Midzonal lesions in yellow fever: A specific pattern of liver injury caused by direct virus action and in situ inflammatory response

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Summary  Yellow fever is an acute infectious, non-contagious disease characterized by intense vasculopathy and lesions in different organs. In the liver, one of the main targets of the virus, the infection induces a characteristic midzonal injury characterized by hepatocyte necrosis, apoptosis and steatosis. This characteristic pattern of liver injury in yellow fever is also observed in conditions of low-flow hypoxia and other infections such as dengue and Rift Valley fever. There are no reports in the literature explaining the genesis of this peculiar histopathological pattern in yellow fever. Some hypotheses have been proposed to explain the mechanism of this midzonal distribution pattern observed in the liver such as low-flow hypoxia and tropism of the virus toward hepatocytes in this area. These hypotheses are discussed in view of more recent findings regarding the pathogenesis of yellow fever and regarding hepatic physiopathology, and a new hypothesis is proposed: the midzonal necrosis is consequence of action of combined factors mainly the direct cytopathic effect of YFV associated with a potent immune response in which CD4+ and CD8+ lymphocytes and the cytokines, especially TGF-β, but also TNF-α and IFN-γ play an important role.

Introduction
Classically, the pattern of host–parasite interaction is a determinant factor of cell injury and essential in the progression and prognosis of infectious and parasitic diseases. In hepatic viral infections, the resulting lesions are generally not only the product of interactions between the infectious agent and host cell, but are also due to factors inherent to systemic alterations, with important secondary involvement of the liver [1]. Viral hemorrhagic fevers such as yellow fever, dengue and Rift Valley fever present as severe acute febrile syndromes, with hemorrhagic phenomena being their most prominent manifestations due to the effects of infection on the endothelial cell [1,2]. Some of these infections frequently share a
common histopathological substrate, a fact leading to cellular alterations that are found in more than one of these diseases such as apoptosis observed in yellow fever and dengue [2].

Regarding yellow fever, like in other viral hemorrhagic fevers, the hepatic involvement is characterized by intense injury to the midzonal area, with hepatocyte steatosis, apoptosis and necrosis. Several studies have been conducted in an attempt to elucidate the etiopathogenic mechanisms underlying the infection with yellow fever virus; however, some questions continue to be extremely controversial and the hypotheses raised require experiments for their final objective confirmation [2,3]. Among these aspects, the preferential midzonal injury has gained interest because several data indicate the presence of distinct mechanisms for the genesis of these characteristic lesions. The pattern of midzonal hepatic injury has been described for different pathological conditions including states of hypoxia and viral infections such as yellow fever, dengue and Rift Valley fever [2,4]. Several hypotheses have been proposed to explain this preferential lesional pattern, including viral tropism, immune response activation and low-flow hypoxia-induced injury. Although characteristic, the intensity of midzonal injury varies among the different diseases and yellow fever is a model of viral infection in which this peculiar aspect of the liver represents a marked feature.

The existent hypothesis

The pattern of midzonal injury is a characteristic histopathological finding of yellow fever when considered together with the other alterations that are observed in the liver during the course of infection. On the other hand, the same characteristic pattern is observed with relative frequency in infections induced by other viral hemorrhagic fevers such as dengue and Rift Valley fever viruses. Therefore, two hypotheses have been raised to explain the occurrence of this characteristic pattern. The first hypothesis proposes that midzonal injury is the consequence of preferential tropism of the virus toward hepatocytes in the midzone region, inducing greater injury in this region compared to the other regions of the hepatic lobule due to the effect of the virus on the cell and the action of cellular immune factors and cytokines able to induce cell death of infected hepatocytes. The second hypothesis suggests the possibility that the characteristic midzonal pattern of hepatic injury is the result of low-flow hypoxia as a consequence of marked alterations in endothelial cells, associated with a greater susceptibility of midzonal hepatocytes to states of low oxygen concentration.

Evaluation of the hypotheses

The classical studies of Councilman [5] have originally described the main histopathological alterations observed in yellow fever liver, which were later complemented by Rocha-Lima in 1912 [6]. In these studies, the authors identified steatosis and hyaline degeneration as characteristic alterations of yellow fever when associated with a peculiar topographic pattern of more intense midzone distribution [6,7]. In subsequent studies, Rocha-Lima emphasized the importance of this histopathological picture of midzonal injury for the diagnosis of yellow fever and regarded it as a feature of this disease. Other authors later confirmed these findings [7–9].

Recent studies have demonstrated that the anatomopathological aspects of the liver in yellow fever are characterized by the severity of the lesions throughout the lobule. However, the samples were characterized by a clear preferential involvement of zone 2 (midzonal area), permitting the identification of narrow bands of preserved hepatocytes around portal spaces and the centrolobular vein. Semiquantitative investigations showed that the centrolobular and the midzone regions are, in terms of the degree and extent of involvement the most affected areas, with a greater preservation being observed for zone 1. As also shown in other classical studies, this latter zone generally maintains a narrow band of preserved hepatocytes around the portal space, showing variable degrees of degenerative and regenerative alterations [7].

Despite the large number of studies reporting this selective predisposition for zone 2 in liver lesions of yellow fever, any adequate explanation for this particular type of involvement have not been described. On the other hand, later studies [10] have demonstrated diversity in different acinar regions of the liver, characterizing the biochemical and ultrastructural heterogeneity of the hepatic parenchyma and its susceptibility to lesions induced by systemic conditions of ischemia and hypoxia which, to some extent, may explain the presence of selective lesions during the course of yellow fever or other infections and also other situations of impairment of blood flow to the liver [4,11].

It should be added that the studies published by De la Monte et al. [4] and Suematsu et al. [11] have
demonstrated a midzonal lesional pattern secondary to low-flow hypoxia and also to increased susceptibility of zone 2 hepatocytes to nociceptive stimuli which caused marked alterations in mitochondrial function and cell death.

**Our hypothesis**

Considering the data in the literature [1,4] and our recent findings [2,3] we propose that multiple factors are responsible for the midzonal lesion in yellow fever. Such involvement seems plausible since the direct viral cytopathic effect in the hepatic cells and the cellular (CD4+ and CD8+ lymphocytes) and immune responses including cytokines action principally of TGF-β, but also of TNF-α and IFN-γ, together with marked vascular alterations (especially the blood low-flow), should be responsible for the set of histopathological alterations observed in yellow fever.

**Discussion**

Monath [12] have emphasized that during the course of yellow fever, the frequent vascular alterations are the cause of lesions in some organs, where they are not necessarily the direct consequence of the cytopathic effect of the virus. This vascular component causing hepatic hypoflow, associated with the known characteristic blood flow in Rappaport’s acinus and the biochemical heterogeneity of the parenchyma, may lead to the characteristic histopathological picture consisting of prominent midzonal lesions followed by centrolobular involvement and preservation of hepatocytes close to the portal space which, theoretically, are under higher oxygen tension. It should be emphasized that microcirculation disorders of the liver are one of the causes of fulminant liver failure and are likely to be part of a broader vasculopathy [11]. This model of midzonal injury secondary to blood hypoflow, which theoretically occurs in the severe hemorrhagic forms of yellow fever, reaches its peak in the final phase of the disease when it could be associated with cardiovascular disorders and a picture of shock, aggravating the lesions in the midzone region and also markedly intensifying those observed in zones 1 and 3. Few studies are available in the literature that describe step-by-step the progression of this characteristic midzonal involvement during the different phases of the disease in order to correlate it with underlying cardiovascular disorders. Vieira et al. [7] analyzed, among 10 cases studied, two biopsies from a patient with altered aminotransferases and without underlying hemorrhagic disorders and observed characteristic midzonal injury even in the absence of evident hemodynamic disorders. Therefore, the influence of other factors should be taken into account, including a possible viral tropism toward zone 2, also demonstrated in immunohistochemical studies with yellow fever-specific antibodies [13], and a cellular and cytokine-triggered immune response during infection, which may equally contribute to the characteristic progression of the lesions.

The more intense immunostaining pattern for yellow fever antigens in the midzone region described by Monath et al. [13] and De Brito et al. [14], together with the demonstration of a higher concentration of inflammatory cells and cytokines at this site reported in various recent studies [2,3], suggest a role of specific viral tropism toward hepatocytes in zone 2 and, consequently, the occurrence of lesions induced by a direct cytopathic effect of the virus associated with a cellular and cytokine-specific immune response as observed in other forms of hepatitis [15]. This interpretation is supported by studies involving a larger series which quantified the distribution of these cells in the hepatic acinus and showed a characteristic midzonal distribution of cytotoxic lymphocytes, activated macrophages and IFN-γ, immune system components that play an important role in the response to viral infections. In contrast to the idea of tropism and viral specificity for the midzone region, it should be remembered that the pattern of midzonal injury is also observed, although at lower intensity, during the course of other viral hemorrhagic fevers that are also characterized by intense vasculopathy such as dengue and Rift Valley fever [12]. These data contradict in part the idea of viral specificity as a single determinant factor of midzonal injury and call attention to a common substrate shared by these infections, i.e., vasculopathy.

In conclusion, we believe that the midzonal lesion in yellow fever is caused by several factors, but the direct virus action and the marked immune response by CD cells and the role of cytokines especially TGF-β are important actors in the genesis of midzonal lesion. It is also clear that a role of the blood low-flow secondary cardiac lesion should be considered. Therefore, only when new experimental studies are performed in non-human monkeys using the new immunologic and pathologic tools available, we will understand in full the pathogenesis of yellow fever including its characteristic hepatic tissue injury. Thus, new studies in this direction are welcomed.
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References