IgA in the horse: cloning of equine polymeric Ig receptor and J chain and characterization of recombinant forms of equine IgA

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INTRODUCTION

After the discovery of antibodies over a century ago, early studies in the horse made important contributions to the understanding of the mammalian adaptive immune system. However, large gaps still remain in our knowledge of the equine immunoglobulin (Ig) system and this is hampering development of specific vaccines and immune-based therapies for many major infectious diseases of the horse. Given the economic importance of the horse globally, it is vital to build a more detailed understanding of equine Ig function, as a key first step toward more effective options for treatment and prevention of equine diseases. A better understanding of the equine IgA (eqIgA) system would seem especially important given the numerous equine infections that are manifest in, or gain a foothold at, the mucosal surface. In addition, a wider knowledge of IgA systems in different mammals will provide invaluable insights into both the variety of functions mediated by this Ab class, and the evolution of the IgA system. Moreover, because there are limitations with mouse models of the IgA system (e.g., the mouse lacks the main Fc receptor (FcγRI) responsible for IgA effector function), it is worthwhile developing a wider knowledge of the IgA systems of other mammals so that relevant animal models may be identified. For these reasons, we sought to establish systems to facilitate molecular characterization of eqIgA.

IgA is present in both the serum and mucosal secretions of the horse, and it is the principal Ig in milk, tears, and secretions of the upper respiratory tract. In common with most other mammalian species, the horse has a single IgA heavy chain constant region (IGHA). In contrast, humans, along with chimpanzees, gorillas, and gibbons, express two subclasses of IgA (IgA1 and IgA2) encoded by distinct heavy chain constant region genes. In mucosal secretions, IgA exists primarily as secretory IgA (SIgA). Transepithelial transport of IgA onto the mucosal surfaces is mediated by the polymeric Ig receptor (pIgR), a type I transmembrane glycoprotein. The pIgR, which is expressed on the basolateral surface of epithelial cells, binds to IgA, which has been produced by plasma cells in mucosal effector sites. This IgA is polymeric (pIgA), comprising two or more IgA monomers joined together by an additional 17 kDa polypeptide, the J chain. On binding, both receptor and ligand are internalized and transcytosed across a series of vesicular compartments to the apical plasma membrane. Here, the extracellular portion of the pIgR is cleaved to form secretory component (SC), which

As in other mammals, immunoglobulin A (IgA) in the horse has a key role in immune defense. To better dissect equine IgA function, we isolated complementary DNA (cDNA) clones for equine J chain and polymeric Ig receptor (pIgR). When coexpressed with equine IgA, equine J chain promoted efficient IgA polymerization. A truncated version of equine pIgR, equivalent to secretory component, bound with nanomolar affinity to recombinant equine and human dimeric IgA but not with monomeric IgA from either species. Searches of the equine genome localized equine J chain and pIgR to chromosomes 3 and 5, respectively, with J chain and pIgR coding sequence distributed across 4 and 11 exons, respectively. Comparisons of transcriptional regulatory sequences suggest that horse and human pIgR expression is controlled through common regulatory mechanisms that are less conserved in rodents. These studies pave the way for full dissection of equine IgA function and open up possibilities for immune-based treatment of equine diseases.

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remains bound to pIgA as an integral part of the SIgA molecule. SC provides SlgA with increased resistance to bacterial proteases, and through its N-glycans mediates anchoring to the mucosal surface, which enhances the protective role of SlgA. SlgA acts as the "first line of immunological defense" against mucosal infection mediating defense through (i) immune exclusion at the mucosal surface, (ii) pIgA-mediated neutralization of pathogens during the process of transcytosis, and (iii) pIgR-mediated excretion of IgA-containing immune complexes formed within the submucosal tissues. In addition, SlgA participates in antigen presentation and sampling within mucosal tissues, regulation of commensal bacteria, and maintenance of mucosal integrity and homeostasis. In the horse, mucosal IgA responses seem to have an important role in protection from upper respiratory tract pathogens such as equine herpesvirus-1, equine influenza virus, and Streptococcus equi.

The presence of J chain in pIgA is essential for interaction with pIgR and, in turn, delivery of pIgA onto mucosal surfaces is dependent on pIgR-mediated transepithelial transport. Hence, J chain and pIgR are critical components of the mucosal IgA system. Proteins consistent with the properties of an equine J chain and SC have been described, but neither these components nor their interaction with equine IgA has been well characterized. In this study, we describe the cloning of equine J chain and pIgR, the generation of recombinant forms of eqIgA and SC, and analysis of their interaction. Furthermore, through genomic sequence analysis we provide an insight into both the chromosomal location and gene arrangement of J chain and pIgR, and the potential factors involved in regulation of pIgR expression.

RESULTS
Equine J chain and pIgR complementary DNA (cDNA) clones
The equine J chain cDNA isolated has an open reading frame of 474 bp (Genbank accession no. GQ981317) (Figure 1). The first 22 amino acids are predicted to encode a leader sequence, suggesting that the mature protein begins at Gly23 and comprises 136 amino acids. The equine J chain amino acid sequence showed a high degree of identity to other mammalian J chains, with 79, 80, and 74% identity to human, cow, and mouse proteins, respectively (Figure 2a). It shows features typical of...
J chain such as a preponderance of acidic residues, a single conserved N-glycosylation site, and eight Cys residues, involved in intra- and interchain disulfide bond formation, conserved in all known mammalian J chain sequences. In common with a J chain protein isolated from reduced and alkylated equine IgM, the predicted amino acid sequence for mature equine J chain lacks methionine. Phylogenetic analysis (Figure 2b) showed equine J chain to segregate with other mammalian sequences but to form a separate branch, reflecting the fact that the family equidae is a member of the order perissodactyla, rather than the order artiodactyla (even-toed ungulates), which includes cattle, sheep, and pigs.

EqplgR cDNA comprised an open reading frame of 2,292 bp (Genbank accession no. GQ981318) (Figure 3a). The first 18 amino acids are predicted to encode an N-terminal leader sequence, indicating that the mature protein begins at Lys19 and comprises 746 amino acids. A comparison of eqplgR with those of other mammalian species (Figure 4) reveals a high similarity in amino acid sequence and structural organization. Similar to other plgRs, the eqplgR comprises an N-terminal extracellular region of five Ig-like domains (D1–5) and one non-Ig-like domain (D6), a short membrane spanning region, and a long C-terminal cytoplasmic tail. Furthermore, the Ig-like domains of eqplgR show the conserved Cys residues involved in intradomain disulfide bonds observed in plgRs from other species. The number of potential N-glycosylation sites differs between species and none of the sites are conserved in all species. There are four putative N-glycosylation sites in the eqplgR compared with seven in human, eight in mouse, four in rat, three in bovine, and two in rabbit plgR. The overall amino acid identity of the eqplgR with human, bovine, and mouse proteins is 65, 63, and 57%, respectively. However, regions with functional significance, such as those involved in interaction with IgA, and regions of the cytoplasmic domain that govern receptor endocytosis and trafficking are more highly conserved (see Figure 4). Phylogenetic analysis (Figure 3b) showed the eqplgR to cluster with the cow, pig, and dog plgR, but to form a separate branch, again illustrating phylogenetic distance from the even-toed ungulate family.

Purification and analysis of recombinant eqIgA (reqIgA)

An expression vector to drive expression of an α heavy chain (HC) comprising a mouse V_{H} domain followed by the Cα1, hinge, Cα2, and Cα3 domains of eqIgA was constructed. Transfection of mouse λ light chain (LC)-expressing CHO-K1 cells with this HC vector resulted in the expression of eqIgA, which bound its cognate antigen 3-nitro-4-hydroxy-5-iodophenylacetate (NIP) and was recognized by anti-mouse λ LC and anti-horse IgA antibodies. Analysis of affinity-purified reqIgA by size exclusion chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Figure 5a–c) was consistent with covalently stabilized monomers (H_{2}L_{2}). Despite the absence of a Cys residue within the CH1 domain with which to form a HC–LC disulfide bond, LC and HC dimers were not observed. However, unlike human IgA, three Cys residues are present within the hinge region of eqIgA, the most N-terminal of which may form a covalent bond with the LC. EqIgA purified from serum, which was included for comparison, ran predominantly as dimer with some monomer and higher polymer. This observation is consistent with previous reports that equine serum IgA is predominantly dimeric. Human IgA1 and IgA2 have two common N-glycosylation sites, one within the CH2 domain and the other within the C-terminal 18 amino acid tailpiece. These conserved N-glycosylation sites are present within eqIgA and treatment of recombinant monomeric eqIgA (reqmlgA) with N-glycanase to remove N-linked sugars (Figure 5c) resulted in a decrease in the size of the IgA HC, suggesting that these sites are occupied in eqIgA.

Analysis of recombinant eqSC (reqSC)

To produce a recombinant form of the plgR equivalent to SC, the eqplgR cDNA was truncated by introduction of a stop codon immediately after that encoding Asp590 in D6. A potential cleavage site has previously been identified here (see Figure 4) and used for the truncation of recombinant human and mouse plgR in earlier studies. Transfection of CHO-K1 cells with eqSC cDNA resulted in the secretion of eqSC protein into supernatant. Positive clones were selected on the basis of reqIgA binding capacity, providing preliminary evidence that the recombinant form of eqSC was able to bind its dIgA ligand. SDS-PAGE analysis revealed that purified reqSC had a molecular mass of approximately 75 kDa (Figure 6) and was recognized by a mouse anti-human SC antibody (Figure 6b), reqSC migrated slightly faster than the recombinant form of hSC (rhSC), probably because of differences in glycosylation, as mentioned above. The recognition of reqSC by concanavalin A (data not shown) confirmed its glycosylation status. Both reqSC and rhSC were able to bind reqdlgA (Figure 6c) and recombinant human dlgA1 (rhdlgA1; Figure 6d), consistent with earlier studies that have described the interspecies binding of SC and dlgA from human and other mammals.

Surface plasmon resonance analysis of dlgA–SC interactions

Injection of 20 nm of reqdlgA or rhdlgA1 over immobilized NIP–bovine serum albumin gave a response of ~400 RU with a variation between cycles of < 10 RU. Subsequent binding of reqSC to both the equine and human form of dlgA fitted a 1:1 Langmuir binding model (Figure 7a–c), in keeping with
Figure 3  Polymeric Ig receptor (pIgR) sequence analysis. (a) Nucleotide sequence and amino acid translation of equine pIgR. Start of the mature protein at Lys19 is highlighted and indicated by D1. The start of each of the extracellular domains, D1–6 and the transmembrane (TMB) and cytoplasmic (Cyt) domains are highlighted and labeled. Putative N-glycosylation acceptor sites are underlined. (b) Phylogenetic analysis of the amino acid sequences of various mammalian pIgRs. Accession numbers of the sequences used are as follows: human, X73079; cow, X81371; pig, AB032195; dog, AY081057; rabbit, X00412; mouse, NM_011082; rat, NM_012723; silver brushtail possum, AF091137; tammar wallaby, AF317205; chimpanzee, XM_514153; orangutan, CR859163; rhesus macaque, XM_001083307; chicken; XM_417977; African clawed frog, EF079076; Fugu, AB176853; and zebra fish, XM_689741. The sequence for common carp was from Rombout et al. 53
Figure 4  Alignment of the amino acid sequences of mammalian polymeric Ig receptor (pIgR). Accession numbers and species abbreviations for the sequences used are as follows: human (hu), X73079; cow (bo), X81371; pig (pig), AB032195; dog (dog), AY081057; rabbit (rab), X00412; mouse (mo), NM_011082; rat (rat), NM_012723; silver brushtail possum (bp), AF091137; and tammar wallaby (tw), AF317205. Cysteines involved in intradomain disulfide bonds are shown in bold. The Cys residue that forms a disulfide bond with IgA is highlighted. N-glycosylation acceptor sites are underlined. Complementarity determining region (CDR)-like loops in D1, secretory component (SC) cleavage site in D6, and regions involved in basolateral and androgen responsive-element (ARE) targeting and endocytosis are boxed and labeled.
the known 1:1 stoichiometry between human plgA and SC. Consistent with studies in other species, we found that eqSC was unable to bind to mlgA, regardless of whether it was equine or human. This finding supports early reports that a molecule consistent with SC associated only with high-molecular-weight species of IgA (> 350 kDa) in equine secretions.10 Equine SC bound to reqdIgA with a $K_D$ of $1.4 \times 10^{-9}$ M, consistent with earlier reports $^{18}$ of the binding of human plgA to SC from various species (human, bovine, and rabbit) with $K_D$ values ranging from $1.3 \times 10^{-9}$ to $3.2 \times 10^{-9}$ M. The slightly lower affinity of reqSC for reqdIgA was almost entirely because of a slower rate of association with the equine ($2.5 \times 10^7$ M$^{-1}$ min$^{-1}$) when compared with the human ($5 \times 10^7$ M$^{-1}$ min$^{-1}$) ligand.

Figure 5  Analysis of recombinant lgA. (a) Size exclusion chromatography (fast protein liquid chromatography (FPLC)) analysis of recombinant equine lgA affinity purified from supernatant of CHO-K1 cells transfected with heavy chain (HC) and light chain (LC) vectors, showing a single peak corresponding to monomeric lgA. (b, c) Immunoblot analysis of equine serum lgA (slgA) and recombinant monomer lgA (mlgA) probed with goat anti-equine lgA under nonreducing and reducing conditions, respectively. (d) Size exclusion chromatography (FPLC) analysis of recombinant equine lgA affinity purified from supernatant of CHO-K1 cells transfected with HC, LC, and J chain vectors, showing peaks corresponding to mlgA, dimer (dlgA), and larger polymers (plgA). (e, f) Immunoblot probed with goat anti-equine lgA and Coomassie stain of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, respectively, of affinity-purified recombinant equine lgA (rlgA) from cells transfected with HC, LC, and J chain, before size exclusion chromatography (FPLC) showing bands corresponding to mlgA, dlgA, and plgA, and after FPLC separation into mlgA (rmIgA) and dlgA (rdIgA) fractions. slgA is included for comparison. In a and d, minor peaks eluting at ~7 ml correspond to antibody aggregated during the purification process.

Figure 6  Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of recombinant equine secretory component (SC). (a) Gel stained with PageBlue. (b–d) Immunoblots probed with (b) mouse anti-human SC, (c) recombinant dimeric equine lgA (reqdIgA), and (d) recombinant human dIgA1 (rIgA1). For each panel, the recombinant form of human SC (rhSC) is shown in the left lane and recombinant equine SC (reqSC) in the right lane.
Preincubation of reqSC with polyclonal serum eqIGM was found to inhibit the binding of reqSC to immobilized reqdIgA (Figure 7d), indicating that reqSC can also bind to eqIGM with appreciable affinity.

Analysis of the equine J chain and eqplgR genomic DNA

Searches of the equine genome with equine J chain and eqplgR cDNA sequences identified sequences on Equus caballus chromosome 3 (ECA3) and ECA5, respectively. Human and mouse J chain and PIGR genes have been localized to HSA4 (Homo sapiens chromosome 4) and MMU5 (Mus musculus chromosome 5) and HSA1 and MMU1, respectively.19–22 Comparative mapping of the human, mouse, and equine genomes has aligned regions of HSA4 and MMU5 to ECA3 and regions of HSA1 and MMU1 to ECA523,24 providing support for our assignment of the equine genes. Close to the human PIGR gene on chromosome 1q31–q42 are the Fc receptors for IgG (FcyR), IgE (FceRI), and IgA/IgM (Fce/μR). We located a receptor homologous to human and mouse FcεRIII as well as the equine FcεRI γ-chain are located on ECA5, suggesting comparable arrangement of genes in the horse.

Exon number, length, and exon–intron boundaries of the equine J chain and PIGR genes bear a close resemblance to those of human and mouse,22,25–27 with the coding sequence of J chain distributed across 4 exons and that of plgR across 11 exons (Figure 8).

The proximal promoter region and exon 1 of the eqplgR gene were compared with human (Y08254), rat (AF039920), and mouse (U83426) sequences to identify conserved binding sites for transcription factors.28,29 DNA elements previously implicated in regulation of basal transcription of plgR mRNA were identified (Figure 9a), including an E-box motif, activator protein 2 binding site, and an inverted repeat motif in the proximal promoter region. Binding sites for inducible factors included a conserved interferon-sensitive response element in exon 1 and steroid-responsive elements, including an androgen responsive-element in exon 1 and a glucocorticoid DNA-responsive element in the proximal promoter region. Interestingly, two interferon-sensitive response elements approximately 100 bp upstream of the transcription start site are conserved between the horse and human but are lacking in the rodent plgR genes. As the interferon-sensitive response element motif is a binding site for the interferon regulatory factor family of cytokine-inducible transcription factors, these evolutionary differences of rodent from both human and horse may reflect differences in plgR regulation by proinflammatory cytokines such as interferon-γ and tumor necrosis factor.

Intron 1 of the eqplgR gene was compared with those of the human (accession no. X95880) and mouse (accession no. AB001489) to identify a conserved intronic enhancer (Figure 9b). This enhancer region contains at least seven target elements for DNA-binding factors.28,29 All seven elements were identified within intron 1 of the eqplgR, including conserved binding sites for tissue-specific (hepatocyte nuclear factor-1) and cytokine-inducible transcription factors (signal transducer and activator of transcription 6 and nuclear factor-κB).
**DISCUSSION**

SIgA provides a “first line” of immune defense, critical for the protection of mucosal sites that are vulnerable to attack by infectious microorganisms. In this study, we report the cloning of equine J chain and pIgR, thereby completing the cloning of all three specific components of equine SIgA, namely IgA HC, J chain, and SC. In addition, we document the first expression of these three components in recombinant form, with subsequent functional characterization.

The recombinant version of eqIgA appeared representative of its native counterpart and assembled as anticipated. An early study suggested that not all eqIgA molecules have covalent links between LC and HC, but this was not observed to be the case with reqIgA. It is possible that these different observations reflect allotype differences between the IgAs analyzed, given the example in mouse IgA, in which different allotypic forms differ in their ability to form H-L disulfide bonds. However, the degree of polymerization seems sufficient to promote dimerization when heterogeneously expressed with human IgA. However, the degree of polymerization
is variable, suggesting that subtle amino acid differences between species may influence the efficiency of interaction. Indeed, additional residues within the tailpiece and the Cα3 domain of IgA seem to have a role in polymerization. Through incorporation of equine J chain, rather than J chain from another mammalian species, our expression system offers the advantage of producing authentic versions of polymeric eqIgA, suitable for in-depth analysis from which reliable conclusions relevant to the horse may be drawn.

Separate to its role in IgA polymerization, J chain seems to be required for the binding of dIgA to pIgR through direct, noncovalent interactions involving a C-terminal loop comprising Cys109 to Cys134 (human numbering) and two other regions (boxed in Figure 2). These elements are observed to be well conserved in equine J chain.

Turning to the role of pIgR, the binding of dIgA to pIgR involves both covalent and noncovalent interactions. Three loops within pIgR D1 that are analogous to the complementarity determining regions of Ig variable domains are critical for noncovalent interaction with dIgA. After noncovalent binding between IgA and pIgR, a covalent bond is formed between the pIgR and one of the IgA monomer subunits. In the human system, the Cys residues involved in this covalent bond are Cys468 in the pIgR D5 and Cys311 within the Cα2 domain of IgA. These Cys residues are conserved in eqpIgR (Figure 4).
Of the HC domains of IgA, the Cα3 domain shows the highest degree of identity across species and seems to be the most important for noncovalent interaction with plgR. Motifs within the Cα3 domain of IgA required for plgR binding are centered on three regions. In human IgA these include a loop region comprising residues 402–410, adjacent residues 411–414, Lys377, and residues Pro440–Phe443, the so-called “PLAF” loop. In the horse, 15 of these 18 amino acids are conserved or highly conserved substitutions, suggesting a common mode of binding.

The plgR from certain species (human and cow) can bind and transport plgM as well as plgA, whereas plgR from rabbits and rodents transports only plgA. Our preliminary results with a polyclonal preparation of eqIgM suggest that plgR in the horse also binds plgM. However, further detailed studies, ideally using recombinant eqIgM, will be required to ascertain the precise affinity of eqSC for eqIgM.

In humans, transcytosis of plgR occurs in the absence of its IgA ligand, resulting in release of free SC into secretions in which it acts to inhibit the binding of bacteria and bacterial toxins to intestinal cells. In addition, free SC produced by bronchial epithelial cells has a regulatory role by complexing with and sequestering soluble interleukin-8. Thus, SC may form part of a feedback mechanism that downregulates interleukin-8–mediated recruitment of neutrophils to the airway and attenuates the inflammatory response. The presence of free SC in equine milk suggests that constitutive release of free SC into the secretions also occurs in horses. Given the upregulation of interleukin-8 and its role in promoting airway neutrophilia in horses with recurrent airway obstruction, it would be interesting to investigate the existence of this feedback mechanism in horses.

Efficient export of plgA into the secretions requires coordinated transcriptional regulation of plgR expression by a number of mediators. We found that many of the binding sites for transcription factors identified within the proximal promoter region, exon 1, and intron 1 of the human PIGR gene are conserved in the horse PIGR gene. These include the interferon-sensitive response element (exon 1), nuclear factor-κB (intron 1), and signal transducer and activator of transcription 6 (intron 1) sites that are required for interferon-γ, tumor necrosis factor, and interleukin-4–mediated upregulation of plgR mRNA. Thus, it seems likely that these cytokines similarly have a role in the horse, in regulation of plgR expression at sites of infection and inflammation.

The effector functions of equine IgA are not yet well characterized. However, evidence of strong opsonophagocytic activity, for example, against the important pathogen S. equi, suggests that equine IgA is able to mediate killing through FcR on phagocytes or through the complement pathway. Indeed, a receptor for eqIgA homologous to human CD89 (FcαRI) is readily detected in equine polymorphonuclear neutrophils and is able to bind equine serum IgA and SlgA. Interestingly, comparisons between human, bovine, and equine CD89 suggest that although their interaction sites for IgA are related, each has distinct features. Thus, to gain insights into this interaction in a particular species, it is essential to investigate the IgA and receptor in that species, rather than making assumptions based on studies in other species. The availability of reqIgA now opens up the possibility of both defining the precise interaction site on eqIgA Fc for eqCD89, and further elucidating the killing mechanisms that eqIgA is capable of triggering.

A mucosal IgA response in horses makes a key contribution to immunity against viral (equine influenza virus and equine herpesvirus-1) and bacterial (S. equi, Rhodococcus equi, Salmonella enterica, and Clostridium botulinum) infections. Our studies pave the way for development of pathogen-specific reqIgA suitable for therapeutic intervention in the horse, mirroring developments in the human system in which recombinant hIgAs targeting various bacterial, viral, and parasitic antigens are under analysis. Mucosal administration of reqIgA could prevent or treat equine infectious disease in which effective vaccines are unavailable or provide only partial protection, such as R. equi pneumonia in foals.

IgA also acts as an architect of the mucosal immune response by participating in antigen presentation to mucosal dendritic cells and induction of appropriate T-cell responses and immunological memory. In addition to a role in protection against pathogenic microorganisms, a significant role for IgA in shaping and regulating the population of commensal bacteria within the mucosa has been recognized. The availability of defined recombinant versions of eqIgA should now facilitate studies into these phenomena in the horse.

In conclusion, we have produced and characterized the first reqIgA to reflect the different molecular forms found in nature, namely polymeric and secretory eqIgA. These show both similarities with other mammalian IgA, and important species-specific distinctions. The latter underline the value of detailed investigations within particular species, and caution against simple extrapolation of findings in one species to another. The recombinant eqIgA, J chain, and SC described in this study are valuable sources of pure and homogenous material that can be used as reference proteins for the production and screening of equine-specific monoclonal antibody reagents. Furthermore, production of reqIgA of defined specificity and molecular form will permit delineation of the precise functions of IgA in equine immunity, and open up possibilities for development of pathogen-specific reqIgA suitable for therapeutic intervention in equine infectious disease.

METHODS

Cloning equine J chain and plgR. cDNA synthesized from equine ileal total RNA using the ImProm II Reverse Transcription System (Promega, Southampton, UK) was used as template for PCR amplification of J chain and plgR using degenerative primers based on the known nucleotide sequences from other mammalian species (detailed in Table 1). After sequencing of J chain and plgR PCR products, 5′/3′ RACE was carried out using total ileal RNA and a 5′/3′ RACE kit (Roche, Manheim, Germany). Forward and reverse primers (Table 1) were designed according to the 5′/3′ RACE sequences and the complete J chain and plgR coding sequences were amplified from cDNA. J chain and plgR PCR products were cloned, respectively, into pcDNA3.1 (Invitrogen, Paisley, UK) and pcDNA3.1/Hygro (Invitrogen) and sequenced as before. Amino acid sequence analysis was carried out using ClustalW (http://www.ebi.ac.uk/clustalw/index.html). Phylogenetic analysis was performed using the neighbor-joining method with 1,000 bootstrap replications available in MEGA3 molecular evolutionary genetics analysis software.
Expression of monomeric and dimeric equine IgA in CHO-K1 cells. Genomic DNA for the horse IGH A gene was amplified by PCR and subcloned as a BamHI fragment downstream of a mouse V H gene specific for NIP in the vector pcDNA3.1V Nip. To produce reqmIgA, CHO-K1 cells expressing a mouse λ LC specific for NIP were transfected with the IgA HC vector, using previously described protocols.\(^5\) Supernatant from individual resistant CHO clones was screened for dIgA production by dotblot on nitrocellulose, detecting bound reqIgA with HRP-conjugated goat anti-mouse λ LC (0.2 μg ml\(^{-1}\)) in phosphate-buffered saline with Tween 20.

Interaction of IgA with reqSC. Surface plasmon resonance experiments were carried out on a BiacoreX instrument (GE Healthcare, Little Chalfont, UK) with NIP-derivatized bovine serum albumin immobilized onto a CM5 chip. Either reqdIgA or hdlIgA (100 μl, 20 nm) was applied at a flow rate of 30 μl min\(^{-1}\); reqSC at concentrations up to 133 nm (100 μl, flow rate of 30 μl min\(^{-1}\)) was then applied and binding to the prebound IgA analyzed. To determine whether reqSC can bind eqIgM, the effect of preincubating reqSC with polyvalent serum eqIgM (0–200 μg ml\(^{-1}\))\(^5\) on its ability to bind to prebound reqdIgA was assessed. BLAevaluation 3.2 software (GE Healthcare) was used for data analysis.

Analysis of equine J chain and plgR genomic DNA sequences. Equine J chain and plgR cDNA sequences were used to search the equine genome (available at http://www.ncbi.nlm.nih.gov/Genbank) for corresponding genomic DNA sequences. The putative transcriptional start sequences and intron–exon arrangements were identified by comparison of cDNA and genomic sequences. Conserved transcription regulatory elements in the equine plgR gene were identified by comparison with human, mouse, and rat PIGR genes.

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DISCLOSURE

The authors declared no conflict of interest.

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