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Review

Recombinant proteins of helminths with immunoregulatory properties and their possible therapeutic use

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ABSTRACT

The inverse relationship between helminth infections and the development of immune-mediated diseases is a cornerstone of the hygiene hypothesis and studies were carried out to elucidate the mechanisms by which helminth-derived molecules can suppress immunological disorders. These studies have fostered the idea that parasitic worms may be used as a promising therapeutic alternative for prevention and treatment of immune-mediated diseases. We discuss the current approaches for identification of helminth proteins with potential immunoregulatory properties, including the strategies based on high-throughput technologies. We also explore the methodological approaches and expression systems used for production of the recombinant forms of more than 20 helminth immunomodulatory proteins, besides their performances when evaluated as immunotherapeutic molecules to treat different immune-mediated conditions, including asthma and inflammatory bowel diseases. Finally, we discuss the perspectives of using these parasite-derived recombinant molecules as tools for future immunotherapy and immunoprophylaxis of human inflammatory diseases.

Contents

1. Introduction

Helminthic parasites, by co-evolution with mammals, developed mechanisms to modulate the immune system of their hosts ins order to survive for long periods and, consequently, cause impact in the development and manifestation of human chronic inflammatory diseases (Ditgen et al., 2014; Bashi et al., 2015; Maizels and McSorley, 2016). The increasing prevalence of immune-mediated inflammatory diseases including asthma, rhinitis, intestinal inflammatory disease, diabetes and several others has been attributed to modern lifestyles in industrialized countries, in particular to the lack of exposure to infectious agents such as microorganisms and parasitic helminths (Strachan, 1989; Parker and Ollerton, 2013; Maizels et al., 2014).

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In fact, helminth infections have been used experimentally to treat inflammatory disorders, with some encouraging results. For example, *Trichinella spiralis* infection has been shown to down-immunoregulate type 1 diabetes, ulcerative colitis and experimental autoimmune encephalomyelitis in experimental models (Ashour et al., 2014; Gruden-Movsesijan et al., 2010; Hübner et al., 2012), whereas *Schistosoma mansoni* was also able to suppress airway inflammation (Pacífico et al., 2009).

Likewise, *Trichuris suis* eggs and alive *Necator americanus* infective larvae have been reported to treat human inflammatory bowel disease and allergic rhinitis in humans (Croese et al., 2006; Summers et al., 2003). However, evaluation of these therapies by most reliable meta-analysis studies concluded that there was not sufficient evidence to ensure their efficacy and safety (Croft et al., 2012; Garg et al., 2014; Maizels, 2016). Thus, prior to considering clinical treatments using these biological products, the helminths or their products require careful selection and the elucidation of mechanisms underlying their immunomodulatory capabilities to avoid the effects related to this ability such as reducing the effectiveness of vaccines, increased susceptibility to co-infection and inhibit the immune response against tumors (Maizels and McSorley, 2016).

Important advances have occurred in understanding the parasite-host relationship, and some molecular and cellular mechanisms are already well defined. These mechanisms include the induction of regulatory cytokine production (IL-10 and TGF-β) (Shiny et al., 2011); CD4+ CD25+ Foxp3+ T cell (Treg cells) recruitment (Layland et al., 2013); alternative macrophage activation inducing an anti-inflammatory phenotype (Du et al., 2014) and an immune response shift (Hübner et al., 2012). However, these mechanisms are variable depending on the parasite species or its products, experimental model, treatment protocol, among other factors (Bashi et al., 2015; Maizels and McSorley, 2016).

Native proteins isolated from helminths have been described as potent immunomodulators. The ES-62 glycoprotein from *Acanthocheilonema viteae* has been shown to interact with several different cells and molecules of the immune system to decrease the inflammatory response, such as in arthritis (Pineda et al., 2012). Ideally, proteins isolated from the parasites could be purified and evaluated for their immunomodulatory potential. However, the processes for obtaining the native molecules are often laborious, time consuming and not effective for production complying with required purity standards, reproducibility, and in large-scale to biophysical and biochemical characterization and clinical use (Fernández-Robledo and Vasta, 2010; Vedadi et al., 2010).

These proteins can be produced through recombinant DNA technology in different expression systems such as bacteria, yeast, protozoa, insect cells and mammalian cells (Frenzel et al., 2013). Some parameters such as growth characteristics, folding, purification and posttranslational modifications are crucial for the choice of the expression system (Pfeifer and Khosla, 2001) and are likely to have an impact on biological activity, stability and pharmacokinetics (Fujihara et al., 2008).

Despite the high cost, most of the recombinant protein pharmaceuticals currently in use are produced in mammalian cell lines, because folding, secretion and posttranslational modifications are more similar to those of human native proteins, a requirement particularly important to proteins whose suitable glycosylation is essential for biological activity (Frenzel et al., 2013). For example, the most therapeutic glycoproteins require suitable sialylation, which is more likely to be achieved by mammalian cells, despite this expression systems have disadvantages such as heterogeneity of glycans and, particularly, handling difficulties to produce specific changes (Hamilton et al., 2006; Kallolimath et al., 2016). In addition, other eukaryotic expression systems as yeast have advantages such as higher yield, shorter fermentation times, growth in chemically defined media and, especially, ease of handling which provides flexibility to insert appropriate biochemical machinery for required post-translational modifications (Hamilton et al., 2006; Schaefer and Plückthun, 2012). These characteristics can be useful for the biosynthesis of helminth recombinant proteins, which are not yet well characterized biochemically and demand specific post-translational modifications.

On the other hand, *E. coli* expression systems have been most useful in producing helminth recombinant proteins to test for immunomodulation with some encouraging results in vitro and in experimental models. This preference is due to the advantages of low cost, ease of handling and high yielding, despite the lack of post-translational modifications and inappropriate folding that may be important for their biological function (Anné et al., 2012; Assenberg et al., 2013).

2. Rational strategies for obtaining recombinant helminth molecules for therapeutics or immunoprophylaxis purposes

Nowadays, multi-technological approaches involving parasitology, genomics, transcriptomics and proteomics methods, associated with a variety of bioinformatics strategies, have contributed enormously to the identification of helminth proteins with potential immunomodulatory properties (Du et al., 2011; Cantacessi et al., 2011; He et al., 2014; Ebner et al., 2014).

Several in vivo techniques using established experimental animal models for asthma (Baqueiro et al., 2010; Navarro et al., 2016), colitis (Brenna et al., 2013) and autoimmune diabetes (Viehmann Milam et al., 2014) are currently available to test helminthic molecules for further use as immunotherapeutic agents of human inflammatory diseases when their mechanisms of action and safety became well recognized.

Many genomic sequences of helminth parasites that are considered to modulate the mammalian host's immune system have become recently publicly available; these include the genomes of *Ascaris suum* (Jex et al., 2011), *Brugia malayi* (Ghedin et al., 2007), *Fasciola hepatica* (Cwiklinski et al., 2015), *Necator americanus* (Tang et al., 2014), *Schistosoma mansoni* (Berriman et al., 2009), *Strongyloides spp* (Hunt et al., 2016), *Toxocara canis* (Zhu et al., 2015), *Trichinella spiralis* (Mitreva et al., 2011), *Trichuris suis* (Jex et al., 2014), and *Trichuris trichiura* (Foth et al., 2014). A number of proteins have already been predicted through *in silico* analysis of these genomes that could be considered as potential immunomodulatory molecules for further evaluation (Ghedin, 2014; Foth et al., 2014; Jex et al., 2014).

Through transcriptomics, using high-throughput sequencing technologies, it is now possible to study all the protein-encoding transcripts produced by specific helminthic tissues during different developmental stages of these parasites (Choi et al., 2011) and also during contact with the host (Foth et al., 2014), allowing for the evaluation of genes involved in different cellular functions and mechanisms such as immunomodulation (Gasser, 2013). The integration of the information gathered from transcriptomic studies with data obtained at the proteomic level will not only contribute toward improving our understanding of parasite biology and of the host-parasite interplay, but might also aid in the identification of novel immunotherapeutic helminthic molecules. For this, novel dedicated databases and bioinformatic pipelines for integrated analysis of helminth "-omics" data, such as the WormBase Parasite, HelmDB and Heminth.net, were made available in recent years (Mangiola et al., 2013; Martin et al., 2015; Howe et al., 2016; Korhonen et al., 2016).

Fig. 1 shows a diagram describing the steps for a general approach to identify helminthic molecules for immunotherapy and immunoprophylaxis of inflammatory diseases. The informa-