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PRODUCTION OF MONOCLONAL ANTIBODIES.

A JOINT EFFORT IN LATIN AMERICA.

DP/RLA/83/003 PROGRAMA REGIONAL DE BIOTECNOLOGIA

PNUD/UNESCO/ONUDI PARA AMERICA LATINA Y CARIBE.

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FINAL REPORT.

1. PARTICIPATING LABORATORIES:

Laboratories from twelve countries,-Argentina, Bolivia, Brasil, Chile, Colombia, Costa Rica, Cuba, Equador, Guatemala, Mexico, Uruguay, and Venezuela-, were involved in the project. A detailed list can be found in ADDENDUM 1.

2. BACKGROUND:

When the representative of the twelve participating laboratories listed above met in 1988 to discuss and elaborate a common project dealing with the large-scale production of Monoclonal Antibodies (MAbs) using cell culture technology, it came clear the diversity in development levels, both in terms of facilities and existing know-how, and even in the immediate aspirations of each one. At the time, some laboratories did not even dare to dream of achieving a technology of this kind, but the final common denominator was the will of each one to participate and make the necessary efforts. The possibility of working together was also a strong motivation that drove all the countries to get involved in the project.

At that point it also became clear that to bridge the technical and scientific gap, the best strategy was to align with the most advanced laboratories, and to propose ambitious but nevertheless realistic goals, that would necessarly "force" the development of a more homogenous technological level in the region.

Two out of the twelve countries had by that time initiated efforts, using other funding sources, for the construction of adequate facilities, the acquisition and installment of equipment, and the formation of human resources for the development of high density hybridoma culture technology and the purification of MAbs. These two laboratories, the Hybridoma Department of the Centro de Ingenieria Genética y Biotecnologia, in Habana, Cuba, and Centro de Biotecnologia, of the Instituto Butantan, in Sao Paulo, Brazil, were designated as reference units, and committed to receive trainees from the other ten countries. Even more prepared than the rest, Cuba and Brazil were not yet ready to produce MAbs "in vitro" in the order of grams, and such a project would also bring benefits to these laboratories.

The other participating laboratories could be divided in two categories: the ones already generating hybridomas and producing very small amounts of MAbs for research purposes, and a second group with interest in the field, but very little, or no previous experience at all, in hybridoma generation and MAbs production.

3. OBJECTIVES OF THE PROJECT:

The project had the following general and specific objectives:

3.a. General:

3.a.1. To promote the cooperation among Latin American countries in order to achieve the necessary technological development for the large-scale production of MAbs.

3.a.2. To motivate:
- the cooperation in research and obtention of products
- the development of assays for the use and validation of such products.
- the regional integration for production and commerce

These objectives intended to increase the region's technological autonomy and provide resources which could result in the solution of inherent problems in areas of human, plant and animal health.

3.b. Specific:

3.b.1. To optimize the technological procedures for the large-scale production of MAbs and to compare the available methodologies.

3.b.2. To transfer the technical information derived from the fullfillment of the aforementioned aspect, in the form of technical reports.

3.b.3. To rationalize the installed regional capacities by creating two reference laboratories for large-scale culture production of MAbs.

3.b.4. To strenghthen the participating laboratories in dependence of their development status.

3.b.5. To produce, in large scale, MAbs with social and/or economical importance in the following areas:
- the diagnosis of tropical and infectious diseases.
- tumor diagnosis and treatment, and transplantation therapeutics.
- the diagnosis of plant and animal pathogens.

4. DEVELOPMENT OF THE PROJECT:

4.1. Trainings and production phases:

The project was conceived as to be developed in two phases, a first one for training, and a second for production.

4.1.1. Training phase:

Before the initiation of this phase, the reference

laboratories of Brazil and Cuba exchanged acquired experience, with reciprocal visits of scientists.

In this phase, the reference laboratories had the responsability of receiving and training investigators coming from the ten countries, in groups of two to four. Each country would send one same or different trainees, for a three month period, to both Brazil and Cuba.

It was agreed that the trainee(s) would already have some basic knowledge of hybridoma technology or, at least, cell culture training. As Bolivia and Guatemala had not two investigators with such qualification, it was agreed that these countries would send students to receive basic training in Argentina and Mexico, respectively, instead of travelling to Brazil and Cuba. With this arrangement Bolivia and Guatemala had the opportunity of receiving a more basic training while participating in the original project. The choice of Argentina and Mexico as receiving countries was due to geographical reasons, thus reducing travel costs and saving funds for laboratory needs.

At the end of this training phase, Cuba and Brazil had received students from nine countries each (in three groups).

The strategy for this phase involved the work in the problems inherent to the technology of scaling up cell culture, the downstream processes and the evaluation of purity grade of antibodies after chromatography. This phase also included regular seminars in specific themes as:
- MAbs and their application in biotechnology
- cell culture devices and bioreactors (spinners, hollow fibers, alginate entrapment, airlift)
- purification methods for MAbs
- human and second generation MAbs
- quality control for therapeutic MAbs
- dissolved oxygen in cell culture
- antibodies with enzymatic activity
- serum free media for cultivation of hybridomas
- anti-idiotypic antibodies

Besides these, various other scientific themes were covered, including particular research projects in course in Brazil and Cuba.

4.1.2. Production phase:

For this phase the strategy was that each one of the ten countries would send one person, already trained in the first phase, to Cuba or Brazil, for the actual production of selected MAbs. The following schedule was defined:
- Countries sending persons to Cuba: Chile, Ecuador, Guatemala, Mexico and Uruguay (2 groups, 3 months each).
- Countries sending persons to Brazil: Argentina, Bolivia,

Colombia, Costa Rica and Venezuela (2 groups, 3 months each).

It should be mentioned that one of the groups to work in Brazil (Colombia and Costa Rica) did not participate in the production phase. Colombia did not sent a person for a particular reason of their own, and Costa Rica sent a person that had not participated in the training phase or had basic cell culture knowledge. As this scientist had other interests in the Butantan's Institute, it was agreed with the director of the participating laboratory that this person would receive training in a sera production unit. In the case of Colombia, one scientist will be sent to Butantan to work in high density cell culture for vaccine production.

The MAbs produced in Cuba were specific for CD3 and alpha 2b interferon. The MAbs produced in Brazil were against CD3 and the A human blood group. The anti-alpha 2b interferon hybridoma had been generated in Cuba, the anti-A hybridomas in Argentina and Brazil, and the anti-CD3 hybridomas were bought from ATCC.

4.2. Meetings:

Four meetings took place between September 1988 and September 1991. In the first meeting the representative of the 12 participating laboratories discussed and elaborated the project to be submitted to UNIDO/UNESCO. The training schedules were defined in the second meeting, in the Spring of 1989.

In the third meeting (August 1990) the project was critically elavuated, with very positive results. This meeting was meant to be the last one but there was a consensus to continue the collaboration, as the project had created bonds between countries and others kinds of cooperation were initiated. This finally led to an extraordinary fourth meeting with two main purposes: to make a final evaluation of the project and to elaborate a "Phase II" project, to be submitted in the near future.

5. RESULTS:

As a general result, the laboratories representative of ten countries received the proposed training (training phase). The production phase had some minor problems with two countries (Colombia and Costa Rica), but the results were judged as very satisfactory by the countries who attended to two phases. On the whole, the objectives originally proposed were accomplished.

The trainees learned how to grow hybridomas from the small, to the medium, to large scale, in several culture devices, and bioreactor models, how to handle MAbs, how to assay and to verify production, how to purify, and how to evaluate the whole process. In the production phase they manipulated the bioreactors by themselves, are now apt to start to work in this field in their countries, and even to choose which equipment is more adequate to

their particular purposes.

Each training and production group elaborated technical reports that were distributed amongst all participating laboratories. A preliminary evaluation of several bioreactors for large-scale production of MAbs in culture was done in the reference laboratories, and some related information can be found in ADDENDUM 2.

One important consequence of the Project was that the participating laboratories gained conscience that, when deciding upon large-scale culture for the production of MAbs, the bioreactors are only part of the problem, as a whole backup unit dealing with media preparation and control, establishment of a cell bank with adequate requirements, the design of an efficient downstream process, and process and quality control measures, can be even more resource-, and time-consuming than the operation of the bioreactors.

6. CONCLUSIONS:

6.1. A significant technological improvement was achieved in the participating laboratories.

6.2. Latin American countries have the capability and conditions to evaluate advanced technology and diffuse know-how.

6.3. Reference laboratories for large-scale culture production of MAbs were created in the region.

6.4. The strategy of defining goals of high technological level, as starting point for the project, prooved to be correct.

6.5. Scientific and technological cooperative bonds were created as a by-product of the project.

6.6. Apart from the direct impact of the technological development achieved by each laboratory, the project helped the units in their effort to obtain additional national and international support for their activities (see ADDENDUM 3 for examples).

6.7. There is a strong need for a further continuation of the technological development, and the scientific links, created by this project. Such future efforts should take the form of:

(a) A "Phase II" Project (ADDENDUM 4)
(b) An information network dealing with the MAbs in development or production in the region (ADDENDUM 5)
(c) The organization of basic and advanced courses on MAb technology (ADDENDUM 6), and the edition and publication of related Manuals.

ADDENDUM 1.

PARTICIPATING LABORATORIES AND REFERENCE NAMES.-

Argentina.-
Dr. Alberto Horenstein, Centro Oncológico de Medicina Nuclear, Inst. A.H. Roffo, Buenos Aires.

Venezuela.-
Dr. Gustavo Ortega, Instituto de Biomedicina, Caracas.

Bolivia.-
Dr. Heriberto Cuevas, Instituto de Investigaciones Biomédicas, Academia Nacional de Ciencias, La Paz.

Brazil.-
Dr. Isaias Raw, Dr. Ana M. Mcro, Centro de Biotecnología, Instituto Butantan, Sao Paulo.

Chile.-
Dr. Luis A. Ferreira V., Facultad de Medicina, Universidad de Chile. Santiago de Chile.

Costa Rica.-
Dr. Bruno Lomonte, Instituto Clodomiro Picado, Universidad de Costa Rica, San José.

Cuba.-
Dr. Jorge V. Gavilondo, Centro de Ingeniería Genética y Biotecnología, La Habana.

Ecuador.-
Dr. Luis E. Plaza, Instituto Ecuatoriano del Seguro Social, Guayaquil.

Guatemala.-
Dr. Ricardo Luján, Instituto de Investigaciones, Universidad del Valle de Guatemala, C. Guatemala.

México.-
Dr. Lourival D. Possani, Centro de Investigación sobre Ingeniería Genética y Biotecnología, UNAM, Cuernavaca

Uruguay.-
Dr. Alberto Nieto, Facultad de Química, Universidad de la República, Montevideo.

Colombia.-
Dr. Oscar Orozco, Instituto Nacional de Cancerologia, Bogota.
Dr. Luis Guillermo Parral, VECOL, Bogotá.

ADDENDUM 2.

SOME EXAMPLES OF CULTURE PROCESSES IN BIOREACTORS FOR THE PRODUCTION OF MONOCLONAL ANTIBODIES.-

Introduction.-

Several culture devices (roller bottles, "spinner" flasks bearing encapsulated or free cells, and dialysis tube high density culture), and four types of bioreactors, were employed in the Project. Among the latter, three models of hollow-fiber bioreactors (Endotronic's Acusyst "R" and "Jr." models, and Amicon's Vitafiber II), and one airlift bioreactor (Bellco's) were studied.

These four bioreactors belong to the so-called "high-density" systems for cell culture, which have gained increasing general acceptance for MAb production by institutions and private companies. Each equipment has its advantages and drawbacks, that depend on the hybridoma to be grown, the level of production desired, the design of the downstream process, and the final application of the MAb.

Hollow-fiber reactors, disregarding the differences between manufacturers, mimetize the growth of cells in tissues. Very high densities are obtained, and the process that leads to the supplement and removal of metabolites and catabolites is similar to what occours in the body (difussion through a capillary network), and defines extracapillary (cells) and intracapillary (culture medium) spaces. The provision of convenient molecular weight cut-offs in the hollow fibers makes it possible to harvest the antibodies in high concentration and low volumes. The high cell densities achieved, produce an extracapillary environment in which the serum requirements can be reduced. This same high cell density makes critical any problem in the operation of the medium renewal system, as cell death rapidly occours.

In all models tested, some cells escape from the extracapillary space unto the harvest. Preliminary experiments indicate that the DNA levels of the supernatants can be very high, as the culture "matures", the bioreactor cartridge fills up with cells, and cell death increases. This can be a drawback for MAbs to be used in humans, and additional purification measures have to be implemented.

Even though high cell densities can be achieved in the airlift model studied in the Project, this system belongs to a more conventional type of fermentor, more like the "homogeneous" types of bioreactors in which there is no physical separation between media and cells. It is based in the growth of hybridoma cells settled on the surface of hundreds of small glass cylinders,

deposited in a 3-liter vase. Complete medium is pumped through-all the vase, and aeration is provided by a sparging device. Thus, the harvest volumes are large, the total need of serum increases, and the antibodies are more diluted. The latter makes necessary the use of a concentration step before chromatography.

The way in which the cells colonize the growing space allows a more "relaxed" control of the equilibrium of the system, as operational problems have a slower impact in cell viability.

The objective of this report is only to exemplify several successfull productions performed in these types of bioreactors. The Project provided each participating laboratory with the preparation to decide in their selection, based upon their needs and conditions.

From a general point of view, our experience is that the information supplied by all manufacturers is incomplete, in the sense that not an enough number of examples representative of all the spectrum of possible behaviours of different hybridomas is supplied. This situation leads to the need of some previous testing, that is not usually available for Latin American laboratories.

Also, the operating manuals are complex, and insufficient in contents, specially in relation to trouble-shooting, and process interpretation, essential for the control of the parameters that determine cell growth, as the scaling-up is not simple, nor can the data obtained from conventional culture can be readily extrapolated.

Example # 1.- Airlift Bioreactor.

(1) Manufacturer and Model: Bellco, Programmable Airlift Bioreactor
(2) Hybridoma: OKT-3 (ATCC)
(3) Culture medium and additives: DME (Sigma), supplemented with beta-mercaptoethanol, antibiotics, and 0.2% ethanol. Fetal bovine serum (Cultilab).
(4) Inoculum: 10^9 cells
(5) Total days of the process: 30 days
(6) Total harvest days: 30 days
(7) Total harvested volume: 69 liters
(8) Total consumption of medium: 50 liters
(9) Total consumption of serum: 20 liters
(10) Total amount of MAbs after purification (Lowry): 3.15 grams

Example # 2.- Hollow Fiber Bioreactor.

(1) Manufacturer and model: Amicon, Vitafiber II
(2) Hybridoma: anti-human B blood group (Brazil)
(3) Culture medium and additives: RPMI 1640 (Sigma), supplemented with beta-mercaptoethanol and antibiotics. Fetal bovine serum (Cultilab).
(4) Inoculum: 4.4×10^8 cells
(5) Total days of the process: 25 days
(6) Total harvest days: 20 days
(7) Total harvested volume: 165 ml
(8) Total consumption of medium: 20 liters
(9) Total consumption of serum: 1.1 liters
(10) Total amount of MAbs: undetermined. The hemaglutination titer of the crude supernatant reached 4,000,000 after 16 days of harvest.

Example # 3.- Hollow Fiber Bioreactor.

(1) Manufacturer and Model: Endotronics, Acusyst-R
(2) Hybridoma: CB-IFNA2.4 (Cuba)
(3) Culture medium and additives: RPMI 1640 (Gibco), supplemented with 3 g/liter of glucose, L-glutamine, sodium pyruvate, and antibiotics. Colostrum-free newborn bovine serum (CubaVet).
(4) Inoculum: 150×10^6 cells
(5) Total days of the process: 76 days
(6) Total harvest days: 62 days
(7) Total harvested volume: 4.132 liters
(8) Total consumption of medium: 560 liters
(9) Total consumption of serum: 233 ml
(10) Total amount of MAbs after purification (Lowry): 7.0 grams

Example # 4.- Hollow Fiber Bioreactor.

(1) Manufacturer and Model: Endotronics, Acusyst-Jr.
(2) Hybridoma: CB-IFNA2.4 (Cuba)
(3) Culture medium and additives: DME (Gibco), supplemented with L-glutamine and sodium pyruvate, and antibiotics. Colostrum-free newborn bovine serum (CubaVet).
(4) Inoculum: 1×10^9 cells
(5) Total days of the process: 60 days
(6) Total harvest days: 53 days
(7) Total harvested volume: 4.0 liters
(8) Total consumption of medium: 300 liters
(9) Total consumption of serum: 400 ml
(10) Total amount of MAbs before purification (ELISA): 10 grams

ADDENDUM 3.

IMPACT OF THE PROJECT IN THE DEVELOPMENT OF THE PARTICIPATING LABORATORIES.-

Apart from the direct impact of the technological development achieved by each laboratory, the project helped the units in their effort to obtain additional national and international support for their activities. The following information was provided by different laboratories.

GUATEMALA.-

The Project promoted the allocation of funds from the National Committee on Biotechnology (CONBIOTEC) to this laboratory, for the development of trainings in nearby countries and the organization of a national seminar on Electrophoretic Techniques. Tight research links were established with the Center for Genetic Engineering and Biotechnology of Cuernavaca, Mexico, that has offered this laboratory possibilities for post-graduate training.

BOLIVIA.-

The development of such Project created an atmosphere of support that led to the allocation of additional funds, and the promotion of courses.

CHILE.-

The Project helped to get the approval of additional funding sources (WHO, SAREC, and CONACYT) that amount some USD 50,000, and to gain support from the University and the government for the presentation of a Project to the BID. The achievement of a superior technological status by the participating laboratory has made it possible to establish interactions with other research centers and enterprises.

URUGUAY.-

The participating laboratory received support for the construction of a 80 sq.m. unit for cell culture, with defined areas for bioreactors and purification. The scientists that assited to the training and production phases of the Project have been able to teach new personel, in order to gain the acceptance of a fellowship at the Biomedical Center of the Uppsala University, in Sweden.

ARGENTINA.-

The participating laboratory gained access to additional funds from CONICET, CABBIO, and IAEA. The Project promoted the

definition by institutions and medical centers of the need of a scaling-up unit for the production of MAbs to be used in diagnosis and treatment of infectious, neoplastic, and hematological diseases.

MEXICO.-

The laboratory was awarded USD 10,000 for additional facilities for cell culture, and USD 20,000 for equipment. The UNAM also approved a research project for the use of stirred tank technology for the culture of hybridomas, and contracted an experienced Mexican scientist, trained in the USA. A scientific collaboration was developed with the Universidad del Valle, Guatemala, as a collateral result of the training of a scientist in Mexico for the production phase of the Project.

VENEZUELA.-

The Project gave speed to the investigations dealing with the generation of MAbs for Mycobacterium leprae, and the establishment of collaborative links with a Colombian institution. Also, the theme of large-scale culture of hybridomas and MAbs production was elected as Doctoral thesis for one of the co-workers of the laboratory.

ECUADOR.-

The Project promoted a superior technical status for the laboratory, that has led to its participation as host in basic immunology courses.

BRAZIL.-

The Project helped to obtain additional substantial funds from a Brazilian Agency (FINEP). Also, accelerated the improvement of the facilities, and the development of the human resources of the participating laboratory.

CUBA.-

The Project led to the allocation of four new laboratories for large-scale culture, and purification of MAbs. Funds for new equipment were also approved, after the technology tested in the Project demonstrated its usefullness. Other Cuban institutions profited from the acquisition of know-how, through direct training (Nat. Ctr. for Bioreagents), or advisorship (Ctr. for Molecular Immunology). The success in training foreign students in the reference laboratory enhanced the teaching capabilities of the unit for future courses organized by the Center.

ADDENDUM 4.

PROJECT TITLE

Production of Monoclonal Antibodies. A Joint Effort in Latin America. Phase II.

BACKGROUND

In 1989, representatives of 12 laboratories from Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, Guatemala, Mexico, Uruguay and Venezuela met in Sao Paulo to elaborate the details of a Project entitled to increase the technological capability of Latin American countries for the production of Monoclonal Antibodies (MAbs).

The Project, denominated "Large-scale Production of Monoclonal Antibodies. A Joint Effort in Latin America", took into account the relative differences in development in this field amongst the participating countries, and designed a strategy to achieve the following main objectives:

(a) optimization of technological procedures for the large-scale production of MAbs, based essentially in a variety of cell culture methods, and including the use of bioreactors

(b) transference of technology and know-how, through technical protocols and direct training

(c) rationalization of existing capacities in the region, by the creation of reference laboratories that would evaluate, develop, and transfer technology

(d) pilot production of mouse MAbs with social or economical relevance for the region

(e) transference of basic know-how on the generation of mouse MAbs

With UNIDO/UNESCO funds, the Project was developed for 30 months (1989 to 1991): (a) reference laboratories for medium-scale (10 or more grams per month) production of mouse MAbs using bioreactors were created in Brazil and Cuba, (b) scientists from Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Uruguay, and Venezuela were trained in the use of different culture technologies for MAbs production, (c) gram quantities of relevant mouse MAbs were produced, and (d) additional know-how for the generation of mouse and human MAbs was diffused.

Important scientific interactions developed as a by-product of the intense exchange of researchers that amounted

to 15 visitors to each reference laboratory, in groups of two to four each.

As a whole, a significant increase in the technological capabilities of the participating laboratories was achieved, when compared with the start-up level. These results have prompted the group to agree in the convenience of a "Phase II" Project that would represent a further step in providing the region with a self-sufficiency status in the fields of generation and production of MAbs intended for "in vitro" and "in vivo" diagnosis, and human therapy.

The group also has considered essential the accelerated acquisition of new technological advances related to the modification of antibodies by genetic engineering, that will determine in the immediate future the quality of the MAbs to be used in humans. The existence of a laboratory in the region that has already acquired such basic know-how is seen as a very positive step for the fast implementation of this task.

STRATEGY AND OBJECTIVES

The basic strategy of the "Phase II" Project runs along the same lines as the one used for the previous one, that is to say, to differentiate and further support laboratories in accordance with the achieved level, so as to rapidly provide the region with a self-sufficient status for production capability and know-how transfer, and, at the same time, to promote the fast achievement of a minimum scientific and technological level in all countries, essential for interaction and potentiation.

To achieve these goals, the "Phase II" Project has the following objectives:

1. To provide Bolivia, Guatemala, Costa Rica, and Ecuador with full technical and know-how capabilities for the generation and characterization of mouse MAbs.

2. To furnish Argentina, Chile, Uruguay, Venezuela and Mexico with laboratory-scale (up to 1 g/month) capability of producing MAbs in culture, using bioreactors.

3. To achieve in the reference laboratories of Brazil and Cuba, and in Argentina, the necessary levels for the production of MAbs for human therapy and "in vivo" diagnosis, according to national and international regulations.

4. To create a reference lab in Cuba for the difussion of basic know-how in the techniques for the modification of antibodies using genetic engineering.

ACTIONS

The aforementioned objectives can be detailed in terms of "actions" to be taken, and indicators of success:

1. To complete the reference laboratories in Cuba and Brazil with the requirements and know-now for the production of MAbs intended for human use, according to national and international regulations.

Such technological status would be demonstrated with pilot productions of at least one mouse MAb per laboratory, approved for a national clinical pilot study. The reference laboratories would diffuse the necessary know-how, and be willing to negotiate trainings, and discuss productions with other countries or groups from the region, on collaborative or contractual basis.

2. To create a reference laboratory in Argentina with the requirements and know-how for the radiolabelling of antibodies and antibody fragments for "in vivo" diagnosis.

Such technological status would be demonstrated with pilot productions of MAb fragments radiolabelled with Tc, In, or I, approved for a national clinical pilot study. The reference laboratory would diffuse the necessary know-how, and be willing to negotiate trainings, and discuss productions with other countries or groups from the region, on collaborative or contractual basis.

3. To complete the laboratories in Argentina, Venezuela, Chile, Mexico, and Uruguay with the facilities for laboratory-scale (up to 1 g per month) production of MAbs in bioreactors.

Such status would be demonstrated by pilot productions of at least one MAb. The laboratories would be willing to negotiate trainings with other countries or groups.

4. To complete the laboratories in Costa Rica, Ecuador, Bolivia and Guatemala with the facilities and know-how for the generation and characterization of mouse MAbs.

Such action would involve the immediate development of joint tasks and trainings in more advanced laboratories from Colombia, Mexico, Argentina, Uruguay, Brazil, Chile, and Venezuela. These supporting laboratories would receive scientists from the four aforementioned countries for six months.

5. To complete the facilities of the laboratory in Cuba so as to increase the speed of development of new "second generation MAbs", using genetic engineering techniques, and allow the start of technology transfer programs.

This laboratory will accept trained scientists for six-month periods, so as to provide them with knowledge related

to the PCR cloning of immunoglobulin variable regions, expression of antibody fragments in bacteria, and expression of modified antibodies in eukaryotic cells.

6. To celebrate two technical and administrative meetings (additional to the one required for the final formulation of the Project). As an additional way to guarantee a critical evaluation of the technical advance of the Project, it has been thought convenient to organize a "satellite" workshop in one of these meetings, in which a scientist representative of each laboratory will expose and discuss in detail the course of the respective tasks.

EXTENSION

The Project is foreseen to be developed in two years (24 months).

PARTICIPANTS

(1) Argentina.- Dr. Alberto Horenstein, Centro Oncológico de Medicina Nuclear, Inst. A.H. Roffo, Buenos Aires.
(2) Venezuela.- Dr. Gustavo Ortega, Instituto de Biomedicina, Caracas.
(3) Bolivia.- Dr. Heriberto Cuevas, Instituto de Investig. Biomédicas, Academia Nacional de Ciencias, La Paz.
(4) Brazil.- Dr. Isaias Raw, Centro de Biotecnología, Instituto Butantan, Sao Paulo.
(5) Chile.- Dr. Luis A. Ferreira V., Facultad de Medicina, Universidad de Chile. Santiago de Chile.
(6) Costa Rica.- Dr. Bruno Lomonte, Instituto Clodomiro Picado, Universidad de Costa Rica, San José.
(7) Cuba.- Dr. Jorge V. Gavilondo, Centro de Ingeniería Genética y Biotecnología, La Habana.
(8) Ecuador.- Dr. Luis E. Plaza, Instituto Ecuatoriano del Seguro Social, Guayaquil.
(9) Guatemala.- Dr. Ricardo Luján, Instituto de Investigaciones, Univ. del Valle de Guatemala, C. Guatemala.
(10) Mexico.- Dr. Lourival D. Possani, Centro de Investigación sobre Ingeniería Genética y Biotecnología, UNAM, Cuernavaca
(11) Uruguay.- Dr. Alberto Nieto, Facultad de Química, Universidad de la República, Montevideo.
(12) Colombia.- Dr. Oscar Orozco, Inst. Nac. Cancerologia, Bogota. Dr. Luis Guillermo Parral, VECOL, Bogotá.

REQUIRED FUNDS

The Project would require approximately USD 1,000,000 from an international source.

TENTATIVE DETAILED DISTRIBUTION OF FUNDS
(see ACTIONS in document)

Action 1.-- Subtotal: USD 120,400
Comments:
(1) For laboratory equipment, reagents and expendables: USD 55,000 for each reference laboratory.
(2) For exchange of scientists:
a.- Trips, board, and lodging (one reciproque visit of two weeks per year, per laboratory; USD 2,200 for ticket, USD 200 per week, per person): USD 10,000

Action 2.-- Subtotal: USD 30,000
Comment: For laboratory equipment, reagents, and expendables.

Action 3.-- Subtotal: USD 300,000
Comment: For laboratory equipment, reagents, and expendables: USD 60,000 for each laboratory.

Action 4.-- Subtotal: USD 276,200
Comments:
The following preliminary scheme of visits was designed: Colombia for Costa Rica, Mexico for Guatemala and Ecuador, Argentina for Ecuador and Bolivia, Brazil for Bolivia, Uruguay for Ecuador, Chile for Bolivia, Venezuela for Guatemala.

(1) For supporting laboratories (Brazil, Argentina, Colombia, Venezuela, Uruguay, Chile, and Mexico).-
a.- Bench fees (USD 1,000 per visitor, per month).
Total: USD 54,000
b.- Expendables (USD 10,000 per lab.). Total: USD 70,000

(2) For the laboratories to receive training (Ecuador, Bolivia, Costa Rica, Guatemala).-
a.- Trips, board and lodging (an average of USD 1,000 per ticket, USD 800 per visitor, per month).
Total: USD 52,200
b.- Expendables and minor equipment (USD 25,000 per lab).
Total: USD 100,000.

Action 5.-- Subtotal: USD 172,000
Comments:
(1) For reference laboratory (Cuba).-
a.- Bench fees (USD 1,200 per visitor, per month).
Total: USD 28,800
b.- Minor equipment and expendables. Total: USD 60,000

(2) For the laboratories sending visiting scientists (a maximum of four visitors, to be selected by the group from candidates presented by Venezuela, Brazil, Argentina, Chile, Uruguay, and Mexico).-

a.- Trips, board, and lodging (an average of USD 1,000 per
ticket, USD 800 per visitor, per month).
Total: USD 23,200

b.- Expendables and minor equipment (USD 15,000 per lab.).
Total: USD 60,000

Action 6.-- Subtotal: USD 76,000
Comments: three meetings (including the "satellite" workshop as part of the second meeting) of the 12 laboratories. USD 66,000 for trips, board and lodging of visitors, and USD 10,000 (USD 2,000 per the host laboratory, per meeting, plus an additional USD 4,000 for the laboratory hosting the workshop) for organization expenses.

Information Exchange for the Control of Project Development.
-- Subtotal: USD 24,000
Comments: USD 1,000 per year, per laboratory.

TOTAL: USD 998,600

ADDENDUM 5.-

PROPOSAL FOR THE CREATION OF A MINIMAL DATABANK REGARDING MONOCLONAL ANTIBODIES IN DEVELOPMENT OR PRODUCTION IN LATIN AMERICAN COUNTRIES.-

BACKGROUND AND OBJECTIVES

The lack of information dealing with Monoclonal Antibodies (MAbs) in development and production in the Latin American region seriously affects the possibilities of scientific and technical interaction and complementation, as well as exchange and commerce of these reagents.

This Proposal involves the creation of a simple structure that would help in the recollection, processing, and diffusion of a minimum of information that would aleviate the aforementioned problems.

The structure would be based on the existent interaction of 12 laboratories in the same number of countries, that has resulted from the development of a Project supported by UNIDO/UNESCO for the last 30 months (1989-1991). These laboratories (see below) will gather nationally as much as possible information to feed the database (see spreadsheet below), and will be responsible for the diffusion of the processed data in the country.

One of such laboratories will act as "COORDINATING CENTER" where the information will be submitted, processed and edited. Another of such laboratories will be responsible for the printout and initial regional distribution of the edited data.

The database will be renewed yearly. The success and impact of this proposal will be evaluated after a period of time of two years.

PARTICIPANTS

Coordinating Center.-
Dr. Jorge V. Gavilondo, Centro de Ingeniería Genética y Biotecnología, La Habana.CUBA

Reproduction and Distribution Center.-
Dr. Lourival D. Possani, Centro de Investigación sobre Ingeniería Genética y Biotecnología, UNAM, Cuernavaca, MEXICO

Representative Laboratories.-
Argentina.-
Dr. Alberto Horenstein, Centro Oncológico de Medicina Nuclear, Inst. A.H. Roffo, Buenos Aires.

Venezuela.-
Dr. Gustavo Ortega, Instituto de Biomedicina, Caracas.

Bolivia.-
Dr. Heriberto Cuevas, Instituto de Investigaciones Biomédicas, Academia Nacional de Ciencias, La Paz.

Brasil.-
Dr. Isaias Raw, Centro de Biotecnología, Instituto Butantan, Sao Paulo.

Chile.-
Dr. Luis A. Ferreira V., Facultad de Medicina, Universidad de Chile. Santiago de Chile.

Costa Rica.-
Dr. Bruno Lomonte, Instituto Clodomiro Picado, Universidad de Costa Rica, San José.

Cuba.-
Dr. Jorge V. Gavilondo, Centro de Ingeniería Genética y Biotecnología, La Habana.

Ecuador.-
Dr. Luis E. Plaza, Instituto Ecuatoriano del Seguro Social, Guayaquil.

Guatemala.-
Dr. Ricardo Luján, Instituto de Investigaciones, Universidad del Valle de Guatemala, C. Guatemala.

México.-
Dr. Lourival D. Possani, Centro de Investigación sobre Ingeniería Genética y Biotecnología, UNAM, Cuernavaca

Uruguay.-
Dr. Alberto Nieto, Facultad de Química, Universidad de la República, Montevideo.

Colombia.-
Dr. Luis Guillermo Parral, VECOL, Bogotá.

SPREADSHEET

The following spreadsheet is envisaged as the basis for the recollection of information:

MONOCLONAL ANTIBODIES. MIDB (Minimal Information Database)

Please Type.- These data are intended to provide other researchers with minimal information for interaction and

exchange.

1. Laboratory (complete address with teleph., fax, telex, name of reference investigator)

2. Specificity of the Antibody (add also three illustrative key words)

3. Denomination of the Hybridoma

4. Origin (circle):
   4.1. mouse x mouse
   4.2. mouse x human
   4.3. human x human
   4.4. human x hetero
   4.5. other species (specify)
   4.6. bi-specific (circle also previous)

5. Denomination of Monoclonal Antibody, Subclass

6. Status and Availability (circle as many as necessary)
   6.1. In commercial production or
   6.2. Production in development
   6.3. Fully characterized or
   6.4. In characterization
   6.5. Available as ascites
   6.6. Available as purified immunoglobulin
   6.7. Available as culture supernatant
   6.8. Hybridoma available

FUNDS

1. Coordinating Center: USD 20,000
   Comments: USD 10,000 will be used for equipment (PC, printer, and start-up kit), USD 3,000 per year for expendables, and USD 2,000 per year for communications.

2. Reproduction and Distribution Center: USD 20,000
   Comments: USD 10,000 per year for contract of printout and mailing costs.

3. Participating Laboratories: USD 24,000
   Comments: USD 1,000 per year, per laboratory, for the gathering of information and mailing costs

TOTAL: USD 64,000

ADDENDUM 6.-

BASIC AND ADVANCED COURSES ON MONOCLONAL ANTIBODY TECHNOLOGY

(a) Course #1.-

TITLE:
Generation and Production of Mouse Monoclonal Antibodies

DATE AND VENUE:
August-September 1992 (to be defined)
Centro Oncologico de Medicina Nuclear, Inst. A.H. Roffo, Buenos Aires, Argentina.

EXTENSION:
Two weeks (10 to 12 working days)

CHARACTERISTICS:
Theoretical lectures and practical demonstrations. Maximum attendance of 20 students from the Latin American region. The course will be given in Spanish.

OBJECTIVES:
(1) To provide a state-of-the-art view of the technology for the obtention and application of monoclonal antibodies (MAbs).
(2) To show through practical demonstrations the conventional methods employed for the generation of mouse hybridomas, and the screening, and production of MAbs.

PROGRAM (tentative):
(1) Theory.-
1.1. Basic elements of the immune response. Antibodies.
1.2. Murine MAbs. Overview.
1.3. Obtention of hybridomas using chemical fusion.
1.4. Techniques in the selection of specific hybridomas.
1.5. Production of MAbs in cell culture.
1.6. Production of MAbs in mice.
1.7. Purification of MAbs.
1.8. Human and bispecific MAbs.
1.9. Modification of MAbs by genetic engineering.
1.10. Applications of MAbs in human diagnosis and therapy.

(2) Practical demonstrations.-
2.1. Immunization of mice.
2.2. Chemical fusion of mouse myelomas and lymphocytes.
2.3. Growing and cloning myelomas and hybridomas.
2.4. Characterization of antibody specificity using ELISA, RIA, agglutination, and immunofluorescence.
2.5. Culture of encapsulated hybridomas.
2.6. Obtention of ascitic fluid from mice.

2.7. Purification of MAbs by low pressure chromatography.
2.8. Preparation of radiolabelled antibodies.

STAFF:
All speakers will be chosen from laboratories of the region. Practical demonstrations will be in charge of the host laboratory and some invited speakers.

INSCRIPTION QUOTA:
Amount to be defined

REQUIRED FUNDS FROM INTERNATIONAL SOURCE:
USD 30,000. To be used for the acquisition of expendables for the practical demonstrations, plus travel, board, and lodging of invited lecturers, and support of a limited number of students with regards to lodging.

COORDINATOR:
Dr. Alberto L. Horenstein
Centro Oncologico de Medicina Nuclear, Instituto A.H. Roffo,
Av. San Martin 5481, CP 1417
Buenos Aires, Argentina
Teleph.: 501-80-54

(b) Course #2.-

TITLE:
Antibody Modification by Genetic Engineering

DATE AND VENUE:
October or November, 1992 (to be defined).
Division of Immunotechnology and Diagnostics, Center for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba

EXTENSION:
Six days (Monday-Saturday)

CHARACTERISTICS:
Theoretical conferences and practical demonstrations. Maximum attendance of 15 students from the Latin American region (knowledge of English required for lectures by foreign invited speakers).

OBJECTIVES:
(1) To provide a state-of-the-art view of the application of genetic engineering techniques for the modification of antibodies and their expression in bacteria and eukaryotic cells, and the impact of such molecules in Biomedicine.
(2) To show through practical demonstrations the use of PCR for the cloning of immunoglobulin variable regions, the cloning of such regions into bacterial expression vectors, and basic manipulations for the transfection of modified

immunoglobulin genes into receptor animal cells.

PROGRAM (tentative):

(1) Theory.-

1.1. Overview of the modification of antibodies by genetic engineering.

1.2. Applications of second generation monoclonal antibodies in basic research, and in human diagnosis and therapy.

1.3. PCR cloning of immunoglobulin genes.

1.4. Expression of modified antibodies in animal cells.

1.5. Expression of antibody fragments in bacteria.

(2) Practical Demonstrations.-

2.1. RNA extraction from mouse hybridomas.

2.2. cDNA preparation from total RNA.

2.3. PCR amplification of mouse immunoglobulin variable regions using degenerate synthetic primers.

2.4. Cloning PCR amplified DNA into sequencing vectors.

2.5. Expression of mouse immunoglobulin variable regions in bacteria, as antibody fragments.

2.6. Transfection of myelomas and hybridomas with modified immunoglobulin genes.

STAFF:

Speakers.-

(1) Dr. Jorge V. Gavilondo, CIGB, Havana

(2) Dr. Sherie Morrison, UCLA, Los Angeles (*)

(3) Dr. Sally Ward, Dallas (*)

(*) to be confirmed

Practical demonstrations.-

Drs. Marta Ayala, Javier Vázquez, Alicia Pedraza, all from CIGB.

INSCRIPTION QUOTA:

Amount to be defined

REQUIRED FUNDS FROM INTERNATIONAL SOURCE:

USD 20,000. To be used for the acquisition of expendables for the practical demonstration, plus travel, board, and lodging of invited lecturers, and support of a limited number of students with regards to lodging.

COORDINATOR:

Dr. Jorge V. Gavilondo
Jefe, Division de Inmunotecnologia y Diagnostico
Centro de Ingenieria Genetica y Biotecnologia
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