Virus Research 182 (2014) 71–77

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

The immune response of ruminant livestock to bluetongue virus: From type I interferon to antibody

N. James Maclachlan[a,*], Christine Henderson[b], Isabelle Schwartz-Cornil[c], Stephan Zientara[c]

[a] Department of Pathology Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
[b] Merial Inc., Athens, GA 30601, USA
[c] ANSES Alfort, UMR 1161 ANSES/INRA/ENVA-UPEC, 23 Av du Général de Gaulle, BP 63, 94703 Maisons-Alfort Cédex, France

ARTICLE INFO

Article history:
Available online 10 October 2013

Keywords:
Bluetongue virus
Immune response
Innate
Adaptive

ABSTRACT

Infection of ruminants with most (but not all) serotypes of bluetongue virus (BTV) leads to a highly blood cell-associated viremia that may be prolonged but not persistent. Furthermore, recovered animals are resistant to reinfection with the homologous virus serotype, which is the basis for vaccination strategies to prevent BTV infection and the clinical disease (bluetongue) that it causes in domestic livestock. BTV infection is initiated at the site of virus inoculation and the associated draining lymph node, from where the virus is then spread in lymph cells to the systemic circulation and secondary sites of replication. Replication of BTV in target cells, notably mononuclear phagocytic cells (dendritic cells and macrophages) and endothelium, leads to the generation of the innate and adaptive immune responses that mediate both initial virus clearance and subsequent resistance to infection with the homologous virus serotype. The goal of this review is to summarize current understanding of these innate and adaptive immune responses of animals to BTV infection.

1. Introduction

Bluetongue (BT) is an arboviral disease of domestic and wild ruminants characterized by vascular injury that results in tissue necrosis, hemorrhage, and edema (Hutcheon, 1902; Maclachlan et al., 2009; Spreull, 1905; Verwoerd and Erasmus, 2004). The causative agent of BT is bluetongue virus (BTV), the prototype member of the genus *Orbivirus*, family *Reoviridae* (Borden et al., 1971; Matsuo and Roy, 2013; Murphy et al., 1971; Schwartz-Cornil et al., 2008). The BTV virion consists of a diffuse outer protein coat and an inner icosahedral core that encloses the transcriptase complex (Stewart et al., 2012; Verwoerd et al., 1972). The genome includes 10 distinct genome segments of double-stranded RNA (dsRNA). Each gene segment encodes at least 1 protein, specifically 7 structural (VP1-7) and 5 nonstructural (NS1-3/3A, 4) proteins (Grubman et al., 1983; Mertens et al., 1984; Ratinier et al., 2011). Some 26 BTV serotypes are now recognized globally (Hofmann et al., 2008; Maan et al., 2012).

BTV infection occurs throughout temperate and tropical regions of the world coincident with the distribution of specific species of vector *Culicoides* biting midges, however the global distribution of BTV has recently altered markedly, particularly in the northern hemisphere (Gibbs and Greiner, 1994; Maclachlan, 2011; Maclachlan and Mayo, 2013; Saegerman et al., 2008; Tabachnick, 2010). Thus, BT is viewed currently as a globally emerging disease (Gibbs et al., 2008; Guis et al., 2012; Jiminez-Clavero, 2012; Maclachlan and Guthrie, 2010; Purse et al., 2008; Weaver and Reisen, 2010). Outbreaks of BT can be economically devastating to livestock production and the presence of BTV in a country can adversely impact the trade and movement of livestock (Maclachlan and Osburn, 2006; Velthuis et al., 2010). Control of BTV infection and BT disease can be difficult, and prevention of the disease is typically reliant on vaccination of susceptible livestock (reviewed: Maclachlan and Mayo, 2013). Sound vaccination strategies should logically be based on a thorough understanding of the ruminant immune response to BTV, which is the focus of this review.

2. Innate immunity

The innate immune response constitutes the first line of defense of animals against viral infections. Innate immune mechanisms are also critical to transition to an effective adaptive immune response (humoral and cellular). Natural BTV infection of ruminants typically occurs following the bite of any one of the multiple species of hematophagous *Culicoides* midges that serve as biological vectors of the virus (Barratt-Boyes and Maclachlan, 1995; Gibbs and

* Corresponding author. Tel.: +1 530 754 8125.
E-mail address: njmaclachlan@ucdavis.edu (N.J. Maclachlan).

0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.09.040

Greiner, 1994; Mellor et al., 2000; Tabachnick, 2010; Verwoerd and Erasmus, 2004). After cutaneous instillation of BTV following the bite of an infected midge, the virus is quickly translocated in dendritic cells to the regional lymph node where initial replication occurs (Barratt-Boyes and Maclachlan, 1994; Ellis et al., 1991; Hemati et al., 2009; Pini, 1976). Conventional dendritic cells are recruited in large numbers to the skin following virus infection, facilitating further replication of the virus at the site of initial infection in the skin (Hemati et al., 2009). Interruption of lymphatic flow to the regional lymph node adjacent to the site of virus inoculation markedly delays the onset of viremia (Barratt-Boyes and Maclachlan, 1994).

Initial replication of BTV in the regional lymph node likely occurs in dendritic cells, macrophages, endothelium and possibly lymphocytes, as these are major cellular targets of BTV (Maclachlan et al., 2009; Darpel et al., 2012). *In vitro* BTV infection of ovine conventional dendritic cells from skin lymph leads to the production of proinflammatory and immune cytokines (interleukins [IL-1β, IL-6, IL-12]) as well as to expression of co-stimulatory molecules that promote the proliferation of BTV-specific CD4+ and CD8+ T cells (Hemati et al., 2009). Similarly, *in vitro* BTV infection of bovine monocyte - derived macrophages leads to their activation with production of tissue necrosis factor (TNF-α), interleukins (*e.g.* IL-1β, IL-8) and inducible nitric oxide synthetase (Drew et al., 2010a). Replication of BTV in the regional lymph node stimulates a marked cellular response characterized by increased numbers of B cells within the node itself and the appearance of virus-specific antibodies and activated CD8+ T cells in the draining efferent lymph (Barratt-Boyes et al., 1995).

Following initial replication of BTV at the site of inoculation and within the adjacent draining lymph node, the virus is then disseminated to a variety of tissues throughout the body where secondary replication occurs in mononuclear phagocytic cells (macrophages and dendritic cells), endothelial cells, lymphocytes including γδ T cells, and perhaps other cell types (Barratt-Boyes et al., 1992; Barratt-Boyes and Maclachlan, 1994, 1995; Darpel et al., 2012; DeMaula et al., 2001, 2002; Ellis et al., 1991; Hemati et al., 2009; Lee et al., 2011; Maclachlan et al., 1990, 2009; Mahrt and Osburn, 1986; Umeshappa et al., 2012; Whetter et al., 1989). Viral RNA (especially dsRNA) is detected by pattern recognition receptors (PRR) in the cytoplasmic and endosomal compartments of host cells, notably toll-like receptors (TLRs) or members of the retinoic acid inducible gene (RIG-1)-like family of cytoplasmic receptors (Levy et al., 2011). Different PRR utilize distinct signaling pathways to trigger the production of Type I and Type III IFNs and other proinflammatory cytokines.

It has long been recognized that BTV infection is a strong inducer of Type I IFN, both *in vivo* and *in vitro* (Foster et al., 1991; Fulton and Pearson, 1982; Huismans, 1969; MacLachlan et al., 1984; Maclachlan and Thompson, 1985; Russell et al., 1996). Whereas adult mice are resistant to productive BTV infection, Type I (α/β) IFN receptor knockout mice (IFNAR [−/−]) are highly susceptible to lethal infection with the virus (Calvo-Pinilla et al., 2009a, 2010; Franceschi et al., 2011). It recently has been shown that BTV induces Type I IFN production in plasmacytoid dendritic cells *via* a MYD88-dependent TLR7/8-independent sensing and signaling pathway, whereas cytoplasmic helicases (RIG-1, MDA5) mediate sensing and signaling in BTV-infected epithelial cells (Chauveau et al., 2012; Ruscanu et al., 2012). The BTV NS3 protein specifically interferes with IFN production in epithelial cells by inhibiting the RIG1-like receptor mediated signaling pathway (Chauveau et al., 2013). Plasmatocytoid dendritic cells and Type 1 IFN may exert a central role in triggering B cell responses and promoting BTV-specific antibody synthesis (humoral immunity), as has recently been shown for rotavirus, which is another dsRNA virus of the family *Reoviridae* (Deal et al., 2013).

Infection of dendritic cells, macrophages, endothelial cells and others during BTV infection of ruminants leads to the production of a variety of proinflammatory and vasoactive mediators including Type I IFNs, TNF-α, and various interleukins and prostanoids, but the response can vary depending on the cell types that are infected as well as their tissue location (Channappanavar et al., 2012; DeMaula et al., 2001, 2002; Drew et al., 2010a; Hemati et al., 2009; Ruscanu et al., 2012, 2013; Umeshappa et al., 2012). Furthermore, although these virus-induced cytokine and chemokine mediators may serve to limit/control the infection during its initial stages and to promote the development of a strong acquired immune response, they may also contribute to the pathogenesis of the capillary leakage syndrome and coagulopathy that characterizes clinical BT in ruminants-the so-called “cytokine storm” of hemorrhagic fevers (Fig. 1) (Drew et al., 2010b; Maclachlan et al., 2008, 2009; Umeshappa et al., 2012). Thus, the virulence to sheep of different strains/serotypes of BTV is correlated with the severity of the vascular lesions they induce and to serum concentrations of acute phase proteins, and not to viral loads (Sanchez-Cordon et al., 2013). Similarly, DeMaula et al. (2002) showed that vascular injury and enhanced coagulation, as assessed by the ratio of plasma thromboxane to prostacyclin, were significantly greater in sheep than cattle inoculated with the same strain of BTV, consistent with the sensitivity of sheep and relative resistance of cattle to expression of clinical BT.

3. Cellular immunity

Cell-mediated immunity (CMI) typically limits viral spread during the initial stages of acute viral infections by the destruction of virus-infected cells (virus factories). Although BTV infection of ruminants clearly results in changes in lymphocyte populations and dynamics both locally (adjacent to the site of infection) and systemically (Barratt-Boyes et al., 1995; Ellis et al., 1990, 1991; Hemati et al., 2009; Umeshappa et al., 2012), the CMI response of ruminants to BTV infection remains poorly characterized. CD8+ cytototoxic T lymphocytes (CTLs) have been identified following BTV infection of both mice and sheep, although their activity is apparently transient and their precise role in mediating virus clearance is poorly defined (Andrew et al., 1995; Calvo-Pinilla et al., 2012; Jeggo and Wardley, 1982a,b; Jeggo et al., 1984, 1986). Adoptive transfer of CTLs harvested from the thoracic duct of a sheep previously infected with BTV partially protected recipient sheep against challenge infection with either homologous or heterologous serotypes of BTV (Jeggo et al., 1984, 1986).

The BTV NS1 and VP2 proteins have been proposed to be major targets of the ovine CMI response to BTV, although the viral protein-specific CTL response of individual sheep can vary markedly (Andrew et al., 1995; Janardhana et al., 1999; Takamatsu and Jeggo, 1989). Ovine CTLs specific for the non-structural NS1 protein are cross reactive amongst different BTV serotypes. Similarly, Calvo-Pinilla et al. (2012) described generation of NS1-specific CD8+ T cells and heterotypic immunity in IFNAR mice that were immunized with recombinant BTV proteins, and Jones et al. (1996, 1997) earlier showed that the BTV nonstructural proteins, especially NS2, can induce cross-reactive CTLs in mice. Together these findings offer some promise that a polyvalent vaccine strategy to prevent BTV infection is potentially feasible, as the NS1 and NS2 proteins are relatively conserved among field strains of BTV regardless of serotype. However, neither homologous nor heterologous protection was obtained with a recombinant adenovirus expressing only the NS1 protein of BTV-2 despite strong induction of protein-specific CD8+ T cells in the vaccinated sheep (unpublished data). Similarly, although BTV core-like particles that include only VP3 and VP7 have been reported to induce partial protection against