# IMMUNIZATION FOR JAPANESE ENCEPHALITIS

Edited by

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# Immunization for Japanese Encephalitis

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# I

# FOREWORD

DORLAND J. DAVIS

It is a pleasure to welcome you to the U.S.-Japan Cooperative Medical Science Program Conference on Japanese Encephalitis Virus Vaccine and it is a particular pleasure to welcome our Japanese colleagues to our Nation's Capital.

The first conference sponsored by the Joint Panel on Virus Diseases was held in December 1967 in Honolulu on smallpox. The second was in Tokyo in December 1968 on the myxoviruses and this is the third conference. We are privileged to hold this conference in the Pan American Health Organization Building which is an appropriate location for this meeting with scientists from Japan, United States and other countries.

The U.S.-Japan Cooperative Medical Science Program, now under the aegis of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, has completed 4 years of activity and one can readily see its influence. For instance, in 1965 the NIAID supported 8 research grants dealing with rabies, encephalitis and smallpox, subjects of primary interest to the Program. This year there are 16 grants, twice as many, and one contract. I am also aware that the Japanese scientists have increased substantially the amount of research on these diseases. There is every reason to believe that continuing interchange between scientists of our 2 countries will accelerate our knowledge of these virus diseases which are of such special importance to Southeast Asia.

# II

# INTRODUCTION

WILLIAM MCD. HAMMON

At the combined meeting of the Panels on Virus Diseases, of the United States-Japan Cooperative Medical Science Program, held in Kyoto on December 5, 1968, the topic for the annual working conference of 1969 was selected. The last, on Myxoviruses, had just been held on December 2, 3 and 4, 1968 in Tokyo. The title tentatively decided upon for that of 1969 was "Working Conference on Viral Vaccines with Special Emphasis on Japanese Encephalitis." The meeting, on the basis of the customary rotation would be in the United States. It was felt that since so many aspects of so many virus diseases had been discussed at the just completed conference, it had not been possible to deal in adequate detail with a lot of important applied aspects, and that an attempt should be made in the future one to deal in adequate depth with a more limited subject, one aspect, control, of one disease.

Immunization against Japanese encephalitis was a topic of great, immediate importance for much of Asia, and many of the Pacific Islands. Two partially purified mouse brain vaccines had gradually come into rather extensive use in Japan and one of these to a lesser degree in Taiwan. Elsewhere, none was used or available from a local source. Research on vaccine was in progress in many laboratories in Japan, Taiwan and the United States. These efforts involved live, attenuated vaccines and inactivated tissue culture vaccines, both types claimed to have potential advantages over those in use. Field trials were being planned in Korea for one of those already in use in Japan, and for an American developed tissue culture vaccine. Only one controlled field trial of a currently available or approved vaccine had ever been conducted and results of this were not accepted by all, the data never having been published. The time appeared appropriate to bring together as many of the scientists as possible who were working on such problems to share their knowledge and obtain additional guidance from other distinguished virologists, experienced manufacturers of other viral vaccines, epidemiologists, and those responsible for licensing and approving new vaccines.

The recommended subject was heartily approved by the 2 panels and planning began immediately. Two chairmen from the American Panel and one from the Japanese were appointed to plan the conference, together with the assistance of the Panel Chairmen. This planning had to be accomplished largely by mail.

The Chairmen agreed to try a rather novel conference procedure. Instead of assigning broad major topics to a few selected speakers, a detailed outline of the subject matter was

prepared as a unified, systematically organized coverage of the total subject, as it might be prepared for a monograph to be written by a single person. The next step involved selection of appropriate persons to present data on each main topic and the many subtopics in the outline, through several descending orders. For a number of main topics one or more scientists were readily recognized as having outstanding qualifications, but these were not all residents of Japan or the United States. United States as the host country invited most of these as their guests. The World Health Organization was kind enough to provide for one of their invited staff members. Acceptances were received from Switzerland, Sweden, the United Arab Republic and the Republic of China. Ten others were selected in the same manner from Japan and the United States. Each of these was requested to present a 10 minute, formal, written paper to introduce, or in a few instances to cover completely the topic assigned. Their manuscripts were to be available in advance to the interpreters, so accurate and effective simultaneous translation would be available for all participants.

The topical outline of the total conference was mailed, following an invitation, to a specially selected list of all American and Japanese scientists whom the chairmen thought might have significant contributions to make, either because of known involvement with Japanese encephalitis immunization vaccine development, or other related experience. Certain limits had to be placed on the number of these invitations, particularly for the Japanese, because of the great travel distance and limited government funds available for such. It was felt that the total number of participants should not exceed 75. Each was requested to study the outline and indicate to what topic or topics they could contribute significant data, and would be willing to present them, and to submit to their respective country's program chairman a brief abstract of their contribution for advance evaluation. The response was very rewarding and the chairmen selected one or several volunteer discussants for each topic outline and encouraged a few to broaden their coverage. Each of these was allotted 5 minutes for each topic, with a few exceptions where it was obvious that more time was needed. Many had contributions to make on a number of specific topics. These participants were requested to prepare an informative abstract with tables and charts similar to those to be used for projection, or complete manuscripts, if preferred, for distribution at the meeting to all attendees and to interpreters. All participants were assured that no publication of the proceedings was intended.

Invited participants were also informed that time was planned, at intervals during the program, for free discussion from the floor and those who did not choose to be formally listed for topics on the program were urged to take part in spontaneous question asking or discussing. Some chose to restrict their participation in this manner.

The conference was held in Washington, D. C. at the Pan American Health Organization's (PAHO) Building, under the auspices of the Geographic Medicine-Branch of the National Institute for Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service on August 4, 5 and 6, 1969.

The list of the 62 attending participants and their affiliations may be found on pages vii-ix. With the excellent projection and simultaneous translation facilities made available by or through PAHO, the staff work of the 2 panel secretariats, the excellent preparation and cooperation of the many participants and the expert handling of sessions by the 12 selected session chairmen, the meeting appeared to have succeeded well in what it attempted to accomplish. As a result, Dr. Yasuiti Nagano, Chairman of

the Japanese panel made a formal request, before the close of the final session, that the proceedings be published, with Dr. William Hammon as chief editor, assisted by Dr. Masaki Kitaoka and Dr. Wilbur Downs.

Dr. Hammon then explained to the participants the obligation each of the many who had prepared only discussion abstracts would have in preparing complete papers, indicated that many papers already submitted would be more useful if accompanied by references and pointed out that a number of the tables and charts projected would require modification for publication. He agreed to accept the proposed responsibility placed on him, provided no participant of a major topic objected to have his contribution published and a great majority of those who had submitted abstracts would provide full manuscripts. The feeling was so prevalent that the data that had been presented during the 3 days was available from no other source and had been so well brought together and was of such great usefulness that one could not afford to do otherwise than to make it permanently available, not only to the participants but to all interested scientists, students and libraries.

Dr. Nagano suggested that it be published as a monograph in English, either in the United States or Japan. If it was desired to have it done in Japan he had already made inquiry and had an interested publisher. This latter suggestion was formally and enthusiastically approved. An early publication date in 1970 was urged.

Within the limited period of time to collect the manuscripts, associated with travel and other committments by many of the participants, a very few contributions to one or more topics could not be included, or have been included only as abstracts. As a whole, however, this compilation is more complete and useful than that presented originally, since translation from Japanese into English has been more carefully and accurately accomplished, the tables, charts and graphs can now be studied better and correlated more effectively with the text than during the conference, and the selected references add greatly to the coverage of a number of topics, particularly making up for the many scientists who for various reasons were unable to participate, and to summarize their valuable contributions. Unfortunately, there was no way to include the many discussions and questions presented from the floor, since there had been no recording.

Now that the compilation is complete and ready for the publisher, the editor wishes to thank all the contributors and co-editors for their enthusiastic and effective cooperation. This is an excellent example of Japanese-American cooperation in the field of medical sciences—the objective of those who envisioned and brought this organization into being, i.e., the Prime Minister of Japan and the President of the United States.

# III

# BASIS FOR SELECTION OF VIRUS VACCINE STRAIN

## A. GENERAL INTRODUCTION

Masami KITAOKA

The Japanese encephalitis (JE) strain for preparation of the vaccine should, needless to say, be selected in general from among many strains kept in the laboratory and isolated in nature on the basis of the following criteria; (1) antigenic characteristics and immunizing potential as much to produce both detectable humoral and tissue immunities as to protect the vaccinees against disease with current, wild strains of JE virus, (2) virus yield from the host animal or tissue culture cells suitable for the mass-production of the vaccine, (3) stability and preservation of the liquid vaccine for at least one year, and much longer in its freeze-dried state, and (4) a safe vaccine without including any component in its preparation which might produce an adverse reaction, local or general, following a course of vaccination, especially after repeated boosters.

During the period 1935 to 1945 a formalin-killed vaccine, mouse-brain type, was prepared by Japanese investigators using the Kalinina strain and tested for its immune response in man[1] and animals,[2] and its protective effect had been established by direct challenge of mice[3] and horses.[4,5] The Kalinina strain was used because it was first isolated by several Japanese investigators from a human brain in 1935,[6] and JE strains were thought to be almost the same in antigenicity, even though a little antigenic difference was found by Mitamura[7] in cross-mouse neutralization tests. After the war the Nakayama strain was obtained again from the U.S.A. and mouse-brain type vaccine was prepared mainly using this strain, for man[8] in small field trials and for horses[9-11] especially used country-widely in horses[12] in 1948 and 1949. The fact that this vaccine prepared with Nakayama-NIH was rejected for animal use by the national assay potency test in immunized mice challenged with the Nakayama-Yakken strain,[13] a strain even originating from the same Nakayama strain, focused our attention again on the variation of antigenic characteristics and immunizing potential of various JE strains. Thus, it has been made clear that the antigenic characteristics and immunizing potential of many strains so far tested are not all the same *in vitro*, by cross HI,[14] CF,[14,15] NT,[14,16] and other tests [17,18] with homologous and heterologous immune sera, and also by the cross-challenge test [13,14,19] *in vivo*, in im-

munized mice against the homologous and heterologous strains. However, the differences found were not as great as seen among influenza viruses, A, A1, A2 and C. Dr. Watanabe[20] pointed out the variation of JE strains due to selective host passage and Okuno[17] of our study group found additional, definitely detectable differences in antigenic characteristics and immunizing potential between both G1 early and G1 late strains due to the mouse passage level. There can be no doubt but that 3 sublines, NIH, Yakken and RFVL, originating from the Nakayama strain isolated by Kasahara[21] in 1935 have been found by laboratory testing to differ from each other, to some extent, in antigenic characteristics and immunizing potential due to extended passage in 3 different laboratories. On the other hand there are some strain differences in antigenic characteristics and immunizing potential found in viruses in nature, as pointed out first by Hale.[15] From the foregoing it can be seen that the antigenicity and biological characteristics of JE virus are, to some extent, changeable by adaptation and selection by passage in the host animals in nature and laboratory, but not as markedly as occurs with some other viruses. At present, JE strains so far isolated can be divided roughly into the Nakayama-NIH type, and the JaGAr type as mentioned by Drs. Yoshioka, Nakamura, Watanabe, Takaku and our group. The former type is classical and a laboratory strain, while the latter is current and a wild strain. Almost all current isolates are classified as the latter type, though minor antigenic difference can be observed among them. By the way it is pointed out that the antigenic variation in 3 strains described by Yaoi[23] is not so conclusive from data obtained by i.p. challenge and a host-dependent antigenic variation was not so easily confirmed by Murakami[13] and our group as described by Watanabe.[20]

Table I presents a summary obtained by cross-neutralizing antibody and cross-protection tests against direct challenge of mice immunized with the classical Nakayama-NIH strain and a current one, JaGAr-01.

A good immune response and high level of protection against the homologous virus, and a low immune response and poor protection against the heterologous can be observed

TABLE I

Comparison of Cross-neutralizing Antibody Response and Cross-protection Value against Direct Challenge in Mice Immunized with Classical Nakayama-NIH and Current JaGAr-01 Strains

| | | Challenge virus | |
|---|---|---|---|
| Host | Strain of vaccine | Nakayama-NIH classical strain | JaGAr-01 current strain |
| | | Cross-neutralizing antibody response | |
| Mice | Nakayama-NIH | High | Low |
| Man | JaGAr-01 | Low | High |
| Rabbits | Nakayama-NIH | High | Low |
| Guinea-pigs | JaGAr-01 | Low<br>Relative high at hyper immunization | High<br>High at hyper immunization |
| | | Cross-protection value | |
| Mice | Nakayama-NIH | High | Low |
| Mice | JaGAr-01 | Low | Relative high |
| | | Cross-kinetic neutralization test | |
| Rabbit hyper immune serum | Nakayama-NIH<br>JaGAr-01 | almost the same kinetic curve and dose response curve but not on anti-JaGAr-01 serum | |

by both cross-NT tests and cross-protection tests. Also, the immunizing potential of the latter strain was found to be less than that of the former, even though as given in Figures 1 and 2, the kinetic curve and dose response curve of neutralization of both strains on anti-Nakayama-NIH serum are apparently similar to each other but not on anti-JaGAr-01 serum (Figures 3 and 4). Table II indicates that the $EID_{50}$ obtained by cross-challenge

TABLE II

$EID_{50}$ of Japanese Encephalitis Vaccines Prepared with 3 Nakayama Strains, NIH, Yakken and RFVL

| Vaccine | | Challenge virus dose: NIH 13 $LD_{50}$ | Challenge virus dose: Yakken 10 $LD_{50}$ |
|---|---|---|---|
| NIH | 107 mg/dl* | 1.75** | 0.43 |
| Yakken | 110 mg/dl | 0.78 | 1.67 |
| RFVL | 93 mg/dl | 1.09 | 0.88 |

*Each virus used for vaccine was 3 times cloned and N-content of each vaccine was adjusted to roughly 100 mg/dl.
**$EID_{50}$ = Log 4.

tests in mice immunized with each of the 3 sublines of Nakayama is not high against the heterologous one, except for RFVL. To select a better strain for vaccine than Nakayama-NIH, a comparative study continues on antigenic characteristics and immunizing potential of strains selected at random from old and new isolates from man and mosquitoes in various areas, such as Thailand, Peking, and Japan. Table III indicates the $EID_{50}$ obtained by cross-challenge tests in mice immunized with each vaccine prepared from suckling mouse brain suspension, in which the virus titer was adjusted to $10^{8.5}LD_{50}$, infected with Nakayama-NIH, JaGAr-01, Chieng-Mai and Q-180. As a result the Q-180 strain might be a candidate for the vaccine strain because its antigenicity seems to be similar to that

TABLE III

Comparison of Cross-protection Values of 4 Vaccines against Direct Intracerebral Challenge with 4 Japanese Encephalitis Virus Strains

| Challenge virus | Vaccines used: Nakayama-NIH | JaGAr-01 | Chieng-Mai | Q-180 |
|---|---|---|---|---|
| Nakayama-NIH human strain Japan 1935 | *7.1* | 5.3 | 5.4 | 7.1 |
| JaGAr-01 mosquito strain Japan 1958 | 6.0 | *6.4* | 6.4 | 6.7 |
| Chieng-Mai human strain Thailand 1964 | 6.8 | 6.3 | *7.0* | >7.0 |
| Q-180 mosquito strain Thailand 1957 | >7.0 | 5.5 | 5.8 | *>7.0* |

Each virus titer before preparation of vaccine was roughly $10^{8.0}$—$10^{8.5}$ $LD_{50}$.
Each vaccine was suckling mouse brain type.
Vaccine used: 1:10 dilution of each vaccine.
Challenge dose: $10^5$, $10^6$ and $10^7$ $LD_{50}$.