REPORT OF THE SIXTH WHO CONSULTATION ON MONOCLONAL ANTIBODIES
IN RABIES DIAGNOSIS AND RESEARCH

The Wistar Institute, Philadelphia, Pennsylvania, USA, 2-3 April 1990

The participants were welcomed by Dr H. Koprowski, Director of the Wistar Institute of Anatomy and Biology, WHO Collaborating Centre for Reference and Research on Rabies. Participation in the "monoclonal antibody group" has been expanded to include Drs Selimov and Khodzinski, (WHO Collaborating Centre for Reference and Research on Rabies, Institute of Poliomyelitis and Viral Encephalitides, Moscow), Dr Alonzo Ruiz from the WHO Regional Office for the Americas, Washington, and Dr J. Cox (WHO Collaborating Centre for Rabies Surveillance and Research, Federal Research Institute for Animal Virus Diseases, Tübingen, Federal Republic of Germany), who attended in place of Dr L.G. Schneider. The list of participants is given in Annex 1.

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The results of the tests carried out are summarized in the following table:

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Les opinions exprimées dans les documents par des auteurs cités nommément n'engagent que lesdits auteurs.
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Performance of Selected MAba against European Viruses

A = Terrestrial Isolates
B = Serotine Isolates
C = Myotis isolates/Finland human isolate

<table>
<thead>
<tr>
<th>Mab no.</th>
<th>Pasteur</th>
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<th>Wistar</th>
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Note: Number(s) in parenthesis indicate the number of isolates examined
a) MAb W187.11.25 performed as anticipated. It reacted strongly with the common terrestrial animal rabies variants found in Europe and was negative with known bat variants isolated from E. serotinus and Myotis.

b) MAb 187.6.1, did not react with all terrestrial animal rabies variants in Europe. M. Lafon, reported negative results for tests of six isolates from Yugoslavia, making this MAb unsatisfactory for broad surveys of terrestrial animal rabies in Europe.

c) MSA 6.3, did not perform as anticipated. Although it reacted with both bat variants in Europe, it also reacted with terrestrial animal rabies isolates from Yugoslavia.

d) DUV 6.15.13, performed as anticipated in 3 of the 5 laboratories. CDC will obtain a fresh sample of this MAb and re-test the samples, as will Weybridge.

e) S62.1.2, performed as anticipated.

In summary, MAbs 187.11.25, DUV 6.15.12 and S 62.1.2 should prove valuable to those researchers who wish to test for bat rabies variants in terrestrial animals.

It was agreed that further work, including the exchange of viruses and Mab DUV 6.15.13 between the CDC and the Wistar Institute, would be essential. It was decided that Mab S 62.1.2 should be included in the reduced WHO MAb panel in order to differentiate the two subtypes of European bat lyssavirus. In addition, isolates of Myotis origin should be tested by the Tubingen group for reactivity with DUV 6.15.13. Based upon these evaluations, viruses of European bat origin were grouped into a new viral taxonomic type: type 5. This type 5 includes two subtypes, Euro-bat 1 (serotina) and Euro-bat 2 (Myotis), as well as the Yuli and Finnish human isolates.

3. MAbs in rabies research in the USSR

Papers were presented by Drs Khodinsky and Salimov, which detailed the history, geographical locations and results of examination of brain smears from 300 animals in the Soviet Union (61 of which were of Arctic origin) with MAb obtained from the late Dr T. Wiktor of Wistar Institute and Dr L.G. Schneider of Tubingen. The MAb P 41 reacted with 28 isolates from non-Arctic regions of the USSR. It was suggested that the isolates could be divided into five distinct groups based on MAb reactivity. Examination of 28 of these viruses isolated in tissue culture (at Weybridge) suggested that 2 bats from Omek resembled type 1 viruses, and the Yuli, as well as two bat isolates from the Ukraine, resembled type 5 viruses. A virus from a ground squirrel (Suslik) was different from these other isolates and should undergo further examination by the group.

4. Examination of non-rabies rhabdoviruses with anti-lyssa MAbs

Following the observation that Centocor anti-rabies conjugate reacts with Adelaide River Virus, a non-rabies rhabdovirus, 70 of 85 rhabdoviruses (kindly supplied by Dr C.H. Calisher, CDC, Fort Collins, USA) were examined with a panel of 158 anti-lyssa MAb made available to Mr King by the "monoclonal antibody group". Considering the above panel in its entirety, positive reactions were observed with 15 viruses. As shown in the following table, seven virus isolates reacted with Centocor conjugate. Centocor anti-rabies conjugate, composed of two MAb (502-2, 103-7), is widely used as a rabies diagnostic reagent. Therefore, positive results when testing material from hosts not normally associated with rabies or from rabies-free areas should be further investigated.
Table 2

Fort Collins non-rabies rhabdoviruses tested against Centocor anti-rabies conjugate.

<table>
<thead>
<tr>
<th>FC #</th>
<th>Virus</th>
<th>Origin</th>
<th>Source</th>
<th>Reactivity</th>
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<tr>
<td>4</td>
<td>Bahia Grande</td>
<td>C.A.R.</td>
<td>Mosquito</td>
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<td>Gossas</td>
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<td>C.A.R.</td>
<td>Bird</td>
<td>4</td>
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<td>73</td>
<td>Rochambeau</td>
<td>Fr. Guiana</td>
<td>Mosquito</td>
<td>2</td>
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</table>

* 4 = ++++; 2 = ++ immunofluorescence quality.
5. **Standardization and availability of a limited MAb panel**

Centocor Inc., has agreed to prepare an FITC-conjugate from MAbs C15-2 and W422-5. Large quantities of ascites fluid containing these MAbs were prepared by CDC and Wistar and will be sent to Centocor. Evaluation of the FITC-conjugates will be performed at CDC. If satisfactory, the product will be lyophilized and packaged at Centocor. The final product will be re-tested at CDC and Wistar and then distributed through WHO, or a WHO collaborating centre.

6. **Preparation of a manuscript summarizing the work of the WHO collaborating centres (1981-1990) on the identification of lyssaviruses with a limited panel of MAbs**

A collaborative paper is under preparation that would effectively summarize the results of the WHO Consultations on MAbs in Rabies Diagnosis and Research.

An initial draft of this paper was distributed and commented upon.

Subjects discussed in the paper include the identification of lyssavirus types by their reaction with 10 MAbs (anti-nucleoprotein), a presentation of the core of the identification scheme which comprises 3 MAbs:

- **502-2** - positive with all lyssavirus types
- **C15-2** - positive with all type 1 ("classical" rabies viruses)
  - negative type 2, 3, 4, 5
- **422-5** - negative type 1, and 5
  - positive type 2, 3, 4

Modalities for specific identification of each Lyssavirus type which requires 7 additional MAbs is given. A proposed list of prototype isolates for each Lyssavirus type is also presented in the draft. It is underlined that laboratory strains can be identified and distinguished from field strains by reactivity with 4 MAbs-(anti-nucleoprotein) and further identification requires neutralization tests with 5 MAbs-(anti-glycoprotein).

Final comments should be submitted to Dr. M. Lafon by mid-June 1990.

7. **Final conclusions & recommendations**

The above mentioned panels allow identification of the various lyssavirus types, subtypes, and the differentiation of major virus strains used for vaccine production from field virus isolates. An additional panel of MAbs was assessed by the group during 1989, in order to differentiate virus isolates of terrestrial animal species from those circulating in European bat species. Furthermore, individual laboratories belonging to the group developed specific panels of MAbs allowing differentiation of rabies virus isolates from major terrestrial and bat species from rabies enzootic areas of the United States, Western Europe and to a lesser extent eastern Europe, Latin America, Asia and Africa.

The group therefore considers that after 8 years of WHO-coordinated collaborative research (see list of consultations in Annex 2), most objectives defined during the first WHO consultation on the subject held in Geneva in September 1982 (see Annex 3) have been fulfilled. In this context the group recommends that priority be given to:

1. Distribution of the reduced panel of MAbs to selected laboratories and training of laboratory staff in the use of limited monoclonal antibodies for rabies diagnosis;
2. Procurement of technical support to laboratories which have shown interest in MAbs and have demonstrated their ability to initiate preliminary screening of isolates (this refers especially to USSR, China and Nigeria);

3. Promoting the collection and proper storage of virus isolates in Asian, African and east European countries and facilitating their examination by the laboratories belonging to the group.

Specifically, further investigation of bat isolates from the Ukraine and bats as well as Suslik isolates from Siberia is required. These isolates have been distributed to the various WHO collaborating centres. Scientists from the Rabies Unit of the WHO Collaborating Centre for Reference and Research on Rabies at the Institute of Pulionmyelitis and Encephalitides, Moscow, should be provided with the reduced panel of 10 MAbs for screening new isolates and should be regularly invited to group meetings.

There is also a need to increase the geographical coverage of the group to improve the knowledge of rabies epidemiology in Asia.

Under these conditions, WHO should consider organizing the next meeting of the group in Moscow, during summer 1991. The possibility of inviting a scientist from China to this meeting should be investigated.

Part II. Monoclonal Antibodies for Post-Exposure Treatment of Rabies

Dr. H. Koprowski was the moderator of the round table and Dr Dietzschold led the discussions. In his introduction Dr Koprowski gave a historical perspective on the classical rabies post-exposure treatment of humans, and the advantages and disadvantages of the various techniques. In addition, he described the potential utility of murine MAbs for the future. For example, there is good evidence from cancer patients in whom they were used that mouse MAbs are extremely safe and that up to 12 gm of mouse protein could be given with few to no side-effects. Moreover, cases of humans sensitized by mouse protein are rare. Chimeric (mouse/human) antibodies were being used in passive therapy of cancer patients. Human antibody was produced in EBV-transformed cells, but it was unlikely that this type of treatment would be licensed for use in rabies, in view of the oncogenic properties of the cells.

Since rabies post-exposure treatment failures are still reported and human immunoglobulin is not available worldwide in sufficient quantities, it is encouraging to try to produce MAbs free of side effects for rabies post-exposure treatment in humans.

1. Development of a MAbs Cocktail

Experiments carried out at the Wistar Institute showed that neutralizing antibodies were most effective in the early stages of infection, and before invasion of the nervous system. Once the nervous system has been invaded the efficacy of the MAbs is questionable. Rabies MAbs have clear advantages over the products now in use:

1. They are highly specific
2. Their production can be closely controlled
3. Their quality can be easily monitored.

Since one antibody alone could not be used, because of the possibility of escape variants, the strategy followed by Wistar's scientists was to choose MAbs with different specificities and to pool them into a "cocktail" of mouse MAbs. Five MAbs were empirically selected. All selected antibodies have high binding affinities and the present cocktail contains in addition to 3 C protein specific antibodies, two MAbs with anti-N activity. Although it was difficult to explain the action of the latter two MAbs, it can be demonstrated that anti-N MAbs are effective with activated macrophages.
In addition the Wistar Institute has prepared hybridomas that secrete rabies virus-specific human MAb generated by somatic cell hybridization techniques from peripheral B lymphocytes of vaccinees, immunized with HDCV. Among these human MAb six reacted with the rabies virus ribonucleoprotein and three with the glycoprotein. One of these glycoprotein-specific MAb effectively neutralized in vitro all fixed rabies virus strains as well as six street rabies isolates, but did not neutralize European bat lyssaviruses nor Nokola virus.

It was identified that a MAb cocktails, whether composed of mouse, human or chimeric MAb, must be tailored to the antigenic profile of viruses in each region.

2. Protection experiments in animal models with MAb

In preliminary experiments, individual mouse MAb gave differing degrees of neutralizing activity and protection against challenge. Therefore, the in vivo protective action of the cocktail is complicated, and not easily predicted based upon in vitro study. From experiments with the mouse MAb cocktail, using different concentrations given to mice, followed by challenge at different times, it was shown that the amount of antibody necessary for protection is a function of time (because the virus invades the nervous system very shortly after infection) and of the site of challenge; ten times more antibody was needed to protect experimental animals when the challenge was given into the masseter muscle rather than into the gastrocnemius muscle. It was recognized that the mouse is a relatively poor model for these studies.

Protection experiments with human MAb in rabies-infected mice and hamsters revealed that the virus-neutralizing MAb are effective not only in preventing lethal rabies virus infection when administered before the virus challenge, but also in post-exposure situations. In addition, treatment of hamsters with 100 international units (I.U.) of the MAb cocktail 24 hours and 72 hours after virus inoculation resulted in complete protection from lethal rabies virus infection. These results indicate the potential of generating human MAb that can be used in rabies post-exposure treatment in humans. The low number of virus-neutralizing human MAb makes it very difficult to obtain a MAb cocktail. Another obstacle in the utilization of human MAb is the potential contamination with the EBV genome.

Animal models for human post-exposure treatment were discussed. Studies in female Syrian hamsters yielded data on duration of immunity in an heterologous host. Protection is seen with 20 to 40 I.U. of MAb according to the peripheral route of challenge. In addition, the time after injection of virus during which treatment is effective is dependent upon the route of inoculation (inferior with masseter inoculation than with gastrocnemius muscle).

3. New techniques for MAb production

A report was made on genetically engineered MAb. Recombinant DNA techniques are now available which permit the conversion of murine to human MAb. One method is to create chimeric antibodies. The rearranged DNA encoding the mouse variable region (VH, VL) from a hybridoma is cloned initially into bacteriophage lambda, then fused to the DNA encoding the human constant heavy and light chains. Upon co-transfection into mouse myeloma cells, an antibody is produced which retains the binding specificity of the original antibody but is considerably less immunogenic to human patients.

A procedure for producing MAb without using the time consuming procedure of creating hybridomas was described. In essence, RNA is isolated from the spleen of an immunized mouse. The variable regions of practically all light and heavy chain mRNA are amplified using the polymerase chain reaction (PCR). The amplified VL and VH DNA are
next cloned into separate bacteriophage lambda vectors to produce libraries of light and heavy chain V regions. These two libraries can then be combined so that heavy and light V regions are made in the same E. coli cells and the presence of Fv specific for rabies virus glycoprotein can be identified using 125 I glycoprotein. Once such Fv has been detected, the V regions can be used to construct complete light and heavy chain genes. This approach can be adapted to produce completely human MAbs.

At present, murine MAbs are produced from hybridomas in mice as ascites fluid or in tissue culture, but both methods are expensive and limited in capacity. Recent advances suggest that it will soon be possible to produce high yielding antibodies in yeasts much more economically.

4. Recommendations and future study

a) The group discussed the various topics concerning the development and evaluation of MAbs in post-exposure treatment and came to several recommendations:

- The efficacy of a mixture of MAbs against epidemiologically relevant street virus should continue to be evaluated.
- The MAb "cocktail" should consist of at least 3 antibodies to minimize the probability of treatment failure due to escape variants.
- Only MAbs of IgG isotypes should be considered.
- MAbs should be of high affinity and broad specificity.
- The composition of the "cocktail" should be adjusted to regional needs (e.g., Southeast Asia, Latin America, etc.) dependent upon local street virus types.

b) Future testing should include these points:

- Testing should continue in small animal models, preferably Syrian hamsters (female, 100-200 grams).
- The HRIG should be used as a standard.
- Potency testing in primate models should be performed in addition to other laboratory animal models especially using MAbs and the combination of MAbs and vaccine.
- Dependent upon primate trials, initial tests in humans must conform to basic FDA/IABS suggestions before initiation.
- Depending upon local needs and epidemiological circumstances, the efficacy of post-exposure treatment of captive animals (e.g., companion animals, livestock, exotic animals in zoos, etc.) with MAbs (and vaccine), must be individually evaluated.
- Future research should continue to evaluate the various biotechnological advances (e.g., antibody chimeras, "humanization" of murine MAbs, recombinant-based and produced antibodies, etc.). This type of research should be strongly encouraged.
Continued collaboration between the various WHO centres should be fostered in the applications of MAbS in the post-exposure treatment of humans and animals.

Note: In addition to the participants of the Sixth Consultation on Monoclonal Antibodies in Rabies Diagnosis and Research (see Annex 1), the round-table discussion was also attended by Dr. H. Ertl, Dr. B. Dietschold, Dr. R. Lerner and Dr. P. Curtis, all of the Wistar Institute.
ANNEX 1

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LIST OF CONSULTATIONS ORGANIZED ON THE SUBJECT


2. Meeting on Monoclonal Antibody for Rabies Diagnosis and Research, organized by the Wistar Institute of Anatomy and Biology, 1983.


5. Monoclonal Antibody in Rabies Diagnosis and Research, Fourth report, St Simons Island, Georgia, USA, 26-28 May 1986 (VPH document, unnumbered).


ANNEX 3


The WHO collaborative study should be continued and expanded with the active participation of more laboratories in different parts of the world. For this kind of study a basic panel of monoclonal antibodies should be agreed upon. The same techniques should be used in all laboratories and a rapid exchange of results should be ensured. Isolates that have unusual antigenic characteristics should be carefully preserved.

Special attention should be given to virus isolates from victims of rabies who die following a complete postexposure treatment and from animals that succumb to rabies despite previous vaccination.

Further use of monoclonal antibody should be devoted to the following areas:

1. The study of the antigenic characteristics of rabies virus strains from different geographical areas should continue. This will allow for clarification of epidemiological events.

2. Efforts should be made for the preparation of monoclonal antibodies specific for selected field viruses representing rabies reservoirs from different parts of the world.

3. Until the host range of rabies-related viruses is more accurately established, routine diagnosis of all rabies-suspected cases in areas where rabies-related viruses could be present should include a panel of monoclonal antibodies capable of identifying the viruses.

4. Monoclonal antibody reactivity patterns of all viruses used for vaccine production should be established. This will allow differentiation of vaccine-induced rabies in animals.