INTERACTION BETWEEN MENINGOPNEUMONITIS ORGANISMS (MP) AND VESICULAR STOMATITIS VIRUS (VSV) WITH POLYMORPHONUCLEAR LEUCOCYTES (PMNL) AND MONOCYTES

Final Report

by

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Final Report of Studies Carried out, in Part, under

Contract NONR 3127 (00)

by

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I. Description of Study

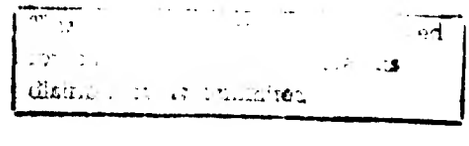
A. Objectives

To investigate the nature of interaction between meningopneumonitis organisms (MP) and vesicular stomatitis virus (VSV) with polymorphonuclear leucocytes (PMNL) and monocytes.

B. Summary of Results

1. Interaction of MP with PMNL and monocytes.

In the course of this study it was demonstrated that fluorocarbon extract of concentrated meningopneumonitis organisms (FEM) contained multifunctional subunits exhibiting precipitating, leucoagglutinating and complement fixing activities. FEM showed 7 precipitinogens in immunodiffusion plates, of which 6 were viral in origin and 1 normal egg factor. Immunological analysis of FEM, using enzymatic treatment with Ouchterlony's technique, as well as Tiselius and acetate membrane electrophoresis, showed 6 viral subunits of which 4 were lipo-DNA-protein, 1 lipo-RNA-protein and 1 RNA-protein



in nature. However, 11 components, of which one was the egg factor, were observed when this extract was studied by immunoelectrophoretic technique. Nucleic acid analysis of FEM showed DNA:RNA in the ratio of 1:1, and optimal densities 260:280 mu in the ratio of 1:83. Comparative immunological, biological and biophysical behavior of these antigenic preparations was analyzed and described.

It was demonstrated that MP agglutinated rabbit pseudoeosinophils. Strongest MP-leucoagglutination occured at 37°C. Leucoagglutination was inhibited by the presence of 0.005 M sodium azide, and by pre-treatment of MP with 0.25% trypsin, $5x10^{-5}$M-$MgCl_2$, or $1x10^{-4}$M-p-chloromercuribenzoic acid. It has been estimated that a minimum of approximately one LD_{50} of MP, or 62 MP particles, would be required for MP-leucoagglutination. Active leucoagglutinin was demonstrated in FEM, an ether extract of FEM and MP wall preparation. Only two out of seven precipitinogens were involved in leucoagglutination, these have been identified as lipoproteins conjugated with DNA and an RNA.

MP organisms were phagocytosed by rabbit pseudoeosinophils in vitro within 30 minutes and by mouse peritoneal monocytes in vivo in 15 minutes. Although the in vitro phagocytosis occured even when the (MP-LD_{50} doses:leucocyte) ratio was 10 in 1, maximum multiplication in the leucocytes took place when the ratio was 1 in 100. Multiplication of MP was demonstrated by serial passages of the organisms in leucocytes, by inhibition of growth by the nucleic acid analogues,

5-fluorodeoxyuridine and 5-fluorouracil, and by microscopic examination of leucocytes stained with fluorescein-conjugated MP-antibody. Phagocytosed and free organisms were transported to the liver and spleen, where they were found until 4 weeks after infection, the duration of the experiment. The agents appeared in the kidney 60 hours after infection (log LD_{50} doses of MP per gram = 3), and their concentration increased until 7.3 log LD_{50} doses of MP per gram were present at 4 weeks.

The above results were presented in a series of papers indicated in the attached bibliography.

2. Studies with vesicular stomatitis virus (VSV).

In the course of the early studies dealing with X-ray inactivation of VSV, several morphological plaque type variants were observed among the irradiated survivors. This observation suggested that VSV offers additional material for radiobiological and genetic studies on animal viruses. Consequently, quantitative studies of the plaque forming ability of VSV were undertaken after irradiation of the virus in phosphate buffered saline, and in nutrient broth with 20% lamb serum. Aggregates of virus were formed in buffer, but dispersed on standing. Virus irradiated in broth formed round plaques, while virus irradiated in buffer produced normal, "ragged" and "minute" plaques. It was concluded that irradiation in buffer may provide the most convenient means for obtaining of plaque type variants.

Subsequent experiments dealing with exposure of VSV to ultraviolet radiation allowed also to collect plaque type mutants of VSV. Three types of mutants, based on plaque diameter in CEF were obtained after UV irradiation of the wild type virus: minute (0.5 to 1 mm), small (1-2 mm) and intermediate (4-5 mm). Wild type VSV forms circular plaque 6-7 mm in diameter. The UV sensitivity of these mutants was the same as that of the wild type, and it was inferred that the mutants were radiation derived. Next, the UV sensitivity of VSV-CEF complexes were studied during the latent period of the virus. It was shown that the virus undergoes two phases of radiosensitivity during replication in chick embryo cells. The first phase occured early in the eclipse or latent period and was characterized by exponential inactivation and increased sensitivity of complexes. The second phase took place during the mid and late eclipse period and was characterized by multihit inactivation of the complexes.

General shape of growth curves for extracellular virus was established. VSV had a relatively short latent period, of about one hour when propagated on CEF, HeLa and L cells. A single particle of VSV formed a stable virus-cell complex and multiplied in the host-cell of this complex when suspended in an appropriate organic medium, releasing new progeny in 60 minutes. VSV formed plaques with comparable plating efficiency on monolayers of CEF and HeLa cells. Eight of the UV induced mutants were phenotypically similar to the wild type on CEF but plaqued with low efficiency on HeLa monolayers.

These mutants adsorb but fail to replicate in HeLa cells. Thus, HeLa monolayers provided a potentially favorable indicator strain for recombination studies on these mutants. However, all efforts in this direction did not yield useful results. Intracerebral infection of 4.4 to 7.4 PFU of the wild type virus on the intermediate plaque size mutants killed mice, however, mice did not succumb to infection of about $1x10^6$ PFU of the small plaque type mutants. Plaque mutant p-1a was shown to be antigenically identical to the wild type of VSV, which information is supported by serological tests as well as the results of immunodiffusion experiments. Two antigens have been demonstrated in both the wild type and a small plaque mutant. These agents were morphologically similar under electron microscope.

Under conditions of overwhelming infection, yields of the p-1a (minute) mutant were from 2 to 3 $\log_{10}$ lower in HeLa and L cells than those obtained with the wild type (W+) virus. Fluorescent antibodies disclosed the early accumulation of intensely staining intra-cytoplasmic deposits with both agents which, in this case of W+ in L cells, culminated in the formation of giant multinucleated syncitia. At the time of extensive involvement of the cultures, as judged by immunofluorescence, electron microscopy revealed the virtual absence of any marked alterations in ultrastructural architecture, except for budding of viral particles off the cellular surface and into cytoplasmic vacuoles. Particles were specifically tagged with ferritin-conjugated antibodies. However, established techniques failed to ensure intracellular

penetration of ferritin-linked antibody, short of destroying the morphological integrity of the cells. What appears to be host induced modification of plaque type, occured as a rare mutation-like event with six stable small plaque mutants of VSV. This virus occurs during growth in HeLa but not during growth in L or CEF monolayers of all the mutants, p-1a and its host modified form, the H-60 type, have been studied more extensively. The data are being analyzed at this writing.

Electron microscopy of interaction of VSV by PMNL showed that virus is phagocyted within less than 15 minutes after mixing of the agent with the phagocytes. The engulfment appears to be due to conventional-type phagocytic or pinocytic activity.

Publications and Abstracts of Papers Presented at National Meetings of ASM or American Society of Immunologists (ASI) Work supported, in Part, by the Contract NONR 3127(00)

I. Studies with Meningopneumonitis Organisms

Abstracts of Talks

1. Interaction between meningopneumonitis virus (MPV) and white blood cells. Presented by I.L. Shechmeister and G. Parikh, at the ASI meeting in 1962. Fed. Proc. v. 21, #2.

2. Phagocytosis and multiplication of meningopneumonitis virus (MPV) in white blood cells in vitro and in vivo. Presented by G. Parikh, I.L. Shechmeister and R.S. Yen, at ASM in 1963. Bacteriol. Proc. v. 125.

Articles

1. Interaction of meningopneumonitis virus with white blood cells. Antigenic subunits of meningopneumonitis virus. G. Parikh and I.L. Shechmeister. Virology; 22, 177-185, 1964.

2. Comparative immunological, biological and biophysical behavior of meningopneumonitis viral antigenic preparations. G.C. Parikh and I.L. Shechmeister, Nature; 204, 1013-1014, 1964.

3. Completely adjusted multipurpose agar gel cutter. G.C. Parikh, H. Koike and I.L. Shechmeister. Applied Microbiology; 13, 122-123, 1965.

4. Agglutination of white blood cells by meningopneumonitis organisms. G.C. Parikh and I.L. Shechmeister, J. Path. Bact.; 93, 317-328, 1967.

5. Multiplication of meningopneumonitis organisms in white blood cells. I.L. Shechmeister and G.C. Parikh, J. Path. Bact.; 95, 461-469, 1968.

II. Studies with VSV

Abstracts of Talks

1. The effect of X-rays on plaque forming ability of vesicular stomatitis virus (VSV). I.L. Shechmeister, R. St. John and D. Pittman. Presented in 1962 at ASM. Bact. Proc.; 42.

2. Comparative pathogenicity of plaque-type mutants of vesicular stomatitis virus (VSV) for mice. I.L. Shechmeister, D. Pittman, R. St. John, F. Probstmeyer and J. Streckfuss. Presented at ASI, 1964. Fed. Proc. 23, #2.

3. Procurement and provisional grouping of plaque-type mutants of vesicular stomatitis virus. D. Pittman, I.L. Shechmeister and R. St. John. Presented at ASM, 1964. Bact. Proc. 44, 123.

4. Comparative studies of host range mutants of vesicular stomatitis virus. I.L. Shechmeister, D. Pittman, R. St. John, F. Probstmeyer and A. Hackett. Presented at ASI in 1965. Fed. Proc. 24, #2.

5. Host range mutants of an RNA animal virus. D. Pittman, I.L. Shechmeister and R. St. John. Bact. Proc. 45, 1965.

Articles

1. Effect of X-rays on plaque-forming ability of vesicular stomatitis virus. I.L. Shechmeister, R. St. John and D. Pittman. Nature; 194, 113, 1962.

2. Sensitivity to ultraviolet radiation of vesicular stomatitis virus during replication in chick embryo cells. D. Pittman, I.L. Shechmeister and R. St. John. Rad. Res. 24, 337-349, 1965.

3. Latent period of vesicular stomatitis virus in chick embryo cells. D. Pittman, R. St. John and I.L. Shechmeister. Nature; 206, 1228-1231, 1965.

4. Comparative pathogenicity of vesicular stomatitis virus and its plaque type mutants. I.L. Shechmeister, J. Streckfuss and R. St. John. Arch. ges. Virusf., 21, 128-132, 1967.

5. A comparative study of vesicular stomatitis virus and its plaque type mutant. F. Probstmeyer and I.L. Shechmeister. Arch. ges. Virusf., 23, 79-88, 1968.

6. Studies on multiplication of vesicular stomatitis virus with fluorescein and ferritin-conjugated antibodies. K. Paucker, I.L. Shechmeister and A. Birch-Andersen. In press. Acta Scandinavica.

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13. ABSTRACT

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B. UV derived plaque type mutants of VSV were collected and studied comparatively for pathogenicity for mice and behavior in different cell lines. What appears to be host-induced modification occured with 6 stable plaque mutants grown on HeLa. Electron microscopy of interaction of VSV and PMNL showed that virus is phaocyted in less than 15 minutes.

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