Expressed gene sequence of the IFNγ-response chemokine CXCL9 of cattle, horses, and swine

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This report describes the cloning and characterization of expressed gene sequences of bovine, equine, and swine CXCL9 from RNA obtained from peripheral blood mononuclear cells (PBMC) and other tissues. The bovine coding region was 378 nucleotides in length, while the equine and swine coding regions were 381 nucleotides. Mapping showed that all three sequences were coded for in four exons in the genome, as are the human and mouse genes. The bovine, equine, and swine coding regions shared 83%, 86%, and 84% homology with human CXCL9, respectively, and all three were 74% homologous with mouse CXCL9. Cladogram comparison of the nucleotide sequences of CXCL9 showed that the bovine, equine and swine sequences were more closely related to one another than to either the human or the mouse sequences. However, the human sequence was more closely related to them than it was to the mouse sequence. These relationships were preserved when the deduced amino acid sequences were evaluated and all sequences showed conservation of the characteristic four cysteines. This work sets the stage for further work with these molecules; an integral goal of the U.S. Veterinary Immune Reagent Network is to develop reagents for investigating diseases in livestock species, poultry, and fish.

The interferon-gamma inducible chemokines CXCL9 (MiG), CXCL10 (IP-10), and CXCL11 (I-TAC) are associated with Th1-type immune responses, and mediate their effects through a single receptor, CXCR3 (Loetscher et al., 1996; Cole et al., 1998). However, the chemokines exhibit spatially different expression in cutaneous lupus erythematosus (Wenzel et al., 2007) and in murine cerebral malaria (Campanella et al., 2008). They exhibit temporal differences in expression during the process of human immunosenescence (Shurin et al., 2007) and in anti-viral responses (Larrubia et al., 2008; Durudas et al., 2009).

In addition, they are reported to interact with different domains of CXCR3 (Colvin et al., 2004). Thus, while the chemokines share many similarities, it is likely that they fill different roles in inflammation. The specific roles of CXCL9 in inflammatory disease and protective immunity to infectious diseases and tumors in livestock are not yet known. As a tool to investigate the activity of this chemokine in livestock, we have cloned, sequenced, and categorized bovine, equine, and swine CXCL9 expressed gene sequences. This is the first report for this chemokine in livestock species.

To generate the clones, the NCBI GenBank database (Benson et al., 2008) was queried for bovine and equine CXCL9. In addition, the published human CXCL9 messenger RNA sequence (GenBank accession number X72755; Farber, 1993) was aligned with GenBank’s expressed sequence tag database (dbEST; Boguski et al., 1993) using the Nucleotide Basic Local Alignment Search Tool (BLASTN;
Fig. 1. Comparison of the bovine, equine and swine sequences with known CXCL9 sequences of other species. (A) Alignment of the nucleotide sequences using ClustalW with periods indicating identity with the human sequence and dashes indicating gaps in the alignments. (B) Unrooted neighbor-joining phylogenetic tree comparing the bovine, swine, and equine CXCL9 coding regions with human, mouse, macaque, ferret, and rat CXCL9, as well as other C-X-C motif ligands. The GenBank accession numbers for the non-livestock sequences used are: Human CXCL9, X72755; human CXCL10, X02530; human CXCL11, NM_005409; mouse CXCL9, M34815; mouse CXCL10, M86829; mouse CXCL11, NM_019494; macaque CXCL9, NM_001032936; ferret CXCL9, EF492057; rat CXCL9, NM_145672. Numbers above the branches represent confidence values from a bootstrap analysis with 1000 trials. (C) Alignment of the deduced amino acid sequences using ClustalW with conserved cysteine residues shaded in grey and predicted signal peptide sequences underlined.
Table 1
Comparison of sequences among IFNγ-response chemokines.

<table>
<thead>
<tr>
<th>Sequence compared to</th>
<th>Nucleotide sequencesa</th>
<th>Deduced amino acid sequencesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bt CXCL9</td>
<td>Ec CXCL9</td>
</tr>
<tr>
<td>H. sapiens CXCL9</td>
<td>83%</td>
<td>86%</td>
</tr>
<tr>
<td>M. mulatta CXCL9</td>
<td>83%</td>
<td>86%</td>
</tr>
<tr>
<td>M. musculus CXCL9</td>
<td>74%</td>
<td>74%</td>
</tr>
<tr>
<td>M. putorius furo CXCL9</td>
<td>85%</td>
<td>85%</td>
</tr>
<tr>
<td>R. norvegicus CXCL9</td>
<td>75%</td>
<td>75%</td>
</tr>
<tr>
<td>B. taurus CXCL9</td>
<td>–</td>
<td>88%</td>
</tr>
<tr>
<td>E. caballus CXCL9</td>
<td>88%</td>
<td>–</td>
</tr>
<tr>
<td>S. scrofa CXCL9</td>
<td>87%</td>
<td>86%</td>
</tr>
<tr>
<td>H. sapiens CXCL10</td>
<td>48%</td>
<td>48%</td>
</tr>
<tr>
<td>M. musculus CXCL10</td>
<td>44%</td>
<td>46%</td>
</tr>
<tr>
<td>H. sapeins CXCL11</td>
<td>42%</td>
<td>41%</td>
</tr>
<tr>
<td>M. musculus CXCL11</td>
<td>45%</td>
<td>46%</td>
</tr>
</tbody>
</table>

a Values are the percent identity among sequences.
b Values before the slash are percent identity. Values after the slash are percent similarity, as determined with the BLOSUM62 amino acid substitution matrix (Henikoff and Henikoff, 1992).

Altschul et al., 1990). The database searches yielded bovine and equine mRNA sequences predicted by the NCBI Gnomon tool (NCBI, 2008; GenBank XM_591770 and XM_001491093.1, respectively) and a full-length swine EST sequence (GenBank BP169836). Primers targeting the putative bovine, equine, and swine CXCL9 sequences were designed based on the predicted sequences and ESTs as follows:

5′-GGAGTGATTTCCACTACCAA-3′ (bovine forward primer);
5′-GGTGGAAGTGAGCTATGTA-3′ (bovine reverse primer);
5′-ATGAAAGAAATGGGTGTCTTTTC-3′ (equine forward primer);
5′-TATGGACCTCCTCCTTTGACGAG-3′ (equine reverse primer);
5′-GACTCAAGTAACACCTACAGAAGT-3′ (swine forward primer); and
5′-CAGATACTTGGTGTAACATGTC-3′ (swine reverse primer).

Total RNA was isolated from PBMC of normal cattle and horses, and from mesenteric lymph nodes, lung, and liver of mini pigs with toxoplasmosis (obtained from residual tissues for a separate study; see Solano et al., 2001), using either TRizol Reagent (Invitrogen, Carlsbad, CA) or RNeasy (QIAGEN, Valencia, CA) according to the manufacturers’ protocols. Complementary DNA was synthesized from total RNA with the Reverse Transcription System (Promega, Madison, WI) or Superscript II (Invitrogen) using oligo-dT primers. Polymerase chain reactions (PCRs) were prepared with GoTaq DNA Polymerase (Promega). After heating to 95 °C for 3 min, the reactions underwent 40 rounds of the following cycle: 1 min at 95 °C; 1 min at 55 °C; 1 min at 72 °C. The reactions were then incubated at 72 °C for 10 min and stored at 4 °C until processed further. After analysis via agarose gel electrophoresis, the PCR products were ligated into pCR2.1-TOPO vector using the TOPO-TA Cloning Kit, and transformed in chemically competent Escherichia coli (Invitrogen). Positive clones were selected by blue-white screening, and were incubated in LB broth for 16 h. Plasmid purification was performed with the QiaPrep Spin miniprep kit (QIAGEN, Valencia, CA) and sequencing performed commercially by GeneWiz (South Plainfield, NJ) using M13R and T7 primers in separate reactions. The cDNA sequences for bovine, equine, and swine CXCL9 were deposited in GenBank (accession numbers EU276061, EU438776, and EU364897.2, respectively).

The alignment of bovine, equine, and swine CXCL9 coding sequences with tCXCL9 sequences from other species using ClustalW (Felsenstein, 1989; Hall, 1999) is shown in Fig. 1A. These sequences were also aligned with the coding sequences of other human and mouse C-X-C motif ligands (alignment not shown) to produce an unrooted phylogenetic tree (Fig. 1B) using the neighbor-joining method (Felsenstein, 1989; Hall, 1999). The results revealed that the bovine, equine, and swine sequences had greater homology with known CXCL9 sequences than with other sequences in the C-X-C motif family. The phylogenetic tree is in agreement with pairwise alignments performed between the C-X-C motif family members. The coding regions of bovine, equine, and swine CXCL9 shared 74–86% identity with non-livestock CXCL9, and less than 50% identity with other C-X-C motif ligands (Table 1).

Pairwise alignments of the deduced amino acid sequences revealed a similar relationship as the nucleotide sequences did. The bovine, equine, and swine proteins shared 72–79% identity, and 83–86% similarity, with human CXCL9 (GenBank CA51284), and 63–66% identity and 77–79% similarity with the mouse protein (GenBank AAA39706). Similarity was determined according to the BLOSUM62 amino acid substitution matrix (Henikoff and Henikoff, 1992). Alignment of the three deduced amino acid sequences with the non-livestock CXCL9 proteins using ClustalW are also shown (Fig. 1C). The predicted bovine CXCL9 protein was 125 amino acids in length, whereas the equine and swine proteins consisted of 126 amino acids. All three protein backbones had a predicted molecular weight of approximately 14 kDa. The derived proteins exhibited conservation of the cysteine residues found in the other CXCL9 proteins, and that are characteristic of the C-X-C motif ligand family. Finally, the signal peptide cleavage sites of bovine, equine, and swine
Fig. 2. Comparison of the intron/exon structure of the bovine, equine, and swine genes with human and mouse genes. Numbers at the top of each graphic refer to coding sequence position; numbers below the graphic refer to position with regard to the respective contig. The following contigs were used: Bovine, GenBank NW_001495216; Equine, GenBank NW_001867411; Swine, GenBank CU672252; Human, NW_001838915; Mouse, NW_001030791.

CXCL9, as predicted by SignalP (Bendtsen et al., 2004; Emanuelsson et al., 2007), aligned with the predicted cleavage sites of the other CXCL9 sequences, as shown in Fig. 1C.

The intron/exon organization of the bovine, equine, and swine genes was derived and is shown compared to human and mouse (Fig. 2). The analysis, performed using the NCBI program Spidey (Wheelen et al., 2001), reveals that the coding regions of all the genes consist of four exons spanning three introns. The bovine, equine, and swine genomic contigs used in this analysis were GenBank NW_001495216 (chromosome 6), NW_001867411 (chromosome 3), and CU672252 (chromosome 8), respectively.

As part of the US-VIRN project (www.vetimm.org), whose goal is to address the dearth of available reagents needed for immunology and infectious disease studies in livestock, poultry and fish, these genes are being expressed in Pichia and will be assessed for bioactivity as well as used to produce monoclonal antibodies. Because of the high level of similarity of these three sequences to the human CXCL9 protein backbone it is predicted that they will likely function similarly in inflammatory disease processes in ruminants, horses, and swine as has been shown for other species. The availability of the reagents will allow this to be evaluated experimentally.

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References


Larrubia, J.R., Benito-Martínez, S., Calvino, M., Sanz-de-Villalobos, E., Parra-Cid, T., 2008. Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection. World J. Gastroenterol. 14, 7149–7159.


