

WHO Consultation on a Rabies Monoclonal Antibody Cocktail for Rabies Post Exposure Treatment. WHO, Geneva, 23-24 May 2002

1. INTRODUCTION

Dr F.-X. Meslin introduced the Consultation by reminding the participants of the current WHO Guidelines for Rabies post-exposure treatment (Annex 3 of the WHO Expert Committee on Rabies in its 8th report issued in 1992). The WHO Guidelines defines category 3 exposure as single or multiple transdermal bite/scratches. In addition, a category 3 exposure includes contamination of mucous membranes with saliva. In most instances in a rabies infected area, category 3 exposure should be followed by wound treatment plus the administration of a rabies post-exposure treatment (PET) comprised of both rabies vaccine and immunoglobulin (RIG) for all severe (category 3) exposures. It is estimated that in an area with endemic dog rabies that between 25 and 35% of all patients requiring PET should receive both passive and active immunization.

However, the current situation in developing countries is characterized by the following:

- Less than 1% of all PET are comprised of vaccine and serum;
- Human RIG is available in confidential quantities on specific markets and is too expensive for most people (about \$250 per adult patient, approximately five times more expensive than purified horse serum);
- Cheaper and safe (purified pepsin digested horse serum) equine immunoglobulin (ERIG) is available in limited quantities and in most situations is inaccessible to those that need it most.

In addition this situation is getting worse as:

- More and more international manufacturers are discontinuing ERIG production;
- Where production of purified equine products has been initiated (e.g. Thailand) it remains limited and hardly satisfies national needs;
- Animal protection groups that are becoming more and more influential in developing countries, condemn animal rearing for serum production.

In this context accelerating research and development of alternative products is very timely and using a limited number of carefully selected MAbs for therapeutic purpose is an attractive possibility. MAbs have demonstrated their activity in certain animal models and with the progress of technology their potential ease of production in large quantities at low cost and ease of quality control compared to polyclonal serum is attractive.

This meeting is the first step of a project that should lead, after careful selection of the MAbs to the evaluation and validation both *in vitro* and *in vivo*, of the efficacy of the MAbs antibody cocktail for rabies prophylactics in combination with vaccination. The second phase should lead to the selection of a technology for production that could be eventually transferred to selected developing countries.

This project is a joint endeavour of three different programmes in WHO: Communicable Diseases (CDS), Health Technologies and Pharmaceuticals (HTP) and Tropical Diseases Research (TDR).

Dr S.N. Madhusudana was nominated Chair and Drs. C. Rupprecht and A. Fooks were nominated as rapporteurs for the Consultation.

The absence due to ill health of Dr A. King, former head of the WHO Collaborating Centre (WHO/CC) at CVL, Weybridge, was very much regretted. The Consultation wished Dr King a very prompt recovery

2. SUMMARY OF PRESENTATIONS

3. SELECTION OF MOUSE MAbS CANDIDATES

The group decided that a minimum of two to three different MAbs are needed in a cocktail to replace immunoglobulin (ERIG in developing countries) in rabies PET. These MAbs should be directed against antigenic sites I, II and possibly III of the rabies virus glycoprotein of isotype IgG1, 2a and possibly 3. Data presented by each WHO/CC at the occasion of this meeting indicated that approximately 20 MAbs are available from the WHO/CCs from which the final MAb cocktail can be selected.

3.1 Criteria for MAbs selection

- The history of hybridomas including relative risk of contamination with certain agents (e.g. FMDV, TSE agents) and use of FCS (foetid calf serum) should be available;
- A production of a minimum of 100 IU per ml of crude hybridoma supernatant should be obtained;
- Stability expressed as loss of antibody secretion production should not exceed 10% up to 30 passages should be documented approximately every 10 passages;
- *In-vitro* cross-reactivity should be measured by RFFIT or FAVN on a selection of rabies and rabies-related viruses isolated from the following host species and geographical areas:
 - dogs from Asia (India, Philippines, Thailand ...etc);
 - dogs from Africa (north Africa, sub-Saharan Africa, etc);
 - dogs from the New World;
 - mongoose(s) from South Africa;
 - ABLV (from Australia).

The group agreed that each laboratory should test at least 10 different viruses. The aim is to cross-neutralise genotype 1/serotype 1 virus isolates representing Asia and Africa.

Laboratory (ies) willing to test representative lyssavirus isolates should feel free to contact relevant rabies WHO/CCs to complement their collection for *in-vitro* testing;

Each lab should select one or 2 MAb candidates and perform minimum screening for sterility and adventitious agents (e.g mycoplasma) then initiate passaging.

Rabies laboratories belonging to the following institutions are ready to perform *in-vitro* tests: CDC, Atlanta, Jefferson University, Philadelphia, ADRI, Ottawa, CVL Weybridge, FRIAVD, Wüsterhausen.

3.2 Sources and candidate MAbs

The group agreed that the cocktail should contained MAbs from at least two different laboratories so that it defeats nationalism, egotism and avoids intellectual property claims that could more easily be formulated if the provider was a single laboratory.

The list of institutions and respective candidate MAbs is given below:

- Wistar Institute of Philadelphia, USA is ready to provide MAbs 11-12-1 (IgG 2a and site 2C) and W507-1 (IgG1, site 3B). The former is certainly the best overall candidate;
- CVL, Weybridge, UK is willing to donate MAb D8 (site unknown);
- CDC, Atlanta, USA ready to provide MAb A (IgG 2b) and MAb B (IgG 2 a) (sites unknown);
- The Institut Pasteur, Paris, France could donate: MAb A2 (IgG2a, site IIA) and MAb D1 (IgG1, site III);
- ADRI, Ottawa, Canada could give MAbs: M727 (IgG2a) and M777 (IgG1) – antigenic sites unknown;
- The laboratory in Tübingen, Germany, (WHO/CC; Wüsterhausen) is ready to donate MAb: E559 – antigenic site unknown - and
- The laboratory of Gif-sur-Yvette, France has two MAbs that could potentially be made available to WHO.

3.3 Action needed at laboratory level for *in vitro* testing

- History of hybridomas should be established in writing;
- Use of FCS should be avoided. Low serum or no serum media must be preferred;
- Each laboratory should establish a master cell Bank with a minimum of 10 vials;
- Tests for mycoplasma, bacteria...etc in T25 cm²;
- 30 passages must be performed (with freezing aliquots after every 10 passages)
- Culture batch (500 ml) on roller bottles;
- Purify IgG on protein A column and determine IU/mg;
- Compare supernatant of passages 0,10,20,30 (a 30% variation +/- for naturalisation from test to test is acceptable) and determine isotype at passages 0 and 30;and finally
- Purify IgG at a concentration of at least 1000 IU/ml.

The purified product must be sent to five WHO/CC laboratories for further testing using the same strain of CVS (ATCC).

4. TIME LINES AND MILESTONES OF THE FIRST PHASE

This exercise should end by first week of December 2002 (estimated four months for passaging alone). It should be considered a priority by the laboratories that have accepted to take part in the study. A review meeting could be organized at the occasion of the meeting on “Rabies in the Americas” that takes place in Mexico early November 2002. Agreement was sought from the WHO/CCs that they would attend this meeting.

Dr S. Leung should prepare a cost estimate for production as soon as possible for consideration of the review meeting.

WHO will establish a user group by email and will be assisted by Dr D.Briggs in CDC, Atlanta.

5. IN VIVO TESTING

In vivo testing will be performed only after the initial assessment of the results of this first round of *in vivo* testing.

There is not a clear correlation between *in vitro* neutralization of rabies virus and *in vivo* efficacy.

The group acknowledged the advantages of the hamster model over mice for preliminary *in vivo* tests.

The group suggests testing the final preparation (i.e. the cocktail and not individual MAbs composing it) for safety and measurement of product half-life in chimpanzees by instillation of the product, as currently is serum in PET. A challenge study in macaques should be conducted. The group should determine the best treatment protocol including vaccine administration (route, regimen...etc) for this challenge study.

The use of the cocktail in certain domestic animal species for post-exposure treatment should be considered.

6. TECHNOLOGY OWNERSHIP, TRANSFER AND PROTECTION

WHO should develop in collaboration with its Legal Office a letter of agreement with the donor institutions that would formalize the conditions of the MAbs donation and in particular settle issues related to indemnity. The group agreed that the WHO letter of agreement should indicate that the ultimate goal of the project is the use of the final product in humans. It should be made clear that the donated hybridomas are not the final product.

The cocktail should be kept for WHO in the WHO Collaborating Centre, (WHICH ONE) in Lausanne, Switzerland and possibly in each laboratory involved that has adequate storage facilities. WHO should safeguard the intellectual property of the cocktail once developed.

The final product should be identifiable; for this purpose sequencing should be carried out.

When transferring the technology WHO should make sure that no undue profit is made on the product. For this purpose, formal agreements should be signed with recipient laboratories stating that the final product should be made available to the public sector at a price close to production costs. The signing of a formal agreement between WHO and the government of each country where the technology is transferred to define the conditions under which the product would be made available to patients should also be considered.

The product should not be given to third parties beyond the developer of the technology and recipient production institutions in developing countries.

The WHO/CCs wish to be involved in the entire process beyond MABs donation; consequently they proposed that a Consortium of the donor laboratories is established under WHO aegis. The purpose of the Consortium would be to ensure that the product is made available to those who need it most. The Consortium would assist WHO in post-marketing monitoring and advise on the choice of the recipient institutions and countries. In this regard the rabies WHO/CCs should investigate as soon as possible the willingness of major PET centres (for example in India and Thailand) with whom they collaborate, to use a MABs cocktail in the future.

Although the primary objective is to produce a cocktail of murine MABs for human prophylaxis, by way of contingency planning it was agreed that it may be necessary to humanise the MABs in the future.

The advantages of human against murine MABs are:

- Allogeneic reactions are minimal;
- Better compartmentalisation;
- Longer *in vivo* half-life;
- Improved ability to interact with human Fc receptors.

ANNEX 1 AGENDA

Thursday 23 May 2002

- 09:00 1. Welcome
- 09:15 2.1 Introduction of attendees
- 09:30 2.2 Selection of Chair & Rapporteur
- 09:45 3. Introduction to the problem, objectives, & agenda
- 10:00 3.1 Brief review of each WHO Collaborating Centre's data on pre-existing neutralizing MAbs, in vitro and in vivo
- 10:30 Coffee**
- 11:00 3.1 Brief review of each WHO Collaborating Centre's data on pre-existing neutralizing MAbs, in vitro and in vivo
- 12:30 Lunch**
- 14:00 4 Rationale for selection of a particular MAb candidate
- 14:30 5. Discussion of heterologous vs. homologous MAbs –
Specific make-up of cocktail: minimum number; anti-G only or others, anti-rabies only or others, isotypes
- 15:00 6. Experimental approach to round table testing of candidate MAbs
- 6.1 in vitro models
- 15:30 Coffee**
- 16:00 6.2 in vivo models
- 16:30 7. Perceived utility & problems: theoretical vs. practical
- 17:00 8. Issues of regulatory safety and efficacy

Friday 24 May 2002

- 09:00 9. Institutional willingness and ability for donation of hybridoma to WHO
- 09:30 10 Conclusions and recommendations for future study and meetings
- 10:30 Coffee**
- 11:00 11 Action items and timeline
- 12:00 12 Closing

ANNEX 2 LIST OF PARTICIPANTS

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