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REVIEW ARTICLE
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Overview of Johne's disease immunology

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Abstract

Johne's disease or paratuberculosis is one of the most economically important diseases of the livestock. Most of the economic losses associated with paratuberculosis are related to decreased milk production, reduced fertility and higher rates of culling. Understanding the immunology of the disease is very important for better understanding of the interplay between the host and the causative agent, *Mycobacterium avium* subsp. *paratuberculosis (*MAP). After uptake of MAP by macrophages residing in host's intestinal tissue, two possible scenarios may emerge; MAP may be destroyed or may establish persistent infection within the macrophages. If MAP persists in the infected macrophage, it continuously modulates adaptive immune responses of the animal. In this short review we describe the host-pathogen interactions in Johne's disease and highlights potential protective mechanisms in order for future design of more effective diagnostic method and vaccine.

Keywords: immunology, Johne's disease, paratuberculosis.

Introduction

Johne's disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) [1]. The disease is responsible for economic losses of approximately US$ 250 million to the US dairy industry [2-3]. JD is a chronic disease of mainly ruminants leading to granulomatous enteritis, persistent diarrhea, progressive wasting and finally death [4-5]. The disease is distributed globally among domestic ruminants (e.g. cattle, sheep and goats) and wildlife species, such as white-tailed deer and bison [6-7]. Understanding the pathogenesis and immunology of infectious diseases helps policy makers define control strategies. These control plans become more important in developing nations where control measures are not properly designed and implemented [8-10]. The objective of this review is to provide an understanding of the pattern of the immune response of JD.

Modes of transmission

Major mode of MAP transmission in dairy cattle is ingestion of feces, milk and colostrum contaminated with MAP. In-utero transmission of MAP has also been reported [11]. Most of the previously published studies have reported that young animals (calves) are the most susceptible for infection but recently it has been shown that MAP can also infect adult animals [12]. Infectivity depends on many factors including dose of MAP ingested, host susceptibility and environmental factors [13]. The reason for age -dependent susceptibility is not well understood but it is mostly attributed to undeveloped immune system of young animals and higher level of exposure of MAP in these animals [14-15].

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Stages of JD

JD can be divided into mainly 3 stage – early infection, subclinical infection and clinical infection. Here, we will discuss host-pathogen interactions in each stage of JD, especially focusing on immunological responses.

Early infection: The early stage of MAP infection is initiated by uptake of MAP by intestinal cells. Until recently, it was thought that MAP enters the sub-epithelial tissue only through M-cells; however, Momotani et al [16] reported that other enterocytes also play role in the MAP entry. M-cells were discovered to be the portal of entry by studying increase in the beta integrin molecules and other pattern recognition receptors molecules after infection with MAP. Also, since M-cells lack digestive enzymes, microvilli, and mucous, it provides an easier path for MAP to invade the intestinal tissue [17]. M-cells then transfer MAP into sub-epithelial tissue where the bacteria will be phagocytized by macrophages and dendritic cells. There are 2 types of infected macrophages-persistently infected (PM) and activated macrophages (AM). MAP inside the PM survives by inhibiting the fusion of phagosome-lysosome and also by increased levels of mitogen activated protein kinase (MAPK) [15]. MAPK helps bacterial survival by decreasing expression of interleukin (IL)-10 (anti-inflammatory cytokine) and preventing fusion of phagosome-lysosome. Also, increased level of Toll-like receptors (TLR's)-TLR2 and TLR 4 occurs in cells infected with MAP. The AM cells produce pro-inflammatory cytokines (e.g. IL-1,

TNF-α, and IL-12) which in turn increases IL-6, IL-8 and IL-10. Increase in IL-1 causes activation of IL-2 producing T cells and leads to clonal expansion of CD4+ T helper cells and CD8+T cytolytic cells. CD4+T cells can differentiate into either Th1 or Th2 cells. Th1 response causes release of IFN-γ, IL-2 and TNF-α. In calves (ruminants), γ δ T cells are the predominant T cells before 6 months of age. Under the influence of IFN-γ, these cells release IFN-γ and TNF-α. However, the release of IFN-γ from both γ δ T cells and CD4+T cells is not sufficient for macrophages to kill intracellular MAP. Destruction of intracellular pathogens primarily relies on cytotoxic lymphocytes like natural killer (NK) cells and CD8+ cells. A CD8+T cell response has also been noticed in spite of its requirement of MHC-I presentation which has not been well understood in bacterial infections [18]. Also, γ δ cytotoxic T cells have also been shown to be activated at an early stage of infection. The reason for this activation is not well understood and needs further investigation. In the early stage of MAP infection, a role for B cells has also been suggested. Being in close proximity of macrophages and T-cells, B cells can act as antigen presenting cells for CD4+T cells and further increase the production of IFN-γ. IFN- γ is known to cause an antibody class switch from IgM to IgG_1. If all these cells and cytokines fail to remove PM the animal progresses to subclinical infection [15].

Sub-clinical infection: In the sub-clinical infection, infected animals present no clinical sign, very low or infrequent shedding of MAP in feces, and low level of antibody titers. When examined microscopically, the animals in this stage show formation of small granulomas. These granulomas have macrophages and giant cells with MAP residing inside the cells. These are mostly the PM cells discussed above. Very interestingly, the CD8+T cell and CD4+T cells are surrounding these granulomas, which are formed by the TNF – α produced by macrophages, CD4+T cells and γ δ T cells. In this stage, the most important cytokine is TNF-α, which is secreted by γ δ T cells and CD4+T cells. TNF-α plays a role in recruitment of macrophages to help control the infection but also causes tissue damage. A proportion of PM secretes IL-10, which is classified as a Th2 cytokine along with IL-4 and IL-5, reduces production of IFN- γ through suppression of CD4+T cells. Due to the reduced level of IFN- γ, the newly recruited macrophages become more prone to persistent infection. The cytotoxic T cell population – CD8+ and γ δ T cells - play different roles in this stage. The γ δ T cells suppress proliferation response of CD4 + T cells to MAP antigen while CD8+ T cells prevent this action. There is likelihood that γ δ T cells causes formation of granulomas and limit further inflammatory responses. There is a limited amount of humoral immune response in this stage of infection and is due to infrequent shedding of MAP. The sub type of immunoglobulin is still IgG_1 since the switch from the subtype to IgG_2 cannot occur due to a limited production of IFN-γ [18].

Clinical infection: This stage is characterized clinically by reduced weight loss and intermittent diarrhea. Histologically, there is extensive damage of the intestine and presence of a large number of granulomas. There is also a high level of shedding of MAP in feces, milk and colostrum. This phase is often accompanied by a strong Th2 response with release of IL-4 and IL-10 [19-20]. Also, the occurrence of Treg cells (CD4+CD25+) causes secretion of IL-10 and TGF β which further suppresses CD4+T cell population. Due to the suppression of Th2 cells, there is a reduction in B-cell response. However, the overall B cell response is still measurable and thus the IgG1 response can be found in this stage. Finally, in the last stage of the clinical disease, infected animals shows profuse diarrhea, weakness, emaciation, intermandibular edema and finally death of the animal [21].

Diagnostics based on humoral immune response

Three different tests are used to measure antibody response in JD: complement fixation, agar gel immunodiffusion, and ELISA. The complement fixation and agar gel immunodiffusion tests both suffer poor sensitivity, and so a recent report has suggested that ELISAs are the best of the three methods for controlling JD in dairy and beef herds [22]. Diagnoses of JD using ELISA have been reported in many previous studies using different antigens. The antigens used in these studies have used protoplasmic antigen (PPA) [23], lipoarabinomannan (LAM) [24], culture filtrate of MAP [25], and MAP proteins-1152 and 1156 [26] for testing antibodies against MAP. Bannantine et al. [27] tested 18 purified recombinant proteins in ELISA format for serodiagnosis of ovine paratuberculosis. They found that MAP proteins 0862 and 3786 demonstrated the strongest antibody response and MAP protein 2116c the weakest [27]. The recommended control measure for JD is testing herds by ELISA methods but the current ELISA tests have low sensitivity (28–44.5%) [28]. Our group have previously reported that the surface antigens of MAP are capable of detecting anti-MAP antibodies in serum at early stages of JD [29-33]. There has always been a need to understand the immunology of infectious diseases to implement it for the development of vaccines and diagnostics.

Conclusion

Understanding the pathogenesis becomes more important for JD since there is no efficient vaccine available and the treatment is not practically possible. Thus, better understanding of the pathogenesis for development of more effective vaccine and diagnostic tests will be the keys to control the disease, The present pathogenesis describes a higher Th1 response initially and a shift to Th2 response later in the course of the disease. It has been evident that for an early infection is cell mediated dominated with a later switch to a Th2 type response. This phenomenon has been referred to as Th1-Th2 dominancy shift. Therefore, Th1-based test (e.g. IFN-γ test) can be used for detection of initial

infection and Th2-based testing (antibody detection) has been used for later infection. Our group has shown that anti-MAP antibodies could be detected in experimentally infected calves as early as 150 days post infection. However, the pattern of Th1-Th2 dynamics varies in naturally infected animals. So, a better understanding of immune responses in JD and utilization of the new knowledge for development of diagnostic tests may eventually help control of JD. From the above discussion, it is clear that the misdirected immune response due to host modulation by MAP leads to establishment of this debilitating disease. Inhibition of phagosomal maturation, reduced apoptosis of the infected cells, reduced MHC II expression. Th1-Th2 shift and induction of CD4+ T cell activity are the influential events that leads to persistent MAP infection. Although, we have an initial understanding of the immunology of JD but certain factors like MAP strain virulence and host genetic susceptibility needs to be studied in detail in future.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of their institutions.

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