phase of the disease, immunosuppression or stimulation may be seen.

MATERIALS AND METHODS
Antigen preparation and administration : erythrocytes obtained from a single sheep were first separated from Alsever solution by centrifugation at 2.400 rpm for 10' at 4° C and then washed three times with in 0.9 % NaCl sol. and adjusted to different concentrations.
BALB/c mice were innoculated with 0,2 ml of erythrocyte suspensions and were sacrificed on the fourth day for plaque assay.
Preparation of the spleen cell suspension: individual cell suspensions were prepared from each spleen by homoginization in RPMI 1640 supplemented with 1% FCS .They were washed once and dissociated cells were brought up to the desired volume with RPMI 1640 medium supplemented with 2% FCS. The determination of cell viability was done by Trypan blue sol. Exclusion and cell number was determined by counting in a hemocytometer.
A Hemolytic plaque assay for detecting cells producing antibody to SRBC was performed in a Cunningham chamber. Direct plaque forming cells were detected four days after antigen administration by incubating the spleen cells with the target cells with and without complement (guinea pig serum 1:10), during 1 H at 37°c. The counting was done by microscope. The number of PFC were calculated per spleen and per million spleen cells

RESULTS: See table I;II.
Our future plans are to try this assay with mice infected with **T.cruzi** so we will have many alternatives:
1- Inmunize mice with soluble extracts of epimastigotes and then infect with **T.cruzi.** and perform the PFC assay.

2-Inmunize mice with different proteins of **T.cruzi.** that can be obtained from sucrose gradients or Ag purified with monoclonal antibodies, idem.

3- To look at polyclonal activation.

HISTOCOMPATIBILITY ANTIGENS IN CHRONIC CHAGAS PATIENTS

Ascurra, M.; Pistilli, N.; Ohta, N.

Chagas' disease is an endemic disease in Latin America. Consevative data from the World Health Organization indicates that 12 million people are infected with Trypanosoma cruzi and that 60 million are under risk of infection. Among Chagas' patients, some develop cardiac and/or other characteristic pathologies while the rest of the patients do not. The mechanisms of such clinico-pathological diversity are still unknown, and could be related to the genetic makeup of the individual.

The major histocompatibility complex, the HLA system, is a region of the short arm of chromosome 6, which controls the immune response. Different haplotypes are associated with increased susceptibility to various deseases (1). Given its complex polymorphism, its study is a powerful genetic tool for establishing the association of the system to various diseases, and in many cases this association has already been found, e. g., in leprosy and tuberculosis (2, 3).

In studies done in mice, the differences in susceptibility to infection by T.cruzi were not found to be related to the major histocompatibility complex (4, 5).

In the present study, the existence of immunogenetic factors in the HLA system related to the presence of clinico- pathologic findings compatible with Chagas' disease was investigated, as well as the presence of useful evidence for the understanding of immunopathogenic processes in Chagas' disease.

MATERIALS AND METHODS

22 Patients of both sexes with positive serology for Chagas' disease, selected among patients of the Instituto de Investigaciones en Ciencias de la Salud, were studied. The patients were divided into two groups: the first, made up of 9 patients with clinico-epidemiological evidence compatible with Chagas' disease (abnormal ECG), and the second, composed of 13 patients with seroepidemiological evidence but without any clinical manifestation (normal ECG) compatible with the disease. A record was created for each patient under study, which included personal data, and items relating to the epidemiology of Chagas' disease, with autoimmune disease and/or the HLA system.

Part of this study was performed with the aim of standardizing the technique for detection of major histocompatibility complex antigens. The method used for detection of class I and II antigens was complement-dependent microlymphotoxicity (Terasaki) (see appendix 1).

RESULTS

In the study of the infected populations distributed in both groups, data suggests that phenotype HLA-A2 could be a protective factor for the lesions found in the disease.

However, in order to draw any inference on the putative association of some particular types of HLA antigens and the development of lesions, a larger number of serologically positive patients, both showing lesions and not, needs to be evaluated, as well as a control population with negative serology and parasitemia.

Similarly, concerning the study of the family groups done on 5 families with serologically positive members, some of them showing abnormal ECGs, the size of the sample is not adequate for drawing valid conclusions about any association of the haplotypes and the development of lesions.

ANEXO 1

HLA typing

1. Instruments
   (1) Centrifuge
   (2) Micro centrifuge
   (3) Incubator 37°c
       25°c
   (4) Inverted microscope
   (5) Water bath
   (6) Jet pipet (six- needle dispenser)
   (7) Micro syringe (50 ul, 250 ul)
   (8) Light box
   (9) Improved neubauer haemocyte meter
   (10) Plastic (or silicon-coated glass) tubes
   (11) Microtubes for micro centrifuge

2. Reagents
   (1) Anticoagulant (heparin)
   (2) Ficoll gradient solution (S.G.=1.077)
   (3) Saline
   (4) Hanks solution
   (5) 5% Eosin y solution
   (6) Neutral formaline
   (7) F.C.S.
   (8) Antibiotics (penicillin/streptomycis)
   (9) HLA typing trays for class I
       for class II CDR & DR)
   (10) Nylon coal column
   (11) Thrombin

3. Protocol
   I. Gel separation
   (1) Centrifuge whole blood, 400 g 10 min.
   (2) Take buffy coat (C.A. 1.5 ml/tube), mixed with same volume of saline.
   (3) Layer on Ficoll gradient solution (S.G.= 1,077), centrifuge 400 G , 30 min.
   (4) Take interface, wash with saline x2
   (5) Cell count
   (6) Thrombin treatment
       (i) Transfer cells into microtube (1 microtube for 10 ml blood).
       (ii) Wash with saline, 1000 G, 1 min.
       (iii) Resuspend in saline, then add thrombin 1 drop Hank' sol. Gently shake for 2-3 min.
       (IV) Flash 3 sec. (1000 G)
       (V) Take the supernatant remained pellet
       (VI) Centrifuge 2000 G 1 min * 2
       (VIII) Add 20% FCS RPMZ (37°C). and centrifuge 2000 G 1 min
       (IX) Suspend in 20 * FCS RPMZ
       Cell count

II. T.B. Separation

(1) Cut the top of the column
(2) Wash nylon wool with warm RPMZ-1640 (10%FCS)
(3) Cell apply
Cell suspencion in 20% FCS RPMZ warm medium
(4) Incubate 37°C 30 min.
(5) Column eluate with 7-10 ml. 20% FCS warm medium passed cells = T cells
(6) Wash the column C 7-10 ml 20% FCS warm medium
(7) Milking of adherent cells with 20% FCS warm RPMI medium => 7-10 ml B cell suspencion
(8) Centrifuge T.B. suspension
(9) Resuspend T cells in 20% RPMZ => cell count

(10) B cell suspension => micro tube => flushing => resuspended => cell count
(11) Adjust cell concentration
$2\text{-}3 \times 10^6$cells/ml.

III. Typing

(A) HLA - A.B.C. typing
(1) Dispense T cells into wells of typing trays at 2 3000 cells/1 ul.
(2) Incubate at room temperature (23-25°) 30, min.
(3) Add 5 ul/Well of c' (c' for class I typing)
(4) Incubate at room temperature, 30 min.
(5) Add 2 ul/well of 5% Eosis y sol.
(6) Incubate 5 min.
(7) Add 8 ul/well of neutral Formaline
(8) Place plates in moisture chamber overnight
(9) Cover a glass plate
(10) Observation with inverted microscope

(B) HLA-DR, DQ typing
almost same with protocol (A)
(1) Dispense B cells into
(2) Incubate 60 min. at 37°C
(3) Add (C' for class II typing)
(4) Incubate 120 min.
(5) |
(10) |Same with (A)

F U T U R E   R E S E A R C H

Continue HLA typing of (1) Normal Paraguayan population.
(2) Random Patients With Chagas' disease
(3) Family members of Chagas' disease patients.

I. Population Study.

Comparisons of HLA antigen frequencies between different patient groups or between patients and normal controls.

Test normal controls at least N = 40 (5 were already typed).
random patients at least N = 50 (22 were already typed).

Calculate value, r value, and relative risk.

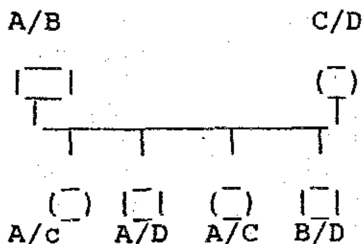
$$X2 = \frac{(ad-cb)^2 \times N}{ABCD} \qquad r = \frac{X2}{N} \qquad \text{relative risk} = \frac{a/b}{c/d}$$

	HLA antigen (+)	HLA antigen (-)	
Disease (+)	a	b	A
Disease (-)	c	d	B
	C	D	N (=A+B)

II. Family study of particular symptoms in Chagas' disease.
Test 10-15 informative families.
Informative family: at least 2 affected patients (siblings) genotypes of HLA can be determined.
Affected sib-pair method:
Analyze how many HLA haplotypes are shared by sib pairs Whose symptoms are same.

	Two haplotype share		one haplotype shape		no sharing
Expected (if there is no linkage between HLA and suscepti-bility)	1	:	2	:	1
Observed	A	:	B	:	C

Compare the ratio 1:2:1 and A:D:C, and calculate value.
Obtain the p value (degree of freedom = 2).
If there is statistically significant deviation from 1:2:1, we can conclude that an HLA-linked gene is involved in susceptibility to a given symptom (ex. cardiopathy) in human Chagas' disease.



( ) : same-symptom
Mode of inheritance: estimated by Morton's most likelihood method.

HLA ANTIGENS IN CHAGAS' DISEASE

I- List of individual patients.

Record Number	Age	Sex	Cardiopathy	HLA-A
2	38	F	+	3,29
8	46	F	+	24,32
10	43	F	+	31,W68
11	46	M	+	23,W19
16	74	M	+	2,31
18	55	F	+	11,31
30	32	F	+	1,30
28	82	M	+	3,24
34	45	F	+	2,31
5	23	M	-	2,-
6	21	F	-	31,W33
12	49	F	-	24,-
13	33	F	-	2,30
19	34	M	-	W68,29
20	49	M	-	2,24
24	21	F	-	25,26
25	21	F	-	2,31
27	11	F	-	2,24
29	84	M	-	2,31
32	16	F	-	1,29
33	20	F	-	69,31
37	28	M	-	2,24

CONTINUATION ----->

HLA-B	HLA-C	HLA-DR	HLA-DRW52/ DRW53	HLA-DQ
39,-	- , -	w15, -	- , -	w6, -
w52, 48	w7 , w8	w15, w6	w52, -	w6, w7
w62, 35	w3 , w4	w15, w12	w52, -	w6, w7
w75, 48	w3 , w6	ND	ND	ND
51, 16	w7 , -	4, w6	w52, w53	w7, -
8, w62	w3 , w7	3, -	w52, -	w2, w3
8, 72	w2 , -	3, w8	w52, -	w2, w4
7, 27	w1 , -	w15, -	w52, -	w6, -
35, 37	w4 , -	4, 5	w52, w53	w6, w7
51, 35	w4 , -	4, 5	w52, w53	w7, -
16, 35	w4 , w7	w15, w12	w52, -	w6, w7
w52, 41	w1 , -	ND	ND	ND
13, 27	w2 , -	w6, w10	w52, w10	w1, -
12, w61	w3 , -	4, 7	w53, -	w2, w3
18, -	w3 , -	w8, -	w52, -	w4, w6
w64, 18	w8 , -	7, -	w53, -	w2, -
w62, 35	w3 , w4	w12, w16	w52, -	w1, w7
18, 45	w4 , -	4, w15	w53, -	w3, w6
w62, 45	w3 , -	w15, -	-, -	w1, w3
8, -	- , -	3, -	w52, -	w2, -
35, w53	w4 , -	w15, -	-, -	w6, w7
61, -	w3 , -	ND	ND	ND

HLA ANTIGENS IN CHAGAS' DISEASE
II. Gene frequencies

HLA	Number of patients	GF	Cardiopathy(+)
HLA-A1	2/22 (9.18%)	0.046	1/9 (11.18)
A2	9/22 (40.9%)	0.231	2/9 (22.2%)
A3	2/22 (9.1%)	0.046	2/9 (22.2%)
A23	1/22 (4.5%)	0.023	1/9 (11.1%)
A24	6/22 (27.3%)	0.147	2/9 (22.2%)
A25	1/22 (4.5%)	0.023	0/9 (0 %)
A26	1/22 (4.5%)	0.023	0/9 (0 %)
A11	1/22 (4.5%)	0.023	1/9 (11.1%)
Aw19*	1/22 (4.5%)	0.023	1/9 (11.1%)
A29	3/22 (13.6%)	0.070	1/9 (11.1%)
A30	2/22 (9.1%)	0.046	1/9 (11.1%)
A31	8/22 (36.4%)	0.202	4/9 (44.4%)
A31	1/22 (4.5%)	0.023	1/9 (11.1%)
Aw33	1/22 (4.5%)	0.023	0/9 (0%)
Aw74	0/22 (0%)		
Aw34	0/22 (0%)		
Aw68	2/22 (9.1%)	0.046	1/9 (11.1%)
Aw69	1/22 (4.5%)	0.023	0/) (0%)
Aw36	0/22 (0%)		
Aw66	0/22 (0%)		
HLA-A Total		1.018	
HLA-Cw1	2/22 (9.1%)	0.046	1/9 (11.1%)
Cw2	2/22 (9.1%)	0.046	1/9 (11.1%)
Cw3	8/22 (36.4%)	0.202	3/9 (33.3%)

CONTINUATION

GF	Cardiopathy (-)	FG	r	rr
0.057	1/13 (7.7%)	0.039		
0.118	7/13 (53.8%)	0.320	0.32	0.25
0.118	0/13 (0%)			
0.057	0/13 (0%)			
0.118	4/13 (30.8%)	0.168		
	1/13 (7.7%)	0.039		
	1/13 (7.7%)	0.039		
0.057	0/13 (0%)			
0.057	0/13 (0%)			
0.057	2/13 (15.4%)	0.080		
0.057	1/13 (7.7%)	0.039		
0.254	4/13 (30.8%)	0.168		
0.057	0/13 (0%)			
	1/13 (7.7%)	0.039		
0.057	1/13 (7.7%)	0.039		
	1/13 (7.7%)	0.039		
1.064		1.009		
0.057	1/13 (7.7%)	0.039		
0.057	1/13 (7.7%)	0.039		
	5/13 (38.5%)	0.216	0.05	0.8

HLA	NUMBER OF PATIENTS	GF	Cardiopathy (+)
HLA-Cw4	6/22 (27.3%)	0.147	1/9 (11.1%)
Cw5	0/22 (0%)		
Cw6	0/22 (0%)		
Cw7	4/22 (18.2%)	0.096	3/9 (33.3%)
Cw8	2/22 (9.1%)	0.046	1/9 (11.1%)
HLA-C		0.583	
HLA-B51	2/22 (9.1%)	0.046	
Bw52	2/22 (9.1%)	0.046	
B7	1/22 (4.5%)	0.023	
B8	3/22 (13.6%)	0.070	
B12*	1/22 (4.5%)	0.023	
B44	0/22 (0%)		
B45	2/22 (9.1%)	0.046	0/9 (0%)
B13	1/22 (4.5%)	0.023	0/9 (0%)
Bw64	1/22 (4.5%)	0.023	0/9 (0%)
Bw65	0/22 (0%)		
Bw62	4/22 (18.2%)	0.096	2/9 (22.2%)
Bw63	0/22 (0%)		
Bw75	1/22 (4.5%)	0.023	1/9 (11.1%)
Bw77	0/22 (0%)		
B16*	2/22 (9.1%)	0.046	1/9 (11.1%)
B38	0/22 (0%)		
B39	1/22 (4.5%)	0.023	1/9 (11.1%)
Bw57	0/22 (0%)		
Bw58	0/22 (0%)		
B18	3/22 (13.6%)	0.070	0/9 (0%)
Bw49	0/22 (0%)		

CONTINUATION

GF	Cardiopathy(-)	GF	r	rr
0.057	5/13 (38.5%)	0.216		
0.183	1/13 (7.7%)	0.039	0,33	6.6
0.057	1/13 (7.7%)	0.039		
0.594		0.588		
0.057	1/13 (7.7%)	0.039		
0.057	1/13 (7.7%)	0.039		
0.057	0/13 (0%)			
0.118	1/13 (7.7%)	0.039		
	1/13 (7.7%)	0.039		
	2/13 (15.4%)	0.080		
	1/13 (7.7%)	0.039		
	1/13 (7.7%)	0.039		
	2/13 (15.4%)	0.080		
0.057	0/13 (0%)			
	1/13 (7.7%)	0.039		
0.057	0/13 (0%)			
	3/13 (23.1%)	0.123		

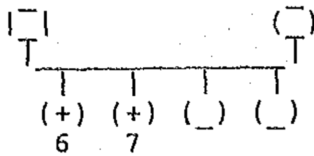
HLA	NUMBER OF PATIENTS		CARDIOPATHY(+)	GF	CARDIOPATHY (-)	GF
HLA-						
Bw50	0/22 (0%)					
Bw54	0/22 (0%)					
Bw55	0/22 (0%)					
Bw56	0/22 (0%)					
B27	2/22 (9.1%)	0.046	1/9 (11.1%)	0.057	1/13 (7.7%)	0.039
B35	6/22 (27.3%)	1.147	2/9 (22.2%)	0.118	4/13 (30.8%)	0.168
B37	1/22 (4.5%)	0.023	1/9 (11.1%)	0.057	0/13 (0%)	
Bw60	0/22 (0%)					
Bw61	2/22 (9.1%)	0.046	0/9 (0%)		2/13 (15.4%)	0.080
Bw41	1/22 (4.5%)	0.023	0/9 (0%)		1/13 (7.7%)	0.039
Bw47	0/22 (0%)					
Bw48	2/22 (9.1%)	0.046	2/9 (22.2%)	0.118	0/13 (0%)	
Bw53	1/22 (4.5%)	0.023	0/9 (0%)		1/13 (7.7%)	0.039
Bw59	0/22 (0%)					
Bw72	1/22 (4.5%)	0.023	1/9 (11.1%)	0.057	0/13 (0%)	
HLA-B	total	0.935		0.985		0.921

* including subtypes
r : value of r.
rr: relative risk.

HLA	Number of patients	GF	Cardiopathy(+)	GF	Cardiopathy(-)	GF
HLA-DR1	0/19(0%)					
DRw15	7/19(36.8%)	0.205	3/8(37.5%)	0.209	4/11(36.4%)	0.203
DRw16	1/19(5.3%)	0.027	0/8(0%)		1/11(9,1%)	0.047
DR3	3/19(15.8%)	0.082	2/8(25,0%)	0.134	1/11(9.1%)	0.047
DR4	5/19(26.3%)	0.142	2/8(25.0%)	0.134	3/11(27.3%)	0.147
DR5	2/19(10.5%)	0.054	1/8(12.5%)	0.065	1/11(9.1%)	0.047
DRw6	3/19(15.8%)	0.082	2/8(25.0%)	0.134	1/11(9.1%)	0.047
DR7	2/19(10.5%)	0.054	0/8(0%)		2/11(18.2%)	0.096
RDw8	2/19(10.5%)	0.054	1/8(12.5%)	0.065	1/11(9.1%)	0.047
DR9	0/19(0%)					
DRw10	1/19(5.3%)	0.027	0/8(0%)		1/11(9.1%)	0.047
DRw12	3/19(15.8%)	0.082	1/8(12.5%)	0.065	2/11(18.2%)	0.096
HLA-DR TOTAL		0.809		0.806		0.824
HLA-DRw52	13/19(68.4%)	0.438	7/8(87.5%)	0.646	6/11(54.4%)	0.325
HLA-DRw53	6/19(31.6%)	0.173	2/8(25.0%)	0.134	4/11(36.4%)	0.203
HLA-DQw1	3/19(15.8%)	0.082	0/8(0%)		3/11(27.3%)	0.147
DQw6	9/19(47.4%)	0.274	5/8(62.5%)	0.387	4/11(36.4%)	0.203
DQw2	5/19(26.3%)	0.142	2/8(25.0%)	0.134	3/11(27.3%)	0.147
DQw3	4/19(21.1%)	0.112	1/8(12.5%)	0.065	3/11(27.3%)	0.147
DQw7	8/19(42.1%)	0.239	4/8(50.0%)	0.293	4/11(36.4%)	0.203
DQw4	2/19(10.5%)	0.054	1/8(12.5%)	0.065	1/11(9.1%)	0.047
HLA-DQ TOTAL		0.903		0.944		0.894

r : Value of r.
rr: Relative risk.

FAMILY I



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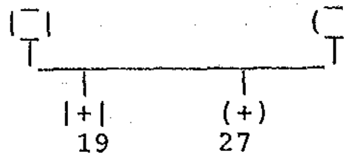

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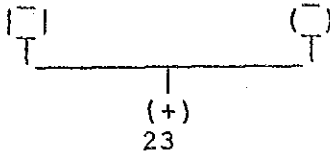

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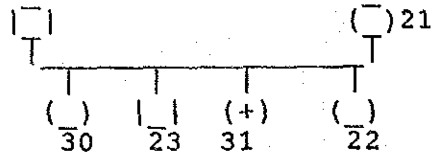

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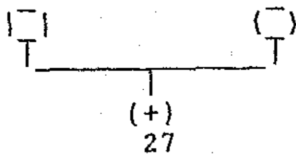

( ) ECG Abnornal

(+) ECG Normal

6- A31Aw33 B16B35 Cw4Cw7
7- A31Aw33 B16B35 Cw4Cw7

13- A2A31 B13B27 Cw3Cw7
14- A2A31 B27B60 Cw2Cw3

15- A2A29 B12B- Cw2-
17- A2A68 B18B48 Cw2Cw3
19- A29A68 B12B61 Cw3C

26- A24A31B62B45 Cw3Cw4
27- A2A24 B18B45 Cw4-

21- A30A- B64B72 Cw2-
22- A1A30- B64B72 Cw2-
23- A1A30 B8B72 Cw2-
30- A1A30 B8B72 Cw2-
31- A26A- B51B64 Cw1Cw8

E ROSETTING IN CEBUS APELLA AND INHIBITION OF ROSETTING BY MONOCLONAL

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ANTIBODY

SUMMARY:

The expression of the epitope detected by monoclonal antibody (MoAb) CD2 and E rosette formation with sheep red blood cells were examined for mononuclear cells from 5 Cebus apella monkeys and 5 human controls. The percentage of CD2 positive cells and of E rosette- forming cells were found to be equivalent and similar to the human percentages. Inhibition of E rosette formation was obtained almost 100 % of the time upom previous binding of CD2 on the E rosette receptor.

INTRODUCTION

It has been demonstrated that non-sensitizad red blood cells from sheep specifically bind thymus-dependent human lymphocytes (T lymphocytes) to form rosettes (6). For this reason, E rosettes have been considered as a specific T cell marker. The nature of the exact specific binding of E receptors for sheep red blood cells (SRBC) is unknown (4). But we know that T cells and many null cells carry receptors for SRBC. This receptor (TP 50) can be studied with monoclonal antibodies (CD2), and has been observed in mononuclear cells obtained from several non-human primates, but not from the prosimians, suggesting that the epitope identified by CD2 is acquired in the course of primate evolution (9, 3, 10).

It is considered that the SRBC receptor is phylogenetically conserved in certain primate species which show E resette-formation. In the present study, E rosette formation and ability of MoAb CD2 to inhibitits formation were investigated in Cebus apella, a New-World monkey used as an experimental model for Chagas' disease (12, 13, 14, 15).

MATERIALS AND METHODS

1. Animals: 5 adult Cebus apella monkeys, 3 females and 2 males, captured in Itacurubí del Rosario, were studied after being submitted to a quarantine period. Blood was taken from the femoral vein under anesthesia with ketamine hydrochloride, 5 mg/Kg,IM. The samples were collected in heparinized tubes.
2. Human controls: 5 healthy adult individuals were used as controls. Their blood was taken by venous puncture and collected in heparinized tubes.
3. Separation of mononuclear cells. the heparinized blood, previously diluted in PBS, was separated on a Fycoll- Histopaque gradient (Sigma), density 1077, and centrifuged at 2000 rpm for 20 min. The cells obtained after separation were transferred to another tube, washed twice with PBS at 1500 rpm for 10 min,and suspended at a concentration of 5 X 10^6 cells/ml.
4. E rosetting assay: 100 ul of the cell suspension is mixed with 100 ul of a 1% sheep red blood cell solution. The mixture is centrifuged at 1000 rpm for 5 min and then incubated for 18 at 4 degrees.The sample is gently suspended and the percentage of rosette- forming lymphocytes (binding of 3 or more SRBCs) is obtained.

5. E. rosetting assay upon incubation with MoAb CD2: 100 ul of the cell suspension was incubated with MoAb Leu 5b (CD2), Becton Dickinson, at different concentration (20 ul, 40 ul, 80 ul and 100 ul) for 30 min. at room temperature. Subsequently, 100 ul of a 1% SRBC solution was added, and the mixture was incubated for 18 h at 4 degrees.
6. MoAb CD2 assay: 100 ul of the cell suspension was incubated with fluorescein bound MoAb Leu 5b (CD2), Becton Dickinson, at different concentrations (20ul, 40ul, 80 ul, and 100 ul) for 30 min. at room temperature, and then counted for fluorescent cells with a fluorescence microscope under epiillumination.

RESULTS

The results are shown in Tables 1 and 2, where it can be seen that the percentage of E rosette- forming monkey cells was practically the same as that obtained in human controls. These tables also show that previous binding of MoAb Leu 5b (CD2) (anti-E rosette receptor) to the monkey lymphocytes inhibits E rosette formation; this inhibition reaches almost 100 % effectiveness. Table 3 shows the percentage of CD2 positive monkey cells at different CD2 concentrations, and compares these results with human controls. No better response was observed with the use of 20 ul.
With these data we can conclude that a SRBC receptor is present in Cebus apella monkeys.

Table 1 E rosette formation and inhibition of its formation by CD at different concentration in Cebus apella

Monkeys	Rosettes	Inhibition with MoAb anti- CD2			
		100 ul	80 ul	40 ul	20 u
114	77 %	0 %	0 %	0 %	0 %
111	80 %	0 %	0 %	0 %	0 %
118	55 %	0 %	0 %	0 %	5 %
33	83 %	0 %	0 %	0 %	0 %
126	84 %	0 %	0 %	0 %	0 %

Table 2. E rosette formation and inhibition of its formation by CD2 at different concentrations in controls.

Humans	Rosettes	Inhibition With MoAb anti-CD2			
		80 ul	40 ul	20 ul	10 ul
F	70 %	-	-	11 %	68 %
F	83 %	-	24 %	13 %	-
H	73 %	1 %	0 %	2 %	-
H	79 %	2 %	1 %	0 %	-
F	50 %	6 %	1 %	2 %	-

TABLE 3. DETERMINATION OF MoAb anti-CD2 receptor in Cebus apella mokeys.

Monkeys	100 ul	80 ul	40 ul	20 ul	Human controls (20 ul)
114	49 %	37 %	42 %	54 %	64 %
111	63 %	62 %	70 %	70 %	65 %
118	60 %	73 %	77 %	75 %	60 %
126	87 %	80 %	85 %	77 %	57 %
33	-	-	-	-	63 %

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PRODUCTION OF MONOCLONAL ANTIBODIES BY HYBRIDOMA FORMATION

Edgar Villagra, Rosa Zárate, Watanabe.

I. INTRODUCTION

In 1975 Kohler and Milstein created a method that permited the production of monospecific antibodies (Ab), derived from single B cell clones. This is achieved by the fusion of Ab-producing cells with myeloma cells (malignant B cells), which yields the so-called "hybridoma". This procedure is based on the principle that a single Ab- producing cell secretes an Ab of a single Known specificity; upon fusion with a myeloma cell, the growth of the "cell clone" is achieved: the Ab thus produced will show a single specificity and will be monoclonal.

II. Objectives of monoclonal Ab production

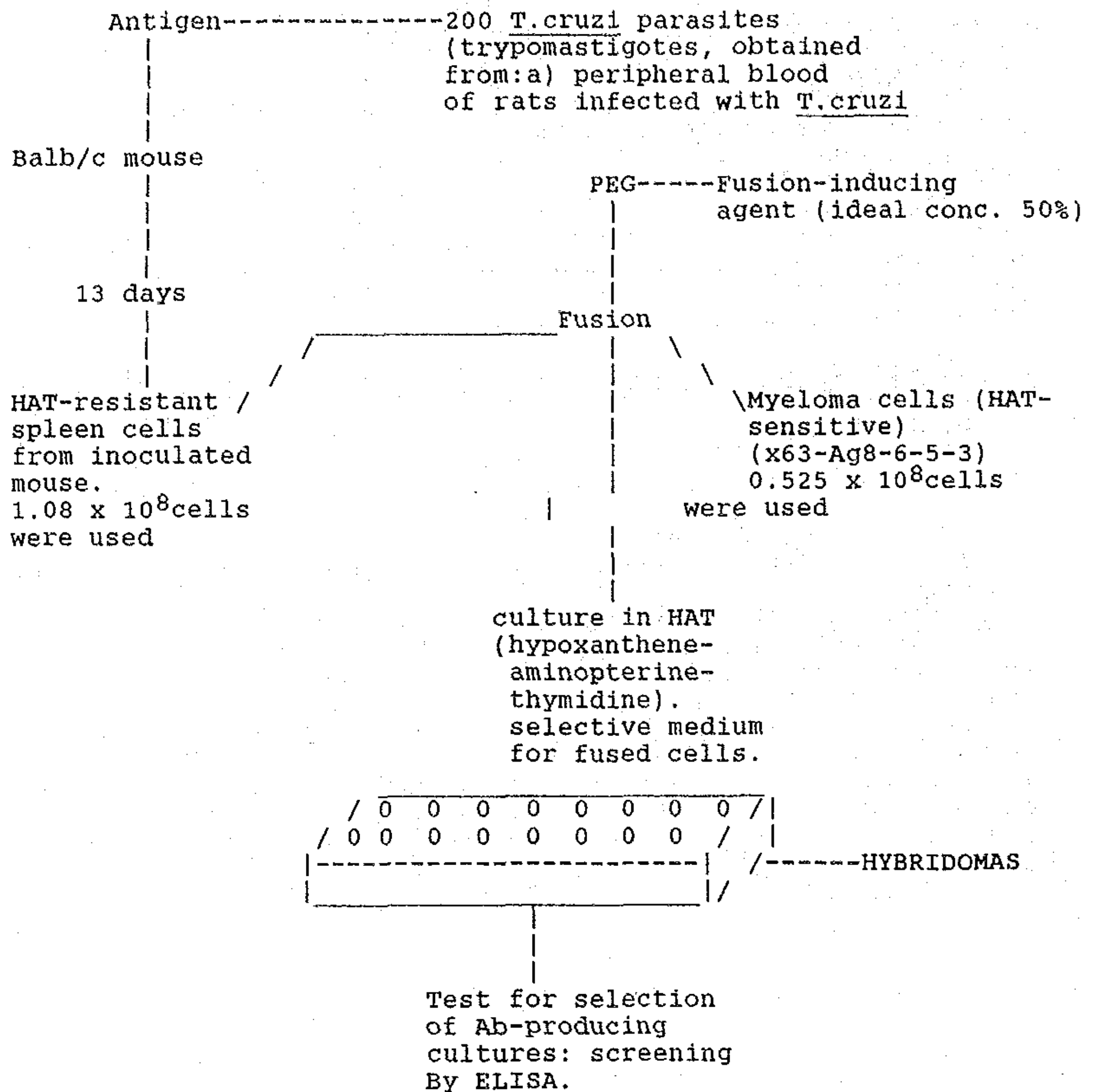
a) As a diagnostic method for detecting infection by T.cruzi.
b) As an aid in identifying T.cruzi strains.
c) As an introduction for developing diagnostic methods for other parasitis diseases.

III. Materials and Methods

1. Establishment of hybridoma cells producing anti-T.cruzi monoclonal Ab.

a) Immunization: the animals most usually chosen are mice or rats. In the present experiments, female Balb/c mice were used.
the inoculation was performed using Freund's complete or incomplete adjuvant.

b) Fusion: Once the inoculated animals produce a good Ab response, their spleens are extracted, and a cell suspension is prepared which will be subjected to fusion with a myeloma cell line, in the presence of polyethylene-glycol (PEG), which favors membrane fusion. Only a small proportion of all the cells in the suspension undergo fusion . After fusion, the cell mixture is cultured in HAT medium.



C) Screening: This is performed by the ELISA test. ELISA (Enzymelinked immunosorbent assay) is a sensitive technique for detecting antigens and antibodies. The antigen used in this experiment was T.cruzi epimastigotes.

TECHNIQUE:

A) Plate sensitization by the Lowry method. The antigen is placed on the plate and incubated 2 hrs. at 37°C, then left overnight at 4°C.

B) Washing (3 times) With PBS-Tween 20.

C) Addition of Block-Ace to prevent cross reastions.

* D) Addition of Ab sample from supernatant of plates where fusion occurred, and incubation 20-30 min at 37°C and 5 % AO2.

E)Rewashing (3 times) with PBS-Tween 20.

F) Addition of second Ab and incubation at 37°C in 5 % CO_2 for 40 min.

G) Washing (5-5 times) with PBS-Tween 20.

H) Addition of chromogene: OPD (O-phenyl diamine). The enzyme peroxidase requires a substrate (in this OPD, which is photosensitive). The enzyme draws an electron from H_2O_2. and the reaction mixture takes on a different color. This reaction is stopped by the addition of H_2SO_4 8 N.

d) Determination of Ab class: this is performed by the Outcherlony test, which determines the class and subclass of monoclonal Ab by immunodiffusion. This test is easy to perform, specific and sensitive. Each test includes 3 plates and 6 antisera (directed against rat and mouse heavy chains, IgG1, IgG2a, IgG2b, IgG3, IgA and IGM). It also includes positive control sera that will immunoprecipitate against each antiserum.

e) Antigen characterization: This was performed by immunoblotting (Western blot). In this technique, antigens or other purified proteins are subjected to electrophoresis on an SDS polycrylamide, gel in order to individually separate the different protein bands, according to molecular weight (MW). The MW of the proteins under study are compared with the electrophoretic mobility of proteins with known MW.

These protein bands are transferred onto nitrocellulose paper, to which they remain fixed. For the detection of the antigen-Ab complex on the nitrocellulose strips, the conjugate goat/hIgG/PoD (goat/ human IgG Ab/ peroxidase), which colors the protein bands, is commonly used.

IV. Results

TOTAL Wells	660 (7 plates)
Hybridoma-positive wells	291 (44.09 %)
MAb-producing hybridoma wells	23

$$\frac{\text{No. MAb (+) Wells}}{\text{Total wells (660)}} \times 100 = 3.48\ \%$$

$$\frac{\text{No. MAb (+) wells}}{\text{No. hybridoma (+) wells (291)}} \times 100 = 7.9\ \%$$

No. of established hybridoma clones = 7
2 Clones: IgG2b
5 Clones: IgM

OBJECTIVES

- Synthesis of MoAb reacting to T.cruzi, and later use of these antibodies for antigen purification .

- Use of these purified antigens in a diagnostic kit, and possible use as a vaccine for Chagas' disease.

MATERIALS AND METHODS

- Spleen cells are taken from Balb/c infected by T.cruzi, these cells are fused with myeloma cells (XG3-Ag8-G5-3); fusion is accomplished in the presence of polyethylene glycol, 50 %.

- The supernatant from the hybridoma culture is incubated with non-purified epimastigote antigens in an ELISA test, and positive cultures are selected.

- The cells producing the MoAb are established by twofold limited dilution.

- Immunodiffusion by the Ouchterlony method is used for identifying antibody class and subclass.

- The molecular weight of the antigen which reacts with the MoAb is determined by eletrophoresis in a 10 % gel and western blotting.

CONCLUSION

- MoAb belonging to the IgG2b and IgM classes were established which recognize T.cruzi antigens.

- As a result of electrophoresis IgM has reacted with different varieties of protein molecules from epimastigotes.

- IgG2b has reacted with low-molecular weight proteins. This area is presenty under active research.

OBJECTIVES FOR THE FUTURE

- Investigate cross-reactivity of these MoAb with Y strain epimastigotes and other epimastigotes.

- Investigate cross-reactivity of these MoAb with other strains besides Y strain, as well as with Leishmania.

- Produce ascitic fluid and assess whether it can supress infection by T.cruzi.

- Purify antigens using ascitic fluid.

④　昭和63年度、平成元年度供与機材リスト

パラグアイ国シャガス病等寄生虫症研究プロジェクト

昭和63年度供与機材

内　訳　書

番号	品名及び仕様	メーカー名	数量	単価	金額
1	高速冷却遠心機 RS-20IV	トミー精工	1式		3,100,000--
	AC220V50Hz 標準附属品付				
	特別附属品				
	アングルローター No.3N 1				
	〃 No.4N 1				
	〃 No.17N 1				
	スイングローター No. TS-7 1				
	バケット 7050-01 1				
	〃 7015-08 1				
	PCチューブ 10mℓ16本組 2				
	〃 50mℓ 8本組 2				
	PPチューブ 500 mℓ 6本組 2				
	ガラスチューブ 15mℓ32本組 2				
	〃 50mℓ 4本組 2				
	バランサー H050 1				
	〃 L050 1				
2	超音波ホモジナイザー 250型	ブランソン	1台		700,000—
	AC220V50Hz				
	スタンド付				
3	ホモジナイザー撹拌装置	池本理化	1台		140,000—
	No.1080 AC220V 用ダウントランス付				
4	微量用高速冷却遠心機KR-1500	久保田	1式		820,000—
	特別附属品				
	アングルローターRA-228 1				
	マイクロアングルローターRA-150AM 1				
	マイクロテストチューブ 2.2mℓ 100入 5				
	〃 1.9mℓ 〃 5				
	〃 1.5mℓ 〃 5				
	38mℓ PPチューブ 10入	1			
	PPキャプ 38mℓ チューブ用 10入 1				

番号	品名及び仕様	メーカー名	数量	単価	金額
5	PHメーター F－13	堀場	1		250,000－
	AC220V50Hz				
	標準附属品付				
6	卓上多本架遠心機 LC06-SP	トミー精工	1式		450,000－
	スイングローター TS-7付				
	特別附属品				
	バケット 7050-01 1				
	〃 7015-06 1				
	バランサー L050 1				
	ガラスチューブ15mℓ×24本 1セット				
	〃 50mℓ×4本 1セット				
7	限外濾過器	アミコン			
	No.8010 10mℓ (25mm膜用)		1		92,000－
	限界処理量 1mℓ				
	No.8050 50mℓ (43mm膜用)		1		101,000－
	限界処理量 2.5mℓ				
	No.8200 180mℓ (62mm膜用)		1		153,600－
	同上用限外フィルター 25m/m 10枚入		1		19,600－
	〃 43m/m 〃		1		40,500－
	62m/m 〃		1		53,000－
8	解剖器具セット	井内盛栄堂	2セット	55,000	110,000－
	小動物用 5-410-01				
9	カラム	ファルマシア			
	Superose 6HR 10/30		1		157,000－
	〃 12HR 10/30		1		157,000－
	HR 16/50 高性能カラム		2	77,000	154,000－
	Superose 6 Pregrade 125 mℓ		1		44,000－
	〃 12 〃 125mℓ		1		56,000－
	XKカラム XK26/40		2	80,000	160,000－
	Alkyl-Superose HR 5/5		1		150,000－

番号	品名及び仕様		メーカー名	数量	単価	金額
	Q-Sepharose Fast Flow 300mℓ			1		32,400−
	S-Sepharose Fast Flow 300mℓ			1		32,400−
	Mono HR 5/15			1		147,000−
10	自動セルハーベスター		ラボサイエンス	1式		789,000−
	LM101 ラボマッシュ	1台				
	吸引ポンプGM-25SS	1				
	5 ℓポリタンク（栓付）	1				
	5 ℓ吸引瓶	1				
	シリカゲル500g	20				
	500 mℓ吸引瓶	1				
	ダウントランスホーマー	1				
11	スライドグラス		松　　浪			
	S2226 水切フロスト3000枚入り			10	33,000	333,000−
	S2215 水磨フロスト 〃			10	40,300	403,000−
12	カバーグラス		松　　浪			
	24×18mm	100枚入		50	6,300	315,000−
	24×24mm	〃		50	8,400	420,000−
	24×32mm	〃		50	10,300	515,000−
	24×40mm	〃		50	15,000	750,000−
13	オベクト整理箱 No.20-836		池　　本	200	980	196,000−
	木製 100枚入					
14	スライドラベル		高　　島	10箱	1,500	15,000−
	セルフラベル（大）	1000入				
15	OCT コンパウンドNo.06441	12本入	ティシュテック	1		19,000−
16	ユニカセットNo.4170	1500入	〃	2	51,000	102,000−
17	包埋皿11〜15号12入	各1	〃	5	10,500	52,500−
18	ミクロトームアダプターNo.4196		〃	1		48,900−
19	ファイリングキャビネットNo.4129		〃	2	35,600	71,200−

番号	品名及び仕様	メーカー名	数量	単価	金額
20	染色バット　タテ5枚用20-864	池本理科	20	850	17,000－
	タテ10枚用20-863	〃	50	1,040	52,000－
	タテ15枚用専用バスケット用	〃	50	1,040	52,000－
21	染色バット金具　15枚用	〃	50	600	30,000－
	20枚用	〃	50	1,250	60,000－
22	障子マッペ　20枚用	高島	500	500	250,000－
22	シャーレ　φ21cm硬質	池本	10	4,500	45,000－
24	替刃式解剖メス、ハンドルF-100	フェザー	1		2,480－
	〃　替刃　No.325　5枚入		20	10,900	218,000－
25	替刃式トリミングナイフ、ハンドルF260	〃	1		3,900－
	〃　替刃No.260 50枚入	〃	3	14,850	44,250－
26	剪刀　替刃付	〃	1		6,100－
27	替刃式解剖メス、ハンドルF60	〃	1		2,470－
	〃　替刃		100枚	600	60,000－
28	全自動洗浄機G7735U	ミーレ社	1式		2,350,000－
	AC220V50Hz三相				
	特別附属品				
	インサート　K104　4				
	〃　K105　4				
	トレー　E332　1				
	直噴式モービルワゴンE329　1				
	洗済N6ℓ　5				
	〃A810kg　5				
29	振とう器	池本理科	1台		345,000－
	ロータリーシェカーN（A型）				
	40-0322				
	容器固定パネル500mℓ用200 mℓ用各1ケ付				
30	マウスゲージTP-106	東洋理工	100ケ	5,000	500,000－
	175×245×125 mm				
	小計				15,184,600－

番号	品名及び仕様	メーカー名	数量	単価	金額
31	電子天秤 L2200S AC220V50Hz シングルレンジ 秤量2220g 読み取り限度0.01g	ザルトリウス	1台		130,000−
32	ダブルビーム分光光度計 U-2000型 AC220V50Hz スペアパーツ 1) 記録紙 20 2) ハロゲンランプ 2 3) D_2ランプ 1 4) 10㎜クオーツセルセット 1 5) ヒューズ3A 5 6) ヒューズ5A 5	日立	1式		1,450,000−
33	電気泳動器具 ペリスタポンプ SJ-1211H (1) パワーサプライ AE-8400 (1) ミニパワー SJ-1082 (1) ディスク電気泳動装置SJ-1060D (1) スラブ電気泳動槽 AE-6200 (1) スラブゲル作製器 AE-6210 (1) スラブゲル乾燥器 AE-3701 (1) ミニスラブ電気泳動セット AES-440(1)	アトー	1式		710,000−
34	ブロッティング装置 トランスブロッド装置 AE-3280 (1) ホライスブロッド AE-6670P (1) パワーサプライ AE-8300 (1)	アトー	1式		290,000−
35	低温槽 MDF-230 −30℃, AC220V50Hz	サンヨー	1台		260,000−
36	ELISAシステム モデル2550, 220V50Hz	バイオラッド	1式		2,100,000−

番号	品名及び仕様	メーカー名	数量	単価	金額
	ELAリーダー　1台				
	ELAウオッシャー　1〃				
	吸引ポンプ　1〃				
	吸引瓶3ℓ　1ヶ				
	スペア光源ランプ　1ヶ				
	スペア感熱記録紙　4ヶ				
37	自動製氷機F200AA-SA	星崎	1台		830,000－
	AC220V50Hz				
38	超音波ピペット洗浄器	ヤマト科学	1台		345,000－
	型式AW-31				
	ダウントランス付				
39	超音波洗浄器	ヤマト科学	1台		270,000－
	型式B5200-J4				
	AC220V50Hz				
	特別附属品				
	タンクカバー　1				
	インサートトレイ（穴明）　1				
40	ウォーターバスインキュベーター	ヤマト科学	1台		163,000－
	型式BT-25, ダウントランス付				
41	電気泳動用消耗品	アトー			
	泳動カラム 7mm×5φ×100mm　12入		10個	3,000	30,000－
	〃 7mm×2.5φ×100mm　12入		10〃	1,500	15,000－
	パッキング　12入		5〃	1,200	6,000－
	プレートA-10 160×160mm		20枚	2,800	56,000－
	〃 B-10 1mm 160×160mm		20〃	2,800	56,000－
	シールチューブ 1.5φ×500mm 2本入		10個	300	3,000－
	ミニスラブ泳動プレートMA-10		20枚	2,500	50,000－
	〃 MB-10		20〃	2,500	50,000－
	シールチューブ 1.5φ×500mm 2本入		10個	300	3,000－
	サンプルコウム C-20-12		2枚	4,000	8,000－

番号	品名及び仕様	メーカー名	数量	単価	金額
	ミニスラブサンプルコウムMC10-12		2枚	3,700	7,400―
	メンブランクフィルターIPVH304FO		2枚	26,000	52,000―
	0.45μm 26×26cm 10枚入				
42	自動固定包埋装置	サクラ精機	1台		885,000―
	RH-12DM-Ⅱ				
	標準附属品付				
43	包埋センター	サクラ精機	1台		1,560,000―
	エンベディングコンソール No.4596				
	ディスペンシングコンソール No.4598				
	サマーコンソール No.4597				
44	クリオスタット CM-501 型	サクラ精機	1台		2,400,000―
	標準附属品付				
45	滑走式ミクロトーム IVS-400型	サクラ精機	1台		580,000―
	替刃式ホルダーセット 240E付				
46	パラフィン伸展器PS-SB型	〃	2台	199,000	398,000―
47	パラフィン熔融器PM-40H型	〃	1台		290,000―
48	インキュベーター IF-2B型	〃	1台		217,000―
	温度上昇防止装置付				
49	マルチデイスカッション顕微鏡	オリンパス	1式		1,510,000―
	BHS-MDO-2(3人用)				
	本体部BHS-F set(220V 用)				
	ﾃﾞｲｽｶｯｼｮﾝ装置：ﾏﾙﾁﾃﾞｲｽｶｯｼｮﾝ				
	鏡筒部指示ﾎﾟｲﾝﾀｰ内臓(BH_2 MDO-B)				
	指示ポインター用トランス (TDO)				
	ﾏﾙﾁﾃﾞｲｽｶｯｼｮﾝ用側視鏡				
	(BH_2-MDO-SV) (1)				
	鏡筒(BH_2-Bi30)双眼 (2)				
	BH_2-TR30 三眼 (1)				
	接眼レンズ：WHK10×(3)				
	WHK10×H (2)				

番号	品名及び仕様	メーカー名	数量	単価	金額
	35WHK10×(1)				
	NFK3.3×(1)				
	対物レンズ：SPLAN APO4×				
	10×，20×，40×，100×（各1）				
	ｽﾍﾟｱﾊﾛｹﾞﾝﾗﾝﾌﾟDOﾗﾝﾌﾟ（各6ヶ）				
50	ディープフリーザー	サンヨー			
	1)MDF-291AT		1台		1,520,000−
	-85℃ 180ℓ AC220V50Hz				
	2)MDF-190AT		1台		1,080,000−
	-85℃ 86ℓ AC220V50Hz				
	3)同上用コンテナー				
	MDF39SC		4		52,000−
	MDF19SC		3		39,000−
51	生物顕微鏡	オリンパス	1式		5,600,000−
	ニューバノックスAHBS-RFL-2				
	落射蛍光システム（220V用）				
	AHBS本体(AHBS-F)				
	落射蛍光投光管(5穴レボ付)(AH_2-RFA)				
	落射蛍光用トランス(AH_2-RFL-T)				
	水銀灯ランプハウス(AH_2-LSRF)				
	吸収フィルターUVBG用				
	励起フィルターBG用				
	蛍光用対物レンズ:DPLAN				
	APO10×UV，20×UV				
	DAPO40×UV，100×UV（oil） 各1				
	ステージ：右下ﾊﾝﾄﾞﾙｽﾃｰｼﾞ(AH_2-SVR)				
	カメラ：35㎜カメラ(C35AD4) 2ヶ				
	ポラロイドカメラ(PH-CP-W) 1ヶ				
	大阪カメラ胴(AH_2-DL) 1ヶ				
	接眼レンズ：SWHK10× 2ヶ				

番号	品名及び仕様	メーカー名	数量	単価	金額
	対物レンズ：SPLAN Fl 1×, Fl 2× SPLANAPO 4×, 10×, 20×, 40×, 100×　　(各1) 標準電動6ケ穴レボルバー AH_2-6RE付 スペア水銀ランプHBD200W　4ヶ スペアハロゲンランプ　6ヶ イマージョンオイル 500cc　2ヶ 無蛍光オイル　50cc　2ヶ				
52	クリーンベンチ PCV-1303 BNGⅢプロパンガス用 AC220V用ダウントランス付	日立	1台		1,100,000―
	合計				24,115,000―

番号	品名及び仕様	メーカー名	数量	単価	金額
53-1	液体シンチレーションカウンター Model LS5000TA 220V 50Hz 1-P	ベックマン	1台		
2	2相モニター	ベックマン	1台		
3	本体設置台 (W100×D80×H60cm)	ベックマン	1台		
4	プリンタ台 (W43×D58×H67.5cm)	ベックマン	1台		
5	CRT棚	ベックマン	1台		
6	プリンタ用紙 (1000枚入／1箱)	ベックマン	3箱		
7	プリンタカートリッジ	ベックマン	1ケ		
8	AVR	ベックマン	1台		
9	ガラスバイアル (500ヶ入／箱)	ベックマン	2箱		
10	カクテル(Rendy Safe)	ベックマン	1箱		
		上記製品	1式		13,000,000−

63年度　供与機材現地調達分

トヨタランドクルーザー　4WD　HJ60型　3,980cc　110 HP
CIF　アスンシオン　US$　28,440.⁰⁰

パーソナルコンピュータシステム（IBM）　一式

・本体PS／2，モデル60-071 ………………………… 1
・ディスプレイ …………………………………………… 1
・外部ディスクシステム（5 1/4 インチ）……… 1
・　〃　（3 1/2 インチ）……… 1
・計算ソフト ……………………………………………… 1
・プリンター ……………………………………………… 1
・フロッピーディスク ………………………………… 50
・印刷リボン ……………………………………………… 50

機材名	仕様	数量	価格
〔医療機材〕			
解剖器具			
ピンセットA	Rochester Ochsner recta de 16cm	4	43,680
〃　B	Crile curva de 14.5cm	4	37,000
〃　C	disección de 16cm	4	15,560
〃　D	Semken	4	22,440
〃　E	Graeffe	4	32,480
〃　F	Halstead mosquito curvo	4	31,860
はさみ	Pecta de 13cm	3	24,450
メス	de bisturí	3	10,950
ガラス器具			
ガラスチューブ	1000cc	2	31,674
〃	500cc	2	19,938
ビーカー	250mℓ	2	6,422
〃	600mℓ	2	10,558
〃	2000mℓ	2	19,200

機　材　名	仕　　　　様	数量	価　　格
さじ		1	3,411
温度計	10-110℃	2	26,338
担子とり器		1	9,880
メスシリンダー	1000ml	4	25,152
〃	2000ml	2	17,930
殺菌灯	220V/50Hz, 300W, 256 ＋365mm	1	1,408,000
液体窒素用タンク	アルミニウム製　CRYOMETAL	1	750,000
〔医薬品〕			
試薬			
ｲﾝﾀｰﾛｲｷﾝ2		1	235,840
ｲﾝﾀｰﾛｲｷﾝ2 RIA用		1	545,600
抗TL2 受信基抗体		1	281,600
抗マウスIgG F-2 抗体		1	224,400
抗マウスIg　抗体		1	293,040
抗マウスIgG 抗体		1	147,840
抗マウスIgG1		1	147,840
抗マウスIgG 2a		1	147,840
抗マウスIgG 2b		1	147,840
抗マウスIgG3		1	147,840
抗マウスIgM		1	124,920
抗マウスIg(L)		1	147,840
抗マウスIg(K)		1	147,840
抗マウスIgウサギ抗体	蛍光標識	1	243,760
抗マウスIgウサギ抗体	HRP標識（吸収済）	1	257,840
抗ヒトIg　ウサギ抗体	HRP標識	1	284,240
抗ﾏｳｽ Ig Fab ｳｻｷﾞ抗体	HRP標識（未回収）	1	243,760
抗ﾏｳｽ Ig Fab ｳｻｷﾞ抗体		1	383,680
抗ﾋﾄ HLA-DR　ﾏｳｽ抗体		1	58,960
抗ﾋﾄHLA-DRｸﾗｽﾏｳｽ抗体		1	58,960

機　材　名	仕　　　様	数量	価　　格
チミジン標識抗体		1	363,440
ウリジン標識抗体		1	166,320
薬品			
MEM	Cat.430-2200	4	2,520,000
MEM	Cat.410-2000	8	896,000
RPMI	Cat.320-1875	8	1,120,000
FCS	胎児牛血清	30	5,460,000
	小　　計		14,797,240
〔一般〕			
エアコン	220V/50Hz,24000BTU/h ﾐｯﾋﾞｼ WRH 4275	7	7,868,000
〃	220V/50Hz,18000BTU/h ﾐｯﾋﾞｼ WRH 1801	3	3,045,000
	（工事費）		600,000
冷蔵庫	220V/50Hz, 420ℓ,Frost-Frec PHILIPS	5	2,795,000
〃	220V/50Hz, 320ℓ PHILIPS	1	308,000
ファクシミリ	CANON FAX 230	1	3,141,600
家具	事務机（大） SILVESTRI	2	335,000
	〃 （小） 〃	4	394,000
	〃 （中） 〃	2	267,000
	椅子（大） 〃	2	290,000
	〃 （中） 〃	10	670,000
	〃 （スツール） 〃	12	510,000
	キャビネット（棚） 〃	1	102,000
	〃 （金庫付） 〃	1	132,000
	ファイリングキャビネット〃	5	540,000
	タイプ用テーブル 〃	2	69,000
	本棚 〃	6	309,000
	ロッカー 〃	2	211,000
	本棚（扉付） 〃	1	108,500
	メタル作業机 〃	2	493,000
	小　　計		21,726,950

パラグアイ国シャガス病等寄生虫症研究プロジェクト

平成元年度供与機材

内　　　訳　　　書

番号	品名及び仕様	メーカー名	数量	単価	金額
1	生物三眼顕微鏡 BHS-313(220V) 対物レンズ：SPLAN 4×, 10×, 20× 40×, 100×　各1 接眼レンズ：WHK 10×(1) 35WHK 10×(1) NFK 3.3×(1) 標準附属品及びスペアーランプ (12V, 100W HAL-L) 6ヶ付	オリンパス	2台	50,000	1,500,000−
2-1	倒立型培養顕微鏡 IMT-2-21型(220V) 構成： 1.本体部（IMT-2-F)　1 2.双眼鏡筒(BH_2-Bi45)　1 3.ステージ(IMT-2-SVR)　1 4.ターレットコンデンサー　2 (IMT-2 LWCD, ULWCD) 5.対物レンズ　各1 PCS PLAM 4×PL, 10×PL, 20×PL, 40×PL 6.接眼レンズ WHK 10×(1), WHK10×-H(1) 7.カメラ(OM-4TI)　1 ケーブルレリーズ(SP-2)付 標準附属品及びスペアーランプ (JC12V, 50W, HAL)6ヶ付	オリンパス	1台		1,310,000−
2-2	全自動写真撮影装置 PM-10-35ADS-2 構成 1.本体露出部(PH-PBSP)　1	オリンパス	1台		690,000−

番号	品 名 及 び 仕 様	メーカー名	数量	単 価	金 額
	2.コントロールボックス(PH-PBSP) 1				
	3.35m/m カメラ(PM-D35A) 1				
	4.35m/m 自動巻上カメラ 1				
	バック (C-35AD-4)				
	5.ビュアー(PM-VSP) 1				
	6.準焦望遠鏡 1				
	(PM-FT-36)				
	7.色温度計(PM-CTR) 1				
	8.フィルター(PM-FILC) 4種				
3	倒立型培養顕微鏡	オリンパス	1台		522,000—
	CK2-TRP-2 (220V)				
	両側固定ステージ (ｻﾌﾞｽﾃｰｼﾞ2ケ付)				
	三眼45° 鏡筒				
	対物レンズ：PCS PL 4×PL				
	PC-D10×PL,				
	LWDCD20×PL				
	LWDCD PL40×PL (各1)				
	接眼レンズ：CWHK10×(2)				
	NFK5×LD(1)				
	位相差装置付				
4	卓上多本架遠心機	クボタ	1台		176,000—
	KN-70 スイングロータ(RS-4)付				
	仕様				
	最高回転数：5,000rpm				
	最大遠心力：4,640×g				
	最大処理量：15mℓ×32本				
	(計 480mℓ)				
	標準附属品付220V50Hz				
	特別附属品				
	1.バケット32×15mℓ, 4ヶ/set	クボタ	1ケ		53,000—

番号	品名及び仕様	メーカー名	数量	単価	金額
	2.バケット 8×50mℓ, 4ヶ/set	〃	1ケ		59,000−
	3.ガラス管15mℓ 10本/set	クボタ	4組	1,250	5,000−
	4. 〃 50mℓ 4本/set	〃	2組	6,500	13,000−
	5.カーボンブラシ10ヶ/set	〃	1組		6,000−
5	クリーンベンチ	日立	1台		1,260,000−
	PCV-1303 BNGⅢ				
	プロパンガス用				
	220V/100V トランス内蔵				
	標準附属品付				
6	CO_2 インキュベーター	サンヨー	1台		1,020,000−
	MCO-175(220V,50Hz)				
	外寸法：770(W)×620(D)×900m/m(H)				
	内寸法：490(W)×505(D)×690m/m(H)				
	内容積：170 ℓ				
	標準附属品付				
	特別標準附属品				
	1.架台 MKD-300T	サンヨー	1台		37,000−
	2.CO_2空ボンベ架台付	〃	1式		60,000−
	3.CO_2ガス圧力調整器	〃	1ケ		30,000−
	4.専用トレー,MCO45ST	〃	6ケ	5,500	33,000−
7	ユニバーサル冷却遠心機	クボタ	1台		513,000−
	5700(220V/50Hz)				
	標準附属品付				
	特別附属品				
	1.スイングローター RS-4/6(4-PLACE)	クボタ	1ヶ		38,000−
	2.バケット 32×15mℓ(4 μm/set)	〃	1組		53,000−
	3. 〃 8×50mℓ(4 μm/set)	〃	1組		59,000−
	4.ガラス管 15mℓ(10 μm/set)	〃	4組		5,000−
	5. 〃 50mℓ(4 μm/set)	〃	2組		13,000−
	6.マイクロプレートローター	〃	1ヶ		114,000−

番号	品 名 及 び 仕 様	メーカー名	数量	単 価	金 額
	RS-96 SA/6	〃			
	7.4×96穴マイクロプレート用	〃	1組		21,000―
	バケット (2μm/set)	クボタ			
	8.カーボンブラシ (10μm/set)	〃	1組		6,000―
8	FPLCシステムA	ファルマシア			
	1)20-0002-01 グラジェントプログラマー GP-250 PLUS		1ヶ		1,172,100―
	2)19-7620-01 クロマトグラフィーテック RA-120		1ヶ		152,000―
	3)18-1000-48 ポンプ P-500		2ヶ	992,000	1,984,000―
	4)19-7500-01 バルブ V-7		1ヶ		142,000―
	5)19-5084-01 プレフィルター		2ヶ	17,500	35,000―
	6)20-0114-01 GP-250 PLUS ケーブルキット		1ヶ		56,000―
	7)19-5079-01 フランジング スタートアップキット		1ヶ		29,000―
	8)18-0425-01 プレフランジド チュービング		1ヶ		11,000―
	9)19-8600-01 FRAC-200 フラクションコレクター		1ヶ		525,000―
	10)19-5164-01 ソレイドバルブ PSV-100		1ヶ		34,000―
	11)18-2200-01 フローリストリクター		1ヶ		29,000―
	12)19-7585-01 スーパーループ 10mℓ		1ヶ		55,000―
	13)19-7850-01 〃 50mℓ		1ヶ		63,000―
	14)18-1000-49 UV-M コントロールユニット		1ヶ		525,000―
	15)18-0604-02 UV-M Hg オプティカルユニット (254mm, 280mmフィルター付)		1ヶ		564,000―
	16)18-0675-01 5m/m フローセル		1ヶ		194,000―
	17)19-8003-01 REC-482 レコーダー		1ヶ		570,000―

番号	品名及び仕様	メーカー名	数量	単価	金額
	18)19-8300-01 PHモニター		1ヶ		423,000−
	(フロースルーPH電極付)				
	19)17-0546-01 MONO Q,HR5/5		1ヶ		142,000−
	20)17-0547-01 MONO S HR5/5		1ヶ		142,000−
	21)17-0548-01 MONO P HR5/20		1ヶ		204,000−
	22)17-0532-01 PEP RPC HR5/5		1ヶ		92,000−
	23)17-0533-01 PRO RPC HR5/2		1ヶ		61,000−
	24)17-0539-01 PRO RPC HR5/10		1ヶ		134,000−
	25)17-0537-01 SUPEROSE 6HR 10/30		1ヶ		152,000−
	26)17-0538-01 SUPEROSE 12HR 10/30		1ヶ		152,000−
	27)17-0519-01 PHENYL-SUPEROSE HR5/5		1ヶ		145,000−
9	ホットプレートφスターラー PC-320	井内	1式		54,000−
	標準付属品及びトランス付				
	(220V/100V)				
10	プラロイド多用途カメラ MP-4	ポラロイド	1式		450,000−
	電気泳動記録用				
	(標準型セット)				
	トランス付 (220V/100V)				
	特別附属品				
	1)フィルター(UV,ﾚｯﾄﾞ,ｵﾚﾝｼﾞ) 各1		1式		6,000−
	2)同上用アダプター, 49m/m		1式		2,500−
11-1	マルチミキサー	井内	1式		38,000−
	M型 (1-266-01)				
	トランス(220V/100V) 付				
11-2	同上用マイクロプレートホルダー	井内	1式		6,000−
	(1-266-03)				
12	ローラーポンプ	井内	1式		110,000−
	RP-NE3(1-658-03)				
	標準附属品付				
13	コンパクト無菌ブース	井内	1式		170,000−

番号	品 名 及 び 仕 様		メーカー名	数量	単 価	金 額
	TY-22型(3-115-02)					
	トランス(220V/100V) 付					
14-1	マントルヒーター		井 内	1式		48,000−
	AFR-20(1-167-04)					
	トランス(220V/100V) 付					
14-2	同上用ガラス器具		井 内	1式		47,000−
	内訳					
	1)80-84 冷却管(19/38-300)	(1)				
	2)80-72 $連結管(19/38 105°)	(1)				
	3)80-78 $平栓(24/40)	(1)				
	4)80-14 $ﾒｽﾌﾗｽｺ(24/40-2ℓ)	(1)				
	5)80-67 $連結管(19/38-24/40)	(1)				
	6)80-64 $連結管枝付	(1)				
	(19/38-19/38-24/40)					
	7)80-116 分留管	(1)				
	(19/38-24/40-200m/m)					
15	エッペンドルフピペット		ｴｯﾍﾟﾝﾄﾞﾙﾌ			
	1)2〜10μℓ 4710			2ヶ	36,000	72,000−
	2)10〜100μℓ 〃			2ヶ	36,000	72,000−
	3)100〜1,000μℓ 〃			2ヶ	36,000	72,000−
16	エッペンドルフスタンダードチップ		ｴｯﾍﾟﾝﾄﾞﾙﾌ			
	1)黄 1000ヶ入			5式	9,500	47,500−
	2)青 1000ヶ入			5式	9,500	47,500−
17	デンシトメーター		コスモ	1式		2,710,000−
	D-607(220V 50Hz)					
	標準附属品付					
	(消耗品約一年間分)					
	1)記録紙 500samples×10Boxes			5式		106,500−
	2)ランプ, 5ケ入			1式		2,900−
	3)カバー			3ヶ	300	900−

番号	品名及び仕様	メーカー名	数量	単価	金額
	4)カセットグラス		2ヶ	4,400	8,800—
18	バイオクロマト自動分析装置	アトー	1式		1,540,000—
	ACC-103C(220-240V)				
	構成：				
	1)AC-5000 バイオ紫外線吸収モーター (1)				
	2)SJ-3462 ミニレコーダー (1)				
	3)SJ-1410 SRミニコレクター (1)				
	4)AC-2300 バイオカラム MP-2590 (1)				
	5)SJ-1215 ペリスタポンプ (1)				
	6)配管部品 (1式)				
19	一般焼却炉	インシナー	1台		302,000—
	A1型				
	寸法：外径φ0.58m				
	内径φ0.48m				
	高さ1.05m				
20	孵卵器，デジタルタイプ	平山	1式		410,000—
	F-100D(220V,50Hz)				
	容量：375ℓ				
	内寸法ぽ：100×75×50cm				
	使用温度：常温～55℃				
21	孵卵器，自然対流式高精度	平山	1式		570,000—
	FTW-502(220V,50Hz)				
	内寸法：50×50×50cm				
	上下2室型				
	使用温度：常温～55℃				
22	自動天秤	日本クレア	1台		114,000—
	CL-4505				
	サイズ:310×400×640m/m				
	秤量：6 kgs				

番号	品名及び仕様	メーカー名	数量	単価	金額
	最小目盛：10g				
23	UVトランスイルミネーター	ﾌﾅｺｼ薬品	1台		140,000―
	TM-15				
	UV波長：302mm				
	UV強度：8000μw/cm²				
	（フィルター表面）				
24	ドットブロッティング	ﾊﾞｲｵﾗｯﾄﾞ	1式		159,000―
	バイオドット				
	オートクレーブ処理可，塩酸				
	塩基使用可，アルコール使用可				
	デンシトメーターの適応：適				
	ｵｰﾊﾞｰﾅｲﾄｲﾝｷｭﾍﾞｰｼｮﾝ可				
	サンプルウェル：96ウェル(8×12)				
	ウェルの大きさ：3mm				
	サンプル量：50～600 μℓ				
25	水流ポンプ	井　内	1ヶ		2,400―
	（アスピレーター）金属製				
	附属品				
	1)ゴムソケット		1ヶ		200―
	2)トラベット（逆流防止用）		1ヶ		1,600―
	合　　計				¥22,720,000―
	消　費　税（3％）				¥ 681,600―
	総　合　計				¥23,401,600―

番号	品名及び仕様	メーカー名	数量	単価	金額
26	電子走査形超音波診断装置	SAL-38B	1式		
	1. 電子走査形超音波診断装置	SAL-38B	1		4,300,000−
	1)本体（参考信号ユニット付）				
	2)コンベックスキャンプローブ	PVB-358M	(1)		
	3)メカニカルセクタプローブ	SM-308S	(1)		
	4)9"モニター		(1)		
	5)標準附属品（ゼリー350g入1本付）		(1)		
	2. 5.0MHzリニア電子プローブ	PLB-505S	1		720,000−
	3. リニア電子スキャンプーブ	PLB-308M	1		720,000−
	4. メカニカルセクタプローブ	SM-508S	1		720,000−
	5. ソノプリンター	TP-8300	1		216,000−
	6. 電源安定器		1		108,000−
	7. ゼリー(5,000g)		10	13,500	135,000−
	8. ソノペーパー(6rolls/box)	U-215	10	8,100	81,000−
				合計	7,000,000−
				消費税(3%)	210,000−
				納入価格	7,210,000−

現 地 調 達 品 目 リ ス ト

	現地調達希望品目		現地での見積額	
	＜医薬品＞			
1	塩化ナトリウム	250g×10	US$	84.^{50}
2	塩化カリウム	1kg×10	US$	309.^{50}
3	リン酸二ナトリウム	500g×10	US$	247.^{10}
4	リン酸一カリウム	250g×10	US$	239.^{00}
5	氷酢酸	1ℓ×10	US$	243.^{50}
6	塩化アンモニウム	100g×10	US$	50.^{40}
7	トルエン	2,500mℓ×10	US$	552.^{10}
8	トリクロル酢酸	500g ×15	US$	721.^{35}
9	油浸オイル	60mℓ×10	US$	44.^{50}
10	エタノール	1ℓ×15	US$	370.^{95}
11	アセトン	1ℓ×15	US$	187.^{65}
12	塩化マグネシウム	1kg×10	US$	506.^{90}
13	EDTA	500g×5	US$	513.^{80}
14	ギムザ酸	500mℓ×20	US$	464.^{00}
15	塩酸	1ℓ×30	US$	284.^{40}
16	トリエタノールアミン	250mℓ×10	US$	42.^{10}
17	乳酸カルシウム	250mℓ×10	US$	374.^{70}
18	硫酸アンモニウム	50×10	US$	194.^{00}
29	ホウ酸	500g×10	US$	174.^{80}
20	重炭酸ナトリウム	5g×15	US$	1,463.^{25}
21	酢酸ナトリウム	250g×10	US$	125.^{00}
22	プロピオン酸	500mℓ×10	US$	461.^{10}
23	2－プロパノール	1ℓ×10	US$	224.^{80}
24	メタ過沃酸ナトリウム	20g×10	US$	505.^{40}
25	ヨード酢酸	100g×8	US$	3,152.^{00}
26	水酸化ナトリウム	250g×10	US$	119.^{20}
27	IgM	5mℓ×60	US$	9,330.^{00}
28	IgA	2mℓ×60	US$	9,330.^{00}

	現地調達希望品目		現地での見積額
29	IgD	×60	US$ 9,330.00
30	C_3 キッド	×60	US$ 9,330.00
31	C_4 キッド	×60	US$ 9,330.00
32	メディウムRPMI1640	100ℓ×10	US$ 4,346.00
33	フィトヘマグルチニン	10mℓ×10	US$ 160.80
34	L－グルタミン	200g×10	US$ 422.40
35	Hepes	200g×10	US$ 512.50
36	牛胎児血清	500mℓ×10	US$ 4,200.00
37	マウス血清	1mℓ×10	US$ 903.60
38	モルモット血清	20mℓ×10	US$ 1,154.50
39	ウサギ血清	100mℓ×10	US$ 489.60
40	トリプシン	20mℓ×10	US$ 102.00
41	トリパンブルー	20mℓ×10	US$ 44.40
42	ゲンタマイシン	10mℓ×10	US$ 171.10
43	ｽﾄﾚﾌﾟﾄﾏｲｼﾝﾍﾟﾆｼﾘﾝGｶﾘｳﾑ	20mℓ×10	US$ 70.80
44	ポリミキシンB	20mℓ×10	US$ 118.40
45	アジュバントインコンプリート	20mℓ×10	US$ 1,184.00
46	アジュバントコンプリート	10mℓ×10	US$ 996.00
47	マイトゲンPWM	5mℓ×10	US$ 205.20
	<医療機材>		
48	液体窒素用タンク	34ℓ×10	G 8,500,000.00
49	フラスコ（細胞培養用）	50mℓ× 5,000	US$ 6,870.00
50	〃	250mℓ× 1,000	US$ 2,840.00
51	遠沈管	50mℓ× 5,000	US$ 4,050.00
52	〃	15mℓ× 5,000	US$ 2,820.00
53	プラスチックピペット	5mℓ× 2,000	US$ 2,300.00
54	〃	10mℓ× 2,000	US$ 2,900.00
55	組織培養デイッシュ	×10,000	US$ 5,800.00
56	マイクロチューブ	15mℓ×50,000	US$ 2,750.00
57	〃	1mℓ×50,000	US$ 2,750.00

	現地調達希望品目		現地での見積額
58	セラムチューブ	×50,000	US$ 6,700.00
59	棚 1×1.8 ×0.32	×10	G 855,000.00
60	キャビネット（小）		
	1.6×1×0.42	×5	G 520,000.00
61	〃 （大）		
	1.1×1.8 ×0.42	×10	G 1,260,000.00
62	作業机スチール製		
	1.9×0.9 ×0.9	×10	G 2,750,000.00
63	収納棚（大）スチール製		
	1×2×0.32	×10	G 530,000.00
64	本棚スチール製		
	1×1.2 ×0.32	×10	G 410,000.00
65	収納棚（中）スチール製		
	1×1.8 ×0.32	×10	G 995,000.00
	小　計		Gs139,252,294
	（追加現地調達品目）		
66	オリンパスCHSシステム顕微鏡	1台	US$ 2,300.00
	平成元年度供与機材現地調達予算額 （示達済元年6月27日決裁）		14,000,000 円

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