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Horse cytokine/IgG fusion proteins – mammalian expression of biologically active cytokines and a system to verify antibody specificity to equine cytokines

Bettina Wagner[a,*,1], Jennifer Robeson[a], Megan McCracken[a], Eva Wattrang[b], Douglas F. Antczak[a]

[a]*Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Hungerford Hill Road, Ithaca, NY 14853, USA*
[b]*Swedish University of Agricultural Sciences, Section of Veterinary Immunology and Virology, Department of Molecular Biosciences, 751 23 Uppsala, Sweden*

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Abstract

Recombinant cytokines are valuable tools for functional studies and candidates for vaccine additives or therapeutic use in various diseases. They can also be used to generate specific antibodies to analyze the roles of different cytokines during immune responses. We generated a mammalian expression system for recombinant cytokines using the equine IgG1 heavy chain constant region as a tag for detection and purification of the expressed cytokine, demonstrated here using equine interferon-gamma (IFN-γ), interleukin-2 (IL-2), interleukin-4 (IL4) and transforming growth factor-β1 (TGF-β1). The resulting IgG1 fusion proteins were composed of the C-terminal heavy chain constant region of the IgG1 (IgGa), and the N-terminal cytokine replacing the immunoglobulin heavy chain variable domain. The fusion proteins were expressed in CHO cells as dimers and their structures had similarity to that of IgG heavy chain antibodies. In contrast to other tags, the IgG1 heavy chain constant region allowed the selection for clones secreting high levels of the recombinant protein by a sensitive ELISA. In addition, the IgG1 heavy chain constant region facilitated identification of stable transfectants by flow cytometry and the secreted recombinant fusion protein by SDS-PAGE and Western blotting. To recover the cytokine from the IgG1 fusion partner, an enterokinase cleavage site was cloned between the cytokine gene and the immunoglobulin heavy chain constant region gene. The purification of the fusion protein by protein G affinity columns, the enterokinase digestion of the cytokine from the IgG1 heavy chain region after or during purification, and the biological activity of the cytokine within the fusion protein or after its isolation was demonstrated in detail

Abbreviations: CHO cells, Chinese hamster ovary cells; ELAW, equine leukocyte antigen workshop; IGHG1, immunoglobulin heavy chain gamma 1 constant gene, encoding the IgG1 heavy chain constant region; IFN-γ, interferon-gamma; IL-2, interleukin 2; IL-4, interleukin 4; MDBK cells, Madin-Darby bovine kidney cells; MHC, major histocompatibility complex; PVDF, polyvinylidene difluoride; TGF-β1, transforming growth factor β1

* Corresponding author. Tel.: +1 607 256 5660; fax: +1 607 256 5608.
E-mail address: bw73@cornell.edu (B. Wagner).
[1] Bettina Wagner is on a leave from the Immunology Unit, Hannover School of Veterinary Medicine, Bischofsholer Damm 15, 30173 Hannover, Germany.

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for equine IFN-γ/IgG1 by up-regulation of major histocompatibility complex (MHC) class II expression on horse lymphocytes. Biological activity could also be confirmed for the IL-2 and IL-4/IgG1 fusion proteins. To test the crossreactivity and specificity of anti-human TGF-β1, and anti-bovine and anti-canine IFN-γ antibodies to respective horse cytokines, the four cytokine/IgG1 fusion proteins were successfully used in ELISA, flow cytometry and/or Western blotting. In summary, equine IgG1 fusion proteins provide a source of recombinant proteins with high structural and functional homology to their native counterparts, including a convenient system for selection of stable, high expressing transfectants, and a means for monitoring specificity of antibodies to equine cytokines.

Keywords: IgG; Fusion protein; Mammalian expression; Recombinant cytokine; Anti-cytokine antibodies; MHC class II; Horse

1. Introduction

During the past decade recombinant equine cytokines have been generated using expression systems employing bacteria (Vandergrifft and Horohov, 1993; Steinbach et al., 2002; Hines et al., 2003), baculoviruses (McMonagle et al., 2001; Wu et al., 2002), and mammalian cells (Vandergrifft and Horohov, 1993; Dohmann et al., 2000; McMonagle et al., 2001; Cunningham et al., 2003). In general, bacterial expression results in high expression levels of the recombinant protein, but it can also result in loss of biological activity, due to differences in protein folding and the lack of glycosylation in bacterial cells. In contrast, mammalian expression provides recombinant proteins with a high similarity to native proteins with regard to their structure and biological functions. Compared with other expression systems, the disadvantage of the mammalian system is the considerably lower amount of recombinant protein produced (Wagner et al., 2002a). Thus, a mammalian expression system is often the method of choice, particularly if only a small amount of the recombinant protein with high similarity to the native molecule is needed, e.g. for functional testing or for the generation of monoclonal antibodies.

However, none of the recombinant cytokines described above have been purified in sufficient concentrations from transfectants, and consequently neither purified equine cytokines nor anti-horse cytokine antibodies are available thus far. The only exception is a single anti-equine IFN-γ antibody. This monoclonal antibody was produced using a synthetic peptide of 40 amino acids, corresponding to the predicted N-terminus of the mature equine IFN-γ as antigen for immunization (Hines et al., 2003). All other reagents which have been described as recognizing selected horse cytokines are crossreactive antibodies from other species (Theoret et al., 2001; Pedersen et al., 2002).

Nevertheless, to obtain optimal expression rates in mammalian cells and to establish stable transfectants, the screening system is very important for the success of the procedure. Without a system to identify rare, high producing clones, many stable mammalian transfectants tend to produce only low amounts of the recombinant protein. Many of the currently available commercial mammalian expression systems offer various tags which are expressed together with the recombinant protein for its detection by FACS or Western blotting. These are adequate systems, but require higher numbers of cells, and thus testing can usually only be performed for a limited number of clones. Faster and more efficient screening systems, such as a specific ELISA, that enable the screening of hundreds of cell clones in a short time and at an early stage of the selection or cloning procedure, increase the chances to select cell clones for high expression of the recombinant protein. An ELISA requires antibodies specific for at least two different epitopes of the recombinant protein. This is not the case for most cytokines and for many other proteins of immunological interest of the horse or many other domestic animals.

Here, we generated a mammalian system to express proteins of immunological interest of the horse as IgG1 fusion proteins. Similar fusion proteins, composed of human IL-2/IgG1 (Landolfi, 1991) or IL-2/IgG2 (Barouch et al., 2000) were previously expressed in mammalian cells. The IL-2/IgG fusion protein mediated the specific effector functions of both the IL-2 and the IgG molecules in vitro (Landolfi, 1991) and