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Catalase epitopes vaccine design for *Helicobacter pylori*: A bioinformatics approach

Niloufar Rashidi, Sharareh Moghim, Jamshid Fagheri and Hajieh Ghasemian Safaei*

Department of Microbiology, Faculty of Medicine, Isfahan University of Medical Sciences, Hezar jarib Street, Isfahan, Iran.

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Bioinformatics tools are helpful for epitopes prediction directly from the genomes of pathogens in order to design a vaccine. Epitopes are sub-sequences of proteins (8 to 10 mer peptides) which bind to MHC to interact with the T cell receptors and stimulate immune responses. Finding a suitable vaccine against *Helicobacter pylori* is necessary, because of high prevalence of the infection (25 to 90%). Moreover, this bacteria has been classified as a grade I carcinogen by WHO since 1994. Catalase, an important enzyme in the virulence of *H. pylori*, could be a suitable candidate for vaccine design because it is highly conserved, which is important for the survival of *H. pylori*; it is expressed in high level and it is exposed on the surface of the bacteria. In this study, we designed epitope-based vaccine for catalase specific regions of *H. pylori* by means of immunobioinformatic tools. *H. pylori* (26695) catalase has been compared with human catalase in order to select specific regions. Afterwards, epitopes of catalase were determined by propred software. Among predicted epitopes, three epitopes were selected including, MVNKDVKQTT, VLLQSTWFL and FHPFDVTKI. Three candidates out of 51 catalase antigen epitopes had the highest score for reactivating with MHC II MHC in propred software. The candidate epitopes for vaccine design should be rather a composition of considering epitopes: MVNKDVKQTTKKVLLQSTWFLKKFHPFDVTKI. In this manner, 39 of 51 alleles of MHC class II were involved and stimulated T-cell responses. We believe prediction of catalase epitopes by the immunoinformatics tools would be valuable for developing new immuoprophylactic strategy against *H. pylori* infection.

Key words: *Helicobacter pylori*, catalase, epitopes.

INTRODUCTION

Importance of epitopes and bioinformatics tools

New genome analysis tools based on bioinformatics and immunoinformatics technologies help us to select the correct antigen or epitope of interest directly from the genomes of pathogens in order to design a vaccine (De Groot et al., 2001; Moise et al., 2008; Zhou et al., 2009).

To develop an epitope-based vaccine, it is necessary to detect immunostimulatory patterns. Protective immune responses may be generated based on a single immune-dominant or more epitopes (De Groot and Berzofsky, 2004). Thus, it is important to choose epitopes derived

from conserved peptide sequences (Khan et al., 2006). T-cell responses are stimulated by the presence of short peptides bound on the surface of antigen presenting cells and binding to major histocompatibility complex (MHC) molecules (De Groot and Berzofsky, 2004).

It is important to consider the protein antigen sequences that function at T-cell epitopes for successful vaccine formulations (Sette et al., 2001; Brusica and August, 2004; Khan et al., 2006). These tools allow us to decide on epitopes or sub-sequences of proteins that interface with the T-cells of the host and predict the MHC binder (De Groot and Berzofsky, 2004). Such tools let the scanning of genome-derived protein sequences for T-cell epitopes and the 8 to 10 mer peptides that bind to MHC and interact with the T cell receptor, stimulate T-cell response. These epitopes prediction tools are proved to be very useful, since they significantly reduce the time and

*Corresponding author. E-mail: ghasemian@med.mui.ac.ir. Tel: 983117922469. Fax: 983116688597.

effort implicated in screening probable epitopes, mainly for pathogens that do not currently present available vaccines and still needs to be further investigated (De Groot and Berzofsky, 2004; Shi et al., 2007; Moise et al., 2008; Zhou et al., 2009). A usual epitope-based vaccine build contains epitopes inserted repeatedly in the construct, with or without intervening spacer amino acids (De Groot et al., 2001; Zhou et al., 2009; Liu et al., 2004). The advantages of this kind of vaccine are: stimulating specific immune responses against conserved sequences, increasing safety and increased strength and extent by the application of practically engineer epitopes (Shi et al., 2007).

Selection of candidate protein sequences can be followed by the use of computerized (*in silico*) software to recognize regions of the proteins that are probable immunogenic. These peptides, representing the epitopes, could be synthesized and their ability to stimulate immune response could be monitored, *in vitro*, by using prepared T-cells from patients (De Groot and Berzofsky, 2004).

***Helicobacter pylori* infection and antigens**

Several microbial agents that cause considerably high rates of mortality and diseases are still waiting for vaccines. One of them is *Helicobacter pylori* (*H. pylori*). There are several problems caused by *H. pylori* infection including: (1) the eradication is not well satisfied; (2) high re-infection rates of patients; (3) high amount of drug dose for treatment; (4) antibiotics resistance (5) high prevalence (80 to 90%) of infected populations in developing countries (Farshad et al., 2009; Ruggiero et al., 2003; Graham, 2000); (6) and classification as a grade 1 carcinogen by WHO (Radcliff et al., 1997; Schistosomes, 1994).

An antigen should have important features to be determined as a vaccine candidate: it should be expressed at the surface abundantly, in order to be easily recognized by immune system, and the sequences should be well conserved and represented as a key virulence factors (Ruggiero et al., 2003). Most of the vaccine of *H. pylori* antigen investigated in mouse model was whole cell of bacteria, urease, heat shock protein, vacuolating cytotoxin and recently, catalase (Ruggiero et al., 2003; Liu et al., 2004).

Catalase could be a suitable candidate for vaccine design. It has very conserved domains, it is expressed in high levels (1% of the cell's total protein) (Harris et al., 2002), it is an important factor for survival of *H. pylori* in an environment rich in toxic oxygen species, found in both the cytosol and the periplasmic space and therefore, it is exposed on bacterial surface and could be easily identified by the immune system (Ramarao et al., 2000; Harris et al., 2002; Radcliff et al., 1997; Guy et al. 2005; Hazell et al., 1991).

Immunity

The mechanisms of defense against *H. pylori* infection are uncertain. Despite strong immune responses, the infection remains for a long time. It is suggested that the *H. pylori* can evade both innate and adaptive immune responses. The immune systems are triggered by *H. pylori* in nature but the responses are not good enough to eliminate this pathogen (Shi et al., 2007; Baldari et al., 2005). There are increasing evidences to hold up that MHCII restricted CD4+ cells play an essential role in defense mechanisms, more than antibodies. A successful immunotherapeutic or immunoprophylactic plan against *H. pylori* should include CD4+ Th cell epitopes (Shi et al., 2007; Ermak et al., 1998; Pappo et al., 1999). Therefore, we believe that stimulating the immune response by vaccine, compared with that of the nature infection could be more effective against *H. pylori* (Shi et al., 2007). In this study, we decided to design epitope-based vaccine according to immunobioinformatic tools for the catalase of *H. pylori*.

MATERIALS AND METHODS

Data collection and identification of specific sequences

Complete sequence of *H. pylori* 26695 catalase were acquired from NCBI; accession code, NP-207669, 505 amino acid and used to identify the specific sequences of catalase in *H. pylori* (26695) and were compared with human catalase (NCBI, accession code: NP-001743) and other strains of *H. pylori* using blast. The sequence of *H. pylori* 26695 catalase had 98 to 100% similarity with the catalase of other *H. pylori* strains (25 strains) which are sequenced in NCBI such as *H. pylori* P12, G27, shi470, J99, ss1, 51,F32, 35A, 908, India 7, etc.

Prediction of T-cell epitopes

Several T-cell epitopes that are identified by more than one MHC and recognized by more than one T- cell clone are called 'promiscuous' epitopes (De Groot et al., 2001; Khan et al., 2006). Different prediction methods according to biological information are considered for epitopes. In this study, we chose ProPred software for prediction promiscuous T-cell epitopes (Singh and Raghava, 2001; Contini et al., 2008; Saltini et al., 2008). The proPred web interface allows users to predict MHC class II binding regions in antigen sequence. 51 MHC class II epitope-mapping matrices were developed at proPred; therefore, it is helpful for the user in locating promiscuous or allele specific binding regions in a query antigen. The peptides (epitopes) predicted in this software, are nanomer. In each nanomer, there is an anchor or a starting residue. The 51 alleles of MHC class II in proPred software were as following : DRB1:0101,0102,0301,0305,0306,0307,0308,0309,0311,0401,0402,0404,0405,0408,0410,0421,0423,0426,0701,0703,0801,0802,0804,0806,0813,0817,1101,1102,1104,1106,1107,1114,1120,1121,1128,1301,1302,1304,1305,1307,1311,1321,1322,1323,1327,1328,1501,1502,1506, DRB5:0101,0105.

The proPred software displayed predicted binders as blue colored region and P1 anchor or the starting residue of each predicted binding frame as red colored. This illustration is useful to highlight the locating promiscuous binding regions (Table 1).

RESULTS

In this study, we selected three epitopes from unique specific and specific regions (Table 1). The first epitope was chosen from 1 to 8, the second epitope from 32 to 44 and the third epitope from 267 to 288 regions of *H. pylori* catalase (Table2).

Of the 51 epitopes, the selected epitopes had the highest score for reactivating MHC class II in ProPred software (Table 3). The first epitope (MVNKDVKQTT) contained 26, the second (VLLQSTWFL) contained 21 and the third (FHPFDVTKI) contained 16 alleles of the 51 MHCII (Table2).

According to blast with human catalase (NCBI, accession code: NP-001743), our result showed that the unique specific regions contained eight amino acids of the initial region from N-terminal domain (MVNKDVKQ) and 19 amino acids of the final region from C-terminal domain (QKMMKDMHGKDMHHTKKKK). Other regions (shown as yellow boxes) were specific (Figure 1) as follows: sequences from 10 to 20 (TAFGAPVWDDN); 32 to 44(LLQSTWFLEKLAA); 209 to 243(ERFWVKFHFHT-MQGVKHLTNEEAEEVRKYDPDS); 267 to 288 (PEEDA-KKYRFHPFDVTKIWIYLQ); 372 to 381(MQNGYYGSLO); 421-431(DDSDYYTQPG); 428-437(QPGDYRSLP).

DISCUSSION

Prevalence of *H. pylori* infection is from 25% in developed countries to more than 90% in developing areas. *H. pylori* is a major cause of gastric carcinoma and lymphoma (Safaei et al., 2008; Rashidi and Ghasemian, 2005; Safaei et al., 2005; Mojtahedi et al., 2007; Safaei et al., 2010).

Therapeutic vaccines against *H. pylori*, which have been assayed so far, are whole bacteria lysate, recombinant subunit antigen vaccines and DNA vaccines but no major advance has been achieved (Zhou et al., 2009).

CD4⁺ T-cells play important roles in producing protective immunity (Shi et al., 2007). Therefore, prediction antigenic epitopes in the context of MHC class II molecules expressed on APC (antigen presenting cell) recognized by CD4⁺ T-cells can be use as a suitable tool for vaccination.

Catalase is a highly conserved enzyme, important for survival of the organism. There is 98 to 100% similarity between catalase of all *Helicobacter* species. This enzyme is expressed in high level and it is exposed on the surface of bacteria; therefore, it is a suitable antigen candidate for epitope vaccine evaluation (Radcliff et al., 1997). Therefore, in this study, we designed epitope vaccine for *H. pylori* catalase with ProPred software. The suggested epitope should be rather a composition of selected three epitopes: MVNKDVKQTTKVVLLQSTWFLKKFHPFDVTKI. This epitope involved 39 out of 51 alleles of MHC class II and it stimulated T-cell responses. Lysines (KK) that is located between epitopes have some

advantages: (1) cathepsine B, a protease that has role in processing antigens for presentation by MHC II, considers KK as a target; (2) it is possible that a new epitope is formed from linkage of some epitopes; therefore, lysines (KK) are an avoiding factor for this undesired event (Zhou et al., 2009). Guy et al. (2005) hypothesized four short fragments in N-terminal domain of *H. pylori* catalase (N1, N2, N3 and N4) and three short fragments in C-terminal domain which have shown a very low degree of sequence similarity with the human enzyme and can be introduced as specific regions for *H. pylori* protein. Our selected epitopes located in N1, N2 and N4 region was based on Guy's study. The first epitope was chosen from the amino acid residue of 1 to 20 which is the specific region of N1, the second epitope was from the amino acid residue of 30 to 44 which is the specific region of N2 and the third epitope was from the amino acid residue of 267 to 288 which is the specific region of N4 (Guy et al., 2005).

Sequences 10 to 20, 372 to 381, 421 to 431 and 428 to 437 are specific regions and 490 to 505 is the unique specific region according to blast (Figure 1). But there was no epitopes in them according to ProPred software and could not be selected as a vaccine candidate.

In the unique specific and specific sequences of *H. pylori* catalase, 1 to 10, 32 to 44, 267 to 288, 209 to 243, MVNKDVKQTT, VLLQSTWFL, FHPFDVTKI, VKHLT-NEEA and WVKFHFHTM epitopes were located (Table 3).

Our first epitope MVNKDVKQTT covered 26 alleles. The first epitope presented a high score (with ranking 1 to 3 among all of the scores in the epitopes) in the reaction with the following alleles: 0301, 0306, 0307, 0311, 0402, 0801, 0802, 0804, 0806, 0817, 1102, 1107, 1114, 1121, 1301, 1322, 1323, 1327 and 1328. Among the selected epitopes, the first one was the only one which could alone react with the alleles: 0301, 0305, 0309, 0801, 0802, 0804, 0806 and 0817, also, the MVNKDVKQTT reacted with alleles 0305, 0309, 0813, 1120 and 1302 and presented acceptable scores.

The second epitope (VLLQSTWFL) covered 21 alleles and in the reaction with six alleles (0102, 0402, 0410, 1301, 1327 and 1328), it presented a high score. It is the only epitope that reacted with 0102, for 0410 to have a high score among all the other epitopes. It also presented acceptable scores in other alleles.

The third one (FHPFDVTKI) contained 16 alleles. It scored high in the reaction with some alleles : 0101, 0408, 0701, 0703 and 1502. It is the only epitope that reacted with 0101, 0408, 0701 and 0703 alleles and had a high score among all the other epitopes. Only this epitope among the five epitopes reacted with allele 1102 and presented acceptable score for it and other alleles.

The fourth epitope, WVKFHFHTM, covered 22 alleles. It presented a high score in the reaction with alleles as also for the first epitope (1102, 1121 and 1322) and the third epitope (1502); presented high score. It had lower

Table 1. Predicted epitopes of catalase enzyme by propped software.

Allele	1-10	31-39	276-284
DRB1_0101	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0102	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0301	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0305	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0306	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0307	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0308	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0309	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0311	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0401	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0402	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0404	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0405	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0408	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0410	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0421	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0423	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0426	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0701	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0703	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0801	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0802	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0804	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0806	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0813	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_0817	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1101	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1102	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1104	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1106	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1107	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1114	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1120	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1121	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1128	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1301	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1302	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1304	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1305	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1307	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1311	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1321	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1322	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1323	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1327	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1328	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1501	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1502	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB1_1506	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB5_0101	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI
DRB5_0105	MVNKDVKQTT	VLLQSTWFL	FHPFDVTKI

Blue region, predicted binders; red color, P1 anchor or the starting residue.

Table 2. Number of reactivating MHC class II for selected epitopes according to ProPred software.

Epitope	Site of amino acid	Reactivating MHC
MV NKDVKQTT	1-10	26
VLLQSTWFL	31-39	21
FHPFDVTKI	276-284	16

Table 3. Comparison scores between the five epitopes according to proPred software.

Allele	1*	2	3	4**	5
DRB1-0101	0	5.33	14.50	4.33	0
DRB1-0102	0	22	14.50	0	8.33
DRB1-0301	40	22.74	—	15.79	—
DRB1-0305	23.8	2.20	9.89	13.19	—
DRB1-0306	36.36	4.55	0	25	10.23
DRB1-0307	36.36	4.55	0	25	10.23
DRB1-0308	36.36	4.55	0	25	10.23
DRB1-0309	29.47	12.21	18.95	—	—
DRB1-0311	36.36	4.55	0	25	10.23
DRB1-0401	—	15.12	8.14	11.63	30.23
DRB1-0402	25	40.62	11.46	23.96	8.33
DRB1-0404	0	25	23.86	4.55	32.95
DRB1-0405	—	23.40	35.11	24.47	20.21
DRB1-0408	—	13.64	35.23	13.64	21.59
DRB1-0410	4.26	34.04	24.47	12.77	30.85
DRB1-0421	—	25.11	17.78	13.33	28.89
DRB1-0423	0	25	23.86	4.55	32.95
DRB1-0426	—	15.12	8.14	11.63	30.23
DRB1-0701	11.21	36.38	49.14	37.93	—
DRB1-0703	11.21	36.38	49.14	37.93	—
DRB1-0801	46.51	—	3.49	11.63	—
DRB1-0802	38.75	—	1.25	0	—
DRB1-0804	51.25	0	0	—	—
DRB1-0806	58.14	6.98	—	—	—
DRB1-0813	29.89	—	33.33	8.05	0
DRB1-0817	45.54	3.96	13.86	16.83	—
DRB1-1101	10.84	—	13.25	6.02	0
DRB1-1102	40.48	33.33	10.47	34.52	1.19
DRB1-1104	22.89	12.05	1.20	4.82	6.02
DRB1-1106	22.89	12.05	1.20	4.82	6.02
DRB1-1107	34.07	—	—	24.18	5.49
DRB1-1114	28.57	21.43	22.62	22.62	—
DRB1-1120	35.23	31.36	31.82	32.95	—
DRB1-1121	40.48	33.33	10.71	34.52	1.19
DRB1-1128	18.39	11.03	22.99	22.99	—
DRB1-1301	46.59	42.73	20.45	25	—
DRB1-1302	35.23	31.36	31.82	32.95	—
DRB1-1304	47.78	42.22	12.22	41.11	—
DRB1-1305	18.39	11.03	22.99	22.99	—
DRB1-1307	4.41	0	—	0	0
DRB1-1311	22.89	12.05	1.20	4.82	6.02
DRB1-1321	20.22	11.24	14.61	17.98	—

Table 3. Continue.

DRB1-1322	40.48	33.33	10.71	34.52	1.19
DRB1-1323	28.57	21.43	22.62	22.62	—
DRB1-1327	46.59	42.73	20.45	25	—
DRB1-1328	46.59	42.73	20.45	25	—
DRB1-1501	11.22	23.06	31.63	39.59	—
DRB1-1502	—	12.86	41.84	49.80	8.16
DRB1-1506	11.22	23.06	31.63	39.59	18.37
DRB5-0101	—	12.24	7.14	15.31	—
DRB5-0105	—	12.24	7.14	15.31	—

1, % highest score of MVNKDVKQTT; * 1, MVNKDVKQTT is a composition of two epitopes: MVNKDVKQT and VNKDVKQTT. Considered scores were related to epitope that had reaction with MHC or had higher score; 2, % highest score of VLLQSTWFL; 3, % highest score of FHPFDVTKI; 4, % highest score of WVKFHFHTMQ; **4- WVKFHFHTMQ, is a composition of two epitopes: WVKFHFHTM and VKFHFHTMQ. Considered scores were related to epitope that had reaction with MHC or has higher score; 5, % highest score of VKHLTNEEA. Threshold is defined as the 'percentage of best scoring natural peptides'. Threshold setting in this study was 3%. It predicts peptides in catalase sequence which belongs to the 3% best scoring natural peptides; yellow box, high score (1 to 3): the highest score with ranking of 1 to 3 among all of the scores in epitopes of specific and non specific regions; blue box, suitable score for the selected three epitopes; gray box, suitable score for the other two epitopes.

GENE ID: 822404 HP0875 | catalase [Helicobacter pylori 26695]
(10 or fewer PubMed links)

Score = 496 bits (1278), Expect = 5e-141, Method: Compositional matrix adjust.
Identities = 261/484 (53%), Positives = 334/484 (69%), Gaps = 17/484 (3%)

Query	28	TTGACNPVCGDKLNVTITVCGPRGPLLVDVVFTDEMAHFDREIPERVVHAKGACAFGYFEV	87
Sbjct	9	TT G PV D NVIT CRRCP+L+Q F +++A FDRERIPERVVHAKG+CA+C F V	68
Query	88	THDITKYSKAKVFEHIGKTKPIAVRFSTVAGESCSADTVRDPGFAVKFYTEDGNWDLVG	147
Sbjct	69	T DITHY+KAK+F +GKKT RFSTVAGE GSAD VRDPGFA+K+YTE+GNWDLVG	128
Query	148	NNTPIFFIIRDPILPSPFIHSQKRNPDTHLKDPPDMVWDFWSLRPESLHQVSLFSDRCGIPD	207
Sbjct	129	NNTPVFFIRDPIFPFIH+QKR+PQT+L + DMVWDFWS PESL+QV+++ SDRGIP	188
Query	208	CHRHMDCYCSHTFKLVNANCEAVYCKFHYKTDQCIGKLSVEDAARLSQEDDPDYCIRDLFN	267
Sbjct	189	RHM+G+CSHTF L+NA GE + KFH+ T QG+K+L+ E+AA + + DPD RDLFN	248
Query	268	AIATGKYPSWTFYIQVMTFNQAETFPFNPFDLTKVWPHKDYPLIPVGLKLVLNRPVNYFA	327
Sbjct	249	AIA C +P W IQVM A+ + F+PFD+TK+W +DYPL+ VG + LN+NP NYFA	308
Query	328	EVEQIAFDPSNMPPGIEASPDHMLQCRLFAYPDTHRHRLCPNYLHIPVNCPPYRANVANYQ	387
Sbjct	309	EVEQAAFSPANVVVPGIGYSPDRMLQCRLFYSYCDTHRYRLGVNYPQIPVWKP-RCPFHSSS	367
Query	388	RDCPMCHQDNQCGAPNTYFNSFGAPEQQPSA-----LEHSIQYSCEVRRFNTANDN--	439
Sbjct	368	RDC M G NY P+S ++ SA L H I+ EV ++ DD+	425
Query	440	VTQVPVAFYVNVLNNEEQKRLCENIA---GHLKDAQIFIQHGAVKVFTEVHPDYGSHIQAL	496
Sbjct	426	TQ +Y + L ++++RL + I H+ +I K +++F + P Y ++	482
Query	497	LDKY 500	
Sbjct	483	LKH 486	

Figure 1. Result of blast search of specific sequences of *H.pylori* catalase (26695) and human catalase. Query, human catalase enzyme sequence; subject, *H. pylori* catalase enzyme sequence.

score or the difference was not significant in reaction with the common alleles in other epitopes.

Although the reaction of the fifth epitope with three alleles was specific (0401, 0421 and 0426) and its score in the reaction with two alleles (0404 and 0423) was high, this epitope, VKHLTNEEA covered only seven alleles which could not be enough for T cell responses in a variety of population.

Therefore, the three epitopes 1, 2 and 3 was selected as: MVNKDVKQTTKKVLQSTWFLKKFHPFDVTKI for laboratory investigation. Prediction of catalase epitopes *in silico* would be a valuable tool for developing new immunoprophylactic strategy against *H. pylori* infection. In addition, it could be helpful for the analysis of other *H. pylori* antigens and other pathogens and provide a novel and usual progress for the design of epitope-based vaccines against many pathogens. It is crucial to study the cytokine production *in vivo* and *in vitro* for evaluation of the designed epitope vaccine. The antigenicity of designed epitopes for elucidating immune response as a vaccine candidate is under investigation in cell lines and animal models.

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