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MINI-REVIEW

Leishmania-based expression systems

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Abstract Production of therapeutic or medical recombinant proteins, such as monoclonal antibodies, proteins, or active enzymes, requires a highly efficient system allowing natural folding and perfect post-translation modifications of the expressed protein. These requirements lead to the generation of a variety of gene expression systems from bacteria to eukaryotes. To achieve the best form of eukaryotic proteins, two factors need to be taken into consideration: choosing a suitable organism to express the protein of interest, and selecting an efficient delivery system. For this reason, the expression of recombinant proteins in eukaryotic nonpathogenic *Leishmania* parasites is an interesting approach which meets both criteria. Here, new *Leishmania*-based expression systems are compared with current systems that have long histories in research and industry.

Keywords *Leishmania* · Expression protein systems · Nonpathogenic parasites

Introduction

Over the past decades, many organisms from prokaryote to eukaryote hosts have been introduced as expression hosts to produce different recombinant proteins, but this has often been hampered by insufficient biological activity of the expressed proteins. This is mainly due to the differences in codon usage pattern and complex post-translation modification process and folding. In case of immunomodulatory proteins, there is still a need for a high-quality expression system from the aspect of safety and protein activity on the immune response.

Leishmania (*L.*) parasites are protozoan single-cell eukaryotes with two morphologically different forms: the promastigote (extracellular) form in sandfly gut and the amastigote (intracellular) form in mammalian macrophages, the latter living as an intracellular parasite. In most *Leishmania* species, promastigotes transmit from animal reservoir (several species of rodents and canines) to mammalian host by a Phlebotomine sandfly, from genera such as *Lutzomyia* and *Phlebotomus*. Recent research has shown that *L. tarentolae*, which is one of the nonpathogenic species of genus *Leishmania*, could be used as one of the best available expression systems. Despite its short history, this species is widely used for gene manipulation, gene targeting, immunogenicity, gene function study, and generation of live vaccines. In addition, these systems facilitate the expression of heterologous proteins or epitopes as antigens from different organisms. The production of recombinant parasites leads to the identification of functional relationships between different proteins and anti-parasitic drugs. This review highlights current strategies to manipulate *Leishmania* genome for future use as an expression system, along with its applications and challenges.

Current expression systems

Advancements in the gene cloning technology have made it feasible to express therapeutic/prophylactic proteins in different hosts. However, one of the main issues is finding a proper

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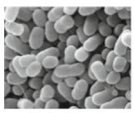
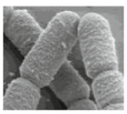
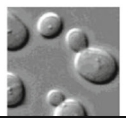
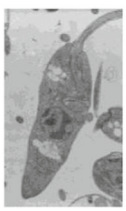
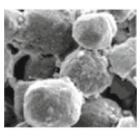
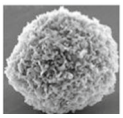
system to produce the protein of interest with the highest biological activity. During recent years, several prokaryotic/eukaryotic expression vectors and hosts have been used to express different types of proteins in recombinant form with various levels of required post-translational modifications. At first, prokaryotic expression systems like different strains of gram-negative bacterium *Escherichia coli* such as BL21 and K12 were widely used to produce recombinant proteins for different applications, such as vaccines, diagnostic tools, drug candidate screening, and functional analysis. As summarized in Table 1, in spite of many advantages such as the simplicity of genetic manipulation as well as fast and inexpensive growth, bacterial expression systems have some fundamental problems, such as absence of post-translation modifications (i.e., glycosylation), misfolding of proteins, and formation of inclusion bodies (Rosano and Ceccarelli 2014). Inclusion bodies (0.2 to 0.6 μm) are insoluble aggregate form of proteins, formed due to incorrect disulfide bond formation, without their natural biological activity (Healthcare and Healthcare 2007). Moreover, another major limitation is contamination of the recombinant protein with remnants of bacterial endotoxin, derived from lipopolysaccharides (LPS) in the outer membrane of gram-negative bacteria, which induces inflammatory responses in both experimental animal models and

Table 1 Advantages and disadvantages of different prokaryotic and eukaryotic expression systems

Expression systems		Advantages	Restrictions	Ref.
Bacteria	***E. coli*** 	Rapid growth (20−30min doubling time) and simple culture, inexpensive, high yield, well-characterized genetics, easy cloning, and large-scale preparation, used for industrial and pharmaceutical protein production.	No post-translational modification, LPS contamination, inclusion bodies, low secretion efficiency, misfolding of protein.	(Rosano and Ceccarelli 2014; Terpe 2006b)
	Bacillus 	Rapid growth and simple culture, high yield, high secretion efficiency, LPS-free.	No post-translation modification, misfolding of recombinant protein, high protease activity.	(Demain and Vaishnav 2009)
Low Eukaryote	**Yeast** 	Easy growth, high yield, post-translation modifications, significant glycosylation, safe.	Low yield, hyperglycosylation, and misfolding in the cell.	(Fernandez and Hoeffler 1998)
	Leishmania 	Rapid and easy growth (5−6-h doubling time), high yield (up to 30mg/ml), endotoxin-free, post-translation modifications, significant glycosylation, inexpensive culture compared to other eukaryotic expression systems, expression with different localizations, nonpathogenic for mammals (about *L. tarentolae*). No LPS contamination.	Long time needed for screening after transfection, probable differentiation of promastigotes to amastigotes.	(Basile and Peticca 2009; Niimi 2012; Sugino and Niimi 2012)
High Eukaryote	**Baculovirus/ insect cells** 	Higher level of gene expression, post-translation modifications, phosphorylation, significant glycosylation, proper folding, easy to scale-up, easier growth compared to mammalian cells, nonpathogenic for mammalian.	Slow growth (18−24-h doubling time), not easy culture, expensive media, not available to all laboratories.	(Airenne et al. 2013; Fernandez and Hoeffler 1998; King 2012)
	Mammalian cells 	Post-translation modifications, significant glycosylation, proper folding.	Slow growth (24-h doubling time), not easy culture, expensive media, risk of contamination with viruses, difficult to make stable cell lines	(Fernandez and Hoeffler 1998; Khan 2013)

human (Fernandez-Robledo and Vasta 2010; Sodoyer 2004; Terpe 2006). Although different promoters and regulation/induction systems have been established to increase the yield of production, bacterial systems remain the best choice only when post-translation modifications are not a major concern.

To circumvent the problem of inclusion body formation caused by cytoplasmic expression, different species of gram-positive bacteria, such as *Bacillus brevis*, *B. megaterium*, *B. subtilis*, and also *Caulobacter crescentus* were used as a substitute to secret large amounts of recombinant proteins out of the cell (Demain and Vaishnav 2009). The other advantage of these species is the absence of endotoxins such as LPS due to the absence of the outer membrane. However, one of the major problems with these species is their production of extracellular proteases like subtilisin, neutral protease, minor serine protease, bacillopeptidase F, and minor metalloesterase (Demain and Vaishnav 2009). Among *Bacillus* species, *B. brevis* has the least extracellular protease activity. For example, a exoprotease-deficient *B. licheniform* strain was engineered to achieve high expression levels, and it was superior in expressing interleukin-3 in comparison to other expression systems such as *E. coli*, *S. cerevisiae*, *K. lactis*, and C127 mammalian cells (Demain and Vaishnav 2009).

To enhance the biological quality of heterologous recombinant proteins, the next generation of expression systems, consisted of low or high eukaryotes such as yeast, mammalian cell lines, and insect cells, was developed (Baculovirus and *Drosophila*). These systems are suitable for protein production in industrial scales due to some special properties such as growth in low pH, high sugar and ethanol concentrations, and high osmotic pressure (Airenne et al. 2013; Fernandez and Hoeffler 1998; King 2012). The main advantages of yeast expression systems such as *Pichia pastoris*, *P. methanolica,* and *Saccharomyces cerevisiae* are their glycosylation pattern, secretion system, chaperon molecules, folding, and proteolytic processing (Fernandez-Robledo and Vasta 2010; Sodoyer 2004). However, low yield and growth rate, high expenses, complexity of culture, hyperglycosylation, and misfolding of the produced proteins inside the cell are some limitations of eukaryotic systems (Khan 2013) compared to bacterial systems.

In the early 1980s, baculovirus/insect cell-based systems were introduced as a new way to produce recombinant proteins with high capacity of eukaryotic-form processing such as phosphorylation and glycosylation. Production of heterologous proteins in cultured insect cells using baculovirus expression vector systems is another safe strategy, since such cells and viruses are nonpathogenic to mammals (Airenne et al. 2013; Jarvis 2009). However, two limitations have restricted the usage of this system: the need to reinfect the cells, and the difference in glycosylation pattern between insects and mammals (King 2012).

Mammalian cells, such as Chinese hamster ovary (CHO) cells, are a suitable host, since they can produce biologically active proteins due to correct folding. Nevertheless, high expenses, low growth rate and complex growth media, and high risk of contamination with viruses make them unfavorable and inaccessible for most laboratories (Fernandez-Robledo and Vasta 2010; Khan 2013; Sodoyer 2004).

Leishmania-based expression systems

Subsequent to discovery and optimization of transfection technique in *Leishmania*, a novel generation of eukaryotic gene expression systems was introduced. *Leishmania* is a low eukaryotic genus and intermediate between eukaryotes and prokaryotes in several aspects (Hightower et al. 1988; Thewes 2014). The development of in vivo expression systems based on nonpathogenic *Leishmania* parasites has brought up new possibilities to find and produce new drugs, therapeutics, and vaccine candidates (Tables 2 and 3). Among these species, *L. tarentolae* is a more attractive species, since it can be cultured in promastigote form in inexpensive media at 26 °C, and in contrast to other spp., *L. tarentolae* can tolerate high cell density in culture and has a fairly short generation time about 5–6 h (Niimi 2012; Sugino and Niimi 2012). In comparison with other systems, this system produces high yields of recombinant protein (0.1–30 mg/L) (Basile and Peticca 2009). *L. tarentolae* is being increasingly used for production of therapeutic proteins or antibodies. These proteins could be stored in cytoplasm or secreted in media. In 1990, for the first time, *L. major* was used to express *L. amazonensis* membrane glycoprotein (GP46A/M-2) from an extrachromosomal DNA (LeBowitz et al. 1990). In parallel, *L. tarentolae* has been applied as a delivery system for production of some proteins or antigens in target cells as if they are intrinsic proteins. In 2005, *L. tarentolae* was introduced for the first time as a live candidate vaccine due to its ability to augment antigen presentation and subsequently enhance the quantity and quality of T cell immune responses after its injection into mice (Breton et al. 2005). Some of the known advantages and disadvantages of using *Leishmania* expression systems are summarized in Table 1.

Nonpathogenic vs. pathogenic *Leishmania*

In addition pathogenic species, *Leishmania* genus also consists of some species that are nonpathogenic to human and some reported animals (Novo et al. 2015; Raymond et al. 2011). These species could be an attractive vehicle for antigen delivery. Nonpathogenic species of *Leishmania* that are

Table 2 List of therapeutic proteins, peptides, or antibodies produced using *Leishmania*-based expression system

Proteins type	Expression mode	Molecular weight (kDa)	Ref.
Therapeutic proteins			
Human Cu/Zn superoxide dismutase (SOD1)	Constitutive/cytoplasm	~24	(Gazdag et al. 2010)
Plasminogen activator (PA)	Integrated, in vitro purified/secreted	66	(Hemayatkar et al. 2010)
Human coagulation factor VII	Integrated, in vitro purified	~50	(Mirzaahmadi et al. 2011)
Recombinant human interferon-gamma (rhIFN-γ)	In vitro purified/secreted	17	(Davoudi et al. 2011)
N-acetyl serotonin methyl transferase (ASMT)	Integrated, in vitro purified/soluble	40	(Ben-Abdallah et al. 2011)
Single-chain fragment variables (scFv's)	Integrated, in vitro purified/secreted	~27	(Klatt and Konthur 2012)
Human IL-29	Integrated, constitutive, secreted	23	(Taromchi et al. 2013)
Fc-region of rabbit IgG	In vitro purified/secreted	28 and 55	(Jorgensen et al. 2014)
CD40 fragments	In vitro purified/secreted	~29.3	(Mlynarcik et al. 2015)
Recombinant human amelogenin (rhAm)	In vitro purified/secreted	19, 30, and 37	(Yadegari et al. 2015)
HeV attachment protein (sHeV G)	In vitro purified/secreted	~ 70	(Fischer et al. 2016)
IP-10	Integrated, in vivo constitutive, secreted	~10	(Taslimi et al. 2016)
Human kallikrein-related peptidase 2 (KLK2)	In vitro purified/secreted	27	(Guo et al. 2016)
Viral proteins			
Human papillomavirus (HPV) L1 VLP	In vitro purified/cytoplasm	60	(Bolhassani et al. 2015)
Hendra virus (HeV) HeV attachment protein (sHeV G)	In vitro purified/secreted	~70	(Fischer et al. 2016)
Leishmanial proteins			
LPG3	Integrated, constitutive/cytoplasm	~97	(Pirdel et al. 2012)

categorized in *Sauroleishmania* genus were discovered in lizards as their main animal host without any mammalian reservoir (Novo et al. 2015; Raymond et al. 2011). Recently, sandflies of the genus *Sergentomyia* are reported as vectors for *L. tarentolae* in the Old World (Novo et al. 2015). Some of the nonpathogenic lizard-hosted *Leishmania* species are *L. adleri*, *L. tarentolae*, *L. hoogstraali*, and *L. agamae*. In spite of many differences between reptilian and mammalian *Leishmania*, there is a close phylogenetic relationship between them (Novo et al. 2015; Previato et al. 1997; Simpson and Holzjr 1988). Morphologically, these parasites are similar to pathogenic species and have the same two typical forms, promastigote and amastigote (Previato et al. 1997). Comparative genome analysis has shown that *L. tarentolae* has more than 90 % homology with other spp., though compared with pathogenic spp. like *L. major*, *L. infantum*, and *L. braziliensis*, it lacks about 250 genes, most of which are needed for intracellular survival (Novo et al. 2015; Raymond

Table 3 List of prophylactic proteins expressed in *L. tarentolae* and used as live vaccine

Prophylactic proteins (as live vaccines)	Expression mode	Animal	Ref.
A2 *L. infantum*	Episomal, in vivo constitutive/cytoplasm	BALB/c mice	(Mizbani et al. 2009)
HPV type 16 E7	Integrated, in vivo constitutive/cytoplasm	C57BL/6 mice	(Salehi et al. 2012)
HPV16 E7 + gp96	Integrated, in vivo constitutive/cytoplasm	C57BL/6 mice	(Hosseinzadeh et al. 2013)
A2 +	Integrated, in vivo constitutive/cytoplasm	BALB/c mice	(Saljoughian et al. 2013)
		Dog	(Shahbazi et al. 2015)
Cysteine proteinase A and B + PpSP15	Integrated, in vivo constitutive/cytoplasm	BALB/c mice C57BL/6 mice	(Zahedifard et al. 2014)
Recombinant influenza haemagglutinins (HA)	Integrated, in vivo secreted and In vitro purified recombinant protein	BALB/c mice	(Pion et al. 2014)
Sandfly salivary antigen PpSP15	Integrated, in vivo constitutive/secretory	BALB/c mice	(Katebi et al. 2015)
MHC class I restricted epitopes	Integrated, in vivo constitutive/cytoplasm	BALB/c mice	(Zandieh et al. 2015)

et al. 2011). Although *L. tarentolae* has the same number of chromosomes as the pathogenic spp. (e.g., *L. major* and *L. infantum*) do (36 chromosomes), its genome is about 5 % smaller. Most of the missing genes are among the amastigote-specific genes in other species (Novo et al. 2015; Real et al. 2013). For example, GP63 gene in *L. tarentolae*, in comparison with *L.* (*Viannia*) *braziliensis* (pathogenic species), is shorter and lacks an extracellular domain, which is responsible for parasite-host interaction (Valdivia et al. 2015). Glycoinositolphospholipids (GIPLs) of *L. adleri* are analogous to the previously characterized GIPLs from pathogenic species of *Leishmania*. LPGs (lipophosphoglycans) have not been found in *L. tarentolae*, while they are present in the flagella pocket region of *L. adleri* (Previato et al. 1997). However, LPG3 is highly conserved and expressed constitutively in *L. tarentolae* (Azizi et al. 2009). Copy number of amastin gene and tuzin-rich region is very lower in *L. tarentolae* compared to that in *L. major* and *L. infantum* (Raymond et al. 2011). In addition, the lizard-hosted *Leishmania* spp. have no Golgi apparatus, though they have a cytoplasmic membrane thickness equal to that of *L. major* (Kazemi et al. 2008). *L. adleri* is able to establish a transient cutaneous infection when experimentally injected to humans, and 5 days after infection, the amastigote form of parasite is detectable at injection site (Previato et al. 1997). *L. tarentolae* is unable to generate any infection symptom in mice (Breton et al. 2005), although traces of *L. terentolae* DNA were detected in the intestines and bone marrow of a 300-year-old adult male (Novo et al. 2015). *L. tarentolae* promastigote is able to transform into an amastigote-like form in the macrophages, but it is not clear whether it replicates (Novo et al. 2015; Raymond et al. 2011). Moreover, *L. tarentolae* lacks some virulence factors such as A2 (Azizi et al. 2009), which are known to change the disease tropism towards visceral organs.

One of the important advantages of *L. tarentolae* species is that it is safe for human and simple to manipulate and work with. Due to its unique properties, *L. tarentolae* has been introduced as a *Leishmania*-based carrier to develop live vaccines (Breton et al. 2005; Katebi et al. 2015; Saljoughian et al. 2013; Shahbazi et al. 2015). In spite of the short survival time of parasite in mice, *L. tarentolae* expressing an exogenous antigen has proved effective in generating protection (Breton et al. 2005; Mizbani et al. 2009).

Genome and regulation of gene expression in *Leishmania*

Completion of the *Leishmania* genome project identified more than 8000 genes, most of which have unknown functions. Around 70 % of these genes code proteins, and among them, 40 % have specific roles in *Leishmania* with a known function (Gongora et al. 2003; Myler et al. 1999). Regulation of gene expression in *Leishmania* has remarkable differences with other eukaryotes due to lack of introns and consensus promoter sequences for RNA polymerase II. Exceptionally, ribosomal DNA (rDNA) genes are the only genes with known promoters, since they are transcribed by RNA polymerase I. Genes transcribed by RNA polymerase II are transcribed into polycistronic precursor RNA molecules, which are later excised into monocistronic transcripts in a process coupled with trans-splicing and polyadenylation. Trans-splicing is consisted of addition of a 39-nucleotide RNA (spliced leader RNA) to the 5′ end of the nascent RNA, forming the mRNA 5′ cap. Poly(A) signals are usually within the 100–300 nucleotides upstream of the trans-splicing site of the next gene. Presence of the poly(A) tail, like in higher eukaryotes, is critical for the stability of the mature mRNA (Beverley 2003; Clayton and Shapira 2007; Clayton 1999, 2002). The necessary regulatory signals responsible for pre-mRNA processing are provided by intergenic untranslated regions (UTRs) or intergenic region (IR) flanking the ORFs (Roy et al. 2000).

Different approaches for gene expression in *Leishmania*

Heterologous gene expression in *Leishmania* can be achieved via both extrachromosomal and chromosome-integrated coding sequences. Different gene manipulation methods lead to generation of different strains, categorized by duration of expression of the heterologous gene into transient and stable strains, expressing protein from episomal or chromosome-integrated DNA, respectively. The intrinsic ability of *Leishmania* to replicate the episomal circular DNA molecules helps maintain exogenous DNA during many rounds of cell division. This has made it possible to achieve relatively long-lasting expression of genes encoded by exrachromosomal DNA molecules. In episomal systems, circular vectors are designed in a way that the protein coding sequence is flanked by two IR sequences, harboring putative trans-splicing and polyadenylation signals.

Fewer limitations in manipulation of plasmid DNA and easier screening of recombinant cell lines make the episomal system more feasible in practice. In addition, high copy number of plasmids per cell leads to higher expression levels in comparison with genome-integrated system. However, gradual loss of plasmid during cell division due to segregational instability is a disadvantage of episomal expression systems (Palomares et al. 2004). Therefore, antibiotic resistance genes are often included in the episomal vectors, and certain concentrations of the relevant antibiotic are added to the cell culture media; this makes the integrated systems, which do not need selectable markers, advantageous especially when presence of antibiotic resistance gene is problematic or the antibiotic inhibits growth or protein synthesis.

In the stable systems, genes could be either integrated into rDNA locus and consequently be transcribed by RNA polymerase I or incorporated into other loci in the genome along with a functional IR and UTR leading to transcription by RNA polymerase II. It is noteworthy that chromosomal integration of the DNA via homologous recombination necessitates the gene being flanked by sequences homologous to the intended genomic locus. In case the plasmid bears both homologous sequences and UTRs, the expression will be possible via both pathways. In any case, Pol II-transcribed genes can be designed to be expressed in either a constitutive or an inducible manner.

On the other hand, efficiency of transfection through electroporation depends on different factors such as parasite species, voltage (Ben-Abdallah et al. 2011), type and number of pulses, (Ben-Abdallah et al. 2011), and composition of the electroporation buffer. Therefore, efficiency of transfection is very different between different strains and species of *Leishmania* (Myler and Fasel 2008). *L. donovani* has the highest transfection efficiencies between *Leishmania* species (Robinson and Beverley 2003). Successful transfection of *L. tarentolae* with episomal plasmids or with the genes integrated into 18S rRNA locus has been reported in several studies (Orlando et al. 2007; Phan et al. 2009; Roy et al. 2000; Saljoughian et al. 2013; Taheri et al. 2014), reporting different levels of expression for reporter genes in these two modes (Magistrelli et al. 2012; Mehta et al. 2008). However, comparing the efficiencies of two different types of expression systems is not easy, since there are several important parameters influencing the expression such as promoter power, constructs, and copy number of genes and plasmids.

Leishmanial vectors

To use leishmanial expression systems, different vectors from non-commercial to commercial are available. In all vectors, the gene of interest should be cloned between a couple IR (homogenous or heterogeneous) to guarantee proper gene regulation. Indeed, the fact that IR sequences are not species-specific enables us to express a wide range of proteins in different parasite strains encoded on the same vector. Depending on the desired expression mode of protein, many types of *Leishmania*-specific plasmid vectors are designed, from constitutive to inducible expression systems with different antibiotic resistance genes such as pX series (carrying DHFR-TS (dihydrofolate reductase-thymidylate synthase gene) as the regulatory elements (Liu and Chang 1992), p6.5 (with a tunicamycin resistance gene as a selective marker, Dutta et al. 2005; Liu and Chang 1992), pGEM (carrying α-tubulin IR Roy et al. 2000), and pLEXSY series (carrying three IR regulatory sequences, aprt, camCB, and a 1.7-kb IR, commercially available from Jena Bioscience). As mentioned above, these sequences are most often acquired from housekeeping genes such as α-tubulin (Boucher et al. 2002; Roy et al. 2000), DHFR (LeBowitz et al. 1990; Robinson and Beverley 2003; Titus et al. 1995), or highly expressed genes like CPB 2.8 (Misslitz et al. 2000). Therefore, the heterologous gene is transcribed by RNA polymerase II, as is the indigenous gene whose UTR is used in the construct. It is also possible to make gene clusters by inserting several ORFs interspersed by more than one IR, such as pLEXSY vector.

In the above-mentioned expression vectors, the exogenous gene is transcribed by RNA polymerase II, which often does not lead to a high expression level. However, increasing the antibiotic concentration in the culture media can increase the yield via excluding the cells with low plasmid copy number. Integration of exogenous gene into rDNA loci can be another solution, which enables reaching high transcription levels by recruiting RNA polymerase I.

Application of *Leishmania* expression systems

Leishmania-based expression systems allow substitution and expression of different genes from other spp. of *Leishmania* (LeBowitz et al. 1990; Mizbani et al. 2009; Mizbani et al. 2011; Taheri et al. 2014) in addition to expression of heterologous genes from other genera (Ben-Abdallah et al. 2011; Katebi et al. 2015; Pirdel et al. 2012; Taheri et al. 2015; Taromchi et al. 2013). *Leishmania* is a special organism that could express proteins with different localizations such as membrane-integrated, cytoplasmic, and secretory (Katebi et al. 2015; Klatt and Konthur 2012; Phan et al. 2009; Soleimani et al. 2006). Moreover, expression levels of heterologous proteins in *Leishmania* may vary depending on the protein, mode of expression (stable or transient), locus and/or intergenic regulatory sequences, copy number of gene (for integrated form), and copy number of plasmid (for episomal form) (Taheri et al. 2014). On the other hand, proteins with different molecular weights might be processed differently depending on the host *Leishmania* species (LeBowitz et al. 1990).

The great applications of these expression systems are establishment and production of active biomedical and prophylactical products. As listed in Table 2, some of proteins or peptides expressed in *Leishmania* can provide alternatives for development of effective drugs such as monoclonal antibodies (e.g., single-chain fragment (scFvs)) (Klatt and Konthur 2012), HeV attachment protein (sHeV G) (Fischer et al. 2016), variable molecules (scFvs), or vaccine development such as LPG3 (Pirdel et al. 2012) and IP-10 as a potential anti-tumor vaccine (Taslimi et al. 2016). Generally, live vaccines are preferred to other types of vaccines due to generation and maintenance of immunological memory. In comparison

with current strategies, live vaccines based on *Leishmania* expression are more efficient due to their multiplication in the host, stimulation of the immune system similar to the natural infection, higher safety, and impossibility to revert to virulent state (Taheri and Rafati 2013). Recently, there have been many studies in animals to develop homologous or heterologous prime-boost vaccination strategies using recombinant live *L. tarentolae* (rlive) and DNA constructs bearing several genes. In many studies, such as rlive expressing A2 alone in BALB/c mice (Mizbani et al. 2009) or fused with cysteine proteinase A (CPA) and B (CPB) in BALB/c mice (Saljoughian et al. 2013) and dogs (Shahbazi et al. 2015) or PpSP15 (a salivary protein from the sandfly *Phlebotomus papatasi*) alone in BALB/c mice (Katebi et al. 2015) or fused with genes CPA + CPB in BALB/c mice and C57BL/6 mice (Zahedifard et al. 2014) have shown that rlive/rlive or DNA/rlive modality of vaccination turned out to be remarkably efficient. Specially, effectiveness of these strategies was more evident when they were compared with other approaches such as DNA/DNA or recombinant proteins alone. In contrast, polytope constructs in DNA/DNA modality were more efficient than DNA/rlive prime-boost regimen against CL (Zandieh et al. 2015). Some of the therapeutic or prophylactic proteins expressed in *L. tarentolae* are listed in Tables 2 and 3, respectively.

Conclusion

Two important factors need to be considered for successful expression of a recombinant protein: the expression host and the gene delivery system. Suitability of an expression system depends on the type of the protein, aim of production of the protein, minimum required purity, activity of the protein, costs, yield, and safety (Fernandez-Robledo and Vasta 2010; Sodoyer 2004). The major drawbacks of most of the heterologous expression systems are the lack of precise post-translation modification that is very critical for biological activity and stability of the product. Interestingly, *L. tarentolae* could be an attractive host or carrier to express antigens in their native state with proper post-translation modifications. As the gene expression techniques in *Leishmania* improve, the range of its applications is widening. Nowadays, different transgenic *Leishmania* strains are developed and used as efficient vaccine carriers or delivery systems against many infection diseases. However, this system is newfound and needs more investigation to demonstrate its potential drawbacks.

Compliance with ethical standards

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