Immunogenicity of Subviral Herpes Simplex Virus Preparations

I. Formation of Neutralizing Antibodies in Different Animal Species
After Administration of Herpes Simplex Virus Solubilized Antigens

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With 3 Figures
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Summary
Production of neutralizing antibodies was followed in guinea pigs, rabbits, hamsters and mice immunized with crude antigen extracts (AM) from human diploid cells infected with herpes simplex virus type 1. The AM induced relatively high levels of neutralizing antibodies in all four species. The antibodies were predominantly complement-requiring and remained so even after administration of repeated AM doses. With the strains used, the antibody response was predominantly type specific and, surprisingly, the type specificity of sera usually increased after administration of repeated doses of AM. Guinea pigs seemed to be the best responsive animal species. They developed the highest levels of antibodies and complement-nonrequiring antibodies were seen in them earlier than in the other animal species. The dose-response experiments carried out in guinea pigs indicated that after a single dose administration the ratio between complement-requiring and complement-nonrequiring antibodies was dependent on the amount of antigen administered. When AM was given without adjuvant less efficient antibody production was observed than after the administration of the same amount of antigen with adjuvant.

Introduction
The development of a subunit type of herpes simplex virus (HSV) vaccine is preferred to whole virion vaccines for reasons of safety (2, 3, 5, 6). However, a number of problems associated with the development of such vaccine, including its immunogenicity, remain to be solved.

In a previous report (6) we described the production and some properties of HSV neutralization antigens extracted from human diploid cells infected with HSV type 1. In the present study, we investigated the formation of neutralizing antibodies in animals immunized with this preparation. A comparison was made...
between rabbits, hamsters, mice and guinea pigs in respect of the development of complement-requiring (CR+) and nonrequiring (CR-) neutralizing antibodies. The type specificity of the antibody response was also examined.

Materials and Methods

Cells

Human diploid cells (LEP) derived from embryo lungs were cultivated as described (8). In a few experiments rabbit embryo fibroblasts were also used. They were cultivated in the same media as the LEP cells.

Virus

HSV-1 (strain KOS) and HSV-2 (strain 196) were kindly provided by Dr. J. L. Melnick, Baylor College of Medicine, Houston. The viruses were grown in LEP cells at a multiplicity of infection (MOI) of 0.1—0.5 PFU per cell. Eagle’s minimal essential medium (MEM) supplemented with 5 per cent heat-inactivated calf serum, 0.075 per cent NaHCO₃ and antibiotics was used as maintenance medium.

Soluble Antigen Mixture

Crude antigen mixtures to be used for immunization were prepared as described in detail elsewhere (6). In brief, LEP cells grown in 1200 ml Roux bottles were infected with HSV-1 at MOI 0.5 PFU per cell and Parker’s i99 medium with 2 per cent of calf serum was added to the cultures after 2 hours of virus adsorption. The infected cells were scraped off the glass after 28-hour incubation at 37° C. They were disrupted by treatment with 0.5 per cent Nonidet P-40 (Shell Chemical Co., Ltd., London, England) in isotonic reticulocyte standard buffer (RSB), pH 7.4. The extract was clarified of cell nuclei and viral nucleocapsids by differential centrifugation. The final mixture of antigens (AM) that had not sedimented after 1-hour centrifugation at 100,000 × g was extensively dialyzed against RSB for 7 days. Absence of infectious virus was verified in LEP cells; one Roux bottle (1200 ml) culture was inoculated with 1 ml of AM and kept for six days at 37° C. Two additional passages were performed. Absence of cytopathic changes was considered an indication of absence of any surviving virus.

51Cr-Release Inhibition Test (CRIT)

The content of HSV solubilized antigens in AMs was determined by a cytotoxicity inhibition test as described previously (7). Twenty complement requiring HSV-1 neutralizing antibody units, 30 units of guinea pig complement and 4 × 10⁴ cryopreserved target HSV-1 infected rabbit fibroblast cells were used in CRIT. The least amount of antigen causing 50 per cent inhibition of 51Cr release was defined as one CRIT antigen unit. On the basis of a correlation between the results of CRIT and the blocking neutralization test it can be concluded that CRIT measures the content of HSV neutralizing antigens (7).

Immunization Procedure

Chinchilla rabbits (about 3000 g), guinea pigs (400 g), young adult Syrian hamsters and white mice, strain H, (9—11 g) were immunized with three doses of AM and the antibody response was tested after every dose of antigen. The amounts of neutralizing antigens used for immunization are shown in Table 1. A mixture of AM with complete Freund adjuvant was administered subcutaneously (s.c.) as the first dose. For the second dose, inoculated s.c. four weeks later, the AM was mixed with incomplete Freund adjuvant. The third dose, without adjuvant, was injected intraperitoneally (i.p.) after another four-weeks interval. Serial serum samples were withdrawn from rabbits and guinea pigs, sera from hamsters and mice were obtained by exsanguinating 5—6 animals at each interval. The sera from each individual rabbit, guinea pig and hamster were kept and tested separately, while mouse sera were pooled.