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Note

Application of reverse vaccinology for the identification of epitope candidates from *Rickettsia rickettsii*

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Rocky mountain spotted fever is a severe disease caused by *Rickettsia rickettsii* that frequently causes the death of the patients. As there are not effective vaccines for this disease, we employed reverse vaccinology to find epitope candidates useful for vaccine development. To apply this bioinformatics, we used the following online software: ProPred1, RANKPEP, and HLA binding, to evaluate 143 amino acid sequences in the genome of *Rickettsia rickettsii* (NC_009882 Sheila Smith). This strategy allowed us to identify 19 epitope sequences with affinity to HLA I alleles: A0201, A24; HLA-B: B3501, B3901.

Keywords: Epitope prediction, Rocky mountain spotted fever, Vaccine

Introduction

Rickettsia rickettsii (R. rickettsii) is a gram-negative bacteria that infects human hosts following its inoculation through the bite of arthropod vectors, producing a disease characterized byendothelial dysfunction, vasculitis, coagulopathy, myocarditis, and organic failure in severe cases[1]. Several efforts have been made to reduce mortality of rickettsiosis through vaccination using whole killed and attenuated bacteria, but also using recombinant proteins composed of single or multiple epitopes; however, even than those have different degrees of success we still lack a vaccine for humans[2]. It is known the critical role of cellular response by Th1 and T CD8+ cell in rickettsiosis which has been demonstrated through diverse epitopes however, experimental and analysis procedures to identify new epitope targets are expensive and time consuming; in contrast with its prediction through *in silico* assays like reverse vaccinology[2,3]. This approach could predict several candidate proteins through mathematical simulation of host-pathogen interaction models like *Rickettsia prowazekii*, with different degrees of success[4,5]. In this study, we did the first complete scanning of the whole genome of *R. rickettsii* (NC_009882. Sheila Smith) to identify epitope sequences related to the cellular response by $CD8^+$ lymphocytes, that could be used for vaccine development, considering its binding capacity and affinity with the most frequent HLA alleles, and its homology with proteins from the bacteria but not from its human host.

Materials and Methods

Individuals within the same species can express different alleles from HLA I capable to recognize and bind different epitopes[6]. There is a direct association between the immunogenicity and the affinity of an HLA peptide complex, however, its stability is equally important, as a low dissociation velocity grants enough stability for the complex in the cell surface to promote adequate interactions with specific T lymphocytes[7]. Considering this, the first step was to evaluate1343 sequences to predict epitopes that could bind to the most frequently expressed molecules of HLA I worldwide (HLA A0201, A24, B3501, B3901) using ProPred1 (http://www.imtech.res.in/raghava/propred1/) and Rankpep (http://imed.med.ucm.es/Tools/rankpep.html). Additionally, the average time of dissociation in min at 37°C for each epitope was obtained with the HLA Peptide Binding Prediction Tool (http://www-bimas.cit.nih.gov/molbio/hla_bind/). Results are shown in (Table 1).

Results

We obtained 19 sequences with at least two promiscuous epitopes that could bind to at least two HLA alleles. Identity analyses employing the BLAST tool did not found an identity with human sequences avoiding the possibility of autoimmunity, but identified up to 98% identity with amino acid sequences from other *Rickettsia* species (Table 1). Among these, outer membrane proteins (Omps), particularly OmpA and OmpB, have been tested with promising results, being able to elicit humoral and cellular immune responses which interestingly can provide cross reactivity against different *Rickettsia* species however, this response is not always protective[2,8-10].

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Table 1 — *In silico* analysis of the chosen amino acid from *Rickettsia rickettsii*

Gene	Access Number	Length in aa	Positions (aa)	Promiscuous epitope	Attachable alleles	Propred1	Rankpep	Propred1/ Rankpep Mean	HLA Binding
Autotransporter outer membrane β-barrel domain-containing protein [*Rickettsia rickettsii*]	WP_012151379	2249	128	KGNLLPVTL	A24	36.16%	4.69%	20.43%	14.4
					B3501	7.8%	22.92%	15.36%	2
			241, 460, 607, 754, 829, 904	KLTNAASVL	A0201	39.17%	67.19%	53.18%	30.6
					A24	28.19%	3.90%	16.05%	8
			266, 485, 632, 779, 854, 929	TGGDNVGVL	B3501	7.88%	34.14%	21.01%	2
					B3901	24.41%	3.11%	13.76%	9
			1279	RARDSVLVL	A24	30.67%	5.23%	17.95%	9.6
					B3501	40.35%	2.76%	21.56%	36
190-KDa cell surface antigen [*Rickettsia rickettsii* str. 'Sheila Smith']	ABV76839.1	2249	241, 460, 607, 754, 829, 904	KLTNAASVL	A0201	19.17%	67.19%	43.18%	30.6
					A24	28.19%	3.9%	16.05%	8
					B3501	7.8%	41.16%	24.48%	2
			266, 485, 632, 779, 854, 929	TGGDNVGVL	B3501	7.8%	34.14%	20.97%	2
					B3901	24.41%	3.11%	13.76%	9
			1279	RARDSVLVL	A24	30.67%	45.31%	37.99%	9.6
					B3501	40.35%	5.23%	22.79%	36
			1449	NSVTAGKKL	A24	25.59%	15.31%	20.45%	6.6
					B3501	18.12%	20.31%	19.22%	5
OmpW family protein [*Rickettsia rickettsii*]	WP_012150330	244		VLRTKYTSL	A0201	7.96%	60.94%	34.45%	4.1
			109		B3501	12.37%	43.87%	28.12%	3
			223	KTMTSKVKL	A0201	7.54%	44.53%	26.04%	3.8
					A24	34.98%	7.35%	21.17%	13.2
					B3501	7.8%	18.04%	12.92%	2
Autotransporter outer membrane β-barrel domain-containing protein [*Rickettsia rickettsii*]	WP_012151219.1	1654	92	LLLNTANNL	A0201	27.44%	60.16%	43.80%	134.3
					A24	26.77%	11.73%	19.25%	7.2
			435	ITFDANGTL	B3501	7.8%	27.12%	17.46%	2
					B3901	27.61%	32.81%	30.21%	12
			1155	TSIETTLTL	A24	26.77%	10.30%	18.54%	7.2
					B3501	25.92%	4.31%	15.12%	10
					B3901	27.61%	17.20%	22.41%	12
			1513	VLVTPMAGL	A0201	24.78%	64.06%	44.42%	83.5
					B3901	24.41%	23.16%	23.79%	9
Endolytic transglycosylaseMltG [*Rickettsia rickettsii*]	WP_012150645.1	339	9	KLFLVIVSL	A0201	36.15%	71.88%	54.02%	636.2
					B3501	7.8%	18.66%	13.23%	2
					B3901	24.41%	7.60%	16.01%	9
			189	KTRLEVLTL	A24	28.19%	0.76%	14.48%	8
					B3501	24.74%	18.66%	21.70%	9
			237	ALTEGKFKL	A0201	34.89%	61.72%	48.31%	507.9
					B3501	7.8%	28.10%	17.95%	2
NAD-glutamate dehydrogenase [*Rickettsia rickettsii*]	WP_012151293.1	1584	89	IENDPAINV	A0201	12.56%	0%	6.28%	9.42
					B4403	15.30%	0%	7.65%	6
			223	LQNDNLVLL	A0201	19.87%	39.84%	29.86%	34.7
					A24	26.77%	4.50%	15.64%	7.2
					B3501	7.8%	10.23%	9.02%	2
					B3901	24.41%	2.45%	13.43%	9
			553	KIYSPKVKL	A0201	19.56%	57.03%	38.30%	32.8
					A24	29.49%	1.66%	15.58%	8.8
					B3501	7.8%	24.74%	16.27%	2
			1037	KLSPEIKKL	A0201	28.83%	58.59%	43.71%	171.9
					A24	31.96%	1.16%	16.56%	10.5
					B3501	12.37%	33.25%	22.81%	3

(*Contd.*)

Table 1 — *In silico* analysis of the chosen amino acid from *Rickettsia rickettsii*

Gene	Access Number	Length in aa	Positions (aa)	Promiscuous epitope	Attachable alleles	Propred1	Rankpep	Propred1/ Rankpep Mean	HLA Binding
Palindromic element RPE1 domain-containing protein [*Rickettsia rickettsii*]	WP_041472420	379	32	KLLSLPISL	A0201	31.49%	65.62%	48.56%	276.643
					A24	36.16%	7.22%	21.69%	14.400
					B3501	7.8%	22.85%	15.33%	2
			192	KVNAESANL	A0201	10.52%	51.56%	31.04%	6.542
					A24	33.69%	11.32%	22.51%	12
					B3501	12.37%	41.38%	26.88%	3
			289	NSINPLVNL	B3501	18.12%	20.24%	19.18%	5
					B3901	24.41%	9.23%	16.82%	9
MULTISPECIES: PIN domain-containing protein [spotted fever group]	WP_012150708.1	128	4	YILDSSALL	A0201	29.81%	69.53%	49.67%	204.973
					B3501	7.8%	14.28%	11.04%	2
					B3901	27.61%	1.19%	14.40%	12
			41	VVAELDKKL	A0201	14.38%	44.53%	29.46%	13.028
					A24	25.03%	4.09%	14.56%	6.336
					B3501	7.8%	18.87%	13.34%	2
Excinuclease ABC subunit UvrA [*Rickettsia rickettsii*]	WP_012151403.1	953	221	DSLESSLNL	A24	26.77%	8.28%	17.53%	7.2
					B3501	25.92%	19.22%	22.57%	10
					B3901	27.61%	4.18%	15.90%	12
			233	ITYLEIVEL	A0201	7.35%	39.84%	23.60%	3.712
					B3901	24.41%	6.73%	15.57%	9
			327	FILETLKAL	A0201	34.47%	64.06%	49.27%	471.437
					A24	26.77%	2.13%	14.45%	7.2
					B3901	32.12%	2.50%	17.31%	18
			342	SIEVPFVSL	A24	26.77%	11.95%	19.36%	7.2
					B3901	24.41%	7.63%	16.02%	9
			538	RLIETLKRL	A0201	29.71%	69.53%	49.62%	201.447
					A24	36.16%	2.14%	19.15%	14.400
					B3501	15.61%	21.09%	18.35%	4
					B3901	32.12%	4.67%	18.40%	18
			817	LIYEKLITL	A0201	28.06%	71.88%	49.97%	150.683
					B3501	7.8%	19.30%	13.55%	2
					B3901	32.12%	15.68%	23.90%	18
MULTISPECIES: hypothetical protein [*Rickettsia*]	WP_012150252.1	77	31	KLQEPIKRL	A0201	36.73%	62.50%	49.62%	705.066
					A24	36.16%	0.61 %	18.39%	14.400
					B3501	15.61%	32.94 %	24.28%	4
					B3901	24.41%	0.21 %	12.31%	9
Acetylglutamate kinase [*Rickettsia rickettsii]*	WP_012150360.1	266	225	FIEEALIKI	A0201	7.74%	70.31%	39.03%	3.982
					B3901	24.41%	5.88 %	15.15%	9
Mrp/NBP35 family ATP-binding protein [*Rickettsia rickettsii*]	WP_012150381.1	319	304	LPLTNLLTL	B3501	33.73%	64.19%	48.96%	20
					B3901	24.41%	22.01 %	23.21%	9
Mrp/NBP35 family ATP-binding protein [*Rickettsia rickettsii*]	WP_012150394.1	239	66	QMVRGVVNL	A0201	19.99%	60.94%	40.47%	35.485
					B3901	24.41%	2.63 %	13.52%	9

(*Contd.*)

Table 1 — *In silico* analysis of the chosen amino acid from *Rickettsia rickettsii*

Sequence	Access Number	Length in aa	Positions (aa)	Promiscuous epitope	Attachable alleles	Propred1	Rankpep	Propred1/ Rankpep Mean	HLA Binding
Hypothetical protein [*Rickettsia rickettsii*]	WP_012150889.1	70aa	29	HSPATIKAF	B3501	18.12%	17%	17.56%	10
					B4403	3.46%	-------	3.46%	0.6
			45	LLYDNAIGL	A0201	34.53%	60.94%	47.74%	476.467
					B3501	7.8%	19.43%	13.62%	2
					B3901	27.61%	7%	17.31%	12
Hypothetical protein *[Rickettsia rickettsii]*	WP_012150747.1	319aa	184	TGEEGRKYL	A24	26.77%	20.93%	23.85%	7.2
					B3901	24.41%	3.51%	13.96%	9
			237	KLGAKVKTL	A0201	28.83%	64.06%	46.45%	171.967
					B3501	7.8%	48.65%	28.23%	2
Hypothetical protein [*Rickettsia rickettsii*]	WP_012150240.1	307 aa	128	FEEDFFKKL	A0201	7.2%	11.72%	9.46%	3.620
					B4403	18.77%	0	9.39%	9
			240	TLIEKLKDL	A0201	29.71%	60.94%	45.33%	201.447
					A24	26.77%	1.50%	14.14%	7.2
					B3501	7.8%	19.83%	13.82%	2
					B3901	32.12%	2.79%	17.46%	18
			250	IPLGSNAWL	A0201	8.81%	14.06%	11.44%	4.824
					B3501	33.73%	34.89%	34.31%	20
hypothetical protein [*Rickettsia rickettsii*]	WP_012150991.1	420 aa	39	KQEENTDFL	A0201	9.4%	17.97%	13.69%	5.361
					A24	36.16%	6.06%	21.11%	14.4
					B3901	32.12%	0.38%	16.25%	18
			181	NLLDQLETL	A0201	33.04%	66.41%	49.73%	365.224
					A24	26.77%	0.39%	13.58%	7.2
					B3501	7.8%	19.08	957.90%	2
					B3901	32.12%	15.82%	23.97%	18
			198	ILNTSENAL	A0201	20.12%	53.12%	36.62%	36.316
					A24	26.77%	0.39%	13.58%	7.2
Hypothetical protein [*Rickettsia rickettsii*]	WP_012150268.1	131 aa	63	GLYENAANL	A0201	31.56%	66.41%	48.99%	280.275
					B3501	7.8%	45.27%	26.54%	2
					B3901	27.61%	5.97%	16.79%	12
			105	YAKEAFNEL	A24	27.5%	17.34%	22.42%	7.603
					B3501	32.54%	0.67%	16.61%	18
Hypothetical protein [*Rickettsia rickettsii*]	WP_012150364.1	966 aa	4	NLLKVLGVL	A24	28.85%	0.06%	14.46%	8.4
					B3901	24.41%	3.47%	13.94%	9
				VSQALPSKL	A24	24.06%	21.74%	22.90%	7.920
			385		B3501	18.12%	20.74%	19.43%	5
				ILNDPGNNL	A0201	28.02%	39.84%	33.93%	148.896
			525		A24	29.24%	2.70%	15.97%	8.640
					B3501	7.8%	39.46%	23.63%	2
			786	VMMAGIIVL	A0201	25.29%	69.53%	47.41%	91.513
					B3901	24.41%	0.55%	12.48%	9
			819	KIPFIGTIL	A0201	8.06%	25.78%	16.92%	4.215
					A24	38.25%	0.69%	19.47%	16.8
					B3501	7.8%	35.21%	21.51%	2

Discussion

In our analysis we found several small regions potentially immunogenic among different Omps, that could be analyzed as sub-unit vaccines looking for stronger and oriented responses as it has been done in other studies[8-11]. Reports related to OmpA have used epitopes from the middle and c-terminal portions of the protein, whereas our study suggest that there are several epitopes within the first 1500 amino acids which could be potential immunogenic candidates[11,12]. This is also the case for the identified epitopes in OmpB, which are contained within the first

1600 amino acids of the protein; a region that experimentally has shown its immunogenic and protective potential, but has not been deeply evaluated in further studies[8-11]. We also identified peptides in 9 proteins related to metabolism, which has not been experimentally analyzed in terms of immunogenicity and could be interesting for future approaches.Further analysis of the sequences using BLAST allowed to assign name and function for several sequences however, 5 of them remained hypothetical. Interestingly, we found that the sequence of the hypothetical protein A1G_00830 contains the motifs of VirB6, a component of the type IV secretion system (TIVSS), a protein complex implied in the exchange of genetic material that, due to its transmembrane localization, is an interesting target for neutralizing and protective immune responses particularly linked by recognition between T $CD4^+$ and β cells, as it has been studied in *Anaplasma marginale*[13,14]. VirB6-like protein are codified by five tandemly arrayed genes in *Rickettsia* species (*rvhB6a-c*), that shows highly divergent and surface exposed N and C sequences flanking the VirB6/TrbL domain[13]. The impact of such potential variability in the structure of TIVSS in the pathogenicity and immunogenicity of rickettsial pathogens remains to be studied, but suggest that could be interesting targets for diagnosis or vaccine development.

As shown by data obtained in this work, reverse vaccinology is a promising strategy that allowed to find several peptides that could be evaluated in vaccine trials, considering not only its immunogenic potential in terms of the cellular response that could elicit but also, its binding strength and affinity for the more abundantly HLA molecules expressed worldwide.

Conclusion

In the present study it was possible to predict new high potential immunogenic sequences for the development of a candidate vaccine, from the complete genome of *R. rickettsii*, through bioinformatic studies of reverse vaccinology. The selection of this limited number of promising peptides makes their evaluation feasible in subsequent *in vivo* studies with animal models to check their immunogenicity and protection.

Conflict of interest

All authors declare no conflict of interest.

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