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VISIT TO PASTEUR INSTITUTE

B. Cuthbertson
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VISIT TO PASTEUR INSTITUTE, 24TH JANUARY

People Contacted - Dr Montagnier)
Dr Chermann) R.E. LAV ASSAYS
Dr Barre)

Dr A N Other) HEPATITIS B ASSAYS

Mr Dela Charriere) FINANCIAL ADMINISTRATOR

1. LAV

Dr Montagnier

Could only spare 15 minutes but following points raised.

1. Keen that Pasteur collaborate with PFC in heat inactivation experiment.

2. Has already performed similar experiment for Travenol but could not discuss these.

3. Willing to let PFC have stocks of virus but only on basis of use for further heat-inactivation experiments. Conditions for release of virus have recently become stricter as they have entered an agreement with Genentech for exploitation of recent sequencing of genome and resultant vaccine possibilities.

   Virus can become available if:-

   I I write to Montagnier detailing precisely our future proposals.

   II We subsequently sign an agreement limiting our activities to above.

4. He was willing to consider the possibility of a Scottish evaluation of the ELISA kits currently being produced for distribution through their commercial subsidiary, Diagnostique Pasteur.

   He also thought it possible that we could get some more fluorescence slides.

   I got the strong impression that he had a low opinion of Jennings, their apparent agent in Scotland.

   Thus, although no specific promises were made, we may be able to deal directly with Pasteur for kits for evaluation (see under Dr Chermann, also).

1.2 Dr Barre

She arrived during conversation with Montagnier and was able to spend most time with me. She would actually oversee the experiments.

1. She and Montagnier agreed that fibrinogen in our product may affect their assays and also agreed that they would want to evaluate this before starting heat experiments. If fibrinogen is/

is a problem, they may have to purify virus by ultra-centrifugation.

2. It was agreed that first priority would be assessment of heat-treatment of FVIII.

3. Outline of experimental protocol would be -

I Send LAV to PFC.

II Freeze-dry + heat treat + return to Pasteur.

III Assess reverse transcriptase activity in -

a. virus control,

b. spiked liquid FVIII,

c. spiked freeze-dried FVIII,

d. heat-treated FVIII,

e. FVIII control.

NOTE: their standard LAV cultures generate approx. 10^6 cpm in their RT-assay but background activity is only 600 cpm.

IV Assess infectivity in above samples (a - d). This is necessary as they know that RT-Negative samples can still infect lymphocyte cultures.

Assessment would be:-

a. Inoculate three dilutions of each sample into cultures of stimulated normal T-Cells in the presence of Human T-Cell growth factor (Interleukin-2). The cultures are re-seeded every 3 days and examined for evidence of infection (by immunofluorescence).

Although their stock cultures, diluted to a RT-Activity of 1,500 cpm, demonstrate infectivity within 5-10 days, she proposed that the cultures should be kept going for one month to ensure that no residual infectivity is missed.

V. I suggested that four heating periods be assessed, these being 2, 12, 24, 48 hours. This was greeted with horror as being unmanageable and we agreed that in the first instsance we would concentrate on two times - 2 hours and that of our second process.

4. Dr Barre showed me around their laboratory and several points emerged.

I Isolation of LAV/HTLVIII is performed in a normal laboratory with large, two-person, recirculating (Class II Type) safety cabinets. Staff wore rear tying gowns, masks and gloves. Security appeared very lax and two gentlemen of the press were in the Lab without wearing any protective clothing and the door remained open throughout my stay.

II/

II All large-scale culturing of LAV is carried out in a second laboratory with category 3 features:- interlocks, changing area, lowered air pressure. Again, however, virus is handled in recirculating type safety cabinet.

III All LAV generation is still carried out by Montagnier's team. Virus is grown in 46cm glass roller bottles. They have still not been able to generate a continuous cell line, unlike Gallo. They, therefore, require a constant supply of fresh, normal T-Cells from the local BTS.

IV Antigen for test kits is generated by detergent-treatment of infected cells. The viral extract is purified by ultracentrifugation through a sucrose gradient. The antigen is also formalin-treated before being given to Diagnostique Pasteur who make the kits.

Control antigen is derived from uninfected cells in an identical manner.

1.3 Dr Chermann

As with Montagnier, I only managed to spend a few minutes with him, in between interviews with a News of the World reporter. He made the following points:-

1. Was also keen to collaborate with PFC in heat inactivation.

2. Explained that ELISA kits were currently being evaluated at five French laboratories, each of which would test 200 known positives and 2,000 blood donors (he did know if donors would be told or whether prospective or retrospective assessment). Positive samples in this evaluation would be followed up by another test for confirmation. No final decision had been taken but he presumed that it would be a western blot or a radioimmune precipitation assay for antibody to the P25 (core glycoprotein) antigen.

   He was unwilling to define the sensitivity of the assay until the assessment had been completed in 3-4 weeks. He was confident that the assay would be very sensitive with a low incidence of false positive results.

   The assay is of conventional design with antigen bound on to the ELISA plate. Samples are incubated in the test wells, in duplicate, and bound antibody is detected with a conjugated anti-serum and substrate. A positive sample is included in the kit and four two-fold dilutions are tested to provide a standard curve. Positive samples give an OD reading of 0.3 or above and a result between 0.2 - 0.3 is borderline and must be repeated.

3. If first evaluation of ELISA kits is satisfactory, further kits will be released for wider assessment and he agreed that Scotland could be included in this assessment. Responsibility for this remains with Pasteur Diagnostique and we will have to contact a Mr Policiard to arrange this.

4. Apparently, their test kits have gained such publicity in France that homosexuals are deliberately giving blood in France in an/

an attempt to discover their AIDS status.

1.4 Mr De La Charriere

This is the financial administrator who will set up any deal with SNBTS for heat-treatment experiments. He proposed the following programme after extensive consultation with Francoise Barre.

1. Pasteur would send us an outline protocol and costing for our approval.

2. If we go ahead, they would expect a payment up front with the balance on completion of the experiment (3-4 months after entering agreement).

3. Was not able to provide prices until lab had calculated work involved but on basis of Travenol deal it could be:-

100,000 Francs (£9,000) Up Front
100,000 - 150,000 F. At Completion

I pointed out that we were not a rich multinational and that a lower price was more likely to be accepted by us. His English was poor and I'm not sure he understood.

2. HEPATITIS B DNA PROBES

Pasteur have developed a P^{32} labelled - DNA-Probe assay for Hepatitis B DNA. They claim that in many cases it is more sensitive than HBsAg testing and is suitable for assaying blood products. Will assay samples for us at 300 Francs (£30 a test). Abbott have obtained the distribution rights for this assay but the person I spoke to did not know when it would be on market or likely price of kit.