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SENSITIVITY OF VIRUS ISOLATION AND IMMUNOFLUORESCENT STAINING IN DIAGNOSIS OF HERPESVIRUS HOMINIS INFECTION. C.T. Cho and K.K. Feng. Depts. of Ped. & Microb. University of Kansas Medical Center, Kansas City, Kansas.

Herpesvirus hominis encephalitis is usually confirmed by virus isolation and immunofluorescent (F-A) staining of the brain tissues. We utilized brain and other tissues from marmoset monkeys infected with H. hominis to evaluate the sensitivity of these two methods.

H. hominis encephalitis and/or disseminated infection in marmosets were established by inoculation of the virus by intracerebral, intramuscular, or intravenous route. Various organ tissues were harvested and prepared for 1) infectivity titration in cultured cells of human embryonic lung fibroblasts, 2) direct F-A staining, and 3) histopathological study. The following tissues were examined: cerebrum, cerebellum, medulla, liver, spleen, kidney, adrenal, lymph node and lung. Data from six marmosets infected with H. hominis type 1 and two infected with type 2 were analyzed.

Our findings indicated that virus isolation in tissue culture system was more sensitive and reliable than the F-A staining method. False negative results by F-A staining were found in two conditions: 1) presence of focal lesions which were missed by the frozen sections, and 2) presence of low concentrations of the virus in tissues (≤ 3.5 Log_{10} $TCID_{50}$/gram). F-A staining method may provide a rapid diagnosis (within 1 to 2 hours), but virus isolation will give a conclusive diagnosis.

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THE ROLE OF MATERNALLY TRANSFERRED ANTIBODIES IN THE RESISTANCE OF NEWBORN MICE AGAINST COXSACKIE B-3 VIRUS INFECTION. Cheng T. Cho, Frank K.K. Feng, and Vincent P. McCarthy. Depts. of Ped. and Microb. University of Kansas Medical Center, Kansas City, Kansas.

Experimental Coxsackie B-3 virus infection in newborn mice was utilized to examine the protective role of circulating antibodies. Subcutaneous administration of specific antiviral antibodies to the infected mice resulted in a significant protection against the lethal infection of Coxsackie B-3 virus.

Exogenous administration of antibodies to pregnant mothers also resulted in an enhanced resistance to Coxsackie B-3 infection in the offsprings. These antibodies are transferred primarily through the placenta. However, intestinal absorption of the antibodies through breast milk may also provide some protection.

Antibodies transferred	No. of mice	Mortality (%)
None	52	100
Milk alone	54	68
Placenta + milk	43	5

These data suggest that antibody-mediated immunity play a significant role in resistance against Coxsackie B-3 virus infection in the newborn.

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DEVELOPMENT OF IMMUNITY AGAINST INFLUENZA A VIRUS INFECTION IN CHILDHOOD BY CONVENTIONAL AND NEURAMINIDASE SPECIFIC INFLUENZA VACCINES. Tina Chow, Karl R. Beutner, Jane Clement and Pearay L. Ogra, Dept. of Peds. and Microbiol., State Univ. of N.Y. at Buffalo.

1000 school children 7 to 14 years of age were immunized with subcutaneously administered inactivated conventional biphasic Port Chalmers H_3ChN_2Ch (Group 1), or Port Chalmers neuraminidase monospecific Heq_1N_2Ch (Group 2) influenza vaccines, or a placebo (Group 3). The response to initial immunization was characterized by the appearance of H_3Ch and N_2Ch antibody activity in Group 1, and high levels of N_2Ch antibody in Group 2. No H_3Ch response was observed in Group 2 and no antibody response was detected in Group 3 subjects. The outcome of subsequent natural infection with live Port Chalmers strain 3 months later and of Victoria strain influenza infection 15 months later was monitored in the study population by careful evaluation of clinical illnesses, rises of H_3Ch or N_2Ch antibody titer and recovery of Port Chalmers or Victoria strain influenza viruses from the subjects during infections. The degree of protection observed against subsequent infections with Port Chalmers and Victoria strains was 46,9% and 59,5% in Group 1 and 11,4% and 43,3% in Group 2, respectively, when compared to the attack rates of infection in Group 3 (placebo) subjects. These data support the role of neuraminidase specific influenza A antibody in protection against infection with influenza A viruses of identical or similar antigenic composition, although the conventional vaccine appeared to be more effective than the neuraminidase vaccine.

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KILLING CURVE ASSESSMENT OF SYNERGY/ANTAGONISM OF AMPICILLIN AND CHLORAMPHENICOL VS. HAEMOPHILUS INFLUENZAE TYPE B. F. Sessions Cole, Donald A. Goldmann, Lynn Teller, and Arnold L. Smith. The Children's Hospital Medical Center, Dept. of Med., Boston, Massachusetts

To assess possible synergy or antagonism of ampicillin (A) and chloramphenicol (C), we performed killing curves on 9 strains of Haemophilus influenzae type B (HITB) isolated from blood or CSF. All were susceptible to A (mean MIC 0.33 ± 0.15 µg/ml, range 0.2 - 0.625) and C (mean MIC 0.79 ± 0.43 µg/ml, range 0.3 - 1.5) by the broth dilution technique at an inoculum of 10^5 CFU. Killing curves were performed with an early log phase inoculum of 10^5 CFU. Bactericidal activity was examined at the following antibiotic concentrations: the MIC of A, 50% of the MIC of A, 25% of the MIC of A, the MIC of C, 50% of the MIC of C, 25% of the MIC of C, and combinations of these concentrations. For each strain, killing was assessed after 0, 4, and 8 hours of incubation at 37°C; bacteria were enumerated by serial tube dilution in cold phosphate buffered saline. No antagonism or synergy were found after 4 and 8 hours. At 4 hours, mean colony counts of all 9 strains incubated at the MIC of A were significantly higher ($2.2 \times 10^5 \pm 0.7$) than those incubated at the MIC of C ($2.1 \times 10^4 \pm 1.5$) ($p < 0.01$) and significantly higher than those incubated at the MIC of A plus the MIC of C ($1.5 \times 10^4 \pm 1.0$) ($p < 0.01$). These data and the rates of killing observed with other concentrations of A and C suggest that C kills HITB more rapidly than A, and that C defines the rate of killing when A and C are present together.

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PRIMARY HUMAN MONOCYTE CULTURE FOR DIAGNOSIS OF ROCKY MT. SPOTTED FEVER (RMSF). Jeffrey P. Davis, Willie Burgdorfer, Laura T. Gutman, Phyllis Melvin, Robert N. Philip, Catherine M. Wilfert. Duke Med. Sch., Dept. Ped., Durham, N.C., & NIAID, Rocky Mt. Lab., Hamilton, Montana.

Culture of human monocytes from patients was used to try to show intracellular Rickettsia sp. early in the course of clinical RMSF. Ten RMSF patients whose diagnosis was confirmed serologically & 9 non-RMSF patients were studied. Glass adherent mononuclear leukocytes were obtained after sedimentation of heparinized blood. Multiple coverslip cultures were established to allow sequential sampling. Five patients were studied prior to receipt of appropriate antibiotic therapy. Intracellular organisms morphologically characteristic of Rickettsia sp. visualized by Gimenez staining were seen in cultured monocytes from 3 of the 5 patients. Twenty-two separate cultures of monocytes from 10 RMSF patients who had received prior antibiotic therapy were negative for Rickettsia-like organisms. All 7 pre-therapy and 14 post-therapy cell cultures from non-RMSF patients were negative.

The organisms seen were present in cells obtained on days 3,7, & 7 of clinical illness but could not be visualized until days 12 10, & 10 of in vitro observation. Thus 15-17 days from onset of clinical illness were required to see organisms. In contrast, serological conversion in 8 patients as measured by microimmunofloresecence & passive hemagglutination occurred 7-13 days after onset of illness. Monocyte cultures did not provide a more rapid diagnosis than serological conversion. Further refinement of this system may circumvent the hazard of culturing rickettsia from clinical materials in embryonated eggs or animals.

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COINCIDENTAL VIRAL INFECTION IN THE PATHOGENESIS OF OVERWHELMING POST-SPLENECTOMY INFECTION(OPSI). James C.Dearth, Gerald S.Gilchrist, Robert L. Telander, E.Omer Burgert,Jr., and Roy E.Ritts. Mayo Clinic and Foundation, Dept. of Pediatrics and Microbiology, Rochester, MN.

In order to explore the pathogenesis and relative infrequency of OPSI, we developed an animal model to test the role of coincidental viral infection in producing transient immunosuppression and predisposing to fatal sepsis in animals later challenged with S.pneumoniae. The role of S.pneumoniae vaccine and partial splenectomy were also evaluated.

Sham(Sh), partial(PS) or total(TS) splenectomy were done on adult rabbits. Herpes simplex virus(HSV) infection was induced 3 weeks later; and 3 days after that a standard inoculum of type I S.pneumoniae was given I.V. As measured by serial lymphocyte blastogenesis, suppression was greater in virus-infected animals compared to matched controls($p<.01$). Suppression tended to be greater in TS animals compared to Sh or PS($p<.10$). Survival at 3 days for virus-infected animals was 46% vs. 67% for control animals($p<.15$). However, only 25% of HSV-suppressed animals survived vs. 77% survival of non-suppressed animals($p<.005$). Overall survival was 64%(Sh) and 60%(PS) vs.33%(TS) ($p<.06$). Vaccination improved survival(68%vs.18%,$p<.004$). The only survivor with titer $<1:32$ had Sh or PS; the only non-survivor with titer $\geq 1:32$ had TS.

The data suggest that coincidental viral infection predisposes splenectomized animals to fatal sepsis, whereas residual splenic tissue or vaccination provides partial protection against OPSI.