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Journal of Immunological Methods 298 (2005) 93–104

JIM
Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

Prolonged and increased expression of soluble Fc receptors, IgG and a TCR-Ig fusion protein by transiently transfected adherent 293E cells

Gøril Berntzen*, Elin Lunde[1], Morten Flobakk[1], Jan Terje Andersen[1], Vigdis Lauvrak, Inger Sandlie*

Department of Molecular Biosciences, University of Oslo, P.O.Box 1041, 0316, Oslo, Norway

Received 12 July 2004; received in revised form 13 December 2004; accepted 14 January 2005
Available online 4 March 2005

Abstract

In studies of the relation between structure and function of proteins of the immune system, there is a continuous need for screening of a large number of protein variants. To optimise the yield following transient gene expression in small or medium culture volumes, several parameters were investigated. First, secretion levels of a soluble form of human Fcγ receptor IIA (FcγRIIA) were measured after transfection of 293, 293E, 293T as well as COS-7 cell lines. The transgene was under cytomegalovirus (CMV) promoter control on the expression vector pcDNA3, which also contains an SV40 origin of replication (SV40 ori). All 293 cell lines secreted more protein than COS-7 cells. Introduction of the Epstein Barr virus (EBV) origin of replication (oriP) greatly increased the protein expression from the 293E cells, both the amount of protein produced per day and the duration of production. At best, 293E cells secreted fully functional protein for 3–4 weeks provided supernatant was harvested every 2–3 days followed by medium replacement. This method was then used for expression of soluble forms of human FcγRI, FcγRIIB, the human neonatal Fc receptor (FcRn), a T cell receptor (TCR)-immunoglobulin (Ig) fusion protein, and human IgG3. With an initial culture volume of 5 ml, the yield was approximately 200 μg for FcγRIIA, 1.5 μg for FcγRI, 5 μg for FcRn, 20 μg for FcγRIIB, 40 μg for the TCR-Ig fusion protein and 850 μg for IgG3. Culture expansion during the 3 weeks of culture further increased the yield. Protein yield was also improved by scaling up the initial volume. This approach can provide sufficient amounts of protein for screening experiments, and in the

Abbreviations: FcγR, Fcγ receptor; FcRn, Neonatal Fc receptor; TCR, T-cell receptor; Ig, Immunoglobulin; T-Ag, T-antigen; SV40 ori, SV40 origin of replication; EBV, Epstein Barr virus; EBNA1, Epstein Barr virus nuclear antigen 1; oriP, EBV origin of replication; CMV, Cytomegalovirus; GST, Glutathione S-transferase; hβ2m, Human β_2 microglobulin; ELISA, Enzyme-linked immunosorbent assay; HRP, Horse radish peroxidase.

* Corresponding authors. Tel.: +47 22 85 46 34; fax: +47 22 85 60 41.
E-mail addresses: goril.berntzen@bio.uio.no (G. Berntzen), inger.sandlie@bio.uio.no (I. Sandlie).

[1] Contributed equally.

0022-1759/$ - see front matter
doi:10.1016/j.jim.2005.01.002

case of antibody, milligrams of recombinant protein for extensive structural analysis can be obtained from one single transient transfection. The approach should be of interest to laboratories that do not have access to a bioreactor but still have a requirement for reasonable amounts of protein to be produced in an easy and cost-effective manner.

Keywords: Prolonged expression; Transient transfection; 293E cells; Lipofectamine; OriP; EBNA1

1. Introduction

Efficient expression of cloned genes is crucial for the elucidation of protein structure and function. Various host cell systems have been developed to express genes of general and immunological interest. The conserved disulfide bridge found in each Ig-domain in members of the Ig superfamily, as well as the presence of unpaired cysteines in several members, considerably reduce the yield of functional protein after bacterial production, denaturation and refolding. Mammalian cells are often chosen when correct protein folding and post-translational modifications such as glycosylation and sulfation are required. However, stable mammalian expression systems based on chromosomal integration of foreign DNA are time-consuming. Therefore, transient gene expression in mammalian cells is becoming increasingly important for the rapid production and functional screening of recombinant proteins. Cationic liposomes, such as LipofectAMINE™ (lipofectamine) and FuGENE™ (fugene), have been shown to mediate transient transfection of DNA with high efficiency and reproducibility into a wide variety of eukaryotic cells (Smith et al., 1993; Zhu et al., 1993; Felgner et al., 1994; Radler et al., 1997; Min et al., 2003; Zhang et al., 2003), and are especially suited for high-throughput efforts where large numbers of transfections are required. To collect sufficient levels of proteins for extensive functional and structural analysis following standard transfection methods, large culture volumes of costly transfection reagent are required. Therefore, optimising the protein yield obtained from one single transfection is of great interest.

In general, the African green monkey kidney COS-7 cell line (SV40 transfected) and the human embryonic kidney 293 cell line (adenovirus transfected) are widely used for recombinant protein production (Durocher et al., 2002). COS-7 cells express the SV40 large T-antigen (T-Ag) that allows episomal amplification of plasmids containing the SV40 ori (Mellon et al., 1981; Chittenden et al., 1991), and so does the 293 genetic variant 293T (Heinzel et al., 1988). The 293 genetic variant 293E expresses EBV nuclear antigen 1 (EBNA1). Plasmids that contain oriP support increased protein expression in cells expressing EBNA1. This increased expression, however, is likely due to the combined effect of the EBNA1-dependent enhancer activity of oriP (Reisman and Sugden, 1986) and the nuclear localization signal in EBNA1 (Ambinder et al., 1991; Langle-Rouault et al., 1998), rather than increased plasmid replication as observed in COS and 293T cells. Moreover, the CMV promoter (Foecking and Hofstetter, 1986) is particularly powerful in all 293 genetic variants where it is trans-activated by the constitutively expressed adenovirus E1a protein (Gorman et al., 1989).

We have studied the effect of a combination of SV40 ori/T-Ag in COS-7 and 293T cells and a combination of oriP/EBNA1 in 293E cells on expression of several genes under the control of the strong constitutive CMV promoter, following lipofectamine induced transfection. Initially, we studied FcγRIIA (CD32A) expression and showed that oriP/EBNA1 in 293E cells was the most efficient expression system, and that secretion of protein peaked 5–14 days after transfection of 293E, but proceeded for up to 26 days. Furthermore, we have shown that frequent medium replacements and also culture expansions lead to an additional increase in protein yield. Finally, the expression level was investigated for another five proteins, namely soluble forms of FcγRI (CD64), FcγRIIB (CD32B), and FcRn, as well as a TCR-Ig fusion protein and IgG3. In all cases, inclusion of oriP in the expression plasmid improved the expression level and also the duration of protein production. The results demonstrate that adherent 293E cells may be