Trypanosoma cruzi Infection; liver involvement

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Currently, in Chagas disease, hepatomegaly is cited in most papers published which either study acutely infected patients or experimental models, and we know that the *Trypanosoma cruzi* can infect multiple cell types in the liver, especially Kupffer cells and dendritic cells. Moreover, liver damage is more pronounced in cases of oral infection, which is mainly found in the Amazon region. However, the importance of liver involvement, including the hepatic immune response, in disease progression does not receive much attention.

infection | Chagas disease | liver | hepatic immune response | Trypanosoma cruzi | cellular immunology

1. Introduction

Hepatocytes compose approximately 60% of the hepatic cells and about 90% of the liver volume [1], and their primary function is formation and excretion of bile; lipid synthesis and plasma lipoprotein secretion; control of cholesterol metabolism; regulation of carbohydrate homeostasis; formation of urea, serum albumin, coagulation factors, enzymes, and other molecules; and metabolism or detoxification of drugs and other exogenous substances [2].

Although previous authors suggested that hepatic stellate cells (HSC) are professional intrahepatic APCs that elicit many T cell responses, in contrast to other liver cells that lead primarily to tolerance, additional results should be considered. For example, it was also demonstrated that HSCs are central in the liver's regulatory response, as they can inhibit T cell responses via B7-H1-mediated apoptosis [3]. Moreover, it was shown that HSCs alone do not present antigens to naive CD4 + T lymphocytes but, in the presence of dendritic cells and TGF-β, preferentially induce FoxP3 + Treg cells [4].

Besides the main hepatic dendritic cells (HDCs) observed, an extraordinary and minor population of DC is also found in the liver, possibly an intermediate population with cytotoxic properties that can efficiently lead to T lymphocyte activation. These NK1.1 + cytotoxic HDCs, also found in other tissues, are inflammatory cells that can produce high levels of IFN-γ via autocrine IL-12 stimulation, leading to the development and activation of cytotoxic T lymphocytes [5][6][7][8].

Reversing or breaking hepatic immune tolerance in persistent infections or cancer is of central importance. This can be demonstrated by the reversion of liver T cell tolerance and viral persistence in the case of hepaitis B virus (HBV) infection after the blockade of inhibitory receptors, such as PD-1 [9][10]. Additionally, in tumors such as hepatocellular carcinoma, PD-1 blockade has good results in inducing a protective immune response [11][12][13].

2. The Participation of the Liver in *T. cruzi* Infection, a More Recent Perspective

Today we know that the liver plays an essential role in the infection, and its importance in parasite clearance and destruction of blood (trypomastigote) forms, for example, is well documented. Experimental murine infections showed that specific antibodies against the parasite plus phagocytic cells are required for extracellular trypomastigote clearance. IgG-coated parasites are phagocytosed by resident mononuclear cells, especially in the liver but also in the lungs and spleen [14]. Moreover, pro-inflammatory cytokines, mainly IFN-γ, potentiate the trypanocidal activity of the phagocytic cells [15], and intact Fc portions of IgGs are required [16]. Sardinha and cols. published in 2010 that the liver is the primary site of parasite accumulation just one hour after intravenous injection of *T. cruzi* trypomastigote forms in chronically infected mice [17]. At this time point, viable parasites and parasite remnants were observed scattered in the liver parenchyma, which considerably diminished after 48 hours, and no intracellular parasites were observed in the liver seven days after the challenge [17]. Moreover, transmission electron microscopy showed platelet thrombi occluding small vessels in the lung, liver, and spleen, and phagocytosed parasites in different stages of destruction were found within macrophages, neutrophils, and eosinophils. Therefore, it seems that not a particular cell population, but different cells, act in concert to destroy the parasites in the liver [14].

The liver is the main synthesis site for the complement system's components, and it has long been evaluated if this lytic pathway could play a role in removing blood parasites. Although trypomastigote clearance is dependent on C3 [18], it is primarily independent of the lytic terminal pathway. A more detailed analysis of the complement system's importance in parasite clearance showed that C1q, C3, mannan-binding lectin, and ficolin molecules bind to trypomastigote forms. Moreover, C3b and C4b deposition assays revealed that *T. cruzi* activates mainly the lectin and alternative complement pathways in non-immune human serum [19]. Experiments using C5-deficient mice showed no difference in parasite clearance compared with wild-type mice [16].

It is long known that blood trypomastigote forms express several complement system inhibitors, such as a decay-accelerating factor expressed by *T. cruzi* (T-DAF) [20], complement C2 receptor inhibitor trispanning [21], complement regulatory protein, and others [22]. Nevertheless, some parasite strains seem to be susceptible to the complement system [19].

It is also known that the infection subverts the host lipid metabolism in multiple ways [23], mainly affecting the low-density lipoprotein- (LDL) and high-density lipoprotein-dependent (HDL) pathways and their receptors. LDL is generated from liver-derived very-low-density lipoprotein (VLDL) and is a potent inhibitor of *T. cruzi* trans-sialidase, an enzyme expressed mainly by epimastigote and trypomastigote forms of *T. cruzi* that transfers sialic acid from

the environment to the parasite surface [24]. Moreover, the addition of LDL and HDL to cells in culture enhances infection by *T. cruzi* trypomastigote forms in a dose-dependent manner [25]. The LDL receptor (LDLr) is one of the molecules used by the parasite during cell invasion, and in vitro infection in the presence of an LDLr blocker resulted in a 42% reduction of intracellular infection [26]. The LDLr is also involved in the trafficking of lysosomes to the cytoplasmic parasitophorous vacuole, and the disruption of the LDLr pathway affects the intracellular parasite load.

Today we know some of the lipid metabolic pathways that led to the first observations of hepatic steatosis by Dr. Chagas. For example, it was recently demonstrated that *T. cruzi* interaction with LDLr leads to the accumulation of LDL cholesterol in host tissue in acute and chronic chagasic patients [27]. Moreover, murine experimental infection revealed a significant increase in the absolute amount of triacylglycerides, cholesterol, and cholesterol esters in liver microsomal membranes [28]. Additionally, *T. cruzi* experimental infection was considered a potent risk factor for non-alcoholic steatohepatitis, associated with strong oxidative stress and metabolic disorders [29].

Regarding the hepatic immune response, we have just recently started to understand the integration and possible interdependency between hepatic and peripheral immunity after infection. As primarily observed at the beginning of the last century, the *T. cruzi* infection leads to inflammatory mononuclear cell infiltration in the liver parenchyma. Today we know some of the main cell types that compose the inflammatory foci and their inflammatory mediators. Briefly, as this topic will be discussed in more detail in the next section, the infection induces an increase in $Mac1^+$, activated $CD8^+$ and $CD4^+$ T lymphocytes expressing CD25, CD69, and/or CD122, natural killer (NK), and NKT cells [17] in the liver. Moreover, one of the significant roles of NK cells and $CD4^+$ T lymphocytes in liver protection and infection control is interferon-gamma production (IFN-γ) [30]. In addition, we observed that experimental murine infection leads to increased hepatic regulatory T (Treg) cell numbers, higher expression of programmed death ligand 1 (PD-L1 or B7-H1) in the liver stroma, increased blood activity of ALT and AST transaminases, and other alterations [31].

3. The Immune Response in the Liver

The liver is the second largest organ in the human body. It performs many essential functions, including metabolic regulation, digestion, production of bile, detoxification (conjugations with sulfate, glucuronic acid, glutathione, acetate, and glycine), and biotransformation of drugs and toxins (oxidations-reductions and hydrolysis) [32][33][34]. Approximately 80% of the blood supply that reaches the liver comes through the hepatic portal vein, consisting of blood that is low in oxygen and rich in nutrients and molecules of the intestinal microbiota. This anatomical characteristic determines that the liver typically meets very high levels of bacterial components that, in the periphery, would be recognized as danger signals and potent pro-inflammatory stimuli. The liver must then be able to individually discriminate pathogenic damage-associated molecular patterns (DAMP) [35] and especially pathogen-associated molecular patterns (PAMP) [36] from harmless DAMPs and PAMPs. This means that the liver must retain its tolerogenic bias and selectively recognize proper danger signals for pro-inflammatory response against infections or tumors, for example. These fascinating properties are just beginning to be elucidated.

Many anatomical, immunological, and environmental aspects play a central role in balancing tolerance versus immune responses in the liver. For example, the organ is highly vascularized, and these hepatic microvessels are known as hepatic sinusoids. The epithelial cells that line the sinusoids are fenestrated, allowing the protrusion of membrane segments and physical interaction between cells flowing in the vase lumen with stromal and parenchymal cells. Between the sinusoids wall and hepatocyte cords, there is the Disse space [37], a space adjacent to the sinusoids that harbors many different cell types in the liver. The very low blood pressure in the sinusoids favors this integrated cellular interaction network, affecting the liver's biochemical and immunological functions [1]. Although the liver is best known for its primary metabolic functions, the organ is of great importance in the local and systemic immune response [5][38], as depicted below.

4. Perspectives

Today we know many of the biochemical pathways and cell populations that sustain hepatic tolerance, and this knowledge should be translated into practical proposals and alternative treatments for autoimmune or chronic inflammatory diseases, for example. A few initiatives have been proposed, such as the induction of hepatic Treg cells by LSEC to control autoimmune encephalomyelitis (EAE) [39]. Previously, the authors showed that LSECs could induce $CD4^+$ $Foxp3^+$ Treg cells in mice [40]. Subsequently, they injected nanoparticles as carriers of autoimmune peptides in vivo, which were selectively delivered to LSECs. This treatment resulted in antigen-specific Treg cell induction, and these cells could completely and permanently prevent the onset of clinical EAE. Moreover, in mice with established clinical EAE, the treatment for Treg cell induction rapidly and substantially improved muscle paralysis and atonia, whereas the control group deteriorated. Similar results were obtained when antigen-specific Treg cells were expanded using neural autoantigen myelin basic protein (MBP) in the liver [41]. In the case of myelin infusion, antigen-specific $Foxp3^+$ Treg cells exerted their effect by diminishing antigen-bearing inflammatory dendritic cells recruitment to lymph nodes and by impairing their function [42]. The induction of Treg cells in the liver or the periphery is reviewed in [43][44].

Other approaches such as those using antigen-specific immunotherapy [45] and neutralization of immunomodulatory molecules in the liver (reviewed in [46]) are being studied or are already in use. Much remains, however, to be understood about the fascinating pathways and immunological cross-talks hidden in the liver immunophysiology, including after *Trypanosoma cruzi* infection.

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