WHO/CDS/CSR/ARO/2005.4

EPIDEMIC ALERT & RESPONSE

WHO Advisory Committee on Variola Virus Research

Report of the Sixth Meeting

Geneva, Switzerland
4–5 November 2004

World Health Organization

Department of Communicable Disease Surveillance and Response

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1. Introduction and report of the Secretariat

1.1 Dr Mike Ryan welcomed participants to the sixth meeting of the WHO Advisory Committee on Variola Virus Research. This was reiterated by Dr Cathy Roth who indicated that the purpose of the meeting was to review the progress of current essential research that depended on access to live variola virus, to advise WHO on the continuing need for this research and to consider proposed revisions to the 1994 recommendations of the Ad Hoc Committee on Orthopoxvirus Infections. Professor Geoffrey Smith was appointed Chairman and Dr Robert Drillien and Dr Peter Greenaway Rapporteurs.

1.2 Dr Daniel Lavanchy then summarized the work of the WHO Secretariat during the past year. He noted that the Ad Hoc Committee on Orthopoxvirus Infections, which advises WHO on public health perspectives, had met on 31 August and 1 September 2004 to consider two main issues.

1.3 The first item considered by the Ad Hoc Committee was a proposed operational framework for the development and management of a smallpox vaccine stockpile and strategic reserve of smallpox vaccine for use by WHO in an emergency. It was intended that this should be composed of two components – a stock of 5×10^6 doses to be held in Geneva and pledged stocks of 200×10^6 doses to be held by Member States. The operational framework governing the generation and use of these stocks was now being considered by WHO.

1.4 Second, the Ad Hoc Committee considered possible revisions to their 1994 recommendations concerning the handling of live variola virus and variola virus DNA. The conclusion was that members of the WHO Advisory Committee on Variola Virus Research were best placed to propose changes to these recommendations and advise on how any changes should be implemented.

2. Update on variola virus strains in the two virus repositories

2.1 Updates were given on the variola virus strains held in the repositories at the Russian State Centre for Research on Virology and Biotechnology (VECTOR), Koltsovo, Russian Federation and the Centers for Disease Control and Prevention, Atlanta, United States of America. Basically, there had been no change in the inventories at either repository. However, a unifying inventory system giving (where known) the derivation of each strain, the material available, virus titre and number of vials was being introduced. These inventories were compliant with the United States Regulations on Select Agents and were lodged with WHO.

2.2 The Advisory Committee was reminded that the Russian collection comprises 120 strains, including 17 primary isolates. It also holds libraries of hybrid plasmids carrying DNA fragments of the complete genomes of eight variola virus strains belonging to two epidemiological types and isolated in different geographical regions of the world. Two internal inspections had been carried out in January and June 2004 that confirmed that the repository was being adequately maintained and that the inventory corresponded to what variola virus cultures were present.

2.3 The Advisory Committee was also reminded that the United States collection contained 451 isolates. The year/date of isolation was known for 229 of these and the geographical area of isolation was known for 238 of them. A total of 49 isolates from divergent geographical origin, year of isolation and low passage number were chosen for in-depth study. Of these, 45 were shown to be viable and have been subject to restriction fragment length polymorphism (RFLP) analysis. Of these, 40 have been sequenced completely. It was noted that a number of requests had been made for the supply of DNA fragments for diagnostic purposes. These have been fulfilled.

2.4 The Advisory Committee was informed that work on the rabbitpox/variola virus chimeric viruses had been carried out to further characterize available diagnostic tests and that additional work on these viruses as well as on the cowpox/variola chimeric viruses was being contemplated before DNA could be isolated from these viruses and archived. Problems with the availability of the BSL-4 laboratory have delayed this work. The Committee maintained the view that retention of the viruses had no scientific justification and repeated the recommendation, made at its fourth and fifth meetings, that these viruses should be destroyed and their destruction recorded in the inventory.

3. Update on diagnostic assays

3.1 Dr I. Damon then presented a "Review and update on reagents and diagnostics: nucleic acids, preparing for FDA review, and monoclonal characterization". The key points were:

- quantitative and qualitative diagnostic assays based on real-time PCR were available and had been field-tested. Reagents and protocols had been refined and standardized. Validated tests had been distributed to public health laboratories;

- the sensitivity of these tests was now down to between 5 and 50 genome copies using DNA prepared from purified virus. The tests were capable of differentiating variola virus from other orthopoxviruses and distinguishing between different variola virus strains;

- IgM capture assays had been used successfully to follow the immune responses to monkeypox virus infections in the United States as well as classifying possible cases. IgM responses to monkeypox virus infection could be detected in those vaccinated previously against smallpox;

- laboratory algorithms covering febrile illness had been developed;

- monoclonal antibodies recognizing conformational epitopes to variola virus have been characterized partially and have some variola virus-specific neutralizing activity. Further analysis of a second set of monoclonal antibodies revealed that they recognize a 41 kDa protein of variola, vaccinia and camelpox viruses.

3.2 Dr H. Meyer presented a paper on the "Detection of orthopoxviruses and simultaneous identification of smallpox virus". He noted that available methods for the identification and differentiation of orthopoxviruses were based on electron microscopy, antigen and antibody detection, isolation and real-time PCR. He noted that:

- the identification of variola virus could be both rapid and specific;
- robust methodologies had been developed and published, and the reagents involved had been produced to good manufacturing practices (GMP) standards;
- tests to differentiate orthopoxvirus strains had been field-tested and these could also differentiate orthopoxvirus infections from other confounding virus infections such as Varicella-zoster virus infections.

3.3 Dr N. Tikunova described her work on the production of "Human combinatorial antibodies against orthopoxviruses". The objective here was to create a collection of recombinant human antibodies against orthopoxviruses and to select those with either genus specificity or variola virus specificity. In summary:

- initial work using a combinatorial synthetic phage display library had yielded a number of antibodies that recognized orthopoxviruses including variola virus, but none of these neutralized variola virus;
- it was decided to create a phage display library from individuals immunized with vaccinia virus;
- antibodies that can be used for orthopoxvirus differentiation and neutralization were obtained and characterized.

3.4 Dr V. Loktev presented a paper on "Cross-reactive neutralizing monoclonal antibodies to the orthopoxviruses". The key points made included:

- hyperimmune sera to different orthopoxviruses are not discriminatory as there are many cross-reactions. This provides a justification for the isolation of neutralizing monoclonal antibodies;
- rat and mouse hybridomas against ectromelia, vaccinia and monkeypox viruses have been produced, or obtained from Dr Ichihashi, and characterized;
- many of these recognize a native 14 kDa protein from variola and ectromelia viruses and may recognize a neutralizing cross-reactive epitope. Some of these were able to neutralize variola and vaccinia viruses.

4. Update on vaccines

4.1 Professor S. Shchelkunov initiated this part of the meeting by describing the "Creation of a live polyvalent vaccine against human immunodeficiency virus, hepatitis B virus, and orthopoxviruses". He noted that such a vaccine would have significant benefits for both biosecurity and public health. The candidate vaccine had been created using coding sequences for T-cell and B-cell epitopes of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) proteins,

which subsequently had been inserted into vaccinia virus. The recombinants produced had the following characteristics:

- were stable in tissue culture over 10 passages;
- induced immune responses to the expected epitopes when inoculated subcutaneously and intracutaneously in BALB/c mice;
- were candidates for a live polyvalent virus vaccine.

4.2 Dr A. Sergeev presented a paper describing studies on the "Reactogenicity, safety and immunogenicity of recombinant variola and hepatitis B bivaccine for oral immunization in humans". This vaccine had been developed because of the risk associated with the use of intradermal inoculations. Tablets “Revax–BT” had been produced. These were stable at –20 °C for 3 years and could be administered orally (held in the mouth while dissolving). He described:

- a clinical trial in which volunteers of different ages and vaccinia immune status were immunized with different doses of vaccine at different times;
- results in which both different degrees of reactogenicity and different levels of immune responses were detected;
- difficulties in obtaining significant immune response to hepatitis B antigens;
- an optimal scheme for immunization.

4.3 Dr D. Ulaeto then outlined "UK research on medical countermeasures to smallpox". The aim of this research is to develop or provide access to effective and licensed vaccines and pharmaceutical countermeasures to smallpox. He indicated that the United Kingdom had:

- purchased vaccine stocks based on the Lister strain grown in tissue culture and had benchmarked these against existing vaccine stocks;
- observed some variation in protective efficacy of the different vaccine stocks in mice;
- undertaken studies on the efficacy of modified vaccinia virus Ankara (MVA) as a second-generation vaccine and that this had performed well in murine lethal