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Review

Review of the recombinant human interferon gamma as an immunotherapeutic: Impacts of production platforms and glycosylation

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ABSTRACT

Human interferon gamma is a cytokine belonging to a diverse group of interferons which have a crucial immunological function against mycobacteria and a wide variety of viral infections. To date, it has been approved for treatment of chronic granulomatous disease and malignant osteopetrosis, and its application as an immunotherapeutic agent against cancer is an increasing prospect. Recombinant human interferon gamma, as a lucrative biopharmaceutical, has been engineered in different expression systems including prokaryotic, protozoan, fungal (yeasts), plant, insect and mammalian cells. Human interferon gamma is commonly expressed in *Escherichia coli*, marketed *as* ACTIMMUNE®, however, the resulting product of the prokaryotic expression system is unglycosylated with a short half-life in the bloodstream; the purification process is tedious and makes the product costlier. Other expression systems also did not show satisfactory results in terms of yields, the biological activity of the protein or economic viability. Thus, the review aims to synthesise available information from previous studies on the production of human interferon gamma and its glycosylation patterns in different expression systems, to provide direction to future research in this field.

Abbreviations: APCs, antigen-presenting cells; BIIC, Baculovirus-infected insect cells; CHO, Chinese hamster ovary; CC, codon context; CoG, cost of goods; DCW, dry cell weight; FDA, Food and Drug Administration; HCDC, high cell density cultivation; HCV, hepatitis C virus; HEK293, human embryonic kidney 293; hIFNγ, human interferon gamma; ICU, individual codon usage; IFN, interferon; IFNGR, interferon gamma receptor; IFNGR-α, Interferon gamma receptor alpha; IFNGR-β, interferon gamma receptor beta; IFNAR, interferon-α/β receptor; IFNG, interferon gamma gene precursor; IGRA, interferon gamma release assays; IRF-1, interferon regulatory factor 1; ISGs, IFN-stimulated genes; IU, international unit; JAK, Janus kinase; LTB, latent tuberculosis; MS, multiple sclerosis; NK cells, natural killer cells; NKT cells, natural killer T cells; PARP, poly (ADP-ribose) polymerase; STATs, signal transducers and activators of transcription; SVR, sustained virological response; T_h1, T helper cell type 1; TB, tuberculosis; TM, transgenic mice; TNFα, tumour necrosis factor alpha.

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Contents

1. Introduction

According to European Union regulations definition, Biopharmaceuticals are proteins or nucleic acid constituents which are formulated using biotechnological approaches for therapeutic *in vivo* use (Borden et al., 2007). Many substances including vaccines, enzymes, antibodies and antibiotics have been commercialised under the biopharmaceutical term, among them, interferons (IFNs) are noticeable due to their therapeutic importance against a wide variety of diseases (see Section 5.1) (Borden et al., 2007; Meager, 2006; Samuel, 2001).

Interferons are macromolecules which were discovered separately by two research groups in the 1950s and named after the aptitude of these molecules to interfere with viral replication of the flu virus in infected cells (Fensterl and Sen, 2009). In the following decades, IFNs have been studied in fine detail including the mechanisms of transcriptional induction, their antiviral properties, mode of action, viral countermeasures and therapeutic applications against a range of diseases (see Section 5.1) (Fensterl and Sen, 2009; Marciano et al., 2004). Subsequently, efforts for cloning and expression of *IFN* genes were carried out in many different protein production systems *viz*., *Escherichia coli*, mammalian cells, yeasts, protozoan and transgenic plants, but only *E. coli* expression systems were at the centre of attention due to high productivities (Chen et al., 2011).

The main IFN genes (α, β, and γ) have been predominantly expressed in *E. coli* at industrial scale. These products have been approved by the FDA (Food and Drug Administration, USA) and are marketed under trade-names of ROFERON-A®, ALFERON-N®, INFERON-A®, and AVENOX® (exceptionally produced in Chinese hamster ovary cells) for human IFNα, BETASERON® for human IFNβ and both ACTIMMUNE® and γ-IMMUNEX® for human IFNγ (hIFNγ) (see Section 5.1) (Jonasch and Haluska, 2001; Panahi et al., 2012).

Notwithstanding the importance of hIFNγ and the presence of many articles about this biopharmaceutical, no review has specifically dealt with the expression of hIFNγ in different host cells. Thus, the objective of this review is to synthesize outcomes of previous efforts on the whole process of expression, optimisation and purification of hIFNγ in different host cells, and the effect of expression host on glycosylation patterns, in order to discern which protein production system might be more desirable for future studies and applications *e.g.* cancer immunotherapy.

2. Overview on interferons

Interferons are cytokines which are expressed by a diverse group of genes and have been cloned from different vertebrates including mammals, birds, fish and even amphibians (Qi et al., 2010). Translated proteins of these genes generally vary in size between 165 and 208 amino acids and the protein moieties are further modified by post-translational glycosylation. IFNs are produced in reaction to viral infections harnessing host cells to non-specifically inhibit viral replication (Samuel, 2001; Takaoka and Yanai, 2006). Mammalian IFNs are broadly classified into three groups, according to amino acid sequence homology and their receptors:

Type I IFNs, also known as viral IFNs, as they are induced by viral infection, contain many subtypes of IFNα (13 in humans originating from leukocytes), one IFNβ (originating from fibroblasts), IFNω (originating from leukocytes), IFNτ (originating from ovine trophoblasts), IFNε, IFNκ and IFNζ. All type I IFN genes are located in a cluster on human chromosome 9 and all interact with the heterodimeric IFNα/β receptor (IFNAR) (Jonasch and Haluska, 2001; Samuel, 2001).

Type II IFNs, also known as immune IFNs, are represented solely by IFNγ, which is distinctly dissimilar to other IFNs and uses a distinct heterodimeric IFNγ receptor (IFNGR) (Samuel, 2001; Takaoka and Yanai, 2006). This type of IFN is induced by either IFNα and β (in the case of viral infection) or IFNγ (in the case of mitogenic or antigenic stimuli) (Samuel, 2001). IFNγ proteins show similar biological activities inherent also to other IFNs; but has the advantage of being 100–10,000 more active as an immuno-modulator than the other IFNs (Farrar and Schreiber, 1993).

Type III IFNs, have lately been identified, containing IFNλ 1, 2, and 3, previously known as Interleukin 29, 28A, and 28B, respectively (Vilcek, 2003). Their genes are located in a cluster on human chromosome 19 and use the heterodimeric IFNλ receptor IL10R2/IFNLR1 (Fensterl and Sen, 2009). This type of IFN is induced directly by viruses or stimulated with IFNα or λ, thus, they are identified as IFN-stimulated genes (Ank et al., 2006).