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PERSPECTIVE

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A hypothesis explaining why so many pathogen virulence proteins are moonlighting proteins

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One sentence summary: This review looks at why so many pathogen virulence proteins are from the primary metabolism and are conserved between pathogen and host.

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ABSTRACT

Moonlighting or multitasking proteins refer to those proteins with two or more functions performed by a single polypeptide chain. Proteins that belong to key ancestral functions and metabolic pathways such as primary metabolism typically exhibit moonlighting phenomenon. We have collected 698 moonlighting proteins in MultitaskProtDB-II database. A survey shows that 25% of the proteins of the database correspond to moonlighting functions related to pathogens virulence activity. Why is the canonical function of these virulence proteins mainly from ancestral key biological functions (especially of primary metabolism)? Our hypothesis is that these proteins present a high conservation between the pathogen protein and the host counterparts. Therefore, the host immune system will not elicit protective antibodies against pathogen proteins. The fact of sharing epitopes with host proteins (known as epitope mimicry) might be the cause of autoimmune diseases. Although many pathogen proteins can be antigenic, only a few of them would elicit a protective immune response. This would also explain the lack of successful vaccines based in these conserved moonlighting proteins.

Keywords: moonlighting proteins; vaccines; virulence proteins; host immune response; epitope; conservation

INTRODUCTION

Moonlighting and multitasking proteins refer to those proteins with two or more functions performed by a single polypeptide chain. Moonlighting proteins present alternative functions (named canonical and moonlighting) that are mostly affected by cellular localization, cell type, oligomeric state, concentration of cellular ligands, substrates, cofactors, products or post-translational modifications (Huberts 2010; Copley 2012; Jeffery 2014). Usually multitasking proteins are experimentally revealed by serendipity. The appearance of a new function within a polypeptide can become an advantage for the microorganism because it permits a lower number of genes and proteins, thus, making its genome more compact. In any case, these proteins complicate the interpretation of knock-outs, DNA arrays, metabolomics, systems biology, drug pharmacokinetics, pharmacodynamics and toxicity assays/analyses. It is remarkable that moonlighting is typically exhibited by proteins that are

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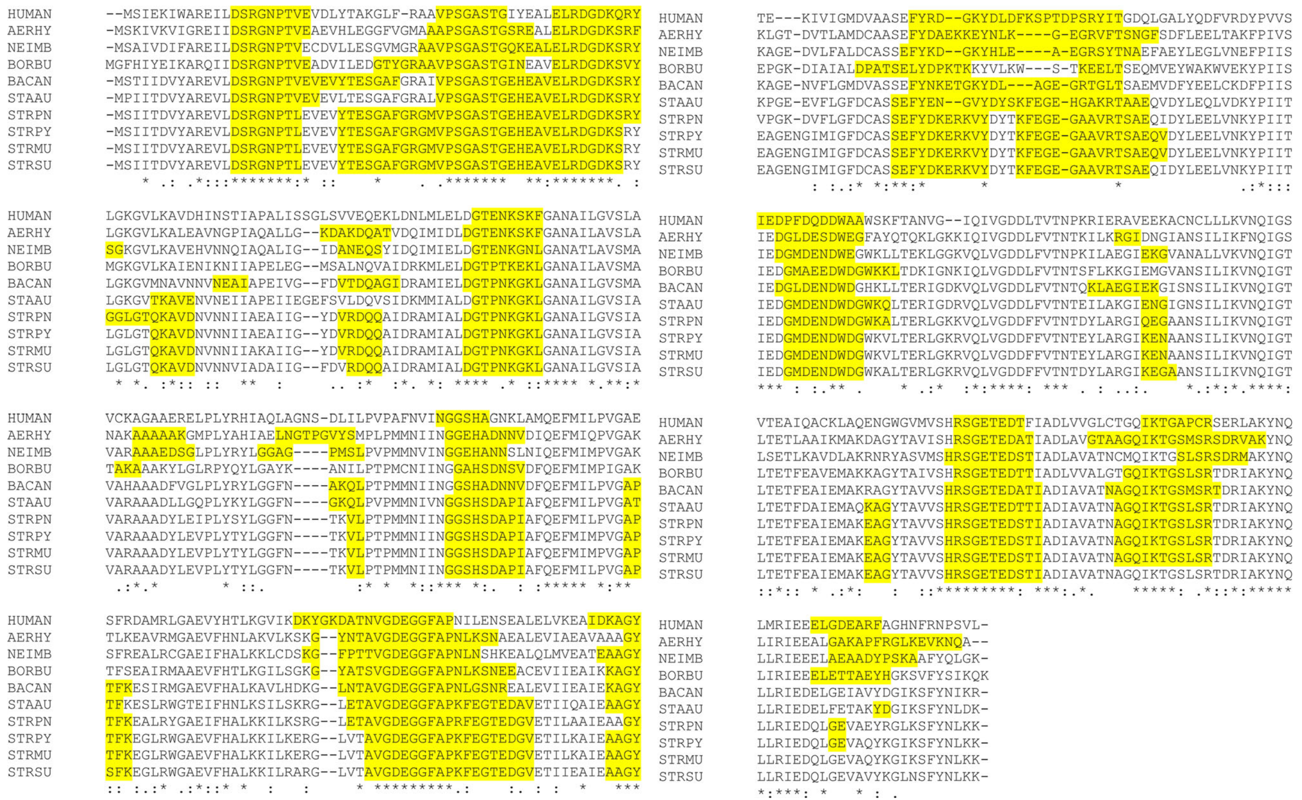

Figure 1. Clustal Omega multialignment of human and pathogen enolases. Highlighted in yellow shows the predicted B-cell epitopes in all the enolase protein sequences. Asterisks depict fully conserved amino acids. Most human predicted epitopes match to highly conserved amino acids stretches. Microorganisms are as follows: AERHY = *Aeromonas hydrophila*; NEIMB = *Neisseria meningitidis*; BORBU = *Borrelia burgdorferi*; BACAN = *Bacillus anthracis*; STAAU = *Staphylococcus aureus*; STRPN = *Streptococcus pneumoniae*; STRPY = *Streptococcus pyogenes*; STRMU = *Streptococcus mutans*; STRSU = *Streptococcus suis*.

ubiquitous and belong to an 'ancestral' function or pathway, for example primary metabolism.

Currently, two updated multitasking/moonlighting protein databases exist: MultitaskProtDB-II (Franco et al. 2018) and MoonProt (Chen et al. 2018). MultitaskProtDB-II contains 694 multitasking proteins. A remarkable fact is that 25% of these known multitasking proteins present a moonlighting function related to the pathogen's virulence activity (Table S1, Supporting Information). Moreover, their canonical function is to be an enzyme of key pathways like glycolysis or Krebs cycle (Henderson and Martin 2011; Amblee and Jeffery 2015). It is remarkable that these proteins are intracellular and, without having a canonical signal peptide or secretion motifs, they go outside the microorganism cell membrane by a still unknown mechanism. These facts rise two major questions:

Why is the canonical function of these virulence proteins mainly from ancestral key biological functions (especially of primary metabolism)? And, why are they shared by many pathogen species?

The high percentage of moonlighting proteins of our database that have been reported as pathogen virulence factors prompts a question: Are most of them true virulence factors? Since the function of pathogen moonlighting proteins is related to key metabolism, hence essential proteins, it is difficult to generate mutants or full-gene knock-outs in order to perform direct experimental demonstrations on their virulence involvement. However, some newly designed and naturally existing mutants do not lose the essential canonical function but become non-virulent in *in vivo* challenges. For example, in anaerobic conditions bacteria lack an operative glycolytic pathway. *Neisseria meningitidis*, which uses fructose-1,6 biphosphate aldolase as an adhesin, loses the adherence capacity to the epithelial and endothelial cells when isogenically mutated, but this capacity returns in the complementation assay (Tunio *et al.* 2010). Another example of modification of a glycolytic enzyme without losing its canonical function is glyceraldehyde phosphate dehydrogenase of *Streptococcus pyogenes*. Here, a small hydrophobic amino acid sequence has been added reducing the amounts of GAPDH at the surface of the microorganism and this mutant loses its virulence in mice (Jin, Agarwal and Pancholi 2011). Finally, another example of a bacterial moonlighting protein acting as a toxin is GroEL, the archetypal category of virulence factors. This protein is an insect neurotoxin in Enterobacter aerogens, but is inactive in *Escherichia coli* when mutated in three amino acids (Yoshida *et al.*2001).

We propose a hypothesis based on our previous work (Amela, Cedano and Querol 2007). In that work, we suggested that, given that one of the most important tasks of the immune system is the differentiation between self and non-selfantigens, this system would discard eliciting protective antibodies against pathogen proteins sharing epitopes with host proteins (epitope mimicry), because this could be the cause of autoimmune diseases (Benoist and Mathis 2001). It means that although many pathogen proteins can be antigenic only a few of them would elicit a protective immune response.

RESULTS AND DISCUSSION

As said before, most of the moonlighting proteins belong to the central metabolism (glycolysis, Krebs cycle...) and their enzyme

amino acid sequences are highly conserved throughout evolution (Henderson and Martin 2011; Amblee and Jeffery 2015). Therefore, they probably share epitopes. Obviously, this has a particular importance for the design and development of successful subunit recombinant vaccines, especially with the advent of reverse vaccinology.

Figure 1 shows a multialignment of nine representatives of one of the most prevalent virulence moonlighting proteins, enolases, versus the human orthologous protein, alpha-enolase. The predicted B-cell epitopes are highlighted in yellow in all the enolase protein sequences. As can be seen, many human epitopes overlap with highly conserved amino acid sequence stretches present in all enolases. According to our hypothesis the human immune system would not elicit protective antibodies against the pathogen's enolases. As we have previously described, there are examples in which even a lower number of shared epitopes can be responsible of an autoimmune response (Amela, Cedano and Querol 2007). Table S2 (Supporting Information) shows other examples of other pathogen's moonlighting enzymes (GAPDH, PDK, PMD…) aligned with the human orthologous counterpart. As can be seen, they also match stretches of amino acid sequence and overlap predicted epitopes.

In fact, it has been reported that streptococcal enolase cross-react with human enolase and may be involved in autoimmune conditions and complications following infection (Fontan et al. 2000; Cole et al. 2005).

An exhaustive inspection of Violinet database shows that no moonlighting protein is, as a vaccine, in the market, which is a good indicator of being a true protective antigen. Few (some chaperones such as GroEL and hsp70) are even in the lower Violinet status (*research*) —some chaperones such as GroEL and hsp70. Moreover, in all the cases involving moonlighting proteins, the assays have been done on mice, guinea pig or rabbit showing quite a low level of protection (≤20%) or the authors merely indicate the presence of 'some degree' of immune response. According to Violinet, the recombinant proteins that have reached the market (or are close to doing it, like an ebola vaccine) are shown in Table 1.

A BLASTP pair alignment of these proteins versus the human and mammal proteomes shows that the output states *No significant similarity found*.

These results agree with our hypothesis that the host, in order to avoid an autoimmune response, avoids eliciting protective antibodies against pathogen proteins with which it shares epitopes. Therefore, pathogen evolution would positively select those virulence proteins whose amino acid sequence is conserved to some degree. Our hypothesis would explain the lack of successful subunit vaccines present in the market that are based on these moonlighting proteins. On the other hand, due to the degree of conserved sequence and shared and epitopes, it should exist cross-strain protective immunity using these moonlighting proteins as subunit vaccines, which is not the case. For all these reasons, a strategy based on designing a vaccine using a moonlighting protein as the main antigen might be unsuccessful.

Table 1. Current recombinant protein vaccines.

Pathogen	Host	Recombinant protiens
Neisseria meningitidis	Human	NHBA, NadA and FHbp
Borrelia burgdorferi	Human	OspA
Bordetella pertussis	Human	fhaB
Human papilloma virus	Human	L1
Hepatitis B virus	Human	Capsid protein
Zaire ebola virus	Human	Vp35
Pig circovirus	Pig	Capsid protein
Pasteurella multocida serotype D	Pig	Dermonecrotoxin

MATERIALS AND METHODS

Pathogen virulence proteins that are moonlighting were collected from MultitaskProtDB-II (Franco et al. 2018), (see Table S1, Supporting Information). The vaccine candidate proteins were obtained from the database Violinet (He et al. 2014), which contains 800 proteins that have been tested as subunit vaccines (recombinant or isolated) and then purified. These proteins can be real marketed vaccines, licensed as successful ones, or having only a 'research' status.

Continuous B-cell epitopes of the human orthologous proteins of the previously said pathogen virulence proteins (i.e. enolases) were predicted using the algorithm BepiPred (Larsen, Lund and Nielssen 2006). Protein sequence alignments were performed with BLASTP of the NCBI server (Altschul et al. 1997), and multialignments were done with Clustal–Omega of the EBI Server (Li et al. 2015). Both analyses were performed under the server default parameters.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSPD* online.

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Conflict of interest. None declare.

REFERENCES

Altschul SF, Madden TL, Schäffer AA *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.

Amblee V, Jeffery C. Physical features of intracellular proteins that moonlight on the cell surface. *PLoS One*. 2015;**10**:e0130575. doi: 10.1371 /journal.pone.0130575.

Amela I, Cedano J, Querol E. Pathogen proteins eliciting antibodies do not share epitopes with host proteins: a bioinformatics approach. *PLoS One* 2007;**2**:e512.

Benoist C, Mathis D. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat Immunol* 2001;**2**:797–801.

Chen C, Zabad S, Liu H *et al.* MoonProt 2.0: an expansion and update of the moonlighting proteins database. *Nucleic Acids Res* 2018;**46**:D640–4.

Cole JN, Ramirez RD, Currie BJ *et al.* Surface analyses and immune reactivities of major cell wall-associated proteins of group A *Streptococcus*. *Infect Immun* 2005;**73**:3137–46.

Copley SD. Moonlighting is mainstream: paradigm adjustment required. *Bioessays* 2012;**34**:578–88.

Fontan PA, Pancholi V, Nociari MM *et al.* Antibodies to streptococcal surface enolase react with human alpha-enolase:

implications in poststreptococcal sequelae. *J Infect Dis* 2000;**182**:1712–21.

Franco-Serrano L, Hernández S, Calvo A *et al*. MultitaskProtDB-II: an update of a database of multitasking/moonlighting proteins/moonlighting proteins. *Nucleic Acids Res* 2018;**46**:D645–8.

Henderson B, Martin A. Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. *Infect Immun* 2011;**79**:3476–91.

He Y, Racz R, Sayers S *et al*. Updates on the web-based VIOLIN vaccine database and analysis system. *Nucl Acids Res* 2014;**42**:D1124–32.

Huberts DH, van der Klei IJ. Moonlighting proteins: an intriguing mode of multitasking. *Biochim Biophys Acta* 2010;**1803**:520–5.

Jeffery CJ. An introduction to protein moonlighting. *Biochem Soc Trans*. 2014;**42**:1679–83.

Jin H, Agarwal S, Pancholi V. Surface export of GAPDH/SDH, a glycolytic enzyme, is essential for *Streptococcus pyogenes* virulence. *mBio*. 2011;**2**:e00068–11.

Larsen JEP, Lund O, Nielssen M. Improved method for predicting linear B-cell epitopes. *Immunome Res* 2006;**2**:2.

Li W, Cowley A, Uludag M *et al*. The EMBL-EBI bioinformatics web and programmatic tools framework. *Nucleic Acids Res* 2015;**43**:W580–4.

Tunio SA, Oldfield NJ, Berry A *et al*. The moonlighting protein fructose-1,6 biphosphate aldolase of *Neisseria meningitidis* adherence to human cells. *BMC Microbiol*. 2010;**76**:605–15.

Yoshida N, Okeda K, Watanabe E *et al*. Protein function. Chaperonin turned insect toxin. *Nature*. 2001;**411**:44.