TITLE: Ebola Virus Replication in Macrophages and its Relation to the Virus Pathogenicity

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57. EBOLA VIRUS REPLICATION IN MACROPHAGES AND ITS RELATION TO THE VIRUS PATHOGENICITY

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INTRODUCTION

Any infectious disease is a result of complicated interplay of both pathogen and organism factors. Identification of the most important events, which determine development of the disease is necessary for understanding of the basic mechanisms of infection. Much attention is paid to the most dangerous viral infections and Ebola hemorrhagic fever is among them. Ebola Zaire infection represents an example of the interaction of host with pathogen, when the virus kills humans and monkeys very rapidly and cruelly (1,2). Mechanisms providing such horrible abilities of Ebola virus are still unclear.

It is known that Ebola virus kills monkeys in 7-9 days post-infection, while guinea pigs develop non-lethal infection (3,4). Sequential passages lead to changes of Ebola virus pathogenicity and guinea pigs began to die. This effect of passages was called “adaptation” of Ebola virus to guinea pigs, and was described in early studies (5). Analogous adaptation of the Ebola virus to adult mice by sequential passages was reported recently (6). Detailed examination of the non-lethal Ebola infection and changes of the infection in the course of passages has been not performed. Meanwhile, comparison of lethal and non-lethal forms of Ebola infection may highlight the mechanisms of pathogenicity and virulence of dangerous Ebola virus. The aim of present study was to analyze differences between fatal and non-fatal infections caused by Ebola virus, and hunt dawn the changes related with transition of non-fatal infection to fatal in experimental animals.

MATERIALS AND METHODS

Ebola virus (Zaire strain), passaged two times in monkeys, was used. Twelve adult green monkeys (Cercopithecus aethiops) were subcutaneously infected with 100 LD$_{50}$ and thirteen adult baboons (Papio hamadryas) were subcutaneously infected with 20-50 LD$_{50}$ of Ebola virus. Eighty outbred young guinea pigs weighing 180-200 g were used for the studies. Animals received Ebola virus in a dose of 10$^6.5$ LD$_{50}$ for newborn mice. Adaptation of Ebola virus to guinea pigs was performed according routine technique of blind passages: animals were infected by 1 ml of 10% hepatic suspension prepared from the liver of guinea pigs on the previous passage. Only feverish animals were used for preparation of the inoculum and for examinations. Nine sequential passages of Ebola virus on guinea pigs were performed. Clinical observations and content of the virus in blood of monkeys and guinea pigs were determined by plaque forming units (PFU) technique or inoculation of newborn mice. Samples of the visceral organs were obtained for light and electron microscopic examinations. Details of the experiments were described may be found in earlier publications (7,8).

RESULTS

Infection of the monkeys with Ebola virus caused fatal disease finishing with death on days 7-9, while guinea pigs developed acute infection without death during the continuance of 28 days of the experiment. Monkeys developed a fever from day 4 postinfection, and showed precipitate drop in the temperature 5-5 h before death. Fever was registered only in
50% of guinea pigs on days 3-8, and on days 12-16 after inoculation with unadapted Ebola virus. The virus was detected in monkey blood from days 3-4 postinfection, and it's content rose up to $10^{7.8}$ PFU /ml at the end of infection. Attempts to find Ebola virus in blood of guinea pigs were unsuccessful even by most sensitive method of the inoculation of newborn mice. Guinea pigs showed anorexia, loss of excitability, weight and tufts of hair during the febrile phases of Ebola infection. Monkeys developed anorexia after fever onset, and 60-70% of baboons inoculated with Ebola virus demonstrated visible signs of the hemorrhagic syndrome: blood vomiting, bleeding from rectum and vagina, prominent hemorrhages into skin and mucous membranes at the infection terminal stages.

Microscopic examination showed clear differences in the pattern of organ pathomorphological changes between the monkeys and guinea pigs. Pathological changes were observed in all visceral organs of Ebola virus infected monkeys, with severe impact to liver, spleen, kidneys and lymphatic organs, and blood system, while guinea pigs showed only local inflammatory process in the liver. Numerous fibrin thrombi and clots were found in blood vessels of green monkeys during last days of the infection, whereas visceral organs of baboons showed multiple hemorrhages of various sizes. The most prominent feature of Ebola infection in guinea pigs was focal inflammatory reaction in the liver (Fig. 1). Visible pathologic changes were not found in another visceral organs except of splenic white pulp and lymphatic nodes, showed slight lymphoid depletion and damage to stromal and macrophage cells.

Examination of the visceral organs of Ebola virus infected animals by electron microscopy revealed different mode of interaction of the virus with host cells. Thus, replication of Ebola virus in all the studied monkeys was observed in macrophage cells, hepatic parenchymal cells, adrenal cortical cells, fibroblasts and endothelial cells. All monkey organs contained Ebola virus infected macrophages and fibroblast cells at the terminal stages of infection indicating the generalized pattern of the disease. In contrast, in guinea pigs Ebola virus replication was restricted to macrophage cells located inside the inflammatory foci in the liver (Fig. 2).

Examination of guinea pigs organs day by day established that Ebola virus was able to replicate only in the cells of macrophage lineage, including Kupffer cells. It was also found that infected cells represented the cells, which induced focal inflammation in the liver. Inflammatory foci in the liver of Ebola virus infected guinea pigs differed in sizes and contained all varieties of leukocytes: large agranular lymphocytes, granular lymphocytes, monocytes, and neutrophils. Fibrin clots and bundles filled the spaces between the cells and bordered the foci around, and thereby prevented the access of Ebola virus into bloodstream. Each foci was isolated from surrounding tissue, and all viral progeny were arrested inside the foci. Ebola virus particles being blocked inside the foci were accessible for all affecting factors releasing by inflammatory leukocytes. It should be noted that hepatocytes were not involved in formation of the inflammatory foci. Hepatocytes remained unaltered even in close vicinity of the foci. Effectiveness of the virus blockage inside the foci was evidenced also by the results of virologic studies. Ebola virus was not found in the blood of a guinea pigs nor at the top of infection, nor in other periods, while hepatic homogenate contained the virus in a concentration of $10^4$ - $10^5$ PFU/ml (Table 1).

Examination of the liver of Ebola virus infected guinea pigs in light microscope revealed rare sole activated Kupffer cells, which were not related to inflammatory foci. Studies by electron microscope found that few of these cells were infected. The cytoplasm contained specific Ebola virus inclusions with straight nucleocapsids (Fig. 3). These infected Kupffer cells produced viral particles having “typical” morphology of Ebola virus, and did not induced inflammatory reaction. No neutrophil or another leukocyte were found in the
vicinity of these infected Kuppfer cells. Thus, these cells represent another kind of replication system for Ebola virus in the liver of guinea pigs providing unrestricted release of the virus into bloodstream and surrounding tissue. Sequential passages of Ebola virus on guinea pigs resulted in rise of the virus pathogenicity for these animals. Concentration of Ebola virus in the liver of guinea pigs was relatively small on first and second passages, then increased, and animals began die on third-fourth passages on days 7-8 after the infection. Liver of guinea pigs at first and second passages showed rare inflammatory foci composed of few leukocytes. We could not find any infected cells in the liver of guinea pigs on first and second passages. Obviously, number of the infected cells was beyond of the sensitivity of electron microscopy, while biological methods showed presence of the Ebola virus in hepatic preparations at the same passages (Table 1). Signs of pathologic changes were not detected in another visceral organs.

Third passage displayed another pattern of the hepatic injury. Ebola virus replication were observed in the liver from days 5-6 postinfection until death. Main feature of the replication was infection of the hepatocytes, not only macrophages. Cells of hepatic parenchyma contained typical Ebola virus inclusions and nucleocapsids (Fig. 3). Total number of Ebola virus infected cells in the liver of guinea pigs on third passage was incomparedly larger than in livers of guinea pigs infected with unadapted virus. Accumulation of leukocytes was evident in the hepatic tissue, but distinct inflammatory foci did not formed. Microcirculatory disturbances were observed in the liver from days 3-4, and small necrosis of hepatic cells were found from days 5-6 postinfection. Microcirculatory disorders were also detected in spleen, lungs, kidney, adrenals and lymphatic nodes in guinea pigs on the third passage of Ebola virus.

The next passages resulted in rise of the level of pathological changes, disease severity and mortality. Pathological characteristics of fatal infection in guinea pigs were in very close similarity to those in monkeys. The set of target cells supporting Ebola virus replication was identical in both guinea pigs and monkeys: macrophages, hepatocytes, adrenal cortical cells, fibroblasts and endothelial cells. The general pattern of pathological changes in visceral organs of monkeys inoculated with Ebola virus, and guinea pigs infected with adapted Ebola virus also was very similar, sometimes nearly identical. However, one significant exception was established: guinea pigs never showed such hemostatic changes as monkeys. Signs of hemorrhages and clotting were not observed in guinea pigs even at passages 7-9. Features indicating impairment of immunity were identical in monkeys and guinea pigs infected with adapted Ebola virus: lymphoid depletion, absence of mitosis in lymphocytes, lack of inflammatory reaction against infected cells. So, in consequence of Ebola virus adaptation to guinea pigs the virus acquired ability to infect additional cellular targets, pathologic changes of visceral organs increased, and the infection became fatal.

DISCUSSION

Pathology of the fatal Ebola infection in monkeys has been described in series of publications (1-4,8,10,11), while data concerning non-lethal infection are very fragmentary (5,6). The present study revealed distinct differences in ability of Ebola virus to replicate in cellular targets in the case of fatal and non-lethal infections. In both monkeys and guinea pigs macrophages were primary targets for Ebola virus, supporting productive infection resulted in formation of viral progeny. However, replication of the virus in monkey macrophages did not induce inflammatory reaction, and progeny viral particles had free access to blood and neighboring cells. In distinction from monkeys, infected macrophages of guinea pigs were surrounded with inflammatory leukocytes and densely encased by fibrin, which blocked Ebola virus progeny inside the foci. Formation of inflammatory foci in the liver prevented
dissemination of the virus and restricted viral replication to macrophages. Thereby, infection acquired the local pattern. Recent studies of murine cytomegalovirus infection showed that local reactions of immune defense may be crucial for the disease development and outcome (9). The results of our study of non-lethal Ebola infection in guinea pigs are in good agreement with this statement. Guinea pigs demonstrated operation of the effective defense mechanism, which provides blockage of Ebola infection in liver and thus determines the course and outcome of disease.

Examination of non-lethal infection found two kinds of interaction of Ebola virus with macrophages of guinea pigs: (1) related and (2) unrelated to development of inflammatory reaction. We suggested that this is an evidence for presence of two kinds of viral particles in initial population of the Ebola virus. The process of adaptation of Ebola virus in its essence is a selection of viral particles, which are able to replicate without induction of local inflammatory response. The initial population of Ebola virus should contain the particles which are able to kill guinea pigs. This is evident from the reproducibility of adaptation experiments. Quantity of these particles increased in the course of passages, and ability to kill guinea pigs became a characteristic feature of the virus population. Our studies traced the changes of infection in guinea pigs during the sequential passages of Ebola virus, and allowed to suggest that differences in interaction of Ebola virus with macrophages were responsible for outcome of the infection.

What a conclusion may be drawn from the results of Ebola infection studies? It is clear that Ebola virus is capable to infect cells of macrophage family in guinea pigs. Replication of the main portion of Ebola virus population induced local events of the immune defense reaction in a form of focal inflammation. Remaining portion of the virus population, very small portion, is able to replicate in macrophage cells without induction of the inflammatory reaction. It seems that immune system can not recognize these infected cells, and Ebola virus may replicate without restrictions. We suggested that just this portion of the viral population is responsible for changes in Ebola virus pathogenicity for guinea pigs during the passages, and organisms of guinea pigs act as a system for selection.

KEYWORDS
Ebola virus, guinea pigs, pathogenicity, passages, macrophages, monkeys.

REFERENCES


FIGURES AND TABLES

Fig. 1. A – a liver of guinea pig infected with unadapted Ebola virus. Day 7 postinfection. Arrows show large inflammatory focus. B – a liver of baboon infected with Ebola virus. Day 7 postinfection. Arrows show hepatocytes containing Ebola virus inclusions. Asterisks are pointed to sinusoids. Note absence of inflammatory reaction to infected cells. Short arrows are pointed to sinusoids. Semithin sections. Light microscopy.
**Fig. 2.** Inflammatory foci in the liver of guinea pig. Day 7 postinfection with unadapted Ebola virus. A – fibrin (thick arrows) deposits between the cells. Thin arrows show viral particles blocked by fibrin. Magnification 10 000. B – part of macrophage cell infected with Ebola virus. Thick arrows show viral inclusion in the cytoplasm, thin arrows are pointed to viral particles. Magnification 18 000.

**Fig. 3.** A- a sole Kupffer cell in guinea pig liver. Day 7 postinfection with unadapted Ebola virus. Thick arrow shows membrane structure specific for Ebola virus replication, thin arrow shows nucleocapsids. Asterisk is pointed to adjacent hepatocyte. Magnification 10 000. B – a portion of hepatocyte infected with Ebola virus. Seventh passage of Ebola virus. Arrows show nucleocapsids in the cytoplasm. Magnification 16 000.