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Report

Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs):

Third Meeting

Geneva, Switzerland
1-2 March 2001

WORLD HEALTH ORGANIZATION
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CONTENTS

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Report

1. INTRODUCTION

The Third Meeting of the WHO Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (WHO TSE Working Group) was held at WHO in Geneva on 1-2 March 2001. The meeting was opened by Dr Yasuhiro Suzuki, Executive Director of the WHO Health Technology and Pharmaceuticals Cluster. Dr Suzuki acknowledged the increasing concern about the troubling possibility that blood products and other medicines and surgical procedures might spread the agent of variant Creutzfeldt-Jakob Disease (vCJD). He welcomed the Group and endorsed their recent efforts to facilitate improved diagnostic testing for CJD by development of internationally agreed-upon reference materials.

Dr Padilla announced with deep regret the untimely death of Dr Clarence Joseph Gibbs Jr. of the National Institute of Health , USA. Dr Gibbs was a founding father of research on TSEs and an early supporter of the WHO TSE Working Group.

2. HUMAN TSE BRAIN-DERIVED MATERIALS: WHO COLLABORATIVE STUDY

2.1 *In vitro* studies

As agreed at the previous meeting of the Working Group, samples of human brain-derived candidate biological reference materials have been distributed by the National Institute of Biological Standards (NIBSC) to the participating laboratories. Those materials were prepared from samples taken from four brains supplied by the CJD Surveillance Unit, Edinburgh, UK. All brains were from persons homozygous for methionine (Met/Met) at the prion-protein-encoding gene (*PRNP)* codon 129; one was a neurologically normal subject, one from a case of vCJD, and two from clinically and histopathologically typical cases of sporadic CJD. The sporadic CJD cases had different PrP isotypes on Western Blotting, corresponding to type 1 and type 2. One hundred grams of each brain were homogenised as a 10% suspension in 0.25M sucrose. The homogenates were distributed into 2000 vials, each containing 0.5ml of suspension.

Seventeen participants agreed to take part in the initial round of a study to compare *in vitro* methods for detection of PrP^{res}**,** specifically immunoblot (15 participants), conformation-dependent immunoassay (CDI) (2 participants) and a DELFIA-based system (2 participants). So far, complete results have been returned from four laboratories carrying out immunoblots, one using CDI and one using the DELFIA -based system. While it is still premature to analyse the data in quantitative terms, the PrP^{res} (the protein resistant form of the abnormal scrapie type prion protein designated PrP^{Sc}) level in one preparation from a sporadic case of CJD appeared to be lower than that from either the other case of sporadic CJD or the vCJD case in most assays reported. The minimal mass of brain tissue required to give a detectable signal differed by an order of magnitude in the different assays, although this result is highly provisional. In terms of dilution end points, the sensitivities varied by more than 2 logs, largely because different sample sizes were assayed.

All immunoblot assays of the vCJD specimen gave the expected pattern of three bands (diglycosylated, monoglycosylated and unglycosylated). However both sporadic CJD specimens unexpectedly gave a double band in the unglycosylated region in some assays indicating that the materials chosen could be a mixture of types 1 and 2 glycotypes.. Possible explanations include

a gel artefact, an artefact of proteinase K digestion, the use of sucrose without buffer as diluent for the samples, that the samples might be a mixture of material from more than one case having different PrP^{res} types, or a reflection of PrP heterogeneity in these cases due to the large amount (100gr) of human-derived material selected.

The gel pattern was reproducible in three laboratories, and the bands migrated at 21KDa and 19KDa, so gel and digestion artefacts seem unlikely. Genetic analyses of the samples by Short Tandem Repeats and by platelet antigen polymorphism indicated that all four preparations were distinct; each pattern was consistent with an origin from a different individual. Professor Gambetti suggested that further efforts be undertaken to find large brain specimens containing only pure 19 KDa or 21 KDa non-glycosylated PrP^{res}.

The Group considered that the vCJD preparation appeared to be suitable for both *in vivo* and *in vitro* assays, while there was some concern that the two cases of sporadic CJD may not represent the most common PrP^{res} glycotype forms of CJD occurring in subjects homozygous for methionine at polymorphic codon 129 of *PRNP*. However, because previous specimens for glycotyping have never been prepared from such large portions of brain, it is not yet known how often two PrP^{res} glycotypes may coexist in different parts of the same brain.

The Working Group requested that all participating laboratories submit results within two months. Completed results will be analysed and a Draft Report of *in vitro* studies, prepared by Dr Minor will be circulated to participants in advance of the next meeting.

2.2 Infectivity assays

The vCJD candidate reference material will be assayed for infectivity in a variety of conventional and transgenic mice, as agreed upon in the Second Meeting of the Working Group. The following laboratories expressed willingness to initiate intracerebral titrations of the vCJD material before the end of 2001: Professor Collinge, Dr Manson, Dr Bruce, Dr Baron and Dr Groschup. Other laboratories may also participate. At least two laboratories may also titrate one or both sporadic CJD candidate reference material preparations.

Dr Bruce reported preliminary results on the measurement of infectivity assayed in RIII mice inoculated intracerebrally with brain, spleen and blood from another case of vCJD. The level of infectivity was about 10^5 LD50 per gram of brain tissue, somewhat less than 10^3 per gram of spleen tissue. No infectivity was detected in blood. These infectivity levels were similar to those Dr Bruce previously measured in tissues of cows with BSE. Based on the infectivity assays presented by Dr Bruce, Professor Ironside suggested collecting spleen tissues from sporadic and vCJD cases as possible additional reference materials.

3. TERMINOLOGY FOR TYPES AND SUBTYPES OF HUMAN PRP^{res}

Professor Gambetti had sent a glycotyping protocol and tissues from 10 different spCJD patients containing various glycoforms of PrP^{res} to NIBSC, and Professor Ironside will provide additional brain samples within two weeks to Professor Collinge who will submit them, together with his protocol, to NIBSC within a month. A list of participants and the final design of a study is in development and will be circulated among the members of the Working Group within two weeks for comment. The participants will be asked to perform the immunoblot test according to both protocols and any other they choose. The results should be available for the next meeting of the Working Group.

4. RODENT-ADAPTED STRAINS OF TSE AGENTS

Dr Minor will discuss with Dr Bostock, Institute for Animal Health, Compton, UK, the possibility of providing certain rodent-adapted strains of TSE agents for consideration as possible biological reference material. Strains of interest will include the following: 263K hamster-adapted scrapie; ME7, 22A, and RML mouse-adapted scrapie; and 301V mouse-adapted BSE. These reference materials will be suitable calibrants for both infectivity and PrP^{res} titres of seed and working stocks of rodent-adapted TSE agents.

5. BLOOD-DERIVED REFERENCE MATERIALS: HARMONIZED PROTOCOL

The Working Group agreed that a simplified protocol, prepared by Dr Rohwer, to collect small amounts of blood from humans with CJD, may be useful for future investigations of blood-

based diagnostic tests. The protocol may not be suitable for studies localizing infectivity in various components of blood. The protocol is attached and will be circulated among representatives of relevant professional and trade associations (PPTA, EPFA, ISBT, AABB) and the European Concerted Action for comments.

Because neither infectivity nor PrP^{res} have been detected in human blood, it is not clear whether blood or any component of blood can provide a useful biological reference material. The rodent-adapted TSE strains discussed above might be considered as reference materials suitable for calibrating spike preparations used in process validation protocols for removal of PrP^{res} and infectivity from blood. Because there was no consensus of what constitutes a relevant TSE spike, the Working Group concluded that it was premature to develop reference materials for the various possible spikes themselves. The Group felt that it would be useful to invite scientists actively conducting research on TSEs in the blood area to join the Working Group.

6. ACTIVITIES IN RELATION TO ANIMAL TSE REFERENCE MATERIALS

Dr Matthews reported that some bovine material potentially suitable for preparation as a BSE reference is available at the Veterinary Laboratories Agency, UK. Dr Minor volunteered services of the NIBSC in support of this activity if needed. Dr Groschup will investigate the possibility of collecting BSE-derived material suitable for candidate biological reference preparations.

Professor Hunsmann reported on progress concerning a pathogenesis study of BSE in monkeys infected via the oral route. Intracerebral titrations of the inoculum in monkeys and mice are already in progress. A small sample of the BSE-infected starting material will remain at the end of the experiment. It may be possible to dilute some of that material slightly to serve as a relevant titrated reference biological material. The participants agreed that this study deserves continued support.

7. OTHER BUSINESS

7.1 Dr M. Ricketts of the WHO Department of Communicable Disease Surveillance and Response asked whether the Working Group considered it appropriate, and if so, if it was willing to review and summarize the role of rapid tests for PrP^{res} in the diagnosis of BSE in animals. The Group considered that the OIE would be the appropriate organization to deal with this matter.

7.2 In conjunction with the Working Group meeting, Professor Pocchiari delivered a lecture to WHO staff on recent events regarding TSEs with emphasis on BSE and vCJD and provided an update on Diagnostic Methods.

7.3 The following dates were proposed for next Working Group Meeting: 26 TO 27 November 2001

Proposal for collection, separation and storage of blood and blood components from TSE-infected humans

1. Collect whole blood anticoagulated with sodium citrate (final concentration 0.5% citrate).

2. Remove a 10% aliquot for storage at ≤-80°C.

3. Centrifuge the remainder at 2280g for 4 minutes at room temperature.

4. Use a transfer pipette to remove the plasma layer, leaving the last 5 mm on the buffy coat. Store the plasma layer as platelet-rich plasma (more strictly designated as "2280g" plasma) at ≤-80°C.

5. Use a separate transfer pipette to collect buffy coat including 5 mm of the underlying red blood cell layer. Store at ≤-80°C.

6. Collect the remaining Red Blood Cells with a third transfer pipette, taking care not to touch the tube walls. Store at ≤-80°C.

WHO Working Group on International Reference Materials for Diagnosis and Study of TSEs
3rd Meeting: 1 - 2 March 2001
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