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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 agents.

23. Mass vaccination against smallpox had been terminated following its successful eradication; an increasing proportion of the world's population was now unvaccinated and susceptible to infection. Current vaccine supplies are extremely limited and yet immunization is the only proven public health measure available to prevent and control a smallpox outbreak. Several countries were therefore contemplating the need to produce more vaccine stocks. If such stocks were produced, issues associated with the preferred vaccine strain, method of production,

[1] The Institute of Medicine was chartered in 1970 by the US National Academy of Sciences to enlist distinguished members of the appropriate professions in the examination of policy matters pertaining to the health of the public.

quality control and the need for further research to produce a less toxigenic vaccine would all need to be addressed.

24. One view expressed was that the only vaccine that could be legitimately deployed at the present time was one that had been used in the original smallpox vaccine programme. All others would have no supporting efficacy data. The key issue was to identify the strain associated with the least toxic side-effects. It was noted that for the US this would probably be the New York City Board of Health strain (the Wyeth production strain) grown in chick embryo fibroblasts.

25. It was argued therefore that from a public health perspective, there was no further need for research into better smallpox vaccines. However, the availability of immune globulin to treat rare cases of disseminated vaccinia infections would be desirable. Some research on the generation of mouse monoclonal antibodies to variola viruses and their subsequent humanization might be justifiable.

26. It was reported that four different drugs had been evaluated for their chemotherapeutic potential against smallpox virus. None had proven to be effective. Two potential prophylactic drugs had been assessed and one had shown significant potential. Further research in these areas could prove beneficial.

27. It was acknowledged that there was a possible conceptual barrier to the development and subsequent deployment of chemotherapeutic or chemoprophylactic drugs.

28. First, therapeutic drugs would only be useful after symptoms had been detected i.e. late after initial infection. These agents would therefore have little benefit for index cases unless morbidity could be effectively prevented after primary clinical symptoms (rash formation) developed. Such drugs would have benefit for secondary cases but if vigilance was assured, this advantage would be negated by the availability of vaccines.

29. Second, prophylactic drugs would only have significant impact for those who were immunosuppressed and susceptible to disseminated vaccinia disease (at-risk populations such as the very young and the old) following vaccination. Since many immunosuppressed individuals were able to tolerate live vaccines, it was considered likely that only a few individuals would fall into this category. Hence the utility and cost-benefit ratio of developing chemotherapeutic agents was questionable. Although these agents could be deployed following a possible bioterrorist release, in the longer term, the availability of vaccines was considered a much better response.

30. One view held that the development of chemotherapeutic or chemoprophylactic agents would be useful but that, from a public health standpoint, the overall benefits were questionable. This view was challenged on the grounds that there was insufficient data available on the true pathology of smallpox virus infections and that the benefits of having suitable anti-viral agents available for treating variola virus infections should not be under-estimated. It was argued that anti-viral drugs were needed to fill the risk gap between initial infection and detection of a clinical condition. The key issue is to ensure that effective measures are available to limit

fatalities due to accidental or deliberate release of variola virus in the future. Whilst no consensus was achieved at this point, it was agreed that whatever drug treatment was available, the earlier during infection it was given, the more likely it was to be effective. This brought into focus the need for effective diagnosis and detection procedures.

4.3 Diagnosis/detection

31. Current methods for detecting and then diagnosing infections with orthopoxviruses were reviewed. It was emphasized that the diagnosis of infection in index cases was dependent on the presence of clinical symptoms. Electron microscopy had a positive contribution to make here and both negative staining (rapid) and thin sectioning (more lengthy) had significant roles to play as they could facilitate differential diagnosis (smallpox from chickenpox). There were limitations as electron microscopic diagnosis required both expensive equipment and trained personnel.

32. Culture-based technologies using both chorioallantoic membranes and tissue cell cultures offered greater flexibility and sensitivity for both detection and differential diagnosis, particularly when coupled with histological and cytochemical analyses using mono- and poly-clonal antibodies.

33. Serological assays (ELISA-based) offered both sensitivity and specificity but were dependent on the presence of pox virus antibodies or antigens in any clinical specimens provided. The availability of DNA detection following the amplification of defined sequences offered great potential for both rapid and early detection and diagnosis. The PCR technology was capable of detecting infection prior to the onset of clinical symptoms and was capable of differentiating between infections with different orthopoxvirus species.

34. It was agreed that this technology had great potential especially for near patient diagnosis and where access to specialized facilities and expertise was limited. More research was needed in this area and any equipment developed, i.e.hand held PCR detectors would need to be evaluated under field conditions. It was agreed that this would be explored but it was also noted that proof of principle had already been demonstrated using scab material present in the CDC repository. Access to live virus would be necessary if these procedures were to be validated.

35. Other diagnostic/detection technologies using phage display techniques, biotinylated nucleotides, RFLP analyses and microchips were described. It was agreed that all these technological developments were useful but, what was needed was clear guidance on the choice of tests to use, how to collect relevant specimens sometimes from remote locations and how to get the correct results back to those people who could make effective use of them. One view was that standardized diagnostic procedures were needed so that appropriate reagents and training in the required methodology were available to all public health authorities throughout the world.

4.4 Immunopathogenesis, immune responses and vaccines

36. Recent work has demonstrated that pox viruses have evolved a number of immune evasion strategies for coping with infection in a range of host organisms. These involve the development of a variety of immunomodulating activities, the synthesis of cytokine receptors, and the induction of a range of growth factors. It was argued that further research using pox viruses can help to dissect the roles and functions of the different components of the human immune system. Work on Ectromelia virus infections of mice was described and parallels with smallpox virus infection in man were identified. This may represent a possible model for smallpox pathogenesis.

37. It was confirmed that vaccinia virus infection resulted in the production of two forms of infectious virus – intracellular mature virus (IMV) and extracellular enveloped virus (EEV). IMV forms stay within infected cells until lysis occurs; EEV represent minor products of infection and contain both virus (not found in IMV) and host-cell specific proteins. EEV is extremely fragile and difficult, if not impossible to prepare in significant amounts. EEV is well suited to spread infection within a host whereas IMV is more likely to spread infection between hosts.

38. IMV and EEV have different properties, they bind to different receptors and enter susceptible cells in different ways. It was noted that EEV can escape neutralization by humoral antibodies produced as a result of natural infection; neutralization is however possible if antibodies are raised against EEV-specific antigens. Virus neutralization by antibodies is, by itself, a complex process and further research is needed to fully understand the mechanisms involved.

39. Possible approaches to producing a new smallpox vaccine were reviewed following an analysis of need. Existing vaccines had proven efficacy but also had an unacceptably high incidence of adverse side-effects. The latter were considered important particularly when the target populations for vaccination had a high proportion of at-risk individuals (the immunocompromised, the elderly, the young, etc.) A moral case could therefore be made for developing a totally new vaccine. However, the effectiveness of any such vaccine could not be adequately assessed because definitive information on the correlates of protective immunity was not available and appropriate field trials would not be possible.

40. A new vaccine would have to be based either on existing live vaccinia strains for which some immunogenicity and safety data were available or on an entirely new formulation. Vaccines based on the existing vaccinia strains had great potential as direct comparison with existing data would be possible. Second generation vaccines using, for example, single variola genes in adenovirus recombinants, Baculovirus produced proteins or DNA vaccines, would all require additional information to satisfy regulatory requirements. Validation of second generation vaccines would require access to live variola virus.

41. A case could be made for the production of an inactivated vaccine using either conventional techniques or more modern technology to develop replication deficient viruses. It was noted that this approach had already been trialed in the Russian Federation using irradiated virus as a primary vaccine and live virus vaccinia as a follow-up. This had produced fewer adverse side-effects in children.

42. The conditions associated with the deployment of either vaccinia-based vaccines or new vaccines was then considered. The discussion revolved around risk/benefit ratios as deployment following a bioterrorist attack would have different implications from the situation where vaccine was to be used for immunization of the general population. Although existing vaccines had possible adverse reactions within immunosuppressed populations, the deployment of vaccines of unproven efficacy was thought to represent the most unlikely scenario in an emergency situation.

43. One suggestion was that research should be done to generate anti-viral agents that would cope with the adverse reactions (progressive vaccinia infection) of the existing vaccines. The moral argument that there was a need to develop less toxigenic vaccines to protect at-risk populations remained unanswered. However, all agreed that better vaccines, able to be deployed in rural areas where access to satisfactory cold chains was difficult, was highly desirable.

44. The use of protective antibodies for passive transfer of immunity was then considered. It was felt that this could play an essential and complementary role in any strategy for protecting populations from infections with variola viruses. The availability of hyperimmune gamma globulin was limited and a case could be made to either prepare more or to isolate and produce monoclonal antibodies as possible alternatives.

45. It was noted that some neutralizing monoclonal antibodies to vaccinia proteins were available but that little effort had been put in to this area. It was unclear which methodology would be most useful to produce these antibodies as this could involve purified virus proteins, recombinant proteins or infected cells. It would also be essential to make neutralizing monoclonal antibodies and polyclonal antibodies to both IMV and EEV. This work would probably involve the generation of mouse antibodies and some consideration would need to be given to their subsequent humanization.

46. Although some doubt was raised over the viability of this approach, it was noted that further work on the production of variola-specific monoclonal antibodies could be done. There had been no known systematic approach to produce these monoclonal antibodies. This work would require access to live virus stocks.

4.5 Animal models to study anti-viral drugs and vaccines

47. The evaluation of antiviral drugs and vaccines against variola virus would be facilitated by the availability of an appropriate animal model for human smallpox disease. There is no animal reservoir of smallpox and a system for directly replicating the systemic disease observed in man has yet to be identified. There are however two types of animal model: indirect (monkeypox in monkeys or rabbits, cowpox in mice) and direct (variola virus in cynomolgus monkeys, suckling or transgenic mice). The merits and limitations of using these models as surrogates of human smallpox infections were discussed.

48. Despite limitations, it was clear that the various regulatory agencies could insist that direct animal models be used to demonstrate the efficacy of anti-viral drugs or new vaccines. Further work may therefore be necessary to validate these models and to produce environments in which the pathogenesis of human smallpox may be further investigated. Alternatively, sufficient data may need to be collected to support the use of animals infected with other orthopoxviruses as suitable surrogates for investigations on possible anti-variola drugs.

4.6 Anti-viral drugs

49. Concerns about possible use of smallpox as a bioterrorist weapon and the re-emergence of monkeypox in central Africa, combined with the recognition that control of vaccinia vaccination in at-risk populations may require re-evaluation has prompted enthusiasm for programmes aimed at the development of new anti-viral therapies. A number of potentially useful drugs, generally developed for the control of other viruses, have been identified.

50. These drugs, Cidofovir – a DNA Polymerase inhibitor – has been shown to have beneficial effects in vitro and in animal models for combating variola virus infections. Although the drug has been approved for the treatment of CMV retinitis in AIDS patients, it does have some associated kidney toxicity. The observed level of toxicity may be deemed acceptable if the drug is used in emergency situations and if the side-effects are carefully monitored.

51. Other potentially useful drugs, some based on the known enzymatic functions encoded by vaccinia/variola viruses, have also been studied. These analyses have demonstrated that different orthopoxvirus strains have different sensitivity profiles and it may be necessary to test potential anti-viral drugs directly on variola viruses either in vitro or in vivo. Some useful work on the development of in vitro cell culture assay systems, based on raft cultures of differentiating epithelial cells was described.

52. It was noted that the current anti-viral drug programme was based on drug exploitation rather than drug discovery, taking drugs with known reactions and asking if these could be usefully used to treat variola (vaccina) virus infections. It was recognized that antivirals deployed in emergency situations would need to be effective when used in conjunction with an immunization campaign. It was also acknowledged that further work was necessary to determine when effective drugs could be deployed – before or after the onset of clinical symptoms.

4.7 Regulatory aspects for licensing drugs and vaccines

53. The procedures for registering and regulating new drugs with the US Food and Drug Administration (FDA) were described. There were five stages of drug development – discovery, non-clinical development, clinical development, registration and post-marketing surveillance. An investigational new drug application (IND) was needed before clinical trials of new drugs, or existing drugs with new applications, could proceed. There were two types of IND: one was

related to commercial applications and had the goal of gaining marketing approval; the other was related to promoting clinical research. Emergency INDs (available for single patients) and treatment INDs (to make promising drugs available to desperately ill patients) were also available.

54. While it was anticipated that INDs would eventually lead to New Drug Applications (NDAs) it was recognized that this did not always happen. There was no time limitation placed on an IND. Some of the specific requirements that would relate to gaining regulatory approval of anti-variola drugs were then explored. It was noted that all drug usage would ultimately depend on an analysis of the risk/benefit ratio (safety versus costs) and that it would be prudent for those involved in drug development to work with the FDA during the early stages of any drug development programme.

55. It was considered likely that the FDA would prefer data on human efficacy studies for drugs targeted specifically at treating variola virus infections. In the absence of this, robust animal model and cell culture data would be needed in which the target pathogen was deployed. The only alternative would be to use approved drugs 'off-label'. Although this was regarded as being within the legitimate practice of medicine, the stockpiling of drugs for this specific purpose may not be acceptable. It was noted that the FDA would regard all data generated during an IND as confidential.

56. Similar regulatory requirements for the licensing of smallpox vaccines were described. However, it was noted that supplies of existing vaccine were limited (and relatively old) and that the 'approved' method of production was no longer considered acceptable. Two issues would need to be considered for approval of new vaccines based on the current vaccinia strains. First, the strain and a suitable seed-lot system would need to be defined. Second, the most appropriate culture system would need to be identified. These issues had been satisfactorily addressed for the current US Department of Defense contract.

57. Approval for new vaccines would depend on the satisfactory completion on phase I, II and III (efficacy) trials and each vaccine would need to demonstrate adherence to a set of common principles (consistency of production, quality of source materials etc.) Standards associated with safety, purity, potency, efficacy, stability and compliance with good manufacturing procedures would all need to be defined.

58. It was considered likely that comparisons with existing information, possibly using bridging studies, would be possible if new vaccinia-based vaccines were developed. This would not be the case if approval was sought for totally new vaccines (recombinant vaccines, single antigen vaccines, DNA vaccines, etc.) which would necessitate the availability of the target pathogen, i.e. access to stocks of live variola virus.

V Summary of arguments and conclusions for research on variola

59. This summary report is provided against the backdrop of the 52nd WHA reaffirming the decision that the remaining stocks of variola virus should be destroyed but that

there should be temporary retention of these stocks until up to not later than 2002. A considerable amount of useful and interesting research to answer a range of questions relevant to orthopoxvirus infections was discussed. Whilst WHO can endorse the value of research on orthopoxviruses other than variola virus, it is beyond the remit of the Advisory Committee to provide detailed comment on much of it and it is for others to determine utility and priority. This report concentrates only on those areas of proposed research that would need access to live variola virus.

Conclusion

- The view of the Committee was that further limited research on variola virus could be justified but members emphasized that this should, under no circumstances, continue beyond the end of 2002.

The need for more DNA sequence information on variola virus

60. Arguments were presented that the current amount of sequence information available was insufficient to provide consensus information across the full range of strains available. From a public health perspective it was felt that further data on strains derived from outbreaks of different severity, at different times and at different geographical locations were needed. It was proposed that further sequences would be needed and that it should be possible to derive the necessary sequences within a short-defined time-scale.

61. Arguments against limitations being placed on gaining sequence information revolved around the need to obtain further information that would facilitate the targeted design of new drugs and the identification of further probes for better discrimination in diagnosis and detection. This information would also be useful for determining evolutionary relationships amongst the orthopoxviruses and for studying changes resulting from different ways of passage.

62. Two related proposals were discussed. First, to identify genes likely to be relevant for drug development and to produce additional sequence information on these specific regions across a broader range of strains in the repository. Second, to undertake RFLP analysis on a wide range of isolates and use this information to identify strains showing significant differences so that corresponding DNA isolations could be prepared and clone libraries obtained.

Conclusions

- Determine full-length genome sequences from additional variola major and minor strains, particularly Congo 70 and Somalia 77
- Prepare additional clone libraries from selected strains
- Establish a work programme with milestones that do not go beyond the end of 2002

The need for novel diagnostic tests for variola virus

63. Modern technology has facilitated the development of new types of diagnostic and detection procedures and some have already been incorporated into state of the art equipment i.e. hand held PCR devices. These procedures and devices are able to detect infections early and with great sensitivity. Commercial devices currently under development will use stable reagents and could be used by individuals with little technical training. These devices still need further validation for use with variola virus under likely field conditions which will require access to the live stocks. With commercial production imminent, this access was likely to be of limited duration.

64. No arguments were raised about the desirability of such tests and equipment; however doubts were raised on the grounds of availability for public health purposes and cost. Assurances were given that because the devices could be configured to accommodate diagnosis and detection of a range of pathogens, it was likely that they would have widespread use for public health monitoring and that costs would be therefore significantly reduced.

Conclusions

- Complete the validation of detection/diagnostic tests and equipment using live variola virus if necessary
- Confirm the sensitivity of the procedures and develop protocols for use in early diagnosis with readily available clinical specimens
- Establish a work programme with milestones that do not go beyond the end of 2002

The need for anti-viral drugs

65. Anti-viral drugs are needed to treat clinical disease and some lead compounds have already been identified although more work is needed to provide better formulations. To gain approval of regulatory authorities in different countries, non-clinical efficacy data from animal model studies and infected cell cultures may be needed for those drugs to be used in smallpox infections. The desirability of obtaining an approved drug drove the need for gaining limited access to live virus.

66. Arguments against supporting this programme revolved around the availability of an effective vaccine reducing the need for such drugs, the logistical difficulties in delivering the drug to the required populations and the cost and likely difficulties associated with maintaining suitable stocks of drug. It was also noted that validation processes could be quite lengthy and that a better strategy might involve the development of drugs to control progressive vaccinia infection, the major adverse side-effects of the current vaccine. The Committee recommended that the reseachers involved in this area work with the FDA and other regulatory authorities to determine a suitable work programme to optimize gaining approval information.

67. There was some discussion on the utility of a broader drug development programme using compounds such as the interferons, chemokines and fusion

inhibitors. It was noted that the future of antiviral drugs was bright and that wider research in this area could be justified, although it was also argued this would merely lead to an open-ended, opportunity-driven programme.

Conclusions

- Encourage work that would lead to the development of drugs capable of treating progressive vaccinial disease
- Complete drug development programme on existing lead compounds and on all work requiring access to live virus with a view to obtaining approval by 2002
- Develop benchmarks against which progress can be monitored by independent observers

The need for monoclonal antibodies with virus-neutralizing activities

68. It was noted that supplies of hyperimmune globulin and neutralizing antibodies to the two infectious forms of variola virus are extremely limited. It is possible that these preparations may have potential therapeutic or prophylactic use. Relatively few monoclonal antibodies are available and access to more could provide additional material for use in diagnosis. Access to live virus stocks would be needed during the initial stages of monoclonal antibody production or if, for example, phage display systems were to be developed.

69. It was argued that the need for hyperimmune serum to variola virus was questionable when hyperimmune serum to vaccinia could be generated with reasonable ease. Similarly, whilst there may be good scientific arguments to prepare additional monoclonal antibodies, the clinical need is less clear. It was also stated that the use of passive immunity was questionable when dealing with an infection where clinical recovery was dependent on developing good cell mediated immune responses.

Conclusion

- Establish a monoclonal antibody production programme that is time-limited

The need for novel smallpox vaccines

70. The arguments for further work on vaccine development were based on the fact that a safer, but similarly efficacious, vaccine was needed. It was noted that new tissue culture-derived vaccine preparations are required as the old method of production (skin scarification) was no longer acceptable in some countries. It was also noted that approval of new or novel smallpox vaccines (replication deficient, recombinant, etc.) by regulatory authorities in different countries would be needed and that this would require validation data using live variola virus.

71. It was questioned whether a novel vaccine was needed as the existing one was so effective. A better strategy would be to work on ways of reducing adverse

reactions using drug therapy. It would also be difficult to justify the deployment of a vaccine of unproven efficacy in emergency situations.

72. It was agreed that production of a tissue culture derived vaccine based on a validated vaccinia strain was the most appropriate way forward but that this should not preclude the development of a secondary vaccine that could be deployed in at-risk populations. The view was expressed that whilst research on these other vaccines should be encouraged, it should be recognized that any developed may not be licensable by regulatory authorities in different countries.

Conclusion

- Further work on vaccine development should be encouraged but this should not be dependent on gaining access to live variola virus stocks

The need for a non-human primate and other animal models to evaluate antiviral drugs and novel vaccines

73. It was argued that regulatory requirements for the introduction of new drugs would require non-clinical efficacy data in animal models that were infected with variola virus. Some work on the development of these, as opposed to surrogate models (ectromelia in mice, monkeypox in monkeys, etc), was therefore needed. Some work was already planned to assess the utility of cynomolgus macaques for this purpose. It was also noted that other animals (suckling mice, transgenic mice) might be suitable hosts to support virus replication. Work to develop an acceptable animal model capable of being infected with variola virus was therefore justified. The availability of a validated animal model would also be useful to evaluate the sensitivity and specificity of diagnostic tests.

74. While the need for research in this area was generally accepted, it was noted that smallpox virus research had been ongoing for decades and a suitable animal model still had not yet been identified. Doubts were raised whether any developed model would produce data that could be directly correlated with human infections.

Conclusions

- Undertake limited exploration of susceptibility of non-human primates and other species to infection with defined variola viruses whose genomic sequences are likely to be determined
- Develop a time-limited work plan defining species, variola virus strain, dose, and inoculation route
- Successful development of an animal model should be completed as soon as possible to facilitate evaluation of antivirals, vaccines and diagnostic tests developed

The need for basic research

75. Some members of the Committee argued that it was essential to continue to support basic research using live variola viruses to further understand all aspects of the pathobiology of this human pathogen. Other members argued that this would have a low priority and meaningful research would need access to a suitable animal model, which could not be guaranteed.

76. One view expressed was that this aspect of a potential research programme should be dropped from further consideration as much information could be derived using other orthopoxviruses. However, it was noted that further research on variola viruses was being proposed and whilst this was being done work of a more fundamental nature might proceed in parallel, provided it did not reflect open-ended research.

Conclusion

- Time limited workplans for basic research with benchmarks and defined end-points should be established.

Annex 1: Agenda

Monday 6 December 1999

8:30 - 9:00 Registration

9:00 - 9:10 Opening remarks
Dr Lindsay Martinez, Director, Department of Communicable Disease Surveillance and Response, WHO

9:10 - 9:30 History of WHA decisions on destruction of variola virus
Dr Lindsay Martinez

9:30 - 10:00 WHA52.10 Resolution 24 May 1999 Background, Contents

10:00 - 10:10 Purpose of the meeting
Dr Lindsay Martinez

10:10 - 10:30 Discussion

10:30 - 11:00 Coffee break

Inventories

Inventory of current vaccine and immunoglobulin stocks
Inventory of variola virus stocks at CDC and Vector (strains, isolates; origins)
Inventory of cloned DNA fragments of variola virus

12:00 - 12:30 ***DISCUSSION OF WORKING PAPERS***

12:30 - 14:00 Lunch

Research performed on variola virus

14:00 - 14:30 DNA sequence information of variola virus
Professor Sergei Shchelkunov, VECTOR and Dr Brian Mahy, CDC

14:30 - 14:50 Lessons learnt from the DNA sequence of variola virus
Dr Geoffrey L. Smith, University of Oxford

14:50 - 15:20 Tea break

DISCUSSION

Tuesday 7 December 1999

State of the art in orthopoxvirus research

09:00 - 09:20 Diagnosis of orthopoxvirus infections
Dr Hans Gelderblom, Robert Koch Institute

09:20 - 10:10 Immunopathogenesis and evasion of the host immune response by orthopoxvirus gene products
Dr Antonio Alcami, University of Cambridge

10:10 - 10:30 Protective antigens of orthopoxviruses
Dr Geoffrey L. Smith

10:30 - 10:45 ***DISCUSSION***

10.45 - 11.15 Coffee break

11:15 - 11:35 Possible approaches to produce novel and safer vaccines
Dr Bernard Moss, NIAID, National Institutes of Health

11:35 - 11:55 Possibility of producing monoclonal antibodies as alternatives to immunoglobulin
Dr Mariano Esteban, Centro Nacional de Biotecnologia, Cantoblanco

11:55 - 12:30 ***DISCUSSION***

12:30 - 14:00 Lunch

14:00 - 14:30 Animal models to study antiviral drugs and novel vaccines
Dr Peter B. Jahrling, USAMRIID

14:30 - 15:00 Antiviral drugs against smallpox: state of the art
Dr John W. Huggins, USAMRIID

15:00 - 15:30 ***DISCUSSION***

15:30 - 16:00 Tea break

16:00 - 16:20 Regulatory perspectives in antiviral drug development
Dr Lauren Iacono-Connors, FDA

16:20 - 16:40 Regulatory requirements for licensing of novel vaccines
Dr Michael Merchlinsky, FDA

16:40 - 17:30 ***DISCUSSION***

Wednesday 8 December 1999

Future Research on Variola Virus

(A brief presentation, of no more than 10 -15 minutes, will sum up the arguments regarding research needs on each topic. The background will have already been presented in the earlier papers, and should be alluded to, but not repeated.) A discussion period , closely kept to time and relevance, will follow each brief presentation. Then the group discussion and vote on whether or not there should be further research, either concluding before the end of the day, or continuing next morning. If the group votes yes to further research, then the next part about how the agenda should be controlled and reviewed should come on day 4. If it is necessary to convene a sub-group as scientific sub-committee, they can meet after that discussion.)

- Need for more DNA sequence information of variola virus
- Need for novel diagnostic tests for variola virus (monoclonal antibodies)
- Need for antiviral drugs
- Need for monoclonal antibodies with virus-neutralizing activity to replace immunoglobulin
- Need for novel smallpox vaccines
- Need for a non-human primate model to evaluate antiviral drugs and novel vaccines
- Group Discussion and Decision on

    (a) should there be further research
    This will either be completed on day 3 or run over into day 4.

Thursday 9 December 1999

If Yes to research:

- Group Discussion and decisions on:

    (b) How should it be proposed, selected, reviewed and evaluated (and funded)

Annex 2: List of participants

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