Veterinary Immunology and Immunopathology 146 (2012) 125–134

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Monoclonal antibodies to equine CD23 identify the low-affinity receptor for IgE on subpopulations of IgM$^+$ and IgG1$^+$ B-cells in horses

Bettina Wagner*, Julia M. Hillegas, Susanna Babasyan

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 25 December 2011
Received in revised form 12 February 2012
Accepted 13 February 2012

Keywords:
Monoclonal antibodies
IL-4 fusion protein
Boost of antibody responses
Low-affinity IgE receptor

ABSTRACT

CD23, also called FcεRII, is the low-affinity receptor for IgE and has first been described as a major receptor regulating IgE responses. In addition, CD23 also binds to CD21, integrins and MHC class II molecules and thus has a much wider functional role in immune regulation ranging from involvement in antigen-presentation to multiple cytokine-like functions of soluble CD23. The role of CD23 during immune responses of the horse is less well understood. Here, we expressed equine CD23 in mammalian cells using a novel IL-4 expression system. Expression resulted in high yield of recombinant IL-4/CD23 fusion protein which was purified and used for the generation of monoclonal antibodies (mAbs) to equine CD23. Seven anti-CD23 mAbs were further characterized. The expression of the low-affinity IgE receptor on equine peripheral blood mononuclear cells was analyzed by flow cytometric analysis. Cell surface staining showed that CD23 is mainly expressed by a subpopulation of equine B-cells. Only a very few equine T-cells or monocytes expressed CD23. CD23$^+$ B-cells were either IgM$^+$ or IgG1$^+$ cells. All CD23$^+$ cells were also positive for cell surface IgE staining suggesting *in vivo* IgE binding by the receptor. Two of the CD23 mAbs detected either the complete extracellular region of CD23 or a 22 kDa cleavage product of CD23 by Western blotting. The new anti-CD23 mAbs provide valuable reagents to further analyze the roles of CD23 during immune responses of the horse in health and disease.

1. Introduction

The molecular structure and function of CD23 have been extensively studied in humans and mice. Besides IgE, ligands of CD23 comprise CD21, MHC class II and the integrin α-chains CD11b and CD11c. CD23 is a type II membrane protein. The N-terminal part of CD23 is composed of the intracellular and transmembrane regions, while the extracellular region contains a C-terminal C-type lectin domain and three membrane-proximal repeats of 21 amino acids. Repeats form a coiled α-helical structure that results in formation of the receptor timer at the cell surface. The C-type lectin domain is responsible for IgE, CD21 and integrin binding. The MHC class II binding region is located in close proximity to the cell membrane. Proteolytic cleavage of membrane-bound CD23 generates a soluble form (sCD23) of 37 kDa and a range of degradation forms of lower molecular weight. All of these retain their lectin head group, can bind IgE and have pleiotropic cytokine-like activities (reviewed by Acharya et al., 2010).

In mice and humans, CD23 is expressed on subsets of B-cells, monocytes (Melewicz et al., 1982) and more weakly on other hematopoietic cells. The latter include T-cells, follicular dendritic cells, eosinophils, NK-cells, Langerhans cells and platelets (Armitage et al., 1989; Joseph et al., 1986; Grangette et al., 1989). In addition, intestinal epithelial cells (Yu et al., 2003) and bone marrow stroma cells (Fourcade et al., 1992) express the receptor. Alternative splicing at the transcriptional level results in two isoforms of the receptor, CD23a and CD23b, which differ in the first nine amino

* Corresponding author at: Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA. Tel.: +1 607 253 3813; fax: +1 607 253 3440.
E-mail address: bw73@cornell.edu (B. Wagner).

0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2012.02.007

acids of the N-terminal cytoplasmic region (Yokota et al., 1988). CD23a is constitutively expressed on resting B-cells and is majorly involved in IgE regulation (Yokota et al., 1988). CD23b plays a key role during transcytosis of IgE and IgE complexes in intestinal epithelial cells (Yu et al., 2003; Montagnac et al., 2005). CD23 expression is regulated by various stimuli such as IL-4, IL-13, IL-5, IL-9, GM-CSF, IFN-γ and CD40L or by infection with Epstein-Barr virus (Wang et al., 1991; Ewart et al., 2002; Rosenwasser and Meng, 2005).

Because of its multiple ligands CD23 plays important roles in the regulation of IgE production, B-cell survival and growth, T-cell and myeloid cell differentiation, as well as in antigen presentation (Gustavsson et al., 2000; Acharya et al., 2010; Platzer et al., 2011). IgE binding to membrane bound CD23 can inhibit the production of IgE in B-cells by a negative feedback loop (Gould and Sutton, 2008; Acharya et al., 2010). Similarly, the interaction of monomeric sCD23 with IgE decreases IgE synthesis in B-cells, while trimeric sCD23 simultaneously bound to IgE and CD21 enhances IgE synthesis by activated B-cells (Hibbert et al., 2005; McCloskey et al., 2007). Other cytokine-like activities mediated by CD23 binding to CD21 or integrins affect B-cell compartments by sustaining growth of activated mature B-cells, promoting differentiation of plasma cells and increasing B-cell precursor survival. In addition, sCD23 promotes differentiation of myeloid precursors, thymocytes and bone marrow $CD4^+$ cells (reviewed by Acharya et al., 2010 and Platzer et al., 2011). Finally, binding of sCD23 to CD11b/CD18 and CD11c/CD18 on monocytes increases the production of inflammatory cytokines and activates nitric oxide production (Platzer et al., 2011). Because of its roles in lymphocyte survival and cytokine release, sCD23 has been associated with chronic lymphocytic leukemia (Hallek and Pflug, 2010) and autoimmune inflammatory conditions in humans, such as systemic lupus erythrematodes (Bansal et al., 1992) and rheumatoid arthritis (Bansal et al., 1994; Massa et al., 1998). Consequently, CD23 has been a target as diagnostic marker in rheumatoid arthritis (Huissoon et al., 2000; Ribbens et al., 2000) and a humanized monoclonal anti-CD23 antibody was tested for therapeutic interventions in patients with leukemia or atopic disorders (Poole et al., 2005; Byrd et al., 2010).

Much less is known about CD23 expression and its regulatory functions on the immune system of the horse. The nucleotide sequence of equine CD23 was described previously (Watson et al., 2000) and mRNA transcripts were found in PBMC and alveolar macrophages (Jackson et al., 2004). CD23 was assumed to be expressed on a subpopulation of equine B-cells because cell surface bound IgE was detected on peripheral B-cells of adult horses (Wagner et al., 2003).

Here, our goal was to produce monoclonal antibodies (mAbs) to equine CD23 and to identify the cell populations in peripheral blood that express the low-affinity IgE receptor in horses. Because of the unusual orientation of the receptor with its C-terminal part representing the extracellular region, we used a novel IL-4 fusion protein system to express equine CD23 in eukaryotic cells. IL-4, formerly known as B-cell stimulating factor (Paul, 1987), initiates B-cell differentiation and immunoglobulin class switching which then results in formation of antigen-specific memory B-cells and IgG-secreting plasma cells (Siebenkotten et al., 1992). We assumed that an IL-4/CD23 fusion protein would boost the immune response to CD23 during immunization and increase the number of equine CD23-specific hybridoma clones during mAb production.

2. Material and methods

2.1. IL-4 expression vector

An expression vector containing equine IL-4 (411 bp) and a sequence encoding an enterokinase digestion site (EK; 24 bp) was generated using the mammalian expression vector pcDNA3.1 (−)/Myc-His, version B (Invitrogen, Carlsbad, CA, USA). The IL-4 expression vector allowed cloning of the gene of interest into the multiple cloning site (MCS) downstream of the IL-4/EK sequence for expression of *r*IL-4 fusion proteins (Fig. 1A). The complete equine IL-4 gene (Genbank Accession GU139701) without stop codon was amplified in two steps with (1) primers containing a *NotI* restriction site (forward – 5′-gcggccgcatgggtctcacctaccaactg-3′) and the partial EK sequence (reverse – 5′-cgtcgtacagatcacacttggagtatttctctttc-3′) and (2) the same forward primer together with a primer for the complete EK sequence with a *BamHI* restriction site (reverse 2 – 5′-ccggatccttatcgtcatcgtcgtacagatc-3′).

2.2. Cloning of equine CD23

For the initial PCR to amplify the complete coding region of the equine CD23 gene (Genbank Accession AF141931, bases 199–1179) from PBMC primers flanking the 981 bp coding region of equine CD23 were used (forward – 5′-atggaggaacatgcatactcag-3′; reverse – 5′-tcagcacgtgaccagccggtc-3′). The resulting PCR product was cloned into pCR4 TopoBlunt (Invitrogen, Carlsbad, CA, USA) as previously described (Wagner et al., 2005). In a second PCR, the CD23/pCR4 plasmid was used as template to amplify the 831 bp extracellular region of CD23 without the stop codon (bases 346–1176 of AF141931). Primers for the second PCR (forward – 5′-ggcggatccggagactgtgcagaagctgaaac-3′, reverse – 5′-cgcgaagcttgggcacgtgaccagccggtcacac-3′) contained *BamHI* and *Hind III* restriction sites (underlined) for cloning of the extracellular region of the CD23 gene into the IL-4 expression vector (Fig. 1B). A partial digestion step was used for expression cloning of the 831 bp CD23 fragment because an internal *BamHI* restriction site. The sequence of the cloned extracellular region of the CD23 gene was 100% homologous to Genbank Accession AF141931 and contained the complete coding sequence for the C-terminal lectin domain responsible for CD23 ligand binding.

2.3. Transfection and protein purification

Transfection of Chinese Hamster Ovary (CHO) cells and generation of a stable *r*IL-4/CD23 transfectant were performed as previously described for other fusion proteins (Wagner et al., 2005). Expression of *r*IL-4/CD23 was