Identification and functional characterization of a bovine orthologue to DC-SIGN

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Abstract: Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) C-type lectin is almost exclusively expressed at the cell surface of DC. In addition to its normal function facilitating contact of DC with T cells, DC-SIGN has been shown to bind a variety of pathogens, including Mycobacterium bovis, and HIV-1 envelope protein gp120. In this study, we identified the bovine ortholog of the human DC-SIGN gene within the bovine genome, which exists as a single copy. PCR amplified a product, showing a 100% match with the predicted sequences as well as a sequence predicted to be similar to that of SIGNR7. Furthermore, a protein with the same molecular weight as human DC-SIGN was detected by Western blot in cell lysate derived from bovine DC. To characterize this molecule functionally, the uptake of FITC-labeled OVA and FITC-labeled gp120 (FITC-gp120) by bovine and human DC was assessed. FITC-gp120 was shown to bind to bovine DC in a time- and temperature-dependent manner. Binding was blocked by a polyclonal anti-DC-SIGN antibody but not by a control antibody. Furthermore, blocking of this molecule also reduced the binding of M. bovis bacillus Calmette-Guerin expressing GFP. Confocal microscopy showed that DC-SIGN was expressed on the surface of bovine DC. Subsequent pulse-chase studies revealed that FITC-gp120 was internalized by bovine monocyte-derived DC as early as 10 min. Thus, there is evidence of a DC-SIGN-like molecule expressed specifically by bovine DC. This molecule may play an important role in the infection of bovine (DC) cells with M. bovis. J. Leukoc. Biol. 83: 1396–1403; 2008.

Key Words: dendritic cells · C-type lectin · gp120 · Mycobacterium bovis BCG

INTRODUCTION

Dendritic cells (DC) are professional antigen-presenting cells (APC) that efficiently capture antigens in the peripheral tissues and migrate to secondary lymphoid organs, where they present the processed antigens to naïve and memory T cells to initiate adaptive immune responses. DC are the only APC recognized as having the ability to prime naïve T cells and to initiate primary T cell-mediated responses [1]. Pathogen-associated molecular patterns recognized by DC through pattern recognition receptors (PRR) are subsequently presented to T cells, thus linking innate and adaptive immunity [1–3]. Immature DC express PRR, including TLR and a range of C-type lectin receptors (CLR) [4]. The majority of CLR expressed by DC have specificity for mannose-containing carbohydrates, through which some of these receptors can potentially be “hijacked” by pathogens [5–10]. An example of one such CLR is the DC-specific ICAM-3-grabbing nonintegrin (SIGN) C-type lectin.

DC-SIGN, a type II transmembrane protein, is highly expressed on the surface of DC [11]. Although only DC were originally found to express DC-SIGN, subsequent studies detected expression on other cells such as decidual macrophages, alveolar macrophages, and B cells [12–14]. In vitro, DC-SIGN expression is found on immature and mature monocyte-derived DC (MoDC). In addition to its role as a cell adhesion molecule, DC-SIGN also functions as an antigen receptor and has been shown to specifically bind Mycobacterium tuberculosis, envelope proteins of hepatitis C, and Ebola virus, as well as the HIV-1 envelope gp120 [5–10]. DC-SIGN is the major receptor for gp120 on MoDC, recognizing the high mannose carbohydrate structure on the molecule [9, 15, 16]. After binding to DC-SIGN, gp120 can be internalized into lysosomes. This is facilitated by two internalization motifs: a dileucine (LL) motif and a tyrosine-based motif (YXXL) in the cytoplasmic domain of DC-SIGN [17].

DC-SIGN-related molecules have been identified in other species, including mouse [18], macaque and chimpanzee [19]. In cattle, only a few CLR have been identified at the mRNA or genome level. For some, such as the bovine mannose receptor (MR), their functional relevance has been confirmed [20].
Recently, DEC-205, dectin-1 and dectin-2 have been cloned and functionally characterized [21–23].

In contrast, no data are available regarding the expression of a bovine ortholog of DC-SIGN. We provide evidence, at the gene and protein level, for the existence of a bovine ortholog to human DC-SIGN expressed on bovine MoDC, which seems to exist in cattle as only one gene, that binds and internalizes HIV-1 gp120 in addition to Mycobacterium bovis bacillus Calmette-Guerin (BCG). Thus, it is likely that this molecule may contribute to the infection of bovine (DC) cells with M. bovis, similar to that described for human DC-SIGN.

MATERIALS AND METHODS

Sequence databases

To identify a bovine DC-SIGN ortholog, the following genome assemblies were used: opossum (January 2006), bovine (March 2006), mouse (February 2006), and human (March 2006). Amino acid sequences of CD209-like peptides were obtained from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) of Mdm_CD209_1 (XP_001377340); Mdm_CD209_2 (XP_001377327); Mmus_CD209a (NP_579973); Mmus_CD209b (NP_579974); Mmus_CD209e (NP_579975); Hsap_CLEC4M (NP_055072). The bovine CD209-like (Btau_CD209L) sequence was the predicted translation product (using the Expasy translate tool http://www.expasy.ch/tools/dna.html) from a combination of two expressed sequence tag (EST) sequences (EH153181 and DY079929).

Prediction of gene structures, organization, and protein domains

Predictions of gene structures and organization were based on Basic Local Alignment Search Tool (BLAST) hits of genome sequences using peptide or DNA sequences to interrogate the University of California Santa Cruz (UCSC; Santa Cruz, CA, USA) genome browser (http://genome.ucsc.edu/). Protein domains within peptide sequences were predicted by Simple Modular Architecture Research Tool (SMART; Version 5; http://smart.embl-heidelberg.de/) [24]. A multiple sequence alignment of peptide sequences was made using ClustalX (Version 1.83; http://biops.u-strasbg.fr/fr/Documentation/ClustalX/) [25] and default parameters. Multiple sequence alignments were edited using Jalview (Version 2.3; http://www.jalview.org) [26].

Calculation of reconciled species and gene trees

Gene trees were determined by the neighbor-joining method [27]. Trees were displayed using TreeView (Version 1.6.6) and GeneTree (Version 1.3.0; http://taxonomy.zoology.gla.ac.uk/software/index.html) [28].

Cell preparation and generation of DC

Human and bovine blood was collected from healthy donors in accordance with home office license and ethical committee approval. Blood was collected into Biosource, Nivelles, Belgium). Medium was changed every 3 days, and cells were harvested after 6–7 days in culture. All incubations were at 37°C in 5% CO2/air unless otherwise stated.

RNA extraction and cDNA synthesis

Total RNA was prepared from bovine MoDC, B cells, and CD4+ T cells following the Qiagen RNeasy mini kit protocol (Qiagen, Crawley, UK). The quality and quantity of the total RNA were determined using a spectrophotometer. RNA (500 ng) was used in the subsequent cDNA reactions using Superscript II RT (Invitrogen, Paisley, UK) and following the manufacturer’s protocol. cDNA was diluted 1:2.5 prior to use.

PCR and sequencing

Primers (MWG Biotech, Ebersberg, Germany) to the 3′-untranslated region of the gene were designed to amplify a 383-bp fragment from either Btau_CD209L sequence to amplify the full-length coding sequence (CDS) as well as an internal fragment (INT). The primers CDS-forward (5′-GAAGAGGATGATGCTGACGGGAGGTTGTTGAGCTGAAAG-3′) and CDS-reverse (5′-TCAGAGGCTGAGGGACGGGAGGTTGTTGAGCTGAAAG-3′) amplified the predicted CDS of 778 bp. Primers INT-FOR (5′-GGAGAGGATGATGCTGACGGGAGGTTGTTGAGCTGAAAG-3′) and INT-REV (5′-CCCAATTCTGAGGGAGGTTGTTGAGCTGAAAG-3′) amplified an INT of 383 bp, representing bases 24–406 of the CDS. These primers where then used in a standard PCR at an annealing temperature of 58°C using cDNA from bovine MoDC, B cells, and CD4+ T cells as template DNA (5 μL). The amplified products were visualized on a 1% agarose gel. Cleared products were then used in sequencing reactions.

Antibodies and flow cytometry

Human and bovine monocytes and MoDC were analyzed for the expression of the cell-surface molecules. The sources of antibodies, their isotypes, and methods for flow cytometry have been described in detail [30]. The following mouse anti-bovine mAb were used: CD14 (C4G33, IgG1), CD40 (IL-1A15, IgG1), MHC II-DQ (CC136, IgG2a), MHC II-DR (CC108, IgG1), CD86 (N32-3, IgG1), and CD11b (all IgG2a). For human cells, the following directly labeled mouse anti-human mAb were used: CD14-PE, CD40-PE, HLA class II-DR, -DP, and -DQ (all IgG2a), CD80-PE, and CD86-PE (both IgG1; all mAb from Serotec Ltd., Oxford, UK). To analyze surface expression of DC-SIGN in both species, a polyclonal anti-human DC-SIGN antibody (pAbs C20, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used. Where necessary, bound mAb was detected using FITC-labeled, isotype-specific secondary reagents (Southern Biotechnology, Birmingham, AL, USA). Immunofluorescent staining of 10,000-gated, live cells was analyzed using FlowJo software, Version 7.1 (Tree Star Inc., Ashland, OR, USA).

M. bovis BCG expressing GFP (BCG-GFP)

BCG-GFP was cultured using methods described previously [31]. Mycobacterial stocks were frozen and stored at –80°C. Stocks were enumerated by serial dilution and spotting onto 7H10 plates.

Western blot analysis for DC-SIGN

Human and bovine MoDC, T cells, B cells, and monocytes produced as described were pelleted by centrifugation at 300 g for 10 min at 4°C. Thereafter, cells were washed twice with ice-cold PBS and lysed in 50 μL M-PER™ lysis buffer (Pierce Biotechnology, Inc., Rockford, IL, USA) per 10^6 cells for 20 min at room temperature. After centrifugation at 16,000 g for 15 min at 4°C, protein extracts were collected and frozen at –20°C. Protein concentrations in cell lysates were measured using the Coomassie Plus Bradfor™ assay kit (Pierce Biotechnology, Inc.) according to the manufacturer’s instructions. Laemmli buffer (5×) was added, and each sample was boiled for 3 min prior to storage at –20°C. For Western blot analysis, 24 μg sample in 20 μL was loaded per well, and proteins were separated by SDS-PAGE. After electrophoresis, 100 V for 90 min, proteins were transferred to a 0.2-μm nitrocellulose membrane (Amersham Biosciences, Germany), which was subsequently blocked with PBS containing 0.05% Tween 20 and 5% milk powder (PBS-TM) for 90 min at room temperature, followed by incubation with mouse anti-human DC-SIGN mAb (diluted 1:1000, clone DCN416, BD Biosciences) or mouse anti-human β-actin (diluted 1:2000, Sigma) overnight at 4°C. Excess antibody was removed by extensive washing in PBS-TM. The membrane was then incubated with HRP-conjugated anti-mouse IgG (Amersham Biosciences)
at a 1:2000 dilution in PBS-TM for 90 min at room temperature. The membranes were washed extensively with PBS-Tween 20, and bands were visualized using the ECL system (Amersham Biosciences) and a Curix 60 processor (Agfa-Gevaert N.V., Mortsel, Belgium).

**Binding assay for DC-SIGN ligands**

Bovine and human MoDC were harvested and adjusted to a concentration of 3–4 × 10^6 ml^{-1}. This cell suspension (100 μl) was incubated with each well of a 96-U-well microtiter plate, which was kept on ice. Binding of FITC-labeled OVA (FITC-OVA) was analyzed as described recently [20]. Briefly, 4 μl FITC-OVA (125 μg ml^{-1}; Molecular Probes, Inc., Eugene, OR, USA) was added to each well and incubated for 30 min on ice or at 37°C. To assess binding of FITC-labeled gp120 (FITC-gp120), FITC-gp120 strain IIIB (ImmunoDiagnostics, Inc., Woburn, MA, USA), at a concentration of 4 μg ml^{-1}, was added to each well and incubated for 5 min or 30 min on ice or at 37°C. To assess the specificity of binding, cells were incubated for 30 min on ice with 40 ng ml^{-1} anti-human CD3 pAb (Sigma), anti-human DC-SIGN pAb (Santa Cruz Biotechnology, Inc.), or anti-DC-SIGN mAb (BD Biosciences) prior to adding FITC-OVA or FITC-gp120. In a second set of experiments, bovine lactoferrin (bLF; Sigma) was used to inhibit the DC-SIGN-gp120 interaction in accordance with a recent publication [32]. MoDC were preincubated with medium alone or 0.01, 0.1, 1, 10, or 100 μg ml^{-1} bLF for 30 min at 37°C, followed by incubation with FITC-gp120 for 30 min at 37°C. After three washes with ice-cold PBS, cells were harvested and analyzed by flow cytometry using a FACSAria. The specificity of gp120 binding was also evaluated using purified bovine CD4 T cells, and the interaction of gp120 with these cells was analyzed as described above.

As DC-SIGN was also described as a receptor for mycobacteria, MoDC were incubated with BCG-GFP at a multiplicity of infection (MOI) of 10 for 2 h at 37°C or on ice as described above. Cells were washed by low-speed centrifugation (300 g) with ice-cold PBS before determining the percentage of cells that bound BCG-GFP by flow cytometry. To assess the specificity of the reaction, DC were preincubated with bLF or pAb to DC-SIGN as described above.

**Confocal microscopy**

Bovine and human MoDC were harvested, adjusted to a concentration of 1 × 10^6 ml^{-1}, and incubated at 37°C with 1 μg ml^{-1} FITC-gp120 for 10 min, 30 min, or 120 min. Cells were washed twice and fixed for 15 min with 2% paraformaldehyde at room temperature. For the analysis of intracellular expression, cells were incubated for 10 min in PBS/BSA/azide/0.02% saponin (Sigma). Cells were stained with anti-DC-SIGN pAbs (C-20, Santa Cruz Biotechnology, Inc.), anti-bovine MHC II mAb (CC158), anti-early endosome antigen-1 (EEA-1; ab2900, Abcam, Cambridge, MA, USA), and Cy3-conjugated cholera toxin (CTX; Quadratech, UK) for 60 min at room temperature. After two washes, cells were incubated for 60 min at room temperature with Cy3-, Cy5-, tetramethyl rhodamine isothiocyanate-, or PE-labeled, isotype-matched secondary antibodies (Amersham-Pharma, Piscataway, NJ, USA; Molecular Probes; and Southern Biotechnology, respectively). Slides were mounted using Prolong-Antifade (Molecular Probes) and analyzed using an LSM510 META confocal microscope and LSM 2.5 image analysis software (Carl Zeiss, Inc., Thornwood, NY, USA).

**Statistical analysis**

Data are presented as mean ± SD, and a representative set of data of at least three repeats is shown. Data were analyzed using GraphPad Prism, Version 4.0 (GraphPad Software Inc., San Diego, CA, USA). Differences between treatment groups were determined by means of a one-way ANOVA, followed by Bonferroni t-test for significant effects. Probability levels used for statistical significance were 95% in all cases.

**RESULTS**

**Analysis of CD209-like sequences in the genomes of cattle and other vertebrates**

Extensive BLAST searches of cattle EST and mRNA sequences using human and rodent CD209-like sequences detected multiple homologies. The longest ESTs, EH153181 and DY079929, overlapped and were predicted (see below) to cover the full CDS of a bovine CD209-like protein (Btau_CD209L), with a predicted translation product of 129 aa residues (Supplemental Figs. 1 and 2). No other sequences were detected in the bovine genome, including the recent prerelease (March 2006), suggesting that only a single gene exists in the bovine genome. To explore the possible evolutionary relationship between these CD209-like molecules, neighbor-joining trees were calculated, and the resulting gene tree is displayed in Figure 1A. (To uncover possible gene duplications/gene deletions, a tree that reconciles species and gene trees was calculated; Supplemental Fig. 4.) Figure 1B shows a prediction of protein domains within the bovine and other CD209-like proteins.
proteins and the typical C-type lectin domain (based on protein sequences shown in Supplemental Fig. 3).

DC-specific expression of Btau_CD209L

As genome analysis suggested the presence of a DC-SIGN-like molecule, primers based on this sequence were used to assess gene expression of Btau_CD209L in RNA extracted from bovine MoDC, B cells, and CD4+ T cells. Both primer sets routinely amplified fragments of the expected size from MoDC, but no products were observed from the B cells or CD4+ T cells (Fig. 2A). Sequence analysis of the INT fragment highlighted 384 bp, which when used to interrogate known published sequences using BLASTN, was a 100% match to a predicted gene “similar to SIGNR7” (GenBank Acc. No. XM_590928.3). The sequence obtained matched that similar to SIGNR7 at a region corresponding to bases 22–408 of the CDS (data not shown).

Cell extracts from bovine MoDC react with a DC-SIGN-specific antibody

As genome analysis suggested the presence of a DC-SIGN-like molecule within the bovine genome, we next evaluated the presence of a corresponding protein in MoDC. Using mAb to human DC-SIGN, a protein band with a similar molecular weight (MW) was detected by immunoblotting in cellular extracts of bovine MoDC, whereas no products were observed from the B cells or CD4+ T cells (Fig. 2B). Two size variants of DC-SIGN were detected in human and bovine MoDC. In human MoDC, the predominant species was 44–48 KDa, with a minor species detected at 42 KDa. In contrast, extracts of bovine MoDC showed bands in the range of 46–48 KDa and a slightly more dominant band at 43–45 KDa. Staining with a mAb to β-actin showed that similar amounts of protein were loaded for each sample.

Cell surface expression of DC-SIGN on human and bovine monocytes and MoDC

As the Western blot results suggested the presence of a DC-SIGN-like molecule expressed by bovine MoDC, we assessed DC-SIGN expression on the surface of freshly isolated CD14+ monocytes and MoDC by FACS analysis. As shown in Figure 3, human and bovine monocytes and MoDC expressed a similar receptor repertoire on their cell surface. Differences observed were that MoDC generated from the human or bovine monocytes (at Day 6 of culture) showed no expression of monocyte marker CD14 and higher expression of the molecules CD40, CD80, CD86, and MHC II. Although surface expression of DC-SIGN was not detected on human and bovine monocytes, strong expression was observed in human MoDC with weaker expression on bovine MoDC.

Binding of FITC-gp120 to bovine MoDC depends on the presence of DC-SIGN

Western blot and flow cytometric analysis suggested the expression of a DC-SIGN-like molecule by bovine MoDC. To assess the function of this molecule, we evaluated its capacity to bind HIV gp120. Initially, we analyzed the ability of HIV gp120 to bind to purified bovine CD4+ T cells in the absence or presence of a pAb to human DC-SIGN. Freshly isolated bovine T cells were able to bind FITC-gp120; however, this binding was not inhibited by the pAb specific for DC-SIGN (Supplemental Fig. 5A). We then tested the ability of human and bovine MoDC to bind FITC-gp120. Human and bovine MoDC were shown to bind FITC-gp120 in a temperature- and time-dependent manner, with an increase of FITC-gp120 binding after 30 min at 37°C (Supplemental Fig. 5B). The specificity of gp120 binding was assessed in the presence of antibodies specific for DC-SIGN or CD3, respectively. Resulting changes in mean fluorescence intensity (MFI) are displayed in Figure 4A. The calculated average blocking percentiles using anti-DC-SIGN pAb in human and bovine MoDC were 52% and 49%, respectively, whereas the anti-DC-SIGN mAb reduced FITC-gp120 binding by 32% and 25%. For control purposes, the binding of FITC-OVA by human and bovine MoDC was analyzed as described [20]. Although both cell types bound FITC-OVA in a similar pattern (Fig. 4B), binding was not influenced by the presence of an anti-CD3 or anti-DC-SIGN

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antibody. To characterize the identified molecule further, we investigated the recently described blocking capacity of bLF on the DC-SIGN-gp120 interaction [32]. A concentration-dependent, inhibitory effect of bLF on FITC-gp120 binding was observed in both species (Fig. 4C). Binding of gp120 to human MoDC was reduced at concentrations as low as 0.1 μM, and the same inhibitory effect was observed with bovine MoDC, even at a concentration of 0.01 μM bLF.

DC-SIGN expressed by DC is involved in BCG-GFP binding

DC-SIGN has been considered to be the major receptor for M. tuberculosis and BCG on immature MoDC. For this reason, we analyzed whether capture of mycobacteria would occur in bovine MoDC after blocking DC-SIGN by a pAb. As shown in Figure 5, preincubation of bovine MoDC with pAb C20 reduced BCG-GFP binding after 2 h of incubation compared with untreated cells. In contrast, preincubation of MoDC with bLF in concentrations showed almost 100% inhibition of FITC-gp120 binding and reduced the binding of BCG-GFP to a lesser extent.

gp120 accumulates within bovine MoDC in a time-dependent manner

To determine whether bovine MoDC not only bound but also internalized HIV gp120, cells were incubated with FITC-gp120 for 10 min, 30 min, and 120 min. As shown in Figure 6, low amounts of FITC-gp120 seemed to be present just beneath the cell membrane after 10 min (Fig. 6B) incubation, accumulating in the cytoplasm after 30 min (Fig. 6C) and 120 min (Fig. 6D). At 30 min incubation, some colocalization (yellow) could be seen between MHC II staining and FITC-gp120. A similar result was obtained for human MoDC (data not shown). At no time-point did FITC-gp120 seem to colocalize with the lipid-raft marker CTX or the early-endosome marker EEA-1 (data not shown).

DISCUSSION

The present study provides the first evidence for a functional bovine ortholog of the human DC-SIGN (CD209) molecule on bovine MoDC. The molecule identified has a similar MW to its human counterpart and is involved in the binding of BCG-GFP and thus, potentially in the uptake of M. bovis. Similarly, this molecule can bind HIV gp120 in a temperature- and time-dependent manner, which was inhibited in the presence of DC-SIGN-specific antibodies or bLF.

Multiple CD209-like sequences were found in opossum (2), mouse (5), and human (2) based on sequence homology and annotation data about the UCSC genome browser (data not shown). Analysis of the cattle genome strongly supports the
existence of a single CD209-like gene, as no other CD209-like sequences were detected. This was based on available bovine and nonbovine sequences (further details shown in Supplemental Figs. 1 and 2). Despite the fact that the cow genome is not yet fully annotated, we are convinced that this is not a result of gaps in the bovine genome assembly. This sequence is predicted to be a direct descendent of an ancestral CD209-like gene. In contrast, opossum, mouse and human genomes contain multiple CD209-like genes, the products of gene duplications/gene deletions. Interestingly, only the human genome sequence shows extensive internal repeats. Our analysis suggests that the opossum sequences are the result of recent gene duplication in this lineage and so, are strictly CD209-like. In contrast to other species, the mouse has five highly divergent CD209-like genes and is therefore unlikely to be the product of any recent gene duplications in the rodent lineage. The most likely explanation is that all but CD209b have been deleted during the past and are no longer present in the human lineage. CD209b is most likely the two human genes CD209 and CLEC4M, which are potentially the product of recent gene duplications and are therefore paralogs and orthologs to the bovine CD209L molecule described here. The SMART analysis also shows that human CD209 proteins are different in that they both contain a repeat domain, which seems to be unique to the human/primate molecules. Interestingly, the predicted and confirmed bovine CD209L molecule seemed to show the highest similarity to the murine SIGNR7. This molecule has recently been described to be one functional homologue to the human DC-SIGN molecule with similar carbohydrate-binding characteristics [33]. The fact that murine SIGNR7 has been described to

**Fig. 4.** Binding of FITC-OVA and FITC-gp120 to human and bovine MoDC, which were generated as described and incubated with FITC-OVA (left columns) or FITC-gp120 (right columns) for 30 min at 37°C in the absence or presence of anti-CD3 antibody or anti-DC-SIGN pAb (A). Open histograms represent untreated cells. Results are from one representative experiment of at least four repeats. Data are expressed as histogram analysis of 10,000 cells. (B) Specificity of gp120 binding to DC-SIGN was analyzed by incubating human and bovine MoDC for 30 min at 37°C in the absence or presence of anti-DC-SIGN pAb or mAb. MFI was converted into percent binding by the following formula: (MFI of gp120 binding in the presence of anti-DC-SIGN antibody) – (MFI background, no gp120)/(MFI of the gp120 binding in the absence of anti-DC-SIGN antibody) – (MFI background, no gp120). A histogram summary of average percentage of gp120 binding from at least four experiments is shown. *, P < 0.05; ***, P < 0.001.

**Fig. 5.** DC-SIGN antibody reduces binding of BCG-GFP to bovine MoDC, which were incubated for 2 h on ice or at 37°C with BCG-GFP at a MOI of 10 (A) or preincubated with pAb to DC-SIGN (B) or 10 µM bLF for 30 min before exposure to BCG-GFP for 2 h at 37°C (C). Filled histograms represent BCG-GFP-incubated cells; open histograms represent cells incubated on ice (A) or incubated with pAb to DC-SIGN (B) or bLF (C), respectively. Results are from one representative experiment of three repeats. Data are expressed as histogram analysis of 5000 cells.
have a shorter neck region compared with human DC-SIGN fits to our observation that the bovine CD209L molecule possesses a shorter sequence as well as potentially a slightly lower molecular weight.

Phenotypic analysis of surface antigens expressed on bovine and human cell subsets revealed that monocytes express lower levels of the costimulatory molecules CD40, CD80, and CD86 when compared with MoDC from both species, supporting the established phenotype of human monocytes and MoDC [20]. In addition, staining with antibodies to DC-SIGN revealed that human and bovine MoDC expressed DC-SIGN, whereas no expression was observed on monocytes from either species. A stronger reactivity of the anti-DC-SIGN antibody was observed with human MoDC than with bovine MoDC. As an antibody generated against the human DC-SIGN molecule was used in this experiment, the less-intense staining observed on bovine MoDC could reflect the level of cross-reactivity of the antibody.

As the initial binding of HIV-1 is mediated by its gp120 envelope protein to DC-SIGN, further investigation was done to show the specific binding of FITC-gp120 by bovine MoDC using human MoDC as controls. Binding of FITC-gp120 to bovine MoDC was DC-SIGN-dependent and was blocked in the presence of antibodies to DC-SIGN or bLF, similar to that described for human MoDC [5, 32]. Although our study does not discriminate between the blocking of the DC-SIGN-gp120 interaction being a result of bLF binding to DC-SIGN or gp120, a recent study performed in the human system suggests that bLF binds to DC-SIGN and not gp120 [32]. In contrast, uptake of FITC-OVA via clathrin-coated pits [20] was unaffected by antibody treatment. Blocking of gp120 binding was higher in the presence of a pAb compared with that observed with the mAb in human and bovine cells. This observation could be explained by the fact that the mAb has a higher specificity compared with the pAb, recognizing fewer epitopes. A further possibility is that gp120 may bind to receptors other than DC-SIGN expressed on the surface of MoDC, such as CD4 and MR [34], and some of these receptors are also present on bovine MoDC [20].

Having established that a functional DC-SIGN ortholog is expressed on bovine MoDC, we then evaluated its role in the uptake of M. bovis BCG. DC-SIGN plays an important role in the functional subversion of DC in mycobacterial infection [8, 35]. In addition, given the observation that DC-SIGN is also expressed on alveolar macrophages [36] and the observed differences in the responses of bovine cells to different mycobacterial strains [37], it is important to understand the function of this receptor in the bovine system. Similar as with gp120, the binding of BCG-GFP to bovine MoDC was reduced in the presence of a pAb to DC-SIGN and to a lesser extent, in the presence of bLF. These data clearly indicate the importance of the bovine DC-SIGN ortholog in mycobacterial infection and may help to explain the production of immunosuppressive IL-10 by bovine alveolar macrophages and DC in response to M. bovis (Lise Schreuder and T. J. Coffey, manuscript in preparation), as well as in vaccinated animals [38]. In addition, the presence of a high-affinity receptor for mycobacterial antigens on DC, such as DC-SIGN, and its known ability to target bound antigens to endosomal/lysosomal compartments may mature these cells. This leads to their migration to the regional draining lymph nodes, where they are able to prime a mycobacterial-specific T cell response. It is worth noting that the role of DC-SIGN and the resulting chemokine response may differ between host as well as mycobacterial species [37, 39].

To verify that the DC-SIGN-bound antigen is internalized and delivered into endosomal/lysosomal compartments, confocal microscopy studies were performed. gp120 was internalized by human and bovine MoDC as early as 10 min after incubation. Our data are in accordance with a recent study showing that DC-SIGN resides within lipid rafts in the cellular membrane but also outside these in the cytoplasm [40].

In summary, our data describe the presence of a bovine ortholog to human DC-SIGN expressed by bovine MoDC, able to bind M. bovis BCG. In addition, this molecule is able to bind and internalize HIV gp120 in a specific manner.

ACKNOWLEDGMENTS

This work was supported by grants from the RVC, the Heptagon Fund, and Pfizer Ltd. (UK) to D. W. We thank K. Triantafilou and M. Triantafilou (Department of Biochemistry, University of Sussex, Brighton, UK) for technical help with confocal microscopy. The BCG-GFP was a kind gift of Martin Vordermeier from the Veterinary Laboratory Agency (Weybridge, UK).