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REPORT OF MEETING OF THE STUDY GROUP ON ORTHOPOXVIRUS RESEARCH ATLANTA, 26-28 JUNE 1979 (CO-SPONSORED BY WHO AND CDC)

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1. Introduction

In December 1978 the Global Commission for the Certification of Smallpox Eradication considered all aspects of the programme. One of the objectives of the Global Commission was "to recommend research activities regarding:

- possible animal reservoirs of variola virus;
- the importance to man of monkeypox and whitepox viruses;
- the possible emergence of new variola-like viruses."

Accordingly, the Global Commission made several recommendations in relation to orthopoxviruses. One of these recommendations was that a "Study Group on Orthopoxviruses" be appointed by the World Health Organization and this group meet periodically. A meeting of a study group on orthopoxvirus research was convened by WHO at the Center for Disease Control (CDC), Atlanta, from 26 to 28 June 1979.

The meeting was opened by Dr Foege, Director of the CDC, who welcomed delegates to Atlanta. Dr Arita welcomed the delegates on behalf of the Director-General of WHO and discussed the objectives of the meeting from the point of view of the smallpox eradication programme.

1.1 Short-term and urgent objectives.

1.1.1 To clarify the situation on white variants of monkeypox virus.

1.1.2 To clarify the situation in respect of "whitepox" viruses.

1.2 Medium- and long-term objectives.

1.2.1 To develop laboratory methods for deeper study of the nature and epidemiology of orthopoxviruses.

1.2.2 To maintain the capability to undertake proper laboratory investigations of any diagnostic problem that may arise in the future.

2. Procedure.

Dr Fenner chaired the meeting, assisted by Dr Nakano and Dr Dumbell as rapporteurs.

3. Participants - See Annex 1.

4. Agenda - See Annex 2 (working papers on file at the Smallpox unit, WHO, Geneva).

5. Topics considered.

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5.1 Background information on the smallpox eradication programme. The present situation with regard to human cases of monkeypox and the programme for global certification was presented by the chairman and members of the WHO Secretariat.

5.2 Recent progress in the analysis of poxvirus genomes and other aspects of the biogenesis and characterization of orthopoxvirus were presented and discussed. Particular attention was focused on the application of the new techniques for studying the structure and organization of poxvirus DNA, and the results of comparative genome mapping of variola, monkeypox and related orthopoxviruses. Analysis at CDC and St. Mary's Hospital of DNA extracted from the six "whitepox" viruses has confirmed the close similarity of these viruses to variola virus which had first been demonstrated by Dr Marennikova and her colleagues, using biological markers.

5.3 White variants of monkeypox.

5.3.1 Several clones isolated in Moscow from stocks of Copenhagen and Congo-8 monkeypox virus strains were shown to have phenotypic properties similar to those of "whitepox" and variola viruses.

5.3.2 Repetition at CDC of the isolation techniques used to obtain these clones has produced several white variants, some of which produce reactions in the rabbit skin and amounts of haemagglutinin in the chorioallantoic membrane similar to those of "whitepox" viruses.

Preliminary studies of restriction endonuclease profiles, using three endonucleases, show that some clones have profiles similar to those given by variola strains; other clones had profiles more closely resembling those of monkeypox.

5.3.3 White variants of Copenhagen and Congo-8 had also been isolated at St Mary's using experimental conditions different from the techniques used in Moscow and at CDC. Seventeen of these clones had been examined phenotypically and by endonuclease analysis; some resembled "whitepox" virus in certain properties but none had all the phenotypic characteristics of "whitepox" virus. Restriction endonuclease analysis showed that some had a deletion near the right end of the map, others had changes involving regions at both ends.

5.4 Specific immunological identification of poxviruses and poxvirus infections.

5.4.1 The potentialities of using staph A protein in order to isolate specific antigens, and the use of monoclonal antibodies as reagents of high specificity and potency were discussed.

5.4.2 Specific antibody detection in sera from the field. Field studies to define better the epidemiology and natural history of monkeypox are progressing satisfactorily in Zaire; sera and organ specimens are being collected from a large number of animals. Earlier studies had shown that a proportion of sera from monkeys and other animals had antibody to orthopoxvirus. A separate study showed that many persons in a relatively unvaccinated human population in Cameroon had orthopoxvirus antibodies. It is important to be able to detect antibody specific to particular orthopoxviruses in animals and humans.

5.5 Hazards associated with native DNA from variola and "whitepox" viruses.

5.5.1 There is no evidence, despite several attempts by experienced workers, that infectivity could be demonstrated in preparations of poxviral DNA. This finding is in accord with the knowledge that a constituent of the virus with polymerase activity is normally involved in the early transcription of the poxvirus genome.

5.5.2 Consequently, preparations of poxvirus DNA should not be regarded as infectious material, and variola DNA should not be subject to the same safety precautions that are required for handling variola virus.

5.5.3 It cannot, however, be taken that such DNA preparations are entirely free of conjectural hazard. Some transcription might occur if experimental conditions permitted access of such DNA to the cell nucleus and it has been shown that some expression of poxvirus DNA may occur in cells concurrently infected with another orthopoxvirus.

6. Recommendations.

6.1 Investigation of white variants of monkeypox virus.

6.1.1 CDC should undertake further endonuclease analysis of the parental monkeypox viruses and designated white variants obtained from them in Moscow and at CDC (See Annex 3).

6.1.2 CDC should be requested temporarily to direct extra resources so that these studies may be completed by the end of November, 1979.

6.1.3 Dr Dumbell should be encouraged to carry out further endonuclease analyses of parental monkeypox viruses and variants derived from them.

6.1.4 Other studies already in progress on white variants of monkeypox should be continued.

6.2 "Whitepox" viruses.

6.2.1 Further work should be done on the comparison of these viruses with variola viruses and monkeypox viruses, by DNA analysis and by two-dimensional analysis of polypeptides. Particular emphasis should be placed on a search for polypeptides or DNA sequences unique to individual viruses.

6.2.2 At the convenience of the laboratories concerned, materials should be made available to laboratories other than the collaborating centres who are willing to work on this problem with due regard to the safeguards listed in 6.3.

6.3 Hazards of native DNA from variola and "whitepox" viruses.

6.3.1 Intact or fragmented variola DNA may be handled outside a maximum containment laboratory provided that:

6.3.1.1 No work with viable poxviruses is concurrently being undertaken in the same room.

6.3.1.2 None of the workers having access to that room have been revaccinated during the previous seven days or are suffering from any poxvirus infection.

6.3.1.3 Experimental materials are properly disposed of at the end of the experiment.

6.3.2 If intact variola DNA is to be transferred from a WHO collaborating centre to another laboratory, the director of the other laboratory should give a written undertaking that.

6.3.2.1 The conditions in 6.3.1 will be observed.

6.3.2.2 The work will be carried out by, or under the direct supervision of, a named and suitably qualified worker.

6.3.2.3 The DNA preparation when not in actual use will be kept in a locked container of which the named supervisor holds the key.

6.3.3 That these recommendations be incorporated into the WHO recommendations for safety measures in laboratories handling variola virus (SME/77.2).

6.3.4 That encouragement be given to the use of the techniques of genetic manipulation to "clone" fragments of variola DNA into a suitable plasmid or other vector in order that adequate amounts of variola DNA for genetic analysis might be provided with reduced hazard.

6.4 Specific immunological identification of poxviruses and poxvirus infections.

6.4.1 CDC should be requested to assign appropriate resources so that after suitable screening selected sera obtained from the field studies in Zaire and Cameroon can immediately be examined by the adsorption-RIA test. Virus isolation from tissues from selected animals should be attempted following these tests.

6.4.2 In the longer term, CDC, in collaboration with the Department of Microbiology, Duke University, should develop monoclonal antibody reagents specific to appropriate orthopoxviruses and devise a test system which can be applied to the screening of a large number of specimens.

6.4.3 Other laboratories should be encouraged to develop and utilize similar techniques.

6.5 Comparative virology of poxviruses.

6.5.1 WHO should encourage the study of the comparative virology of orthopoxviruses and other poxviruses, in order that future isolates, perhaps from novel situations, may be quickly and correctly identified.

6.5.2 Study of the natural history of monkeypox and other animal poxviruses should be encouraged and supported, and the reporting of outbreaks of poxvirus infections in animals, or their transmission to man, should also be encouraged.

6.5.3 Capability should be maintained to undertake proper laboratory investigations of any diagnostic problem that may arise in the future.

6.5.3.1 By ensuring that there are suitable maximum containment laboratories and that variola and variola-like viruses are maintained for comparative studies.

6.5.3.2 By the development of specific and relatively hazard-free diagnostic methods.

6.5.4 The maintenance of interest and expertise in the study of poxviruses should be supported by the establishment of a Poxvirus Study Group which should meet at intervals under the auspices of WHO.

ANNEX 1

Meeting of Study Group on Orthopoxvirus Research

List of Participants

Dr P. Bachmann, Institut für Med. Mikrobiologie, Infektions-und Seuchenmedizin, Ludwig-Maximilians-Universität, D 8000 Munich 22, Federal Republic of Germany

Dr D. Baxby, Department of Medical Microbiology, University of Liverpool, Royal Liverpool Hospital, Prescot Street, Liverpool, L7 8XW, United Kingdom

Dr S. Dales, Department of Microbiology and Immunology, Faculty of Medicine, the University of Western Ontario, London, Ontario, Canada N6A 5C1

Dr W. R. Dowdle, Center for Disease Control, Atlanta, Georgia 30333, USA

Dr K. R. Dumbell, Department of Virology, The Wright-Fleming Institute of Microbiology, St Mary's Hospital Medical School, London, W2 1PG, United Kingdom

Dr J. J. Esposito, Viral Exanthems Branch, Center for Disease Control, Atlanta, Georgia 30333, USA

Dr F. Fenner, Centre for Resource and Environmental Studies, the Australian National University, P.O. Box 4, Canberra, A.C.T. 2600, Australia

Dr E. Hayes, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27707, USA

Dr A. C. Hekker, Rijks Instituut voor de Volksgezondheid, Postbus 1, Bilthoven, Netherlands

Dr J. A. Holowczak, Department of Medical Microbiology, College of Medicine and Dentistry, Rutgers Medical School, Piscataway, New Jersey 08854, USA

Dr D. Hopkins, Center for Disease Control, Atlanta, Georgia 30333, USA

Dr W. K. Joklik, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710, USA

Dr Kalisa-Ruti, Programme Elargi des Vaccinations, B.P. 9638, Kinshasa, Zaire

Dr T. Kitamura, National Institute of Health, 284 Kamiosaki-chojamaru, Hinagawa-ku, Tokyo, Japan

Dr S. S. Marennikova, Research Institute of Virus Preparations, 1 Dubrovskaya 15, Moscow 109088, USSR

Dr B. Moss, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, USA

Dr J. H. Nakano, Viral Exanthems Branch, Center for Disease Control, Atlanta, Georgia 30333, USA

Dr E. Paoletti, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201, USA

Dr R. Wittek, Institut für Virologie, Universitat der Zurich, 8057 Zurich, Switzerland

Dr R. Wyler, Institut für Virologie der Universitat Zurich, Winterhurerstrasse 266 a, 8057 Zurich, Switzerland

Dr I. Arita, Smallpox Eradication Unit, World Health Organization, Geneva, Switzerland

Dr J. G. Bremen, Smallpox Eradication Unit, World Health Organization, Geneva, Switzerland

ANNEX 2

Agenda of Meeting on Orthopoxvirus Research, 26-28 June 1979

Opening remarks		Dr Foege
Introductions, designation of officers		Dr Arita
1.	Objectives of the meeting	Dr Arita
2.	Present status of smallpox eradication and certification	Dr Arita
3.	Human monkeypox update	Dr Breman Dr Kalisa
4.	Summary of previous meetings on orthopoxvirus research	Dr Fenner
5.	Report of the consultation on the justification for retention and use of variola virus in the post eradication era	Dr Fenner
6.	Report of the meeting of officials from laboratories currently retaining variola virus	Dr Arita
7.	Progress in genome mapping of poxviruses	
7.1	Orthopoxvirus DNA: Strain differentiation by electrophoresis of restriction endonuclease fragmented virion DNA	Dr Esposito
7.2	Genome mapping of poxviruses	Dr Wittek
7.3	Genome mapping of selected orthopoxviruses	Dr Dumbell
7.4	Heterogeneity of vaccinia virus genomes from plaque purified virus	Dr Paoletti
7.5	Terminal repeat sequences and heteroduplex genome mapping of orthopoxviruses	Dr Moss
7.6	Mirror image deletions in vaccinia virus DNA	Dr Dales
7.7	Infectivity of poxvirus DNA	Dr Joklik
8.	Progress in research on monkeypox virus as a source of whitepox virus	Dr Marennikova Dr Nakano Dr Dumbell Dr Esposito
9.	Summing up of points 7 and 8: Achievements and opportunities	Dr Joklik
10.	Specific immunological identification of poxviruses and poxviral infections	
10.1	Principles involved in detecting type-specific polypeptides by development of specific reagents for them	Dr Joklik
10.2	Experiences with several viruses including poxviruses	Dr Hayes

Annex 2

10.3	Identification of specific antibodies of vaccinia, monkeypox and variola viruses by radioimmune assay	Mrs Walls
10.4	Immunofluorescent methods	Dr Hekker
11.	Other current poxvirus research	
11.1	Structural polypeptides of orthopoxviruses: their distribution in various members and location within the virion	Dr Baxby
11.2	Variola virus strains of 1960-1975: The range of intraspecies variability and correlation between virus properties and geographic origin	Dr Marennikova
11.3	In vitro transcription and mRNA biogenesis in the poxvirus system	Dr Paoletti
11.4	Biogenesis of poxviruses: Role for the DNA-dependent RNA polymerase II of the host during late functions	Dr Dales
11.5	Studies of the structure of poxvirions and their genomes	Dr Holowczak
11.6	Differentiation of several strains of vaccinia virus by two dimension gel electrophoresis of the outer coat protein	Dr Kitamura

ANNEX 3

"Designated" strains of viruses for endonuclease analysis

1. Used and recovered at CDC, Atlanta, by Dr Shelukhina

   Cop-CDC second pp monkeypox virus

   Clones derived from virus: white 1 (N1, 4-26-79); white 2 (N11, 4-5-79)

   Congo-8-CDC second pp monkeypox virus

   Clone derived from this: white 1 (N7, 4-26-79)

   Benin monkeypox virus

   Clone derived from this: white 1 (N9, 4-19-79)

2. Recovered at CDC, Atlanta, by Dr Nakano:

   Two Copenhagen white variants

   Two Congo-8 white variants

3. Used and recovered at the Research Institute of Viral Preparations, Moscow, by Dr Marennikova

   Cop-Moscow monkeypox virus

   Clones derived from this: MpW_{Ham-1}, MpW_{Ham-2}, MpW_{Cop}

   Congo-8-Moscow monkeypox virus

   Clone derived from this: MpW_{C-8}

4. "Whitepox" Chimp 9

5. Variola Harvey

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