XXVIII MEETING OF THE ADVISORY COMMITTEE ON HEALTH RESEARCH

Montevideo, Uruguay
20-23 August 1991

ACHR 28/91.8
Original: Spanish

PAHO SUPPORT FOR BIOTECHNOLOGY PROJECTS IN LATIN AMERICA

Dr. Elsa Segura
Instituto Nacional de Enfermedad de Chagas
"Dr. Mario Fatala Chaben"
Buenos Aires, Argentina
July 1991

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the Pan American Health Organization (PAHO). Authors alone are responsible for views expressed in signed articles.
I. INTRODUCTION

PAHO organized the Advisory Committee on Health Research (ACHR) in the early 1950s. In 1985 it was recognized for the first time that PAHO needed to step up activity in the field of biotechnology, based on a recommendation by ACHR (1).

That recommendation coincided with an important trend in the field of biotechnology in the countries of the Region. Since that time, PAHO has made considerable progress in promoting such projects. In 1987, PAHO created the Advisory Subcommittee on Biotechnology, which while meeting with its Advisors in Costa Rica recommended the "Regional Program for the Development of Biotechnology Applied to Health in Latin America and the Caribbean" (1). This Program is now in full swing at PAHO.

Following the ACHR's recommendation, PAHO's Research Secretariat began a project called "Priority Technological Developments: Development and Evaluation of Diagnostic Methods for AIDS, Hepatitis B, and Malaria" (PTD) (2) in 1986.

This paper contains an analysis of the results obtained from implementation of the project, its impact on the field of biotechnology, and the advisability of undertaking other projects which--applying knowledge from biochemistry, biology, and process engineering--can provide useful tools for the health and development of the populations of Latin America and the Caribbean.

II. PAHO-SUPPORTED PROJECTS: RESULTS AND SPECIFIC RECOMMENDATIONS

The projects presented at the launching of the PTD were partially funded by PAHO and began in 1986. They ended between 1989 and 1990. Below some of the results they obtained are analyzed.

1. Acquired Immunodeficiency Syndrome (AIDS)

Four projects have been conducted which sought to isolate and characterize the Human Immunodeficiency Virus (HIV). Two projects dealt with the preparation of serum panels to evaluate reagents for the diagnosis of HIV infection. Another project sought to develop a combination of reagents to diagnose the infection.

Isolation and characterization of the virus provides the necessary knowledge to establish a surveillance mechanism for genetic and antigenic variations of HIV.
Therefore, at a meeting in December 1989, the World Health Organization's (WHO) Global Programme on AIDS established the Criteria and World Network for the Characterization and Isolation of HIV (3). It is hoped that this Network will generate information on the geographic distribution and variations of HIV, providing a data base on critical genetic and immunological information to design reagents for immunoprevention or immunotherapy.

The set of projects spurred by PAHO attained the aforementioned objective in the Region. The researchers were able to isolate HIV from different origins in Mexico (4), Venezuela (5), Brazil (6), and Argentina (7). The isolates were made following a methodology very similar to that proposed by the WHO. Viral isolates were made from clinically evaluated patients at Centers with the capacity to conduct specific serologic studies and viral isolation. The patients recruited had different clinical statuses.

The researchers obtained around 30 stabilized isolates. During follow-up on the cultures, parallel monitoring was done for the presence of viral antigens. Characterization of the isolates was done biologically, studying their cytopathogenic effect on well-defined cellular lines (4, 6, 7), although such characteristics could not be related to their geographic origin or the clinical status of the patient from which they came.

From a practical point of view, it was demonstrated how, as in other systems (6), some isolated viruses established a better lymphoid cell line than others (4). Some viral isolates that were observed for 40 days presented low levels of viral antigen expression on the cell surface (4, 6). In the majority of cases, the isolates had to be observed for more than 4 weeks in order to find an expression of the viral antigen.

Virus was detected in all of the lymphoid cell lines with a peak on the 18th day after infection (4, 6), except for one monocyte cell line in which viral detection was minimal and late. These cells also presented little cytopathogenic effect (4, 6).

One finding of epidemiological significance is that the 30 viral isolates corresponded to HIV-1, and some of the sera studied in Venezuela are also reagents against HTLV-1 (5) antigens, as has been reported in other countries (8).

Viral purifications of the isolates from Brazil presented a difference in the antigen pattern observed with strips of commercial nitrocellulose. The authors maintain that this may be due to loss of components of the viral capsule from purification. However, greater density is found in the p55 band than in the commercial immunoblottings. Commercially prepared ones contain native proteins obtained by expression or synthesis, and for that reason it would be very difficult to find an identical pattern.
Further work in the antigenic characterization of local viral isolates is recommended, defining the amino acid sequence of the HIV markers. This information could be centralized to establish a surveillance system on the appearance of viral antigenic variables. Such a system would be of great importance today, taking into account that test vaccines against HIV are being prepared in countries of the Region.

Electronic microscopic characterization of some isolates revealed the presence of viral particles and outbreaks in the cells found to be susceptible (5, 6).

As for genotype characterization, some of the HIV isolates showed polymorphism with regard to the HIV-1 prototype (HTLV-IIIb) and the restriction sites induced by some enzymes (6).

It is recommended that these studies be applied to more viral isolates, to the viral genome sequencing, and that viral DNA probes be obtained. At this stage of the game, the groups in the Region could contribute with probes to study possible variations of HIV from the polymerase chain reaction (PCR) (9). Another area to be developed is application of PCR to confirm pediatric infections in which the specific antibodies do not permit diagnosis (10).

Developing a diagnosis for an infectious disease significantly helps in controlling its transmission, not only on an individual basis, but also in studying populations. The results allow epidemiologists to estimate the size of the population affected, or that will be affected by spread of the disease, as well as the risk factors involved. Serology is useful for the detection of antibodies against serological markers of the clinical status of the HIV infection (8). Recombinant proteins and reagents prepared using purified viral proteins have been used as antigens for the detection of antibodies, both in the initial screening phase and in the confirmation phase (8).

Research on the development of reagents for the diagnosis of HIV infection has gained great momentum. Simple reactions have been described that do not require infrastructure for their implementation, such as the case of passive agglutination which can detect complex HIV antibodies and antigens (10). This type of reaction useful for field work is contributing to the testing of an agglutination reaction with latex particles (Cambridge Bioscience, Inc.) being tested in Venezuela (5), which demonstrates sensitivity and specificity equal to that found with a first generation immunoenzymatic reaction (EIA-Abbott Laboratories). The positive sera were confirmed by Western blot. The sensitivity and specificity studies are preliminary.

Within the set of projects supported by PAHO is one that proposes to develop a combination of recombinant antigens developed in Cuba for the diagnosis of HIV infection (11). The project
proposes to assemble an immunoenzymatic diagnostic system based on
the search for antibodies, using two recombinant proteins—one from
the viral capsule (env)gp41 and one from its core (gag)p24. The
project basically seeks to validate preliminary results which
showed that the reagent was highly sensitive. On the one hand, the
laboratory (11) has attained a sufficient level of development to
produce the two recombinant proteins cited above; on the other, it
has developed technology that allows it to conclude the project
with a product. There are several laboratories in the Hemisphere
that have sequenced the genome of microorganisms, and which have
clones that express certain proteins, some of them of interest to
diagnosis (12). However, there are few with the capability to
convert them into a biotechnological product. The project's final
report contains more information on the procurement,
characterization, and purification of the (env)gp41 and (gag)p24
than on the series of tests that led the laboratory (11) to develop
a combination of reagents. That makes it difficult to assess the
impact that PAHO support has had on the development of the product,
REC-VIH-1 (*TN: Reagent HIV-1). The sensitivity and specificity of
REC-VIH-1 seem encouraging considering the percentages shown,
although the numerical data is difficult to interpret for the time
being. In conclusion, this project contributes to development in
the Region of a reagent using recombinant HIV-1 antigens through a
third generation reaction for diagnosis of HIV infection. More
precise data on its evaluation in the field will be necessary when
deciding how it should be distributed.

The specificity of diagnostic tests for HIV infection have
shown progress since the first ones were authorized in 1985 (8).
Even so, people are often unnecessarily alarmed by the appearance
of false positives. That nonspecificity increases in populations
in which prevalence is very low, causing a corresponding concern
given that to date HIV infection is considered to be the certain
cause of only one death in ten years. On the other hand, a false
negative bears the consequence of permitting possible transmission
by transfusion (90% effective) or not advising an individual of the
risk of transmitting the infection. Information obtained from
epidemiological data and molecular biology have pointed to the
possibility of geographic variations in the etiological viral agent
of AIDS, after the discovery of HIV-2. Putting this data together
explains why there are chances of obtaining inaccurate results, as
occurs with other infectious diseases. In addition to having
accessible reagents capable of detecting smaller quantities of
antibodies or antigens, it is necessary for each laboratory to have
a diagnostic quality control system. Therefore, it is essential
that the laboratories in the Region be able to attain the maximum
specificity and sensitivity offered by the reagent being used. One
of the best strategies proven so far for improving the quality of
diagnosis of an infectious disease, is timely access to a reliable
bank of reference sera.
This was another contribution made by the PTD projects encouraged by PAHO. A project was supported in Brazil (13), and another in Argentina (14), aimed at obtaining panels of well defined sera from non-HIV-infected and HIV-infected individuals, in their different clinical forms.

The existence of these sera is useful for testing new native or recombinant proteins, as well as DNA probes or other HIV elements, and to evaluate products being developed. There are 83 plasma samples obtained in Brazil (13), of which 14 are reagents for immunoblotting, although the persons from whom they were obtained do not present the same clinical status as HIV infection. The existing volumes are greater than 60 ml. each (13). The other plasma bank has 277 samples of more than 130 ml. each, obtained following the ethical recommendations of the Helsinki Convention, in the Argentine cities with greatest HIV prevalence (14). Of the 277, 256 are from asymptomatic patients, of which almost 30% are HIV seropositive. The patients are blood donors and members of different risk groups. In the 277 plasmas, antibodies against Trypanosoma cruzi was applied, and 17 tested positive (14). Thus, more than 300 reference sera are available to the Region, characterized for reactivity against HIV antigens and with detailed clinical data. These sera will be of great retroactive value in the future for development of knowledge of the viral variation of HIV and its impact on the procurement of therapeutic and immunoprotective media.

2. Hepatitis B

The specific markers for infection by the Hepatitis B Virus (HBV) have been known since discovery of the Australia Antigen and its relation to clinical infection by Hepatitis B (15, 16, 17). The antigen HBsAG is produced in noticeable quantities during the acute and chronic stages of the infection. On the other hand, in a small number of patients infected by HBV, HBsAG cannot be detected, in which case the only serological marker is the antiprotein for the core of HBV (18). Beyond the extensive knowledge developed on markers of the HBV infection, is the preventive interest in determining them in blood donors. It has been demonstrated that the risk of post-transfusional Hepatitis B is minimized if the donors are volunteers and are tested for the presence of HBsAG (19).

The contribution made by detection of HBsAG to reducing the risk of infection, is due to the use of third generation reactions (20). PAHO encouraged the development of reagents for the diagnosis of Hepatitis B (21). Argentine investigators had already prepared polyclonal antibodies used to immunize a vaccine and a viral purifier given by Dr. Akira Homma (Brazil). The equipment developed, EIA MIC, was tested along with HBsAG reference subtypes "ad" and "av" and compared to different commercial immunoenzymatic
tests. This made it possible to determine their detection sensitivity, which was between 1 and 5 ug/ml with 100% correlation. The specificity tests were done with 500 sera from hepatopathic patients, comparing them with known tests.

EIA MIC presented 98% specificity, given the appearance of 2% of the positive sera among those that were known to be negative from proven commercial reagents. 10,000 sera were studied with EIA MIC, with similar results. In order to reduce the nonspecificity, the need was raised to work with monoclonal antibodies (MAb) (21). In addition, in this way they could enhance homogeneity between batches and make production of the purified antigen independent. From this emerged PAHO support for the identification and production of monoclonal antibodies for the detection of HBsAG (21).

The MAbs were obtained after fusion with lymphocytes from mice immunized with a first generation vaccine, HBsAg subtypes "ad" and "av" with P3K63 Ag8 and X 563 murine myeloma cells.

For the capture tests over nitrocellulose, they used the purified MAbs in combinations, either alone or with a second antibody marked with a peroxidase, against 100 sera from non-infected persons and 20 from infected persons. The sensitivity found in the tests was not satisfactory. The low speed at which new fusions were produced prevented PAHO support for this project from continuing. In later communication with the group it has been determined that MAbs useful to improve EIA MIC have been developed, and that the reagent is ready to be transferred to the productive sector through the legal instruments in place in Argentina.

There is significant market potential for use of this type of reagent in the Hemisphere. In Argentina approximately 400,000 blood samples to be transfused are tested with commercial reagents, while around 1 million donations are collected (21). In other countries, it is estimated that around 20 million donations of blood are made annually.

Regarding diagnosis of the hepatitis viruses, the development of a diagnosis of infection by the Hepatitis C virus (HCV) should be encouraged in the Region (22, 23, 24), as well as detection of the E virus (HEV) (25), in order to provoke epidemiological studies. For now, HCV is related to transmission by transfusion in the countries of the Northern Hemisphere, while HEV would be significant in the countries of the Southern Hemisphere.

3. Malaria

Plasmodium vivax and Plasmodium falciparum produce the tropical disease of greatest consequence for public health, with an annual incidence of 300 million cases and 1% mortality. It is
estimated that the Region of the Americas accounts for 10% of world incidence. In 1989 Brazil alone reported 1 million new cases (26). In addition to measures to chemically control the vector, diagnosis of the infection is part of the program for surveillance of transmission, given chances for detecting new cases in populations under entomological surveillance. Furthermore, detection of the parasite confirms a clinical presumption of malaria and establishes the course of treatment. The most widespread parasitological diagnosis is microscopic examination of a drop of blood, which is generally performed on persons with fever or samples from residents of the endemic area for purposes of epidemiological surveillance. The system is quite useful, though it has room for improvement given that its sensitivity in treated cases or cases of immunocompetent patients is not sufficient. At any rate, it is the most sensitive method and the one against which all new methods are tested.

PAHO has financed two projects which seek to detect \( P. falciparum \) antigens (26) in the blood or urine of exposed persons or patients. The possibility of detecting \( P. vivax \) and \( P. falciparum \) antigens in sera, blood, and urine is also studied, using MAbs from different sources as a probe (27).

Other investigators had previously developed reactions based on the use of MAb and polyclonal antibodies (PAb), both revealing antigens by radioimmunoassays (RIA) (28) or by EIA (29). One radiometric immunoassay using various MAbs and one PAC in different combinations, showed that it was better to use two MAbs recognizing different antigenic sites, than one MAb plus one PAI (30). The best possibility was the use of one iodized MAb sandwiched with another MAb. This test detected antigenicity after treatment, it did not show crossed results with \( P. vivax \), and the parasites were detected long after microscopic observation became negative (31). In another experience combinations of antibodies were tried against different clinical statuses or epidemiological situation in the patients, and it was found that the two combinations used were complementary, allowing 94.7% detection of antigens in patients with acute malaria. However, they established that detection of antigens is not the most appropriate way to control blood banks, because it detects half of the donors that presented with specific IgG. Even so, the positives through this method presented negative microscopy (32), which means that the use of MAb combinations can improve the classic microscopic method.

In the project of the Sao Paulo researchers, 14 anti-\( P. vivax \) and anti-\( P. falciparum \) MAbs were first used from the laboratories of New York University (NYU), which recognized peptides from 28 to 210 Kd. located in infected erythrocytes, merozoites, and intraerythrocyte forms. These MAbs were tested for EIA in sandwiches, to detect soluble Plasmodium antigens. All of the tests performed attained 78% sensitivity, taking into account infections produced by \( P. vivax \) and \( P. falciparum \). That
sensitivity increased to 80% when considering only patients infected with *P. falciparum* and 3 patients with another, undetermined type of malaria (26). Sensitivity in relation to microscopic detection of parasitemia does not indicate it should be replaced. Sensitivity of detection of antigens in urine was also low (40%).

A contribution to this work has been made by detection through indirect immunofluorescence (IFI) of parasites in 100% of the slides obtained from parasite-infected patients, using a combination of anti-*P. vivax* + anti-*P. falciparum* MAbs obtained at the Sao Paulo laboratory, in the monofluor equipment from the Pasteur Institute (Paris, France).

It is also noted that in the case of malaria, there must be a bank of sera and plasma, with the parasite slides corresponding to each patient, with a complete medical record. There are currently several MAbs in the Hemisphere that could be quickly tested in experienced laboratories such as that of these researchers (26). Testing of more monoclonal antibodies that recognize peptides, from different locations in Plasmodium, could lead to improved sensitivity figures in this work.

Another project that seeks to develop a method for diagnosing malaria was funded in Mexico (27). The researchers developed a strong protocol for procurement of anti-*P. vivax* MAb. They obtained 21 hybridomas and 18 clones that were tested for EIA and IFI against *P. vivax* and *P. falciparum* antigens. Through IFI, 16 reacted with *P. vivax*, 4 with *P. vivax* and *P. falciparum*, and 1 with *P. falciparum*. Five MAbs provided by NYU were used as a pattern. From these they selected one which presented a well defined pattern for EIA and IFI with the parasites from the laboratory. The laboratory MAbs presented a fluorescence and optical density similar to those of the reference MAbs. Five of them presented a less defined pattern which improved through the use of dot blots. The investigators maintain that this would be the technique of choice (27) and propose to continue the project with the testing of serum or plasma from the patient in capture with the MAb. The work requires more time to conclude design of the reagent to detect *P. vivax* antigens. At this point in its development it is recommended that the researchers from Sao Paulo and from Mexico City exchange materials, in order to give scientific strength to their results and convert them into a product sooner.

The third malaria project supported by PAHO (33) proposed development of a test for its diagnosis, which could be used in field studies. It was conducted in three stages. In the first, the investigators standardized an EIA reaction for the detection of antibodies with an antigen prepared with sonicated *P. vivax*. Although the reaction that developed had high sensitivity, it is difficult to obtain the antigen since to date *P. vivax* cultures
have not been developed. At the stage of detecting antigenicity, they obtained 74% sensitivity compared to the parasitological exam. In the third stage, they tested three separate monoclonal antibodies for antigenicity, comparing them with the results obtained with a polyclonal antibody. Through EIA and with the MAbs they obtained less sensitivity than with the polyclonal antibodies. For that reason they developed an immunocytochemical test for the detection of *P. vivax* in slides. With the polyclonal antibody they obtained a level of sensitivity equal to that of the parasitological method, with the advantage that it permits specific identification of *P. vivax* (33).

III. GENERAL RECOMMENDATIONS

Support for research and development of the biotechnology project should continue within PAHO's plans.

This will require a continuing budget for at least three years, which should include activities to complement the work conducted in the laboratories. These are: periodic meetings for coordination, evaluation, or consultation, and at least one visit to each laboratory by experts to evaluate the project during its execution.

At the same time that these projects are conducted, it would be desirable for PAHO's research coordination unit to stimulate a strengthening of ties between the laboratories and the control programs in the Ministries of Health, so that transfer of the results will occur naturally and with ease, and so that the joint endeavors of researchers working according to the scientific method, and technicians facing problems in the field, will be mutually beneficial.

Another possible area of coordination is with groups in the Region working to develop a framework of probes for the diagnosis of malaria, within the Regional Biotechnology Project supported by the UNDP.

PAHO could encourage the current programs of the Secretariat for Research to establish contacts with the national or international system of production, as well as national agencies devoted to that particular area, once they finish their projects.

In future support for research, conditions should be improved under which the materials necessary to carry out the project are delivered.

The laboratories and agencies in the national system of production should be encouraged to bear in mind biosafety factors. PAHO's biotechnology program could incorporate this aspect into its plan for seminars.
Particularly in regard to projects reviewed in this report, the following suggestions are offered.

A. As a whole and for a short period of time, support should be given to projects attempting viral isolation and characterization (4, 6, 7), so as to establish a bank of isolates and a data bank to install a Hemisphere-wide system of surveillance of viral variability. Among other things, it would be useful for providing more appropriate conditions for testing the effectiveness of HIV vaccines.

B. Support should be given as a whole and for a short period of time to the groups in Mexico and Brazil, so that they can finish development of a sufficiently sensitive reagent for parasitic detection, which would be technologically available to the Region for the diagnosis of malaria.

C. Testing should be encouraged, with defined serum panels (13, 14) from the reagents developed (11) or tested (5) in the first stage of the program.

IV. VIEWPOINTS

The definition of health includes the field of application, which encompasses nutrition, water supply, systems for the elimination of wastes, care of the environment, and work.

In the area of biotechnology PAHO could consider new openings to promote the harmonious development of health. Research and development in fields that transcend the framework of diagnosis and vaccines could analyze, among other things, alternatives to improve animal and vegetable nutrients, and access to their conservation, or the degradation of solid wastes.

PAHO could promote the mechanisms needed so that the technological advances obtained in knowledge of the human genome can be applied to study of the genomes of microorganisms and proteins of interest to health in the Region.

Participatory promotion along with the countries of the Region, is the basis for the real contribution that a PAHO regional biotechnology project can make by the end of this century.
2. Programa de subvenciones para la Investigación OPS/OMS DRC 3499u. 1987


