Gold Organs in *Brucellosis*

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Brucella is an intracellular bacterium that causes abortion, reproduction failure in livestock and leads to a debilitating flu-like illness with serious chronic complications if untreated in humans. The “gold organs” for nesting *Brucella,* in which *Brucella* replicates in cells of the reticular endothelial system, include the spleen, lymph nodes, liver, bone marrow, epididymis, and placenta.

Brucella | replication niche | reservoir | persistence

1. The Reticuloendothelial System

The reticuloendothelial system was originally described in 1924 by K. Aschoff as a group of cells able to incorporate vital dyes from the circulation, “reticulo” referring to their propensity to form a network or reticulum by their cytoplasmic extensions and “endothelial” referring to their vicinity to the endothelium. In 1969, a group of pathologists proposed another term, the monocyte phagocyte system (MPS) [1]. Nowadays the reticuloendothelial system or MPS embraces a family of cells that include committed precursors in the bone marrow, circulating blood monocytes, tissue macrophages, and DC in almost every organ in the body [2].

Brucella has a predilection for organs rich in reticuloendothelial cells (including spleen, liver, bone marrow, and lymph nodes) and is able to replicate successfully in any of them. Intracellular replication is directly linked to *Brucella* pathogenicity and it is not a coincidence that in humans, the most frequent clinical features of brucellosis are an enlarged liver in 65% of the cases, splenomegaly in 52% of the cases (from 40 cases), and lymphadenopathies in children [3][4]. Even in the chicken embryo model, replication of *B. abortus* detected within the rough ER of mesenchymal, mesothelial, and yolk endodermal cells, spreads to all tissues, with the liver and spleen being the most severely infected [5].

In tissues, the typical histopathological response to *Brucella* infection is a granulomatous inflammation, which contains representative members of the MPS, including macrophages with an epithelioid shape, i.e., with an increased amount of cytoplasm. Examination of biopsies from humans and livestock animals reveals granulomas in the liver, spleen, bone marrow, and other tissues [4][5][6][7]. As such, the initial replication niche of *Brucella* serves as a platform to establish a chronic infection. *Brucella* infected animals develop granulomatous inflammatory lesions in lymphoid tissues, including the supramammary lymph nodes, reproductive organs, notably the udder, and sometimes joints and synovial membranes. Those granulomas and their intratissular location are responsible for the chronicity of the disease, which can last for months or years [6][8] and in that respect, resemble the granulomas extensively studied in tuberculosis. In fact, in the absence of antibiotic treatment in the acute phase, *Brucella* is

able to persist for months without causing significant morbidity or mortality. In the acute phase of infection in a resistant mouse model, the C57BL/6 mice, the formation of granuloma (comprising NOSII+ monocyte-derived inflammatory DC, T cells, and granulocytes) is mediated by MyD88, IL-12, and IFNγ and essential for the control of the bacteria [6][8]. However, these granulomas were not detected in a susceptible murine model of infection, the BALB/c mice, at that stage [6][8]. In *B. melitensis* acutely infected livers, discrete pyogranulomatous inflammatory areas, characterized by a similar influx of neutrophils, macrophages, and monocyte-derived DC, were detected amongst normal hepatocytes in both mouse models [6][8]. At the chronic phase, infected livers displayed well established demarcated infiltration areas of macrophages, lymphocytes, and neutrophils [6]. In chronic granulomas, the presence of lymphocytes is thought to reflect the former activation of the immune system, whereas recruitment of neutrophils suggests that live *Brucella* is still present. The fact that the granuloma areas were typically found surrounding or associated with liver portal tracts and that neutrophils may function as vehicles for dispersion, according to the Trojan horse model [9], supports a dynamic role of granulomas in the development of *Brucella* chronicity. Remarkably, granulomas provide a rich nutrient source, as shown for the dormant non-replicative *Mycobacterium bacilli* that internalize inside the granuloma, lipids from foamy macrophage lipid droplets [10].

2. Genital-Reproductive Organs: Placenta and Epididymis

Brucella has a pronounced tropism for genital organs in its natural hosts, placenta in females, and epididymis in males. The placenta is one of the paradisiac organs in terms of replication, containing up to 10^{14} Brucellae in the cow [11][12]. This particular environment allows high replication rates, leading consequently to abortion, the most common clinical feature of brucellosis in livestock. As the main route of infection in these farm animals is aborted fetuses, this seems to be a very efficient strategy to spread *Brucella* progeny to new hosts.

Some common properties in these reproductive organs have shed light on *Brucella's* tropism. Firstly, high concentrations of erythritol are present in uterine, epididymal, and fetal tissues from ruminants [12][13][14][15]. Why is this important? Erythritol has been shown to be the preferred carbon/energy source for *Brucella* spp., promoting their massive growth [16]. In addition, the ruminant placenta produces progesterone, which further enhances in vitro *B. abortus* growth [17]. However, *B. abortus* vaccine strain S19 is not stimulated by erythritol [18][19], although it is capable of causing genital infection and abortion [20]. This suggests the existence of other trophic factors. Indeed, the dominance of fructose over glucose takes place in the placenta of cows, sows, ewes, and to a lesser extent in that of other animals [16][21][22]. The same preference applies to the epididymis, seminal fluids, and oviducts of several mammals [16]. As such, both organs play a trophic role and provide effective sources of carbon, nitrogen, and energy for *Brucella* spp. [16][23].

Secondly, the immune-privileged status of the testis and semen, and local immunosuppression at the feto-maternal interface in the placenta might also account for *Brucella* tropism [16].

Thirdly, *Brucella* preferentially replicates within trophoblasts, highly metabolically active cells that adjust their production of proteins and steroids throughout gestation. Intracellular *Brucella* likely induces the synthesis of

steroids and modifies the metabolism of prostaglandin precursors, such as arachidonic acid, which together with the COX-2 enzyme are essential for *Brucella* lymph node persistence and subversion of the immune response [24].

Finally, the high hydrophobicity of the outer-membrane of *Brucella* together with its propensity to replicate within the ER [25][26], may represent an evolutionary adaptation for using hydrophobic substances available within this sub-cellular compartment in trophoblasts [23].

In humans, the genital tropism holds true as *Brucella* induces epididymorchitis [27] and may infect the placenta, even if abortion is very uncommon [28][29].

Therefore, both the localization and abundant multiplication in the reproductive tract of animals is crucial in the biology of this pathogen.

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