Short Communication

CD209/DC-SIGN mediates efficient infection of monocyte-derived dendritic cells by clinical adenovirus 2C isolates in the presence of bovine lactoferrin

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Adenoviruses are common viral pathogens, particularly following allogeneic haematopoetic stem-cell transfer, with potentially fatal consequences (Feuchtinger et al., 2007). There is currently no universally accepted therapy. Adenovirus species C serotype 2 (2C) often causes respiratory infection, but the relevant attachment receptors on cells in the lung have largely not been defined.

Cells that mediate innate immune responses to adenoviruses and adenoviral vectors include alveolar epithelial cells, macrophages, plasmacytoid and conventional dendritic cells (DC) (Muruve, 2004; Wu et al., 2010; Zhu et al., 2007). While great progress has been made in recent years in understanding the innate immune response to adenovirus and adenoviral vectors, particularly through intracellular inflammasomes and retinoic acid-inducible gene I-like receptors (Chiu et al., 2009; Di Paolo et al., 2009; Muruve et al., 2008; Zaiss et al., 2009), little is known about attachment receptors on myeloid cells. A variety of mechanisms and receptors may be used for adenoviral infection (Arnberg, 2009). The majority of adenoviruses initially engage an attachment receptor, following which RGD motifs within the penton base contact integrins that mediate viral internalization. The commonly used attachment receptor CAR (coxackie virus and adenovirus receptor) is poorly accessible in the lung, and has been suggested to be not relevant for infection in vivo (Arnberg, 2009). CD46 is a second well characterized receptor that mediates efficient infection of many cell types including myeloid cells, but only functions for particular species B adenoviruses (Gaggar et al., 2003). Bridging molecules such as dipalmitoyl phosphatidylincholine, a major component of pulmonary surfactant, or lactoferrin, an antiviral protein abundantly expressed in the lung, mediate infection by species C adenoviruses (Balakireva et al., 2003; Johansson et al., 2007; Adams et al., 2009). Although the identity of dipalmitoyl phosphatidylincholine and human lactoferrin receptors on alveolar epithelial cells and monocyte-derived dendritic cells (MDDC) remain unknown, the pattern recognition receptor CD209/DC-SIGN (dendritic cell-specific ICAM-3 grabbing nonintegrin) on MDDC has been shown to function as a receptor for bovine lactoferrin-treated adenovirus 5 (Adams et al., 2009).

We have previously shown that a clinical adenovirus 2C isolate (BB2000-61) infects MDDC efficiently (Kessler et al., 2010) and aimed to identify the relevant attachment receptors on these cells. Given the proposed role of lactoferrin receptors for infection by species C adenoviruses, we first determined whether lactoferrin affects the infection efficiency of adenovirus BB2000-61. Monocytes isolated from PBMC using CD14-labelled microbeads
(Miltenyi Biotech) were cultured for 5 days in granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 to generate MDDC as described previously (Kessler et al., 2010). These cells expressed high levels of CD11c and CD209/DC-SIGN as a DC marker, but not the monocyte/macrophage marker CD14 (data not shown). Infected cells were stained 1 day post-infection for the early antigen E1A (Abcam) with DAPI as a counterstain (Fig. 1a). Infection rates of MDDC in the absence of lactoferrin were relatively high at 15–30 %, suggesting that there is a receptor on MDDC for adenovirus 2C that has yet to be identified. A pronounced enhancement of MDDC infection by adenovirus 2C was observed in the presence of 150 μg ml−1 commercially available bovine lactoferrin (Fig. 1a, b; Sigma). Similar results were observed when cells were stained for the late antigen hexon (data not shown). To determine whether this enhancement effect was specific to the isolate BB2000-61 or more generally applicable, we infected MDDC with a second adenovirus 2C clinical isolate (1320-55) derived from peripheral blood leukocytes. As expected, the infection rate by isolate 1320-55 was lower than that of isolate BB2000-61 using comparable numbers of infectious virions (4.7–6.4 x 10⁴ fluorescence focus units) in the absence of lactoferrin. Nevertheless, the infection rate was similarly enhanced in the presence of bovine lactoferrin (Fig. 1b), suggesting that the enhancement effect of bovine lactoferrin is a more general effect on species C serotype 2 adenoviruses.

To determine whether this enhancement effect may occur in humans, we assayed whether human lactoferrin similarly enhances infection of MDDC (Fig. 1b). In contrast to the results from Adams et al. (2009), there was no detectable enhancement of infection for both adenovirus 2C isolates

Fig. 1. Bovine lactoferrin enhances infection of MDDC by adenovirus 2C isolates but not by HCMV TB40/E. (a, b) MDDC were infected with adenovirus 2C isolates in the absence or presence of 150 μg bovine or human lactoferrin ml⁻¹, and were stained 1 day p.i. for E1A with DAPI as counterstain. Data (b) are means ± SEM of two independent experiments. (c) MDDC were infected with HCMV TB40/E in the absence or presence of 150 μg bovine lactoferrin ml⁻¹, and were stained 1 day p.i. for IE1 with DAPI as counterstain. Data are representative of two independent experiments. (d) MDDC were infected with adenovirus 2C isolates in the presence of the indicated concentrations of bovine lactoferrin. Data are means ± SEM of three independent experiments where infection in the presence of 150 μg lactoferrin ml⁻¹ was set as 100 %. Bars, 50 μm.
(Fig. 1b). These contrasting results may be explained by differences between recombinant adenovirus 5, used by Adams et al. (2009), and the adenovirus 2C isolates used in this study. For example, infection rates of MDDC in the absence of lactoferrin by adenovirus 5 were routinely lower at 3–9% compared with those by adenovirus 2C isolates at 15–30%. It is possible that such higher infection rates mask an enhancement effect of human lactoferrin. To address this, we titrated down the number of infectious virions to obtain infection rates between 3 and 9%, but nevertheless were still unable to demonstrate an enhancement effect of human lactoferrin (data not shown). A second difference is that Adams et al. (2009) used a sensitive assay for transgenic GFP expression, as opposed to staining of viral E1A and hexon in this study. It remains to be shown whether the enhancement of infection by adenovirus 5 mediated by human lactoferrin is a consequence of enhanced viral binding and uptake, or whether human lactoferrin supports viral replication and transcription of the reporter GFP (Adams et al., 2009). Enhancement of infection of corneal epithelial cells mediated by human lactoferrin, however, requires species C fiber knob and occurs at the level of viral binding to cells (Johansson et al., 2007). The reasons for the conflicting results shown in this study and from Adams et al. (2009) are not clear. From our results, we cannot therefore conclude that human lactoferrin enhances the infection of MDDC by adenovirus 2C isolates, suggesting that human lactoferrin may not enhance infection of DC in vivo by such clinical isolates.

Given the described antiviral properties of bovine lactoferrin (van der Strate et al., 2001), we next tested whether bovine lactoferrin inhibits infection by another virus under the same conditions. We made use of the human cytomegalovirus (HCMV) isolate TB40/E that infects MDDC (Riegler et al., 2000). In line with the antiviral effects of lactoferrin, the HCMV infection rate using 1756 Journal of General Virology 15–30%. It is possible that such higher infection rates could be enhanced to approaching 40% (Adams et al., 2009), enhancement of adenovirus 2C infection was more efficient with rates routinely greater than 70% (Fig. 1b).

To determine whether other myeloid cells can also be efficiently infected in the presence of bovine lactoferrin, we compared infection of MDDC and blood monocyte-derived macrophages (MDMØ) from the same donors. Monocytes were cultured in GM-CSF without IL-4 to generate MDMØ in parallel with MDDC. These MDMØ expressed CD14 as a monocyte/macrophage marker, but not the DC marker DC-SIGN (data not shown). In contrast to MDDC, MDMØ were infected at low rates in the presence or absence of bovine lactoferrin (Fig. 2a). Increasing the number of infectious virions 100-fold increased the infection rate of macrophages to a mean 3.6% and revealed an enhanced infection rate of a mean 9.7% in the presence of bovine lactoferrin (data not shown). Thus, there is a receptor expressed on macrophages, which might be Dectin-2 or CD14 for example (McGreal et al., 2006; Baveye et al., 2000), which can enhance the infection rate of these cells in the presence of bovine lactoferrin. Nevertheless, these results additionally suggested that a bovine lactoferrin receptor relevant for enhanced infection of MDDC to higher rates routinely exceeding 70% is expressed on MDDC but not MDMØ.

Bovine lactoferrin reportedly binds directly to adenovirus 2C (Pietrantoni et al., 2003), and contains a high proportion of high mannose glycosylation moieties, depending on factors including species origin (Spik et al., 1988), which serve as ligands for particular C-type lectin innate immune receptors. Such pattern recognition receptors include Dectin-2, Mincl, the mannose receptor and CD209/DC-SIGN (Guo et al., 2004; Lee et al., 2011; McGreal et al., 2006; Yamasaki et al., 2009). Given that MDDC apparently express a relevant bovine lactoferrin receptor, we focussed on CD209/DC-SIGN, which is expressed on MDDC but not MDMØ. Infection of MDDC in the presence of 200 μg mannin ml⁻¹, a reagent commonly used to block CD209/DC-SIGN (and other C-type lectin) interactions, was significantly inhibited, as analysed by one-way ANOVA with Bonferroni’s multiple comparison test, suggesting that carbohydrate moieties are involved (Fig. 2b). As a negative control, infection was not significantly blocked by 100 μg soluble β-glucan ml⁻¹, which blocks interactions of the fungal pattern recognition receptor Dectin-1 (Fig. 2b, Taylor et al., 2007). To further define the receptor we made use of specific blocking antibodies. As controls, infection of MDDC by isolate BB2000-61 was not blocked by 20 μg ml⁻¹ isotype control antibody or antibody to CD46 (B97) (Buchholz et al., 1997), which is known to mediate infection of MDDC by some species B adenoviruses of 150 μg ml⁻¹, in the range of physiological concentrations of lactoferrin in humans (Thompson et al., 1990; van der Strate et al., 2001), for which reason further experiments were performed using this concentration of bovine lactoferrin. A second difference between adenovirus 5 and 2C is that while adenovirus 5 infection rates could be enhanced to approaching 40% (Adams et al., 2009), enhancement of adenovirus 2C infection was more efficient with rates routinely greater than 70% (Fig. 1b).

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However, infection of MDDC by adenovirus BB2000-61 was partially blocked by 20 μg ml⁻¹ antibody to DC-SIGN (Fig. 2c, clone 120507; R&D Systems). Similarly, infection of MDDC by isolate 1320-55 was partially blocked by both mannan and CD209/DC-SIGN antibody, but not by isotype control or CD46 antibodies (data not shown). DC-SIGN antibody-mediated blockade of infection was less pronounced than that by mannan, which may reflect the lower concentration of blocking reagent used or that other receptors are involved. Thus, CD209/DC-SIGN at least partially mediates infection of MDDC by adenovirus 2C in the presence of bovine lactoferrin.

To provide clearer data on CD209/DC-SIGN in adenoviral infection we performed a gain-of-function experiment in U937 macrophages. We ectopically expressed CD209/DC-SIGN on U937 macrophages, which do not express this molecule, and determined whether these transductants could then be infected with adenovirus 2C. For this purpose, we cloned CD209/DC-SIGN into the vector pMXsIP (Kitamura, 1998), transiently transfected phoenix amphi cells using Fugene HD (Roche) to generate replication defective retrovirus, and infected U937 macrophages with retrovirus as described previously (Dennehy et al., 2011). Stable transductants expressed high levels of CD209/DC-SIGN by flow cytometry (Fig. 3a), and could be readily infected with adenovirus 2C in the presence of bovine lactoferrin (Fig. 3b). By contrast parental U937 cells were not infected with adenovirus 2C in the presence or absence of bovine lactoferrin (Fig. 3b, data not shown). These data thus clearly demonstrate that CD209/DC-SIGN functions as an adenovirus 2C receptor in vitro in the presence of bovine lactoferrin.

Lastly, to determine whether cells infected with bovine lactoferrin-treated adenovirus respond appropriately to infection, we measured cytokine responses (Fig. 3c). MDDC treated with bovine lactoferrin alone produced low but readily detectable levels of tumour necrosis factor (TNF), suggesting that either low levels of endotoxins are present in the commercially available lactoferrin or that lactoferrin itself can stimulate low level TNF production consistent with its ability to upregulate CD86 in MDDC (Adams et al., 2009). TNF production was increased after treatment of cells with adenovirus 2C alone, and further increased to high levels following infection with bovine lactoferrin-treated adenovirus. Similarly, MDDC treated with adenovirus 2C alone produced low levels of IL-1β, which was further enhanced following infection with bovine lactoferrin-treated adenovirus. Given that IL-1β is a signature cytokine produced by inflammasomes
following infection with adenoviruses (Muruve et al., 2008), we conclude that MDDC initiate an appropriate cytokine response following infection with adenovirus 2C isolates in the presence of bovine lactoferrin.

The antiviral effects of lactoferrin are well established. In addition to inhibiting infection by HCMV, lactoferrin inhibits infection by human immunodeficiency virus, hepatitis C virus, herpes simplex virus, poliovirus, rotavirus and respiratory syncytial virus (van der Strate et al., 2001). It is therefore paradoxical that bovine lactoferrin enhances infection of MDDC by adenovirus 2C. Given that human lactoferrin did not enhance infection of MDDC by adenovirus 2C isolates, we cannot conclude that lactoferrin plays a role in the infection of human DC by clinical adenovirus 2C isolates. The most likely explanation for the different effects of bovine versus human lactoferrin is that the latter does not contain high mannose carbohydrate moieties that are required for interaction with CD209/DC-SIGN (Spik et al., 1988; Guo et al., 2004). It remains to be determined whether lactoferrin and DC-SIGN orthologues play a role in infection of DC by adenoviruses in species in which lactoferrin does contain high mannose moieties. The identity of the receptors that mediate enhanced infection of MDDC by recombinant adenovirus 5 in the presence of human lactoferrin (Adams et al., 2009), and of corneal epithelial cells by species C adenoviruses in the presence of human lactoferrin (Johansson et al., 2007), as well as infection of MDDC by adenovirus 2C isolates in the absence of lactoferrin remain to be determined.

Given the high efficiency of infection of MDDC by adenovirus 2C in the presence of bovine lactoferrin, these data nevertheless may provide a useful tool to dissect the initiation of innate and adaptive immune responses by myeloid cells to adenovirus in vitro. For example, both high infection rates and high numbers of cells are required to identify adenoviral peptide epitopes presented on MHC class I and II. The use of MDDC, infected with high efficiency by clinically relevant adenovirus 2C isolates, from leukapheresis preparations may provide sufficient material for such approaches. By contrast, myeloid cell lines such as U937 and K562 cannot be efficiently infected with adenovirus 2C (Fig. 3b, data not shown), even though K562 is partially positive for CAR expression (Yotnda et al., 2004). However, DC-SIGN transduced cell lines such as U937 or K562-HLA-A*2 can readily be infected by adenovirus 2C in the presence of bovine lactoferrin (Fig. 3b, data not shown), and may thus provide a method to identify epitopes from clinically relevant adenovirus 2C isolates.

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