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WHO Advisory Committee on Variola Virus Research

Report of a WHO meeting

Geneva, Switzerland, 6-9 December 1999

WORLD HEALTH ORGANIZATION

Department of Communicable Disease
Surveillance and Response

Contents

I. Introduction

1. Dr Lindsay Martinez, Director, Department of Communicable Disease Surveillance and Response, welcomed participants and indicated that the Advisory Committee on Variola Virus Research had been convened to comply with Resolution WHA 52.10, adopted by the 52nd World Health Assembly (WHA) on 24 May 1999. The purpose of the meeting was to :
   - establish what research, if any, must be carried out in order to reach global consensus on the timing for the destruction of existing variola virus stocks;
   - commence, if appropriate, the development of a research plan for priority work on the virus.

2. Dr Martinez stated that members of the Committee had been chosen to provide continuity with the Ad Hoc Committee on Orthopoxvirus Infections, and to obtain adequate regional representation. Independent advisers and observers were attending to ensure that sound scientific advice was available from active scientists and experts active in all related areas of interest. Legal counsel was also available to provide initial advice on the interpretation of the Resolution, if required.

3. Dr Andre Plantinga was appointed Chairperson following a pre-meeting consultation; Dr Peter Greenaway was appointed Rapporteur. Meeting participants are listed in Annex 1.

II. Background and history

4. It was noted that the WHA had declared the successful global eradication of smallpox in 1980. Post-eradication policies were subsequently overseen by a formal WHO Committee on Orthopoxvirus Infections (meeting annually until 1988) and then by an Ad Hoc Committee which had met in 1990, 1994 and 1999. A recommendation of the Ad Hoc Committee was that all live virus stocks held at the two collaborating Centres in the Russian Federation and the United States should be destroyed. This was agreed by the WHA in 1996 and a destruction date was set for June 1999.

5. The 1999 WHA reviewed this recommendation partly because of scientific and public health concerns and partly because of issues relating to the potential use of smallpox virus by bioterrorists. There was no consensus amongst Member States on the way forward but the view was taken that further research might be necessary before destruction occurred. The WHA therefore agreed to delay the destruction of remaining stocks of live virus but authorized the temporary retention of stocks, subject to annual review, for the purpose of further international research into antiviral agents and improved vaccines, and to permit high-priority investigations of the genetic structure and pathogenesis of smallpox. This Advisory Committee was convened to implement this WHA Resolution.

6. Some clarification of the Committee's remit was sought. The Committee is to make recommendations and a report is to be prepared for the WHA's next meeting. If further research is recommended then some of the recommendations could have long-term (beyond the year 2002) implications. The terms of reference for the two collaborating centres were requested and WHO agreed to make these available. Ownership of the stocks held by these centres was then questioned. The legal opinion was that they had been left for safe keeping under the supervision of WHO; ownership therefore remained unclear.

7. Dr Martinez then reviewed WHO activities since the WHA resolution. She noted that funds had been mobilized for programme management activities, that an action plan up to May 2000 had been prepared, that a Programme Manager had been appointed, that scientific discussions had been held between US and Russian scientists, that a site inspection of the facilities of the WHO Collaborating Centre, VECTOR, Koltsovo, Russian Federation, had been done and that a corresponding inspection of the facilities at the Centers for Disease Control and Prevention, (CDC), United States was planned for February. Dr Martinez agreed that the report of the scientific meeting would be made available to the Committee.

III. Inventories

8. Inventories of the live virus stocks held by the two collaborating centres were tabled.

9. CDC indicated that it may be possible to obtain further (clinical) data on the samples that they held and the Committee considered that this should be encouraged. No pathogenicity studies had been done; viability had not been systematically checked and whilst the virus had been successfully grown from three samples, some contamination was observed. A physical examination of the repository has not yet been done to confirm the inventory.

10. Koltsovo indicated that they held some 120 strains and that some work had been done on the relative pathogenicity for mice and chick embryos. Different strains clearly had different properties.

11. Members of the Committee indicated that it might be useful to identify any overlap between the two collections. It was also noted that the clinical data associated with each specimen is likely to be limited. It was therefore questioned whether the collections would be useful for either epidemiological purposes or for studies on virulence/pathogenicity.

12. Information on the availability of cloned DNA fragments was requested. It was noted that WHO should have a register of which laboratories hold cloned fragment stocks and that transfer between laboratories should be reported to WHO. It was confirmed that there were no reported random libraries of different strains.

13. The current inventory of smallpox vaccine held by different Member States was reviewed. It was noted that some 60 million doses were registered but that the status (storage conditions, revalidation etc) of many was uncertain. It was unclear how many countries held stocks of hyperimmune gamma globulin; it was noted that the US army held stocks of source plasma and that it planned to process these.

IV. State of the Art in Orthopoxvirus Research

4.1 DNA sequence information on variola virus

14. Three papers on the current status of DNA sequence information on variola virus strains were presented. The entire sequence of Bangladesh 1975, including the terminal hairpin loops was available. Entire genome sequences, apart from the terminal loops, were available for India 1967 and Garcia 1966. Sub-genomic sequences from Somalia 1977, Congo 1970, Harvey 1944, Sierra Lione and Butler 1952, plus some selected gene sequences were also available.

15. This sequence data had confirmed that the central core of all orthopoxviruses was highly conserved but that divergence increased towards the terminal fragments. There was considerable similarity between major strains (India and Harvey); greater variation was observed when comparisons to minor strains were made and most variation when variola strains were compared with either vaccinia or monkeypox virus. An analysis of the 200 open reading frames contained within the Bangladesh and India sequences indicated that 122 were identical, 42 had only one amino acid substitution, 11 had two changes and only 25 were more divergent. This variation was more apparent when the sequences of these strains were compared to Garcia and then to different vaccinia strains. Significant variation in the A33 – A52 region was observed.

16. It was noted that analysing restriction fragment length polymorphisms represented a good way of comparing different strains and that this technique could be used as a means of identifying interesting regions. Members of the Committee indicated that it might be useful to compare isolates from different regions, years or from different people from the same outbreak. The robustness of the clinical information held by the repositories was again questioned.

17. Comparisons between different variola and vaccinia isolates demonstrated that sequence variations towards the genomic termini often resulted in the fragmentation of coding regions. The corresponding proteins were therefore either non-functional or had an altered function. It would be difficult to identify those genes involved in determining virulence on the basis of sequence information alone. However, it was noted that some variola virus gene products have immunomodulatory functions and stimulate a range of host cell responses. These could have implications for pathogenesis and virulence.

18. It was concluded that sequence information would provide data on evolutionary relationships between orthopoxviruses and that this data would facilitate structure/function analyses. It would be difficult to use this data to determine which genes were involved in determining virulence. Nevertheless, screening for genes

homologous to immunomodulatory factors, replication and other enzymes, etc. could produce interesting data and identify possible functions against which drugs might be targeted. It was noted that viruses held by both repositories had not been specifically selected and this placed a limitation on what the generated sequence information could be used for.

19. The need for a good animal model to investigate variola virus pathogenesis was emphasized. Variola virus itself does not grow well in most animal models but some 'natural' animal models already existed – monkeypox in monkeys, ectromelia in mice, myxoma in rabbits. Data on laboratory attenuation of some strains (myxoma) did not always translate across to the field situation. Some caution was expressed over the interpretation of data from current animal models.

20. The need to obtain more sequence information from variola virus strains was questioned. It was agreed that whilst an open-ended sequencing programme to determine evolutionary relationships amongst orthopoxviruses had much scientific merit, there was little clinical or public health justification. However, there was consensus that additional full genomic sequences of two additional strains (one a South African major strain) would prove useful. It was also felt that identifying specific genes that could be useful chemotherapeutic targets (for example, the DNA polymerases) and then sequencing these across a range of isolates could generate valuable data.

21. It was becoming technically easier to acquire DNA sequence information and so the resources needed to undertake this further work should not prove limiting. The key question to address was the amount of sequence information that could be justified in both scientific and clinical terms. It was argued that selection of genomes to be sequenced should take into account variables such as geographical variation, time of specimen collection and disease severity.

4.2 A public health perspective to variola virus research

22. The public health agenda for further research on variola viruses had not been adequately considered during the review on the future scientific needs for live variola virus conducted by the Institute of Medicine[1]. There were three parts to the public health agenda – vaccines, chemotherapeutic agents and chemoprophylactic