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Neutralizing Human Antibodies From Immune Phage Display Antibody Library Recognize p35 Orthopoxvirus Protein

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FSRI SRC VB "Vector"

In order to select neutralizing anti-orthopoxvirus antibodies, a combinatorial immune library of human single-chain antibody fragments was constructed from Vh and Vl genes of peripheral blood lymphocytes isolated from four vaccinia virus immune donors. The library was panned against cowpox virus, and after two rounds of biopanning the library was shown to be enriched with antibodies specific to orthopoxviruses. Enriched population of polyclonal phage antibodies bound orthopoxvirus protein with molecular weight about 35 kDa in Western blot analysis. The same protein was identified as immunodominant in Western blot using sera of vaccinia virus immune donors. A panel of unique antibodies specific to orthopoxviruses were selected from the enriched library. Ten of the selected antibodies were able to inhibit plaque formation of cowpox virus in Vero E6 cells monolayer in PRNT. Among the neutralizing antibodies eight were able to bind the proteins of vaccinia, cowpox and ectromelia viruses with molecular weights about 35 kDa in Western blot analysis. To identify this protein, the J3L ORF of cowpox virus strain Grishak (analog of H3L ORF of vaccinia virus, strain Copenhagen) was cloned. The resulting recombinant protein prJ3L retained native epitopes of p35 viral protein, that was proved by developing of prJ3L with sera of vaccinia virus immune donors in Western blot analysis. Eight neutralizing antibodies that bound orthopoxvirus protein with molecular weight about 35 kDa were able to bind prJ3L. It was also shown that polyclonal phage antibodies from enriched population recognized prJ3L. Thus, our data confirmed that p35 protein is a key orthopoxvirus antigen for humoral immune response in humans.

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New Digital Method of Virus-Cell Interaction Description

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We have used the computer-assisted analysis of diffraction patterns (CAADP) obtained as a result of laser coherent beam scattering on the preparation of sensitive cell culture Hep-2 infected by *Herpes simplex virus* (HSV) strain US-1. The fractal microscope (FM) is a new device developed for the virus-cell interaction monitoring in real time beginning from the very early stages. Samples of Hep-2 cells cultivated for 24 and 48 h were used as an object of further infection with HSV US-1. E-aminocaproic acid (E-ACA) was used for modification of the virus-cell interaction through proteolysis inhibition at the early stages of viral infection. The E-ACA treated and non-treated substrates both were investigated. Main experimental result of our approach was the establishment of the fractal nature of the cell culture specimens fully described by the unique digital parameter—fractal dimension *D*, modified by the addition of the E-ACA and anti-herpetic preparations. We have shown also that *D* is strongly specific for every type of the specimen as well as its prehistory. We have registered instrumentally with the use of the FM that practically all the useful information about virus-cell interaction and structural changes of the virus-cell dynamic system induced by the addition of antivirals is contained in *D* parameter evaluated for the central self-shadowed part of the FM target. The best antiviral QSAR designed, synthesized and studied in the present study was deca-azatricyclo[28,2.2.2^{13,16}]-tetra-trioctane. As it was shown through FM use, its influence on the HSV US-1 reproduction (69%) was almost as large as that of well-known antiherpetic Acyclovir (80%) on Hep-2 model. We have registered in the FM studies that the changes of the smallest fractal cluster element were considerable even when E-ACA was added in the concentration far from cell toxic dosage. The proposed FM approach has demonstrated in laboratory experiment the highest possible sensitivity and could be used widely in antiviral drug design as the feedback signal source at the stage of viral infectious diseases treatment for both drug type and dosage correction.

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Fractal Microscope—Multipurpose Device For Antiviral Research

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Influenza virus A2/Hong Kong/1/68 (A2HK/1/68) was chosen to be a model object for the present study. Chicken embryos were infected by A2HK/1/68 virus. After 48 h, the allantoic liquid was collected and new viral generation was checked up through haemagglutination (HA) reaction. The virus containing material was titrated on the choryoallantoic membranes' fragments and the infectious activity TID_{50} was evaluated in a regular manner. We have used fractal microscope (FM) for the classification of the virus-containing preparations through registration of the fractal dimension *D*. This parameter of the virus-cell interaction was evaluated through computer-assisted analysis of the diffraction patterns (DP) formed on the target as the result of SNF-XXX-780-20-KB Lasiris laser diode radiation with the wavelength of 783.7 nm and output power of 15.09 mW scattered on the virus-cell system. The DP set of bright scattered spots usually contained from 20,000 to 60,000 units and was reg-