RECOMBINANT HUMAN INTERFERON-BETA: CURRENT PERSPECTIVES

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ABSTRACT

Recombinant human interferon-beta (rhIFN-β) is a drug of choice for the treatment of multiple sclerosis and is also a potential candidate for the treatment of several other diseases in humans. However, there are several issues associated with the production of this therapeutic protein. In this review, we have presented the current perspectives on rhIFN-β. In the early part of this review, we have discussed about the characteristics of human IFNs, with a major emphasis on hIFN-β, followed by a brief discussion on the clinical applications of rhIFN-β. The later part of the review elaborates pros and cons of various expression systems used to produce rhIFN-β and various assays used to characterize the properties of recombinantly produced hIFN-β. Understanding the intricacies of the available production and characterization systems for rhIFN-β will have tremendous commercial impact. This may lead to cost effective generation of this therapeutic protein which will benefit both manufacturers and end users (patients).

KEYWORDS: recombinant protein; interferon-β; expression systems, E. coli, inclusion bodies.

INTERFERONS

Cytokines are a family of secreted proteins that act as a communication link between various cells and also their surrounding environment (Chelbi-Alix and Wietzerbin, et al. 2007). Various cytokines include chemokines, lymphokines, monokines, interleukins, tumor necrosis factor, and interferons (IFNs) (Chelbi-Alix and Wietzerbin, et al. 2007). IFNs are glycoproteins in nature and are normally produced in the body in response to infections.
(Tayal and Kalra 2008; Zilberstein, et al. 1986). In 1957, Alick Isaac and Jean Lindenmann discovered IFN while studying the effect of influenza virus on the chorioallantoic membrane of chicken egg, where they observed “interference” on the growth of this virus by IFN (Isaac and Lindenmann, 1957; Chelbi-Alix and Wietzerbin, 2007). Structurally, IFNs possess four-helical bundle topology and belong to hematopoietic growth factor family (also known as helical cytokine family) (Karpusas, et al. 1997; Pestka, et al. 2004).

IFNs act as vital regulatory mediators of the immune response and have been implicated in the defence mechanisms against a variety of infections (Smeekens, et al. 2013). They are produced by almost every cell type in the body, including the cells of immune system, when stimulated by infectious agents (Leader, et al. 2008; Pestka, et al. 2004; Taniguchi, et al. 1980; Chevaliez and Pawlotsky, 2007). IFNs are also produced by certain tumour cells (van Beers, et al. 2011). IFNs exert a myriad of physiological effects including antiviral and antitumor activities. It has been suggested that the anti-tumor properties of IFNs are due to the combination of direct (anti-proliferative) and indirect (immune-mediated) effects (Mager, et al. 2003; Runkel, et al. 2000; Borden, et al. 2007; Gutterman, 1994; Baron, et al. 1991; Karpusas, et al., 1998). Also, IFNs have been shown to exert a strong impact on the metabolism, differentiation and homeostasis of a wide variety of cells (Stefan, et al. 2009; Borden, et al. 2007; Tayal and Kalra 2008; Runkel, et al. 2000).

In the last five decades, there has been a major advancement in biomedicine due to the discovery and molecular understanding of the cellular mechanism and clinical use of IFNs (Borden, et al. 2007). Different IFNs possess a low sequence homology across the species (in terms of their amino acid sequences) and exhibit a range of similar, but not identical biological activities, suggesting species-specific action of different IFNs (Chelbi-Alix and Wietzerbin, 2007). For this reason, even when there is 70% homology between mouse and human IFNs, direct use of human IFNs in mice studies is not advisable (Davis, et al. 1985).

**Classification of IFNs**

Based on their amino acid sequence, physical and biological properties and receptor specificity, different IFNs are classified into three subfamilies (Fig. 1).

**Type I IFNs subfamily**

This subfamily of IFNs contains eight members and out of which only 5 members are reported in humans (Chevaliez and Pawlotsky, 2007; Pestka, et al. 2004). The genes encoding
for type I IFNs are located on chromosome 9p in humans (Pestka, et al. 2004). IFN-alpha (IFN-α) is a 165 amino acids long leukocyte interferon and 13 IFN-α genes, which produce 13 distinct although structurally related IFN-α sub-type proteins, are reported (Rudick and Goelz, 2011; Allen and Diaz, 1996). IFN-beta (IFN-β) (a fibroblast interferon) and IFN-omega (IFN-ω) are 166 and 172-174 amino acids long polypeptides, respectively (Zilberstein, et al. 1986; Rudick and Goelz 2011; Taniguchi, et al. 1980; Capon, et al. 1985, Hauptmann and Swetly, 1985; Adolf, et al.1990). IFN-kappa (IFN-κ) (a keratinocyte-derived IFN) exhibits constitutive expression in many tissues, whereas IFN-epsilon (IFN-ε) is mostly expressed in the placenta and fetal membranes (LaFleur, et al. 2001; Kotenko and Langer 2004). Other type I IFN subfamily members (IFN-delta (IFN-δ), IFN-tau (IFN-τ) and IFN-zeta (IFN-ζ)) are not reported in humans (Chelbi-Alix and Wietzerbin, 2007; Borden, et al. 2007). At amino acid level, IFN-α and IFN-β share 30% sequence similarity while the sequence similarity between IFN-α and IFN-ω is up to 60% (Taniguchi et al. 1980; Karpusas, et al. 1997). Despite their closeness in their structures, type I IFNs exert overlapping and differential physiological effects (Brierley and Fish, 2002; Parker, et al. 2016). Thus, because of their beneficial properties, type I IFNs have been implicated for various therapeutic applications in humans (Parker, et al. 2016; Goldstein and Lazlo, 1988; Leader, et al. 2008; Hartung, 2014; Rudick and Goelz, 2011; Seddighzadeh, et al. 2014).

Type II IFNs subfamily

Type II IFNs are predominantly produced by the cells of immune systems and are also referred to as ‘immune IFNs’ (Schroder, et al. 2004; Gray and Goeddel 1982; Chevaliez and Pawlotsky, 2007; Pestka, et al. 2004; de Maeyer and Maeyer-Guignard, 1998). IFN-gamma (IFN-γ) is the only member of this subfamily. In humans, IFN-γ is encoded by a gene located

**Type III IFNs subfamily**

The subfamily of type III IFNs also contains only one member, IFN-lambda (IFN-λ) (Kotenko and Langer, 2004; Chevaliez and Pawlotsky. 2007; de Maeyer and Maeyer-Guignard, 1998). Humans express three sub-types of IFN-λ; IFN-λ1, IFN-λ2 and IFN-λ3 (Kotenko and Langer, 2004, Sheppard, et al. 2003). Recently, a fourth IFN-λ sub-type (IFN-λ4) has been reported in humans, which is expressed only in a small fraction of the human population (Hermant and Michaels, 2014). In humans, genes encoding for IFN-λ1, -λ2 and -λ3 are located on chromosome 19 (Hermant and Michaels, 2014). Type III IFNs are found to be co-produced with IFN-β, however, it is observed that the antiviral effect elicited by both these IFNs depends upon the species of infecting virus (Hermant and Michaels, 2014). It is proposed that IFN-λ may primarily act as a protection of mucosal entities (Hermant and Michaels, 2014).

**Mechanism of action of IFNs**

Presence of double stranded - RNA of viruses, various microbial products and chemical inducers have been shown to trigger the production of IFNs by a variety of cells (Platanias, 2005). Pathogen associated molecular patterns (PAMPs) present in viruses and microbial products are recognized by the pattern recognition receptors (PRRs) present on the target cells and induce the production of IFNs. The best known PRRs which participate in this action are the toll-like receptors (TLRs). Engagement of PRRs stimulates signalling pathways by activating various transcription factors in the target cells thereby resulting in the production and release of various IFNs (Kang, et al. 2012, Rudick and Goelz 2011, Basu, et al. 2009; Yoneyama, et al. 2004; Borden, et al. 2007; Platanias 2005). The secreted IFNs then act in autocrine and paracrine fashions by binding to the receptors present on the membrane of target cells.
Different types of IFNs bind to different receptors. Type I IFNs bind to a heterodimeric receptor (known as IFN-α/β receptor) which is composed of two subunits, IFNAR1 and IFNAR2 (Jaitin, et al. 2006; Tayal and Kalra 2008; Lavoie, et al. 2011) (Fig. 2). While, type II IFNs bind to a heterodimeric receptor (known as IFN-γ receptor) which is made up of IFNGR1 and IFNGR2 subunits (Mogensen, et. al 1999; Jaitin, et al. 2006; Weerd, et al. 2007). Type III IFNs recognize a specific receptor, called as IFN lambda receptor (IFNLR), on the target cells (Kotenko and Langer in 2004, Tayal and Kalra 2008). Type I and II IFN-receptors are ubiquitously expressed on most of the cells while the expression of type III IFN-receptors is tissue specific (Kotenko and Langer 2004; Chevaliez and Pawlotsky, 2007).

Binding of type I IFNs (for e.g., IFN-β) to the extracellular domain of IFNAR2 subunit results in the formation of a binary IFN-IFNAR2 complex which then interacts with IFNAR1 subunit to form a ternary complex (Jaitin, et al. 2006; Tayal et al. 2008; Lavoie, et al. 2011). This allows the interaction of intracellular domains of the receptor subunits with the downstream protein signalling molecules, Janus kinases (JAK) and tyrosine kinase (Tyk).

Type I and type III IFNs activate JAK1 and Tyk2 in the target cells, whereas, type II IFNs activate JAK1 and JAK2 (Fig. 2). This results in a series of phosphorylation events ultimately activating signal transducer and activator of transcription (STATS) in the target cells (Taniguchi and Takaoka, 2001; Platanias, 2005; Strunk, et al. 2008; Karpusus, et al. 1997; de Weerd, et al. 2013, Rudick and Goelz 2011; Mennechet and Uze, 2006). In case of type I and type III IFNs, activated STATS then interacts with other cytoplasmic proteins and forms...
activator complex, IFN-stimulated gene factor (ISGF3). ISGF3 is composed of STAT1, STAT2 and IRF9, the latter is a member of the family of IFN-regulatory factors (IRFs). ISGF3 complex then translocate into the nucleus and binds to IFN-sensitive response elements (ISRE) present in IFN-regulated genes (Hertzog, et al. 2011; Karpusus, et al. 1997). In case of type II IFNs, the activator complex consists of only dimeric STATS (without the involvement of IRF9) and this complex then binds to IFN- γ activated sequence (GAS). The binding of these regulatory factors to ISREs/GAS elements causes modulation of several hundred of IFN-sensitive genes (ISG) in the target cells (George, et al. 2012, Rudick and Goelz, 2011). The differential activities of various subfamilies of IFNs are believed to be modulated by the stability of their ternary receptor complexes (Thomas, et al. 2011). Also, the signal transduction pathways of type I and type III IFNs are significantly intertwined resulting in cross-talks between these IFN signalling systems (Hermant and Michaels, 2014).

**Human IFN-β**


**Clinical utility of hIFN-β and its adverse effects**

IFNs are the first recombinantly produced cytokines (Meager, 2002) and have been included under group 1b of functional classification of protein therapeutics (Leader, et al. 2008). Recombinant IFNs have been shown to exert a wide range of beneficial effects, including antiviral, antibacterial, antitumor, antiproliferative, proapoptotic and cytotoxic effect, and are thus used clinically for various conditions in humans (Parker, et al. 2016; Goldstein and Lazlo, 1988; Leader, et al. 2008). Recombinant hIFN-β is predominantly prescribed for the treatment of multiple sclerosis (MS) (Hartung, 2014; Rudick and Goelz, 2011; Seddighzadeh, et al. 2014; Reder and Feng, 2014; Kieseier, et al. 2015). MS is a chronic autoimmune disease of central nervous system and is a main cause of non-traumatic disability in young adults (Filippini, et al. 2003; Chofflon, et al. 2002; Chofflon, 2000; Armason, 1999). The exact mechanism of induction of MS is still unclear, however, it is believed that several environmental and genetic factors trigger the activation of autoimmune cells against myelin proteins. These autoimmune cells then permeate blood-brain-barrier (BBB) and cause
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demyelination of neurons subsequently manifesting into the clinical symptoms of MS (Dhib-Jalbut and Marks, 2010; Kieseier, 2011). Currently, there is no cure available for MS and rhIFN-β is the first therapy approved by US Food and Drug Administration (US FDA) for the treatment of MS (Sorensen, et al. 2003; Rudick, et al. 2004; Paolicelli, et al. 2009). The mechanism as to how hIFN-β elicits its therapeutic effect in MS is poorly understood, although, it is proposed that the beneficial effects of IFN-β are due to its antiinflammatory and immune-modulatory properties. It is also proposed that, rhIFN-β reduces the leukocyte migration across BBB and regulates autoimmunity (Dhib-Jalbut and Marks, 2010; Kieseier, 2011).

The utility of rhIFN-β has also been described in case of asthma (Cakebread, et al. 2011). In animal model of asthma, oral rhIFN-β administration has been shown to suppress eosinophilic infiltration into the lung of the animals thereby modulating the late asthmatic response (Satoh, et al. 1999). Such studies have led to the hypothesis that hIFN-β may have a potential therapeutic role in the treatment of asthma in humans. Infact, several clinical studies are currently in progress to assess the utility of IFN-β therapy in chronic obstructive pulmonary disorder (COPD) (Reder and Feng, 2014; Gaajetaan, et al. 2013). Also, rhIFN-β has shown a tremendous promise for the treatment of a other human diseases like viral infections and various cancers (Chevaliez and Pawlotsky. 2007; Sasaki, et al. 2015; Paolicelli, et al. 2009; Saidha, et al. 2012; Cooksley, 2004; Festi, et al. 2004; Joffe, et al. 1997).


Two different forms of rhIFN-β; rhIFN-β1a and rhIFN-β1b, are approved by US FDA for the treatment of MS (Rodriguez, et al. 2010). The rhIFN-β1a is a glycosylated protein produced using eukaryotic expression systems (viz., CHO cells) and possesses identical amino acid sequence to that of naturally occurring hIFN-β (Kagawa, et al. 1988). While, IFN-β1b is a
non-glycosylated form of hIFN-β produced by using prokaryotic expression systems (Mark, et al. 1984). It is reported that the stability and efficacy of recombinantly produced IFN-β is influenced by the level and pattern of post-translational modifications (Karpusas et al. 1997; Conradt et al. 1987; Runkel et al. 1998, Dissing-Olesen, et al. 2008; Antonetti, et al. 2002). Also, it has been shown that recombinantly produced non-glycosylated form (rhIFN-β1b) is biologically active and exhibits activities similar to its glycosylated counterpart (Dissing-Olesen, et al. 2008; Kasam, et al. 1995).

**Expression systems used to produce rhIFN-β - their advantages and disadvantages**

Considering the clinical applications of rhIFN-β, development of a production system which could produce this therapeutic protein in ample yield is a dire need. Towards this, various attempts to develop better production methods are still underway. Overall, the parameters that affect any recombinant protein production includes the choice of the expression system utilized, the conditions used for the growth of recombinant cells and procedure standardized for the purification of recombinant protein. We now briefly discuss below various expression systems that have been used for the production of rhIFN-β.

Previously, hIFN-β for clinical applications used to be isolated from human fibroblasts by exposing these cells to viral antigens (Derynck, et al. 1980). Though, the protein produced by using this system was native, this system of production suffered from serious disadvantages of low yield and lack of safety. With the advancement of recombinant DNA technology, hIFN-β is now being produced by using a variety of heterologous expression systems ([Fig. 3](#)) (Madhavan and Sukumaran, 2016; Paz Maldonado, et al. 2007; Zago, et al. 2009; Rao, et al. 2009; Villela, et al. 2010; Fazeli, et al. 2013, 2014 Moradian, et al. 2013; Skoko, et al. 2003; Yu, et al. 2003; Smith, et al. 1983).
Commercial production of therapeutic proteins by using *E. coli* expression system is preferred over other systems (Gellisen, 2005; Peti and Page, 2007; Sivashanmugam, et al. 2009). This is because of many reasons including well characterized genetics of *E. coli*, which makes genetic manipulations in these cells easy, and high growth rate of *E. coli* cells, permitting high yield production of target recombinant proteins (Gellisen, 2005; Peti and Page, 2007; Sivashanmugam, et al. 2009). These and other characteristics make *E. coli* a workhorse for the production of several recombinant proteins (Baneyx, 1999; Bajaj, et al. 2015; Beladiya, et al. 2015; Iyengar, et al. 2015). Infact, several therapeutic proteins produced by using *E. coli* expression systems have been approved by US FDA (Walsh, 2014). High cell density fermentation of recombinant *E. coli* is normally used to produce recombinant proteins (Riesenberg and Guthke, 1999). However, these techniques still suffer from the disadvantage of low productivity of recombinant proteins. This may be improved by employing various strategies (Tabandeh, et al. 2004; Lim and Jung, 1998). Towards this, a number of studies have investigated the optimization of culture conditions on the cell growth and target recombinant protein expression (Morowvat, et al. 2015; Tabandeh, et al. 2004; Babaeipour, et al. 2008; Yoon and Kang, 1994; Shin, et al. 1998).

Production of rhIFN-β by using *E. coli* expression system is widely reported (Allen, et al. 2015; Paz Maldonado, et al. 2007; Rao, et al. 2009; Villela, et al. 2010; Fazeli, et al. 2012; Moradian, et al. 2013). However, the usage of this expression system suffers from several disadvantages including lack of post-translation modification, low-to-moderate yield and poor biological activity of rhIFN-β. It is known that overexpression of rhIFN-β in *E. coli* leads to aggregation of misfolded proteins in the form of inclusion bodies (IBs) (Kamionka, 2011). Other major disadvantages include presence of toxic material (derivatives of *E. coli* cell wall) in the purified protein preparation. Recombinant plasmid instability in *E. coli* at high cell density growth is also recognized as a major contributor in lowering the yield of target proteins (Daneshiam, et al. 2006; Kamionka, 2011; Sahdev, et al. 2008).

The rhIFN-β is also produced by using eukaryotic expression systems. Several researchers have employed yeast for the expression and production of rhIFN-β (Skoko, et al. 2003; Yu, et al. 2003). Yeast expression system offers many advantages over other eukaryotic systems, including short doubling-time and rapid growth of yeast cells on simple culture media, easy genetic manipulation, and presence of glycosylation machinery in the yeast cell for producing post-translationally modified (glycosylated) proteins (Goffeau, et al. 1996; Feizi, et al. 2013,
Mattanovich, et al. 2004, 2012). However, the use of this expression system is preceded by several disadvantages, including hyper- and/or inappropriate-glycosylation of target proteins, which adversely affects their biological properties (e.g., immunogenicity) when used as a drug in humans (Cregg, et al. 2000; Mattanovich, et al. 2004).

Several insect cell lines have also been used for the production of eukaryotic proteins. Baculovirus-infected insect cells and Drosophila Schneider cells are commonly employed for this purpose (Aumiller, et al. 2003; Stifter, et al. 2014). The production of rhIFN-β has been reported by using baculovirus expression vector in Spodoptera frugiperda cells (Smith, et al. 1983). The use of insect cell lines for the production of rhIFN-β also suffers from several disadvantages, such as slow cell growth and difficult genetic manipulation of insect cells, expensive culture conditions, and significant toxicological and economic issues due to the use of viral transfection in this expression system (Stifter, et al. 2014; Aumiller, et al. 2003).

Various animal cell lines have also been exploited for the production of therapeutically important proteins (Gellisen, 2005). These include, human embryonic kidney 293 (HEK 293), baby hamster kidney (BHK-21), Chinese hamster ovary (CHO), murine myeloma lymphoblastoid cell line, etc (Gellisen, 2005). With more than 50% of the therapeutic protein products now being produced by using the mammalian cell lines, this expression system offers many advantages over other expression systems (Gellisen, 2005; Walsh, 2014). These include the capacity for appropriate post-translational modifications of target recombinant proteins, which makes these recombinant proteins very similar to their naturally occurring counterparts (Aricescu and Owens, 2013). CHO cell line has in fact been used for the production of rhIFN-β (Kay, et al. 2016; Zago, et al. 2009; Villela, et al. 2010). However, the use of mammalian cells to produce rhIFN-β for therapeutic use also have certain limitations, including slow growth rate of recombinant cells, possibility of contamination of oncogenic or viral DNA in the purified protein preparations, low product yield and requirement of complex culture media thereby elevating the cost of finished product (Mohan, et al. 2008; Lai, et al. 2013; Villela, et al. 2010). Other systems that are being exploited for the production of rhIFN-β include plants and chemical synthesis (Sakamoto, et al. 2012; Li, et al. 2007; Edelbaum, et al. 1992).

It is important to note here that, since rhIFN-β is being produced by using myriad of expression systems, the clinical use of these different preparations may also lead to
differential therapeutic effects (Scagnolari, et al. 2014; Runkel, et al., 1998; Bermel and Rudick, et al. 2007). In fact, a significant difference in the biological activity is reported among different rhIFN-β formulations (Scagnolari, et al. 2014). Moreover, a considerable difference in the generation of neutralizing antibodies in patients administered with rhIFN-β produced by different expression systems is also observed (Runkel, et al. 1998; Bermel and Rudick, et al. 2007).

**Overcoming the low-yield issue of rhIFN-β**

As discussed above, *E. coli* is the expression system of choice to produce rhIFN-β for commercial use. However, overexpression of hIFN-β in *E. coli* results in aggregation of the overexpressed protein in the form of IB, thereby affecting overall yield of the target protein (Baneyx and Mujacic, 2004). This is due to the lack of availability of the post-translational machinery in the *E. coli* cells (Kamionka, 2011). Towards this several approaches have been devised to overcome these disadvantages. For e.g., expression of rhIFN-β protein in periplasmic space of the bacteria can address the problem associated with the cytoplasmic environment to an extent (Rao, et al. 2009; Morowvat, et al. 2014). Use of modified culture media with optimal nutrient concentration also helps in improving the stability of recombinant plasmids in the bacterial cells (Rao, et al. 2009). Interestingly, overexpression of rhIFN-β in *E. coli* and subsequent in vitro refolding of protein present in IBs to their active form has emerged as the most lucrative approach to overcome the low-yield issue (Rao, et al. 2009; García-Fruitos, et al. 2012; Eiberle and Junghauer, 2010; Freydell, et al. 2007; Georgiou and Valax, 1999). IBs are aggregates enriched in target recombinant proteins and in vitro refolding permits the refolding (renaturation) of these inactive proteins into their active form (Rao, et al. 2009). In fact, in vitro refolding has emerged as a potential alternative approach over the production of soluble (active) recombinant proteins to increase the yield (Panda, 2005; Singh and Panda, 2005; Clark, 1998; Guise, et al. 1996; Mayer and Buchner, 2004; Gellisen, 2005; Palomeres, et al. 2004; Vallejo and Rinas, 2004). However, the process of in vitro refolding is recognized to be the bottleneck in the protein production scheme, and hence it is suggested that the refolding methods of different recombinant proteins should be developed on a case-by-case basis.

One of the main requirement in the production of rhIFN-β by in vitro refolding is to characterize the refolded protein. A variety of different approaches have been utilized by
different groups for the characterization of refolded rhIFN-β. Several of these approaches are briefly discussed below.

**Approaches used for the characterization of refolded rhIFN-β**

A variety of approaches are utilized for the characterization of refolded rhIFN-β. These include indirect approaches (biophysical and biochemical assays) and direct approaches (cell-based and animal-based assays) (Fig. 4). Various biophysical methods that are used for the characterization of refolded rhIFN-β include spectroscopy, chromatography and ultracentrifugation techniques (Manavalan and Johnson, 1985; Stifter, et al. 2014; van Beers, et al. 2011). Circular Dichroism (CD) and fluorescence spectroscopy provide information about the structure of protein in question and by comparing the results of refolded protein with native (active) counterpart, one can assess the structural similarity between these proteins (Manavalan and Johnson, 1985; Stifter, et al. 2014). Inactive aggregates of rhIFN-β are monitored by using dynamic light scattering and analytical ultracentrifugation (Manning, et al. 2010; van Beers, et al. 2011). Chromatographic techniques, such as reverse phase-high performance liquid chromatography (RP-HPLC), are also used to characterize refolded and misfolded rhIFN-β (Rao, et al. 2009). Biochemical methods are also used to characterize refolded rhIFN-β and enzyme-linked immunosorbent assay (ELISA) is a method of choice in this regard (Yamazaki, et al, 1989).

Numerous direct approaches are also utilized to measure the biological activity of refolded rhIFN-β (Fig. 4). Based on the inherent physiological activities of hIFN-β, different types of
cell-based assays are available for this. The antiviral activity of rhIFN-β can be measured by using cytopathic effect protection assay by using a variety of cell lines (Meager 1996, 2002; Antonetti, et al. 2002; Skoko, et al. 2003; Scagnolari, et al. 2014). To monitor the antiproliferative activity of refolded rhIFN-β, various cell lines have been used (Chen, et al. 1988; Singh, et al. 1995). Apart from these two activities, assessment of immunomodulatory activity of rhIFN-β is also used to characterize the biological activity of rhIFN-β (Luger, et al. 1989; Mastrangeli, et al. 2015). Similarly, various reporter gene assays are also utilized to monitor the activity of rIFN-β (Canosi, et al. 1996; Sato, et al. 2001). In these assays, a transgenic cell line harbouring a reporter gene that is driven by an IFN-β-responsive element is used. Three different transfected cell lines with different reporter genes; African green monkey cell (Vero)/Mx-Luciferase, A549/Mx-Luciferase and A293/ISRE-SEAP (IFN stimulated response element-secreted alkaline phosphatase) have been used (Voigt, et al. 2013; Scagnolari, et al. 2014).

To assess the efficacy of recombinantly produced IFN-β several studies with animal models have been reported (Rudick, et al. 1998; Deonarain, et al. 2000; Samuel and Diamond, 2005). The molecules which have proved to be efficacious and safe in these animal models have progressed further for studies in human subjects. Clinical studies with rhIFN-β manufactured by different pharmaceutical enterprises showing the data about the efficacy, safety, toxicity and biochemical studies are extensively reported in the literature and several such studies are still ongoing (Walther and Hohlfeld, 1999; Choffillon, 2000; de Anders, et al. 2007; NCT02491684; NCT01892722; NCT02364986).

Thus, it seems that all the available approaches for the characterization of recombinantly produced hIFN-β (discussed above) have certain advantages and disadvantages over others. For e.g., though the use of indirect approaches for the characterization of refolded rhIFN-β is simple, fast and cost effective, it does not provide any information on the biological activity of the refolded protein. On the other hand, direct approaches, such as the cell-based assays, provide better approximation of the biological activity of rhIFN-β. However, cell-based assays use different cell lines for assessing specific biological properties of rhIFN-β, and use of a single assay may not provide the indication of overall biological effect of the protein that appears when rhIFN-β is used as a drug in humans. Determination of the biological activity of rhIFN-β by using appropriate animal models is the best approach, as some of the parameters such immunogenicity, pharmacodynamics and pharmacokinetics of rhIFN-β
could be assessed in such studies. However, results obtained from studies may not reflect the approximation of the efficacy which is needed to establish refolded rhIFN-β as a drug for human use. Studies performed in human subjects offer the highest achievable level of results pertaining to the efficacy and safety of recombinantly produced hIFN-β. However, clinical studies require the highest level of sophistication and a large number of trained personnel from varied disciplines. Hence, there lies a tight agreement between the choice of approach utilized and the quality of results desired for the development of recombinantly produced (refolded) hIFN-β as a drug for human use.

DISCUSSION

Recombinant hIFN-β is clinically used for the treatment of MS and the utility of this protein is also being tested for several other disease conditions. Various eukaryotic expression systems are used for the production of rhIFN-β. However, these systems suffer from severe disadvantages of low-yield and molecular heterogeneity of rhIFN-β (in terms of inappropriate glycosylation which affects its biological activity). To overcome these issues, production of rhIFN-β by using E. coli expression system is thus preferred. However, the E. coli expression system suffers from several shortcomings as well. Hence, there lies a tight association between the choice of expression system and the quality and quantity of the finished product. This balance should be carefully maintained in designing the production processes of rhIFN-β which may lay a foundation for the development of cost effective therapy.

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Figure Legends

Figure 1. Classification of human IFNs (Chevaliez and Pawlotsky, 2007; Pestka, et al. 2004; Branca and Baglioni, 1981).

Figure 2. Mechanism of action of human IFNs. Legends: IFNAR 1 & 2- interferon α/β receptor 1 & 2; IFNLR- interferon lambda receptor; IFNGR 1 & 2- interferon gamma receptor 1 & 2; JAK 1 & 2- Janus kinase 1 & 2; Tyk2- tyrosine kinase 2; STAT 1 & 2- signal transducer and activator of transcription 1 & 2; ISGF3- interferon stimulated gene factor 3; IRF9- interferon regulatory factor 9; GAS- interferon-γ activated sequence; ISRE- interferon
sensitive response elements; ISG- interferon sensitive genes (George, et al. 2012; Taniguchi and Takaoka, 2001; Platanias, 2005; Parker et al, 2016).

Figure: 3 Various expression systems used for the production of rhIFN-β.

Figure: 4 Various methods used for the characterization of refolded rhIFN-β. Legends
RP-HPLC- reverse phase high performance liquid chromatography; CD- circular dichroism spectroscopy; DLS- dynamic light scattering; AUC- analytical ultracentrifugation; ELISA- enzyme linked immuno-sorbent assay.

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