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Protective adaptive immunity to *Chlamydophila abortus* infection and control of ovine enzootic abortion (OEA)

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ABSTRACT

Ovine enzootic abortion (OEA) remains a major problem in sheep-rearing countries despite the availability of protective vaccines. The causative agent, *Chlamydophila abortus*, is a Gram-negative bacterium that can induce a persistent, subclinical infection in non-pregnant sheep. The development of a new safe, effective and practical vaccine requires a detailed understanding of host–pathogen interactions and the identification of clear correlates of protection. Since disease (abortion) is only observed during pregnancy, the nature of host immunity to *C. abortus* and the specialised immunological features that permit maternal acceptance of the semi-allogeneic fetus are central to the pathogenesis of OEA. We review the current literature on persistence of *C. abortus*, host immunity to infection and mechanisms of abortion. We identify the key outstanding questions surrounding OEA and discuss the current knowledge gaps with a view to developing improved control strategies.

1. Introduction

Ovine enzootic abortion (OEA) is caused by the Gram-negative bacterium *Chlamydophila abortus* (Aitken and Longbottom, 2007). Despite the existence of commercially-available protective vaccines, OEA is a major problem in sheep-rearing countries and remains the single most common diagnosed cause of infectious abortion in the UK (Longbottom and Livingstone, 2006). There is a clear need for improved control strategies for OEA that include more effective diagnosis, prophylaxis and farm management systems. The development and implementation of such strategies depends on a greater understanding of the biology of *C. abortus* and its transmission, host immunity and how the immunological and physiological changes associated with pregnancy relate to disease pathogenesis. Here, we review the current knowledge of protective host immune responses to *C. abortus* infection in sheep and discuss how this can be applied to future vaccine design.

2. Pathogenesis of OEA

Organisms belonging to the genera *Chlamydia/Chlamydophila* have the potential to establish persistent infections in a wide range of natural hosts, often without overt signs of disease. Sheep infected with *C. abortus* usually have no clinical signs of the infection outwith pregnancy, during the early- to mid-stages of pregnancy, but then abort most commonly in the last 2–3 weeks of gestation (Longbottom and Coulter, 2003). In addition to abortion, ewes may exhibit a vaginal discharge around the time of abortion, but otherwise appear healthy. For these reasons, *C. abortus* infection often goes unnoticed or undetected in sheep flocks until it is too late to implement preventative measures during the lambing period (Aitken and Longbottom, 2007). Following abortion, characteristic signs of OEA are thickened, necrotic placental membranes and

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placental exudate. Aborted fetuses are usually well-developed and do not have gross anatomical abnormalities, suggesting that placentitis is a key element in the pathogenesis of OEA (Buxton et al., 2002).

2.1. Questions surrounding OEA

Primary infection with *C. abortus* is thought to occur mucosally, most likely via the oro-nasal route. The source of infection for naïve ewes is contaminated birth material (placental or fetal) or exudate from infected ewes that shed *C. abortus* at lambing time. These newly-infected ewes are those that are likely to abort in the next season (Entrican et al., 2001). This transmission and disease profile raises some key outstanding questions regarding OEA. Firstly, how do sheep respond immunologically to a primary mucosal infection that leads to sub-clinical persistence of *C. abortus*? Understanding the nature of that immune response is important since it is protective for the ewe so long as she is not pregnant, but is not protective in terms of OEA. Secondly, where is the anatomical site of latency/persistence of *C. abortus*? *C. abortus* is an obligate intracellular bacterium and neither the cell type nor tissue in which the organism resides during a persistent phase in non-pregnant sheep has been identified. Thirdly, what are the factors that dictate invasion of the placenta from the site of persistence? Underlying this question is one of pathogen latency versus persistence and how those terms are interpreted. For example, latency could be regarded as a static phase of the pathogen that is asymptomatic for the host whereas persistence could be either a static or productive phase of the pathogen that may or may not be symptomatic. In this review we will use the term 'persistence' rather than 'latency'. It is not known if *C. abortus* recrudesces from a persistent phase in the ewe at a particular stage during pregnancy, producing infectious, extracellular *C. abortus* elementary bodies (EBs) that invade the placenta, or if EBs are constantly being released at low levels during persistence and opportunistically enter the placenta from maternal blood at a specific time during pregnancy. If the former is true, it suggests a role for alterations in maternal immunity during pregnancy, if the latter is true it suggests that physiological development of the placenta will be a defining factor. This leads on the fourth question: why is the placenta a target for *C. abortus* and why does it grow so well in placental trophoblast? The organism proliferates in the placenta and ultimately causes disease indicating that the immune response mounted by the ewe following primary infection is not adequate to control placental infection after invasion has occurred. Finally, what constitutes a protective, adaptive immune response to *C. abortus*? Ewes that have aborted as a result of OEA develop strong protective immunity and successfully reproduce in subsequent seasons. For future sub-unit vaccines to be effective, they need to mimic this protective state, which in turn demands a clear understanding of the immune mechanisms involved.

There is no detailed literature on the adaptive immune response in sheep following primary mucosal infection with *C. abortus*, nor has the site of persistence been identified, so the first two questions cannot be answered. However, there are data on host immunity to *C. abortus* in sheep and on the specialised immunological features of mammalian pregnancy that can help to address the remaining three questions. These will be the focus of this review.

2.2. Mechanisms of persistence of C. abortus in sheep

A role for the ovine host immune response in establishing and maintaining persistence has been identified. This involves cytokine production and subsequent induction of intracellular biochemical pathways that restrict chlamydial multiplication. Experimental infection of ovine cells with *C. abortus* EBs results in growth of the organism and subsequent cell death. However, if the cells are treated with recombinant ovine interferon-gamma (IFN-γ) at the time of infection, growth of the organism is restricted in a dose-dependent manner (Entrican et al., 1998). It was found during extended studies that infected cells could be maintained in IFN-γ for many weeks and that infectious EBs would cease to be detectable in the cell-free culture supernates. However, subsequent removal of IFN-γ from these cultures resulted in the re-appearance of EBs (Brown et al., 2001). The concentrations of IFN-γ used in those experiments were physiologically-relevant, as demonstrated by the concentrations of IFN-γ found in lymph collected *ex vivo* from an immune sheep challenged with *C. abortus* (Graham et al., 1995). It was established that the anti-chlamydial effects of IFN-γ were distinct from its antiviral effects, since treatment of infected cells with type-I IFN matching the antiviral activity of the IFN-γ, did not restrict *C. abortus* growth (Entrican et al., 1998). Many experiments have demonstrated that the anti-chlamydial effects of recombinant ovine IFN-γ are directly associated with induction of the enzyme indoleamine 2,3-dioxygenase (IDO) and tryptophan deprivation (Brown et al., 2001; Entrican et al., 2002). Confirmation of the dependency of *C. abortus* for host tryptophan came with the sequencing of the *C. abortus* genome, conclusively demonstrating that the organism is auxotrophic for tryptophan since it lacks a tryptophan synthase operon (Thomson et al., 2005). Collectively, these data indicate that IFN-γ controls *C. abortus* growth in a reversible, dose-dependant manner and allows it to become persistent in ovine cells. Any alterations in the balance between IFN-γ production, IDO expression and tryptophan availability could therefore alter the dynamics of a persistent *C. abortus* infection in ovine cells. If such changes occur during pregnancy they could explain the disease pattern of OEA. It is necessary to identify the site of persistence in sheep to adequately address that question.

2.3. Maternal immunity during pregnancy

It was recognised over 50 years ago that pregnancy in eutherian (placental) mammals presents a theoretical challenge to the self-nonself model of immune activation (Medawar, 1953). The self-nonself model predicts that the maternal immune system should reject a fetus that has inherited half of its genes from its father, a prediction that does not hold up. Sir Peter Medawar proposed a several