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INTEREFERENCE BETWEEN HUMAN HEPATITIS A VIRUS AND AN ATTENUATED APATHOGENIC AVIAN VIRUS*

L. K. CSATARY, L. KASZA and R. J. MASSEY

Virginia Cancer Research Institute, Arlington, Virginia and Rush Presbyterian St. Luke's Medical Center, Chicago, Illinois, USA

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The effect of an attenuated apathogenic avian bursa virus on the course of human hepatitis A viral infection was studied in marmoset monkeys. The monkeys were infected with human hepatitis A virus, then superinfected with avian bursa virus one and three weeks after initial inoculation with human hepatitis A virus. The superinfected monkeys did not show the characteristic serum glutamic pyruvic transaminase (SGPT) elevation. Also their liver biopsies showed no pathologic changes. The virus control animals exhibited six times higher SGPT enzyme elevation than the superinfected groups, and hepatitis was detected by histopathology. This experiment, as known to us, is the first in which a definite interference was documented using a nonpathogenic virus against a highly pathogenic and clinically significant human virus. This should be considered a successful experiment demonstrating that the use of an apathogen virus for the cure of a virus-induced disease is a realistic possibility.

Although intensely studied for the last sixty years, viral hepatitis remains a major public health problem. Viral hepatitis is caused by several different viruses. Hepatitis A virus (HAV) accounts for 60–80% of the reported cases, and hepatitis B virus (HBV) is responsible for 10–30% [1]. In addition, based on the lack of HAV and HBV antibodies, the existence of at least a third type of viral hepatitis (non-A, non-B) and possible others were suggested [2]. Hepatitis A is transmitted primarily by the fecaloral route and occurs endemically and epidemically. The transmission takes place by contaminated water, food and drink [3]. Hepatitis B is transmitted by direct inoculation and by close personal contact, and is usually endemic. Hepatitis non-A, non-B is currently recognized primarily in post-transfusion patients [3].

HAV infection remains a major public health problem. There is no vaccine or special treatment available. Marmoset monkeys are a suitable experimental model for studying HAV [4]. The virological, serological and

LASZLO K. CSATARY, LOUIS KASZA
Virginia Cancer Research Institute
1600 South Eads St., Arlington, Virginia 22202. USA

RICHARD J. MASSEY
Rush Presbyterian St. Luke's Medical Center
Chicago, Illinois 60612, USA

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pathological changes that characterize HAV infections and type A hepatitis in human patients can be reproduced experimentally in these animals [5]. However, the signs and symptoms are less remarkable in monkeys than in humans. The aim of this study was to determine whether the infection of marmosets by HAV could be altered by administering an avian bursa vaccine [6] postinfection.

Materials and methods

Human hepatitis A virus. Faecal specimens were obtained during the acute phase of hepatitis A (MS-1 prototype) from an infected chimpanzee. Inocula for intramuscular inoculations consisted of a 5% fecal suspension (w/v) diluted in phosphate-buffered saline (pH 7.2), clarified (300 g for 30 min.), filtered (0.45 nm filter prewashed with Hank's balanced salt solution containing 1% albumin). The inoculum was diluted 1 : 500 and 0.5 ml inoculated per animal. This dose of virus induces hepatitis in inoculated animals usually within five to six weeks.

Avian bursa vaccine. Marmoset monkeys were inoculated with the Bursa Disease Vaccine (Sterwin Laboratories, Inc. Millsboro, Delaware; Bursa-vac No. C-603). This vaccine is used for the prevention of avian bursa disease. Each marmoset monkey was inoculated with 50 units orally and 50 units intranasally on days described below.

Animals. In an exploratory study before the start of the main experiment, the effect of avian bursa virus was studied in four adult, wild- caught and/or colony-born and reared red-bellied marmoset monkeys *(Saguinus labiatus labiatus).* Four monkeys served as uninoculated controls.

In the main experiment, the monkeys were divided into five experimental groups of four animal each. In each animal the baseline enzyme values were determined prior to inoculation. Baseline data of three consecutive weeks included serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Percutaneous liver biopsies were performed on the third week. Each group was treated in this following manner: Group 1, inoculated with saline; Group 2, inoculated with HAV; Group 3, inoculated with HAV and treated with bursa vaccine one week post-inoculation; Group 4, inoculated with HAV and treated with bursa vaccine three weeks post-inoculation; and Group 5, inoculated with HAV and treated with bursa vaccine five weeks post-inoculation. The treatments with bursa vaccina were repeated four consecutive days in each vaccinated animal.

During the experiment, each animal was bled weekly and percutaneous liver biopsies were performed bi-weekly, except for the fifth and sixth weeks. SGOT/SGPT levels were determined for each serum sample. Serum (approximately 0.2 ml) was stored at -20 °C for future evaluations. Tissue sections of the liver biopsies were histopathologically evaluated for evidence of hepatitis. All animals were sacrified at 10 weeks, and necropsies were performed. All major tissues were saved in 10% buffered formalin. In this report, we emphasized the two major parameters of viral hepatitis A, the presence or absence of elevated enzyme level (SGPT) and the presence or absence of histological evidence of hepatitis.

Results

In the exploratory experiment, there was no evidence that the avian bursa virus affected the animals. The SGPT and liver biopsies remained in normal range.

In the main experiment, the baseline data remained within a normal range. The data during the viral exposures are demonstrated in Figs 1, 2 and 3 and are described below.

Group 1. Transaminase levels remained normal and there was no histologic evidence of hepatitis throughout the experiment.

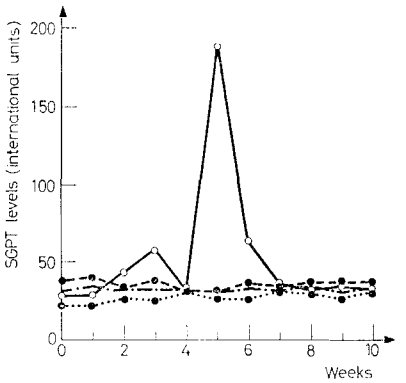

Fig. 1. Interference between human hepatitis A virus and an attenuated apathogenic avian bursa virus. ●——● Group 1, saline control ○——○ Group 2, HAV inoculated, ●····● Group 3, HAV + bursa virus 1 week post inoculation, ·—·—· Group 4, HAV + bursa virus 3 weeks post inoculation

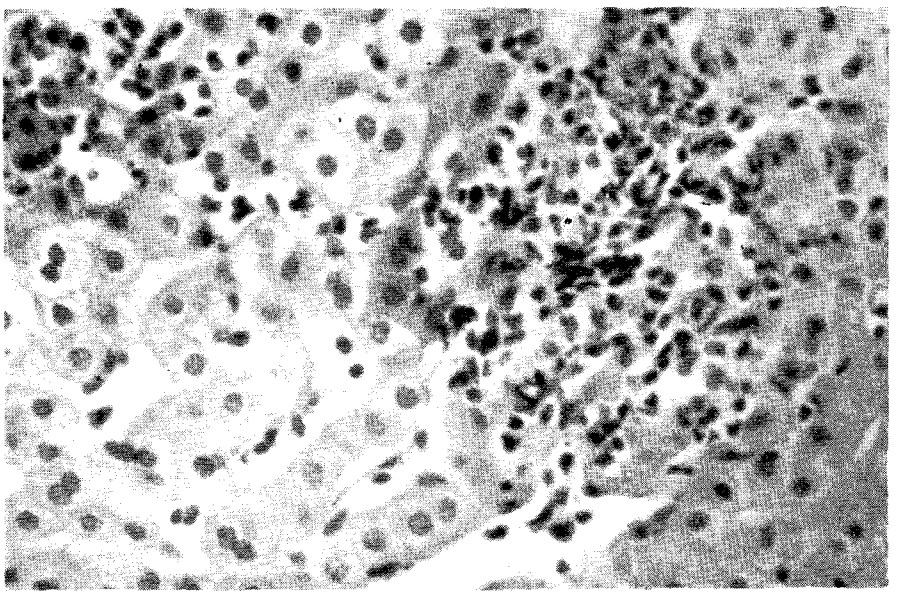

Fig. 2. Liver biopsy from marmoset monkeys inoculated with HAV. Biopsy was done five weeks after inoculation with HAV. There is focal leukocytic infiltration, hepatocellular necrosis and cellular degeneration. Haematoxylin-eosin stain, ×150

Group 2. SGPT levels remained normal until the fifth week post-inoculation. On week five, the average SGPT level was six times the baseline value and there was histological evidence of hepatitis (Figs 1 and 2). On week six, average SGPT levels were twice the baseline, value, and there was still evidence

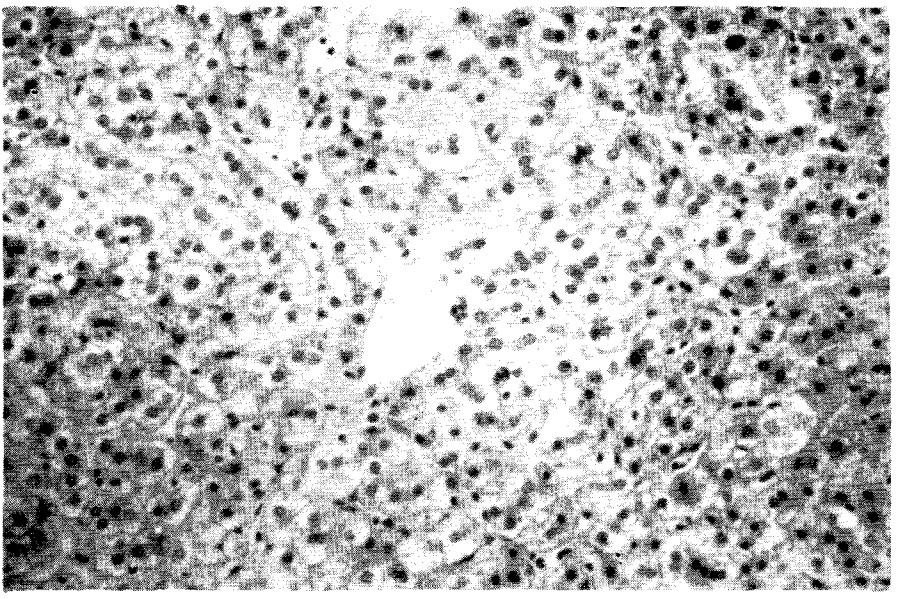

Fig. 3. Liver biopsy from marmoset monkeys inoculated with HAV followed by inoculation with avian bursa virus one week later. Biopsy was done five weeks after inoculation with HAV. The normal architecture of the liver can be recognized. Haematoxylin-eosin stain. ×100

of hepatitis microscopically, although inflammation was not as severe as seen on week five. At seven weeks, the SGPT levels and liver biopsies became and remained normal through 10 weeks.

Group 3. No evidence of hepatitis was observed; SGPT levels remained normal and liver histology remained normal through week 10 (Figs 1 and 3).

Group 4. The results were the same as for Group 3.

Group 5. SGPT levels remained normal until week five at which time there was a fivefold increase. Contamitantly, there was histological evidence for hepatitis. On week six, the SGPT levels returned to twice the baseline, and there was still evidence of hepatitis microscopically similar to that observed in Group 2 at week six. At seven weeks, the SGPT levels remained normal and liver biopsy morphology returned to normal, and it remained normal through 10 weeks.

All animals were sacrificed on week ten and autopsies were performed. No gross pathology was observed in any of the animals. Tissues from liver, lung, kidney, spleen and brain were processed and slides were made for microscopic evaluations. No liver pathology was observed and all other tissues appeared normal.

In addition to the SGPT enzymes, the SGOT enzymes were also determined. The levels of the SGOT enzymes did not show significant changes in

different groups during the course of this experiment. It should be noted that the SGPT, and not the SGOT enzymes are the most sensitive indicator of hepatitis in marmoset monkeys [5].

Discussion

One of us (LKC) made an observation that concurrent viral infections can significantly influence the outcome of viral and hypothetical viral diseases in humans [7, 8]. By systematic data collection, the most remarkable inhibitions were seen in the human herpes [7] and hepatitis viral infections. Under carefully controlled and monitored conditions the apathogenic bursa virus interfered with both the human hepatitis A and B viral infections. The interference was recognized in spite of the fact that HAV is an RNA and HBV is a DNA virus, and they differ from each other in a lot of characteristics. The bursa virus is a double-stranded RNA virus. This peculiar RNA structure of the bursa virus may possibly esplain why both hepatitis viruses are affected.

For evaluation of these observations, suitable animal models were sought to study the interference under experimental conditions. It was found that the effect of an oncogenic animal virus can be favourably influenced by human influenza virus [7]. In a preliminary experiment, it was also found that the avian encephalitis virus markedly (70%) reduced the mortality of mice preinoculated with a strain of rabies virus. The other six viruses in the same experiment — avian Newcastle, duck plague vaccine, avian infectious bronchitis, bursa virus, canine hepatitis and canine distemper — had little or no effect on mortality [9]. From this rabies experiment previously mentioned, it can be concluded than when viral interference is used, the key point is to find the suitable interfering virus which is apathogenic to the host and which is capable of eliminating the harmful effect of the pathogenic virus. In another experiment, interference was also found between Rous' sarcoma, Marek's disease and avian bursa viruses [9]. The mechanisms of interference need a great deal of further exploration. Among others, there are possibilities that the apathogenic virus interferes by (i) inhibition of the attachment of the pathogenic virus on the cellular receptor, (ii) intercellular inhibition of viral reproduction, assembly and maturation n cells by the challenging virus, (iii) interferon production, (iv) stimulation of immuno-response induced by the superinfecting apathogenic virus.

In our most recent in vivo experiment [10], we succeeded enhancing the virus interference phenomenon by adding to the interfering viruses an antihistaminic compound (chlorpromazine), [11].

The fifth group of four animals was superinfected five weeks after HAV infection. This group did not show differences in SGPT and liver histopathology from the positive control group. Very likely, the reason is that in marmoset

monkeys the detectable symptoms are already present at five weeks, similar to the illustration in Figs 1 and 2. In humans, in which the course of the diseas is more severe and lasts longer than in monkeys, the beneficial effects of the interfering virus could last probably much longer. Even when clinical signs are already present, the superinfecting suitable virus is capable of interrupting the pathological process in any phase of the disease. Experiments have been initiated using animal models for the study of the effects of apathogenic viruses on human hepatitis B and non-A, non-B viral infections.

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