HERPESVIRUS

CLINICAL, PHARMACOLOGICAL AND BASIC ASPECTS

Editors
Hiroshi Shiota
Yung-Chi Cheng
William H. Prusoff

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Proceedings of the International Symposium on Herpesvirus held in Tokushima City, Japan, July 27–30, 1981

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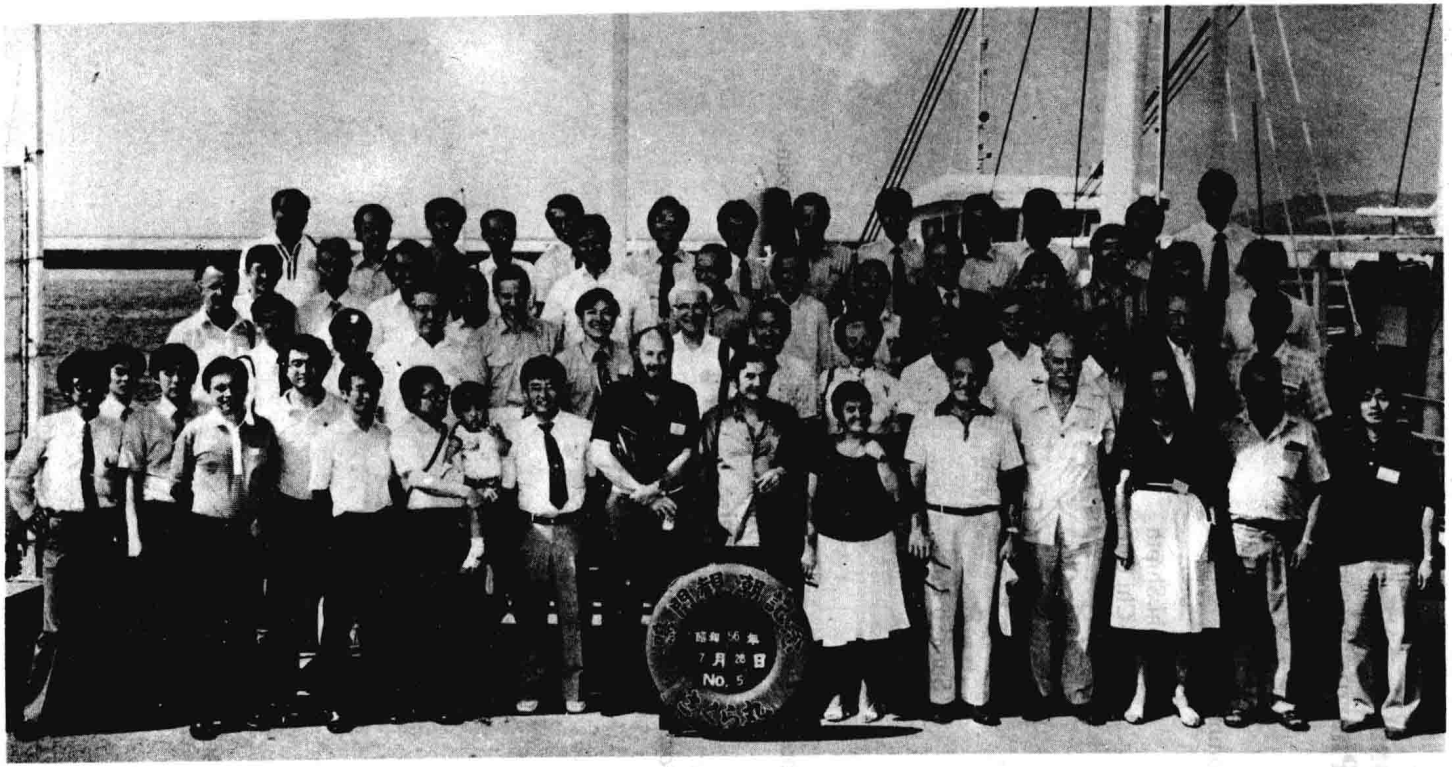

Sightseeing at Naruto – July 28th, 1981

PREFACE

We are very glad that this International Symposium on Herpesvirus could be held in the beautiful country city of Tokushima rather than in a big city like Tokyo. Fortunately, many participants could attend the Symposium, even though the trip to Tokushima is rather difficult.

We were overwhelmed by hearing so many excellent papers at the Symposium. Accordingly, it seemed appropriate that this important material should be published to make it thus more widely available. We thank all the speakers for their cooperation and for sending their manuscripts for publication.

We are very happy that the Proceedings have now been completed and are available. We hope that they will be useful in clinical, pharmacological and basic fields and provide valuable information for future studies on herpesviruses.

Hiroshi Shiota
Yung-Chi Cheng
William H. Prusoff

OPENING REMARKS

It is a great pleasure for us to have the International Symposium on 'Herpesvirus: Clinical, Pharmacological and Basic Aspects' here in Tokushima. As you all know, this is a Satellite Symposium of the 8th International Congress of Pharmacology which was held in Tokyo last week. At this Symposium, pharmacologists, clinicians and virologists can get together to discuss herpesvirus-induced diseases. Usually, pharmacologists have their own congresses and we, clinicians, have our own congresses separately. But here, three types of specialists, pharmacologists, virologists and clinicians, can meet and discuss in detail all overlapping interests. This is one of the most important aspects of this Symposium.

Many foreign speakers as well as Japanese speakers are present. In particular, I wish to mention three outstanding scientists: Professor Prusoff, one of the organizers of this Symposium, synthesized 5-iodo-2′-deoxyuridine (IDU) in 1959. Until then we did not have any effective antivirals. Professor Kaufman of the United States proved IDU to be effective against herpetic keratitis in 1962. Since then it has been used all over the world and is now regarded as the drug of choice in the treatment of herpes simplex virus infections. But, as you all know, although IDU is effective, it has some toxic reactions. However, Dr. Elion and her team synthesized a new compound, called acyclovir, which is a guanine derivative. They found that it has very low toxicity and so can be used systemically. We are delighted to have these three doctors, who established milestones in medical virology, here with us. And not only these three doctors, but many other outstanding specialists are taking part in this Symposium. I would like to thank them very much for attending and I hope that this Symposium will provide you all with important information to combat viral diseases.

Finally, I would like to mention three more doctors who have helped to make this Symposium possible. First of all, Professor Kamesaburo Yoshino of Tokyo University. Professor Yoshino is a pioneer in herpes studies in Japan. Unfortunately he could not come to this Symposium because of another meeting. It was he who selected the main speakers from Japan and gave me his excellent advice. The second one is Professor Uchida of the Virology Department of Tokushima University, who kindly assisted me and gave me his encouragement. The third is Professor Mitsui, President of the Association for Research in Infectious Diseases of the Eye in Japan, who supported this symposium financially and mentally, and encouraged me in every respect.

I hope that all of you will have a very good time in Tokushima and that you will find the Symposium useful as regards the combat against herpesvirus-induced diseases.

Hiroshi Shiota

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TRANSMISSION AND SCANNING ELECTRON MICROSCOPIC STUDIES OF HERPES VIRUSES

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INTRODUCTION

Numerous electron microscopic studies on herpesviruses have so far been presented and at least two excellent reviews, namely by Watson (1973)(9) and by O'Callaghan and Randall (1976)(6) dealt with the morphogenesis of these viruses. For more than 15 years we have been engaged in electron microscopic studies on various herpesviruses, first to reveal their maturation process and second to clarify ultrastructural pathology of cells infected with these viruses. This report is concerned with some of our experimental results related to this research.

ULTRASTRUCTURE OF VIRIONS OF HERPESVIRUSES

In thin sections most virions of herpesviruses appear as spherical particles of an oval or round electron dense core, a capsid surrounding the core and an envelope enclosing the capsid, as illustrated by one particle of type 1 herpes simplex virus (HSV-1) shown in Fig. 1. Occasionally, the tegument, defined as the structure located between the capsid and the envelope (7), is prominently visible, and a typical example is the virion of turkey herpesvirus shown in Fig. 2. The amount of this material is variable according to the kind of herpesvirus and also from virion to virion of any given herpesvirus.

Furlong et al. (1972)(1) hypothesized that the core of the herpes simplex virion consists of an electron-dense toroidal structure penetrated by a less dense cylindrical mass. It should be kept in mind, however, that the morphological appearance of the core varies, being highly dependent on the kind and method of fixation (2). The hypothesis of Furlong et al. developed from observations of infected cell material prepared by fixation in 2.5% glutaraldehyde in phosphate buffer (pH 7.2). When cells productively infected with any given herpesvirus were prefixed with this kind of fixative with or without a slight modification and postfixed with Millonig's fixative, capsids enclosing a toroid structure were easily detectable in the nucleus. In fact we observed the structure

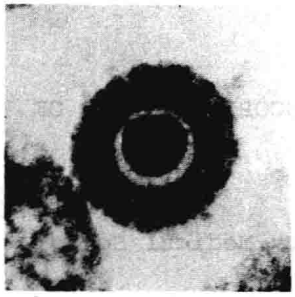

Figure 1. A virion of type 1 herpes simplex virus. x 140,000

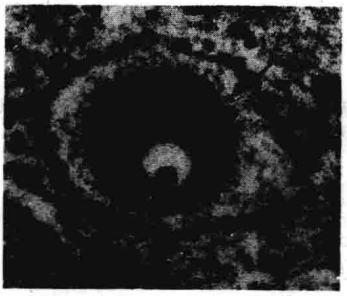

Figure 2. A virion of turkey herpesvirus with abundant tegument material seen in the rough-surfaced endoplasmic reticulum. x 73,500

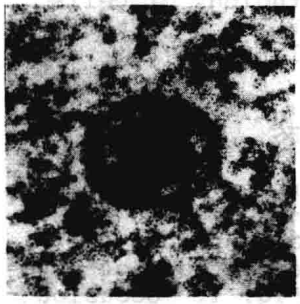

Figure 3. A capsid with a toroid structure detected in a nucleus infected with Marek's disease virus. Fixation: prefixation with 1% glutaraldehyde in Sørenson's phosphate buffer and postfixation with Millonig's fixative. x 170,000

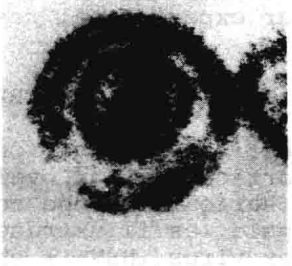

Figure 4. A virion of type 1 herpes simplex virus. The core appears to consist of an electron-dense ball of twine. Fixation: prefixation with 1.5% glutaraldehyde in 0.16 M cacodylate buffer and postfixation with 1% osmium tetroxide in 0.1 M s-collidine buffer. x 150,000

with eight herpesviruses examined (3). Fig. 3. shows a toroid structure in a capsid found in a nucleus infected with Marek's disease virus.

On the other hand, when infected cells were prefixed with glutaraldehyde in cacodylate buffer and postfixed with osmium tetroxide in s-collidine buffer, toroids could no longer be found and instead viral cores resembling an electron-dense ball of twine were observed (Fig. 4). This morphological appearance of the core, which was also observed by Heine and Cottler-Fox (1974) with one of five fixing and *en bloc* staining procedures examined (2), may demonstrate an ultrastructure closer to the native one.

MORPHOLOGICAL VARIABILITY OF INTRANUCLEAR VIRUS PARTICLES CORRELATED WITH THEIR DEVELOPMENT

Capsids with variable appearance of cores were easily detectable in the nuclei of cells infected with any given herpesvirus and this variation in morphology was thought to be mainly caused by the difference in their developmental stages.

To clarify this problem time-sequence electron microscopic examinations of cells infected with type 2 herpes simplex virus (HSV-2) were performed. FL cells were infected with the syn^+ and syn^- variants of the UW 268 strain at an adsorbed multiplicity of approximately 10 and fixed 4, 6 and 8 hrs later. The fixatives used were 1% glutaraldehyde in phosphate buffer for prefixation and Millonig's fixative for postfixation. The following results were obtained. Intranuclear particles appeared as early as 4 hrs after infection and rapidly increased in number, while enveloped particles and budding particles were found 6 hrs after infection. The frequencies of various viral forms in the nuclei at each time were

TABLE 1. Different intranuclear viral forms and frequency of their appearance at earlier periods after infection.

Virus	Hours after infection							
UW268 Syn^+	4	7%	3%	47%	0%	40%	0%	3%
	6	5%	2%	33%	2%	30%	6%	23%
	8	5%	3%	29%	7%	18%	4%	35%
UW268 Syn^-	4	2%	4%	69%	0%	22%	0%	3%
	6	4%	4%	27%	1%	43%	2%	19%
	8	1%	1%	37%	3%	20%	1%	35%