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Ability to differentiate between cp and ncp BVDV by microarrays: Towards an application in clinical veterinary medicine?

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Abstract

Microarray expression profiling provides a comprehensive portrait of the transcriptional world enabling us to view the organism as a 'system' that is more than the sum of its parts. The vigilance of cells to environmental change, the alacrity of the transcriptional response, the short half-life of cellular mRNA and the genome-scale nature of the investigation collectively explain the power of this method. These same features pose the most significant experimental design and execution issues which, unless surmounted, predictably generate a distorted image of the transcriptome. Conversely, the expression profile of a properly conceived and conducted microarray experiment can be used for hypothesis testing: disclosure of the metabolic and biosynthetic pathways that underlie adaptation of the organism to infectious processes; the identification of co-ordinately regulated genes; the regulatory circuits and signal transduction systems that mediate the adaptive response; and temporal features of developmental programmes. The study of viral pathogenesis by microarray expression profiling poses special challenges and opportunities. Although the technical hurdles are many, obtaining expression profiles of an organism growing in tissue will probably reveal strategies for growth and survival of the virus in the host's cells. Here, we show data obtained using a tailored microarray system based on synthetic polynucleotides derived from human sequences (SIRS-Lab GmbH, Jena, Germany) to study the effect of cytopathogenic (cpe) and non-cytopathogenic (ncp) bovine viral diarrhoea virus (BVDV) infection of bovine macrophages, focusing on intracellular signalling molecules. Of the 575 genes present on the array, more than 70% showed a reaction with the oligonuleotides spotted on the array, and 26 genes were differentially expressed comparing cDNA derived from cpe and ncp infected cells. These data will help to further understand our knowledge regarding BVDV infection, and will especially help to understand differences in cellular responses to cpe and ncp biotypes.

Keywords: Microarray; Innate immunity; TLR; BVDV; Bovine

1. Introduction

The pestiviruses – bovine viral diarrhoea virus (BVDV), classical swine fever virus and border disease virus of sheep, together with the flaviviruses

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and hepatitis C virus – are a closely related group of small enveloped viruses, the *Flaviviridae*, with a single-stranded, positive-sense RNA genome of approximately 12.5 kb. The RNA is translated into a single virus polyprotein that is processed by both viral and host proteases to either 11 or 12 virus polypeptides dependent on the virus biotype. The viruses are generally non-cytopathogenic (ncp), although BVDV associated with the development of mucosal disease in persistently infected (PI) animals is a cytopathogenic (cp) biotype, which produces an additional virus polypeptide, NS3, which represents the C-terminal two-thirds of NS2-3 present in ncp BVDV strains (Donis and Dubovi, 1987).

Several groups have recently attributed the persistent infection to the ability of ncp BVDV to interfere with the induction of interferon (IFN) type I production in macrophages (MΦ) (Baigent et al., 2002, 2004; Charleston et al., 2001; Schweizer and Peterhans, 2001). The lack of IFN induction observed with ncp BVDVs and the failure to induce apoptosis might have advantages for the survival of the virus (Schweizer and Peterhans, 1999, 2001). The induction of apoptosis by cp BVDV appears to be an active process of induction since cells infected with ncp BVDV and subsequently infected with cp BVDV still undergo apoptosis (Zhang et al., 1996); these results indicate that ncp BVDV does not inhibit apoptosis. The mechanism of induction of apoptosis by cp BVDV is controversial, but it has been suggested that the accumulation of viral RNA in cells infected by cp BVDV could activate the double-stranded RNA-activated protein kinase (PKR) and initiate apoptosis (Vassilev and Donis, 2000). However, more recent studies on the cp strain NADL have demonstrated that apoptosis induced by cp BVDV can be mediated through endoplasmic reticulum (ER) stress, but the initiating trigger of the apoptotic pathway is not clear (Jordan et al., 2002).

The observation that ncp BVDV specifically interferes with the induction of IFN type I by inhibiting the transcription factors IFN regulatory transcription factor (IRF) 3 and 7 may be relevant in view of the role an innate immune system plays in the establishment of the adaptive immune response, and understanding these differences may help to identify genes specifically activated by ncp or cp BVDV, which can be used as diagnostic markers. Both IRF3 and IRF7 have been shown to be involved in the signalling cascade activated by a specific group of pattern recognition receptors, the Toll-like receptors (TLR). Whereas most TLR seem to be expressed on the cellular surface, TLRs involved in the generation of the innate immune response to viruses, such as TLR3, TLR7 and TLR8 are present within vesicles in the cytoplasm or even free in the cytoplasm. Activation of these TLRs by dsRNA, which is unavoidably formed during the replication of DNA and RNA viruses, is a key trigger of IFN production in virtually all cells and leads to transcriptional activation of IFNβ gene(s) via NF-κB and other mechanisms (Fig. 1).

Dissection of the activation of the TLR/IFN type I pathway becomes increasingly complex with more and more signalling molecules being described to be involved. Despite the fact that intracellular molecules share a high cross-species homology, current reagents to receptors and adaptor molecules do not seem to cross-react convincingly (Werling and Jungi, unpublished observation). However, these investigations may be helped by the development of bovine-specific microarrays, which are currently already in use (Aho et al., 2003; Coussens et al., 2002, 2003; Yao et al., 2001). Despite the undoubted beneficial use of such large arrays for research, their analysis requires extensive bioinformatics input and is often very time-consuming. We therefore investigated the applicability of utilising a commercially available "tailored" microarray, containing 547 probes for human genes involved in TLR signalling, type I IFN production and NF-κB signalling to dissect differences in cellular activation induced by cells exposed to either cp or ncp BVDV. Identifying genes uniquely responding to either virus exposure could lead to the development of new diagnostic tools.

2. Materials and methods

2.1. Animals and cell culture

Blood was collected from Holstein Friesian cattle. For generation of monocyte-derived macrophages (MΦ), PBMC isolated by an adapted Ficoll-metrizomate procedure were sealed in Teflon bags (10–20 ml, 4×10^6 PBMC ml^{-1}) as described previously (Werling et al., 2004), and cultured for 6–8 days at 37 °C in