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Original Article

In Silico Analysis of Mycobacterim tuberculosis Proteins to Understand Their Role in Susceptibility and Protection

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ABSTRACT

Aim: To analyze the epitopes derived from protective and TB pathologic antigen with respect to known protective and susceptible Class I HLA alleles. Method: The sequences of protective and susceptible antigens were first analyzed using bioinformatic tool CTLpred. The top scoring three epitopes from this were then used to analyse their Class I HLA restriction employing the Propred matrix. Result: We found that an increased number of epitopes of antigens involved in pathogenesis were predicted to associate with susceptible alleles than for protective alleles. Conclusion: For selecting an epitope for vaccine design it is not only important to study its ability to induce the protective cytokine IFN-γ but also the cytokine like IL-10 involved in pathogenesis, in the context of a specific HLA class I molecule.

1. Introduction

Bacillus Calmette-Guérin (BCG), has failed to have any significant impact on protection against tuberculosis (TB) [1, 2]. Therefore identification of antigens and epitopes of Mycobacterium tuberculosis (M. tb) as candidates for the development of new vaccines is the need of the hour. The protective role of Th cells in tuberculosis infection has been recognized and several Th cell antigen/ epitopes have also been identified [3, 4]. On the other hand although CTLs have recently drawn some attention, very few CTL specific antigen /epitopes have been described till date.

Secretory antigens of M. tb are important since many studies have demonstrated their potential to induce cellular immunity [5]. Besides these, other antigens of particular interest are proteins encoded by the regions of difference (RD). RD includes 11 genomic regions in M. tuberculosis that are deleted in all vaccine strains of M. bovis BCG and encompass >80kb genomic DNA of M. tuberculosis. RDs however may contribute to protective immune response and/or pathogenesis of the disease. Mustafa and Al- Attiyah have studied all the 11 RDs and found that one group represented by RD1 activates peripheral blood mononuclear cells (PBMCs) to preferentially secrete the protective cytokine IFN-γ and another group represented by RD12 and RD13 activates PBMCs to secrete IL-10 preferentially which in turn suppresses the secretion of IFN-γ in response to peptides of the first group [6].

The *in silico* approach wherein computational algorithms are used to predict epitope association with various human leukocyte antigen (HLA) molecules, has curtailed the time, money and efforts required for vaccine design. Numerous databases and web servers are now available that predict epitopes binding to various HLAs. Also, many studies indicate the role of HLA in susceptibility to tuberculosis [7-10]. Therefore, in order to rationally select sequences that may function as T cell epitopes in vaccine formulation, recognition of peptide by HLA remains an important criterion.

In this study we have used CTLpred for analyzing the association of reported protective antigens and epitopes with HLA alleles found in healthy subjects. Similarly we have also analyzed antigen/epitopes that are reported to be involved in pathogenesis of TB (TB pathologic antigens) in the context of HLA alleles commonly found in TB patients. A large number of epitopes of antigens associated with pathogenesis were predicted to bind to susceptible alleles than the protective alleles which probably enlighten the role of these HLA alleles in susceptibility to tuberculosis.

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2. Materials & Methods

Both protective and TB pathologic antigens were included in this study. Secreted antigens Ag 85A, Ag 85B, Ag 85C, CFP-10 and ESAT-6 are known to enhance IFN-γ production [19,20]. Similarly, antigens Rv1818c, Rv3812 and Rv3018c from the PE/PPE [21] family are also known to increase IFN-γ secretion while M. tb 8.4, M. tb9.9A, 19 kDa protein and EsxG are known to harbor CTL epitopes [22]. Similarly, proteins of RD12 region (Rv2072c-Rv2075c) and RD13 region (Rv2645-Rv2660c) which are known to induce IL-10 and hence are instrumental in pathogenesis have also been included in this study [6].

The sequence of these antigens were retrieved from NCBI entrez Protein database at http://www.ncbi.nlm.nih.gov/protein

Prediction of epitopes with CTLpred

The CTLpred server allows the user to predict epitopes using quantitative matrix (QM), Support Vector Machine (SVM) and Artificial Neural Network (ANN) approaches. Sequences of the antigens obtained from NCBI were used as input sequence. The server allows the user to employ these approaches either individually or by combining ANN and SVM or performing a consensus prediction using ANN and SVM. The consensus approach and the combined approach increase the specificity and sensitivity of the prediction respectively. The user can vary the cutoff score for all prediction approaches. A consensus approach was used to predict the antigenic peptides with a cutoff score of 0.51 (default value) for ANN and 0.36 (default value) for SVM. The number of top scoring peptides to be displayed can also be chosen. We opted to analyze the top three scoring peptides and the results obtained (unpublished) include the nanomer peptides in a descending order of their score. The user is also given the choice of selecting a particular matrix i.e nHLAPred or Propred for finding the HLA restriction of the peptides. We chose Propred to identify the HLA alleles that could bind to the respective epitopes. CTLpred can be accessed freely from URL http://www.imtech.res.in/raghava/ctlpred [23].

HLA alleles

Several class I alleles have been studied in order to understand their association with tuberculosis. These studies are based on the occurrence of the respective alleles found in tuberculosis patients and healthy controls. Those that are found more in tuberculosis patients are considered susceptible while those found more frequently in healthy controls are considered as protective. The alleles included in this study were based on these studies (Table 1) [8-18].

Statistical analysis

Results were expressed as mean ± SEM. The data is Statistical analysis of the data was done using unpaired Student's t test using GraphPad Prism 5 software. A p-value of less than 0.05 was considered to be statistically-significant.

3. RESULTS

For analysis, the protein sequence of selected antigens was submitted to CTLpred. CTLpred allows one to view the results onsite in a tabular form and also to choose the number of top scoring peptides to be displayed. We chose to analyze the top three peptides. This result (onsite) shows the sequence of the peptides, the starting position and the score. CTLpred also states whether the peptide can be considered as epitope or non-epitope based on the comparison of the peptide scores for ANN and SVM with respect to default value of 0.51 and 0.36 for ANN and SVM respectively. The result of HLA restriction of these peptides using Propred matrix is displayed in Tables 2-5, where only the alleles that are known to be protective or susceptible are included. However there were some epitopes that did not bind to any of the selected HLA alleles and hence not shown in these tables. Also, in few cases such as ESAT-6 only two epitopes were predicted as HLA binders.

Table 1: Protective and susceptible alleles based on molecular studies

Protective	Susceptible	Reference No.
HLA-A2	HLA-B62	8
	HLA-B14	9
	HLA-B35	10
HLA-B44	HLA-B60	11
HLA-A3, HLA-B44	HLA-A1	12
HLA-A11	HLA-B40	13
	HLA-B8	14
	HLA-B27	15
	HLA-B7 HLA-B27	16
HLA-B52	HLA-B51	17
HLA-A11	HLA-B*4006(B61)	18

Table 2 and table 4 show epitopes from protective antigens that may bind to various protective or susceptible alleles respectively. Similarly, table 3 and table 5 display epitopes from TB pathologic antigens that may bind to known protective or susceptible HLA alleles respectively.

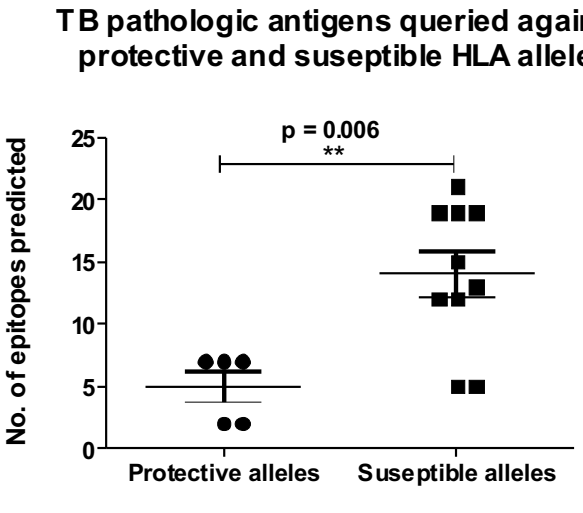
When the protective antigens were queried against protective alleles, the total number of alleles predicted was not significantly different (p=0.0923) from that of susceptible alleles (Figure 2). However, as seen from figure 1, the total number of epitopes from TB pathologic antigens predicted for the susceptible alleles significantly higher (p = 0.006) that for the protective alleles. Protective alleles HLA-A2 and HLA-A*1101 have five predicted epitopes of protective antigens each in contrast to seven and three predicted epitopes of TB pathologic antigen. Similarly, six epitopes each of protective antigens were predicted for HLA-A3 and HLA-B*4403 whereas seven epitopes, of TB pathologic antigens each. HLA-B*5201 was the only protective allele to have more predicted epitopes of protective antigens (4 epitopes) than the TB pathologic antigen (2 epitopes). (Table 2 & 3)

Table 2 : CTLpred analysis of protective antigen association with Protective HLA-Alleles

HLA-A2	HLA-A3	HLA-A*1101	HLA-B*4403	HLA-B*5201
WPYWNEQLV-85C	AMGPTLIGL-85A	WLSANRAVK-85B	AAVPTTTVL-Rv1818c	FQGGGPHAV-85C
RQAGVQYSR-cfp10	WLSANRAVK-85B	RQAGVQYSR-cfp10	SELPAVAWV-Rv3018c	WPYWNEQLV-85C
AVVRFQEAA-cfp10	RQAGVQYSR-cfp10	SANPFPFLR-Rv3812	DPRSNLARF-Rv2350c	HTGPAPVIV-Rv3018c
SELPAVAWV-Rv3018c	SANPFPFLR-Rv3812	ATKDGSHYK-19kda	CENPGIREF-Rv2350c	RVPPRPYVM-M.tb39
AYVPYVAWL-Rv3018c	ATKDGSHYK-19kda	RVPPRPYVM-M.tb39	AAAAKVNTL-ExsG	
	NVNGVTLGY-19kda		AAASTYTGF-ExsG	

Figure 1: Graph showing the number of epitopes predicted of TB pathologic antigens when queried against protective and susceptible alleles using CTLpred software HLA epitope precition tool. Results are expressed as mean ± SEM. Statistical analysis was done using unpaired Student's t test. ($p<0.05$)

Figure 2: Graph showing the number of epitopes predicted of protective antigens when queried against protective and susceptible alleles using CTLpred software HLA epitope precition tool. Results are expressed as mean ± SEM. Statistical analysis was done using unpaired Student's t test. ($p<0.05$)



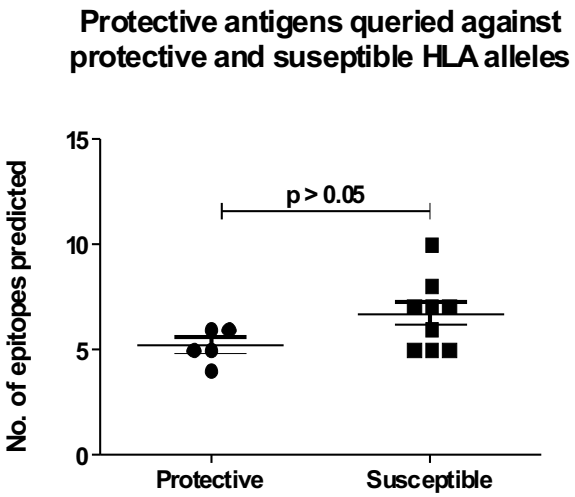


Table 3 : CTLpred analysis of TB pathologic antigen association with Protective HLA-Alleles

HLA-A2	HLA-A3	HLA-A*1101	HLA-B*4403	HLA-B*5201
ALRPMFVAL-Rv2073c	ALRPMFVAL-Rv2073c	RVVDGRVLR-Rv2645	AELRRANAI-Rv2648	RSWPGCTAV-Rv2072c
TLDDGRRQL-Rv2645	RVVDGRVLR-Rv2645	WVDWFNHRR-Rv2649	REGDVIVRV-Rv2651c	STWAGFAYV-Rv2649
AKADRRIEL-Rv2646	TLRHRYATR-Rv2646		GERVRAQVL-Rv2652c	
STWAGFAYV-Rv2649	WVDWFNHRR-Rv2649		AESHGVAAV-RV2654c	
VLVDNAFRV-Rv2650c	VLVDNAFRV-Rv2650c		TEDRAPATV-Rv2656c	
SEAAEYLAV-Rv2657c	ALCLRLSQL-Rv2658c		SEAAEYLAV-Rv2657c	
ALCLRLSQL-Rv2658c	VVAPSQFTF-Rv2660c		CAILGLNQF-Rv2660c	

Table 4 : CTLpred analysis of protective antigen association with susceptible HLA-Alleles

HLA-A1	HLA-B7	HLA-B8	HLA-B14	HLA-B*2705	HLA-B51	HLA-B60	HLA-B61	HLA-B62
AADEVSAAM-Rv1818c	AMGPTLIGL-85A	HVKPTGSAV-85A	QRNDPLLNV-85A	QRNDPLLNV-85A	WLSANRAVK-85B	RPGLPVEYL-85B	WPYWNEQLV-85C	FQGGGPHAV-85C
SANPFPFLR-Rv3812	RPGLPVEYL-85B	RPGLPVEYL-85B	AMGPTLIGL-85A	AMGPTLIGL-85A	IYAGSLSAL-85B	TATELNNAL-esat6	AADDVSIAV-Rv3812	NVASGTAGF-Rv1818c
AADDVSIAV-Rv3812	WPYWNEQLV-85C	TATELNNAL-esat6	KRNDPMVQI-85C	WLSANRAVK-85B	RPGLPVEYL-85B	AANKQKQEL-cfp10	SELPAVAWV-Rv3018c	NVNGVTLGY-19kda
HTGPAPVIV-Rv3018c	AANKQKQEL-cfp10	AANKQKQEL-cfp10	AAVPTTTVL-Rv1818c	RPGLPVEYL-85B	FQGGGPHAV-85C	AAVPTTTVL-Rv1818c	HTGPAPVIV-Rv3018c	HQAIVRDVL-9.9a
NVNGVTLGY-19kda	AAVPTTTVL-Rv1818c	AAVPTTTVL-Rv1818c	HQAIVRDVL-9.9a	KRNDPMVQI-85C	WPYWNEQLV-85C	SELPAVAWV-Rv3018c	AANQLMNNV-M.tb39	AAASTYTGF-ExsG
	APPPQRAAM-M.tb8.4	RVPPRPYVM-M.tb39	RVPPRPYVM-M.tb39	FQGGGPHAV-85C	APPPQRAAM-M.tb8.4	HQAIVRDVL-9.9a		
	HQAIVRDVL-9.9a	AAHARFVAA-ExsG	LRVPPRPYV-M.tb39	RQAGVQYSR-cfp10	LRVPPRPYV-M.tb39			
	RVPPRPYVM-M.tb39			KRGLTVAVA-19kda				
				HQAIVRDVL-9.9a				
				LRVPPRPYV-M.tb39				

Analysis of susceptible alleles not only predicted a large number of epitopes of protective antigens (table 4) but also a high number of predicted epitopes of TB pathologic antigens (table 5). HLA-B*2705 had the highest number of total predicted epitopes (29 epitopes) with nineteen predicted epitopes of TB pathologic antigens and only ten of protective antigens. Similarly, HLA-B14 showed the second highest number of predicted epitopes (28 epitopes) including seven from protective antigens and the maximum of 21 epitopes from TB pathologic antigens. Nineteen epitopes of TB pathologic antigens were predicted for B7, whereas only eight were predicted for protective antigens. Similarly, thirteen and fifteen epitopes of TB pathologic antigens were predicted for HLA-B51 and HLA-B60 in contrast to seven and six epitopes respectively from protective antigens. Twelve epitopes of TB pathologic antigens were predicted for HLA-B8 and HLA-B61 each whereas only seven and five respectively for protective antigens. HLA-B62 had five predicted epitopes of protective and susceptible antigens each.

Table 5 : CTLpred analysis of TB pathologic antigen association with susceptible HLA-Alleles

HLA-A1	HLA-B7	HLA-B8	HLA-B14	HLA-B*2705	HLA-B51	HLA-B60	HLA-B61	HLA-B62
TLDDGRRQL-Rv2645	AARPSVIFL-Rv2072c	AARPSVIFL-Rv2072c	IRVLTLAAL-Rv2072c	RSWPGCTAV Rv2072c	IRVLTLAAL-Rv2072c	AARPSVIFL-Rv2072c	RSWPGCTAV-Rv2072c	ALRPMFVAL-Rv2073c
AKADRRIEL-Rv2646	ALRPMFVAL-Rv2073c	ALRPMFVAL-Rv2073c	IRVRRANYV-Rv2073c	IRVLTLAAL-Rv2072c	IRVRRANYV-Rv2073c	AANKQKQEL-Rv2073c	TPRPNPRRV-Rv2074	VLGIGPAAA-Rv2645
STWAGFAYV-Rv2649	AANKQKQEL-Rv2073c	TPRPNPRRV-Rv2074	RPNPRRVVI-Rv2074	IRVRRANYV-Rv2073c	TPRPNPRRV-Rv2074	RASGARAVL-Rv2075c	NPRRVVIEV-Rv2074	VLVDNAFRV-Rv2650cA
SEAAEYLAV-Rv2657c	TPRPNPRRV-Rv2074	RPNPRRVVI-Rv2074	RASGARAVL-Rv2075c	ALRPMFVAL-Rv2073c	NPRRVVIEV-Rv2074	AELRRANAI-Rv2648	AELRRANAI-Rv2648	LCLRLSQL-Rv2658cC
ALCLRLSQL-Rv2658c	RPNPRRVVI-Rv2074	RASGARAVL-Rv2075c	TLDDGRRQL-Rv2645	NPRRVVIEV-Rv2074	RPNPRRVV I-Rv2074	REGDVIVRV-Rv2651c	STWAGFAYV-Rv2649	AILGLNQF-Rv2660c
	RASGARAVL-Rv2075c	TLRHRYATR-Rv2646	RRIELMIRL-Rv2646	RASGARAVL-Rv2075c	WVDWFNHRR-Rv2649	AESHGVAAV-RV2654c	REGDVIVRV-Rv2651c	
	GERVRAQVL-Rv2652c	AKADRRIEL-Rv2646	AKADRRIEL-Rv2646	RRIELMIRL-Rv2646	WRSIEDVEL-Rv2649	ESHGVAAVL-RV2654c	GERVRAQVL-Rv2652c	
	DPKPGKRRV-Rv2652c	RPKAKQRQR-Rv2647	RKDFTPSEL-Rv2647	WRSIEDVEL-Rv2649	VLVDNAFRV-Rv2650c	AAVELARAL-RV2654c	DPKPGKRRV-Rv2652c	
	RVVPELAAL-Rv2652c	DPKPGKRRV-Rv2652c	WRSIEDVEL-Rv2649	REGDVIVRV-Rv2651c	DPKPGKRRV-Rv2652c	AESHGVAAV-Rv2654c	AESHGVAAV-RV2654c	
	APRRNRVGR-Rv2653c	AAVELARAL-RV2654c	DRVGSTVEL-Rv2650c	GERVRAQVL-Rv2652c	RPAGGHIQM-Rv2658c	ESHGVAAVL-Rv2654c	AESHGVAAV-Rv2654c	
	ESHGVAAVL-RV2654c	ESHGVAAVL-Rv2654c	TRYPVGRAV-Rv2651c	RVVPELAAL-Rv2652c	MRYGELTEL-Rv2659c	AAVELARAL-Rv2654c	TEDRAPATV-Rv2656c	
	AAVELARAL-RV2654c	RPDLRVHDL-Rv2659c	SRSLAEARL-Rv2651c	RRAQRQRDL-Rv2653c	RPDLRVHDL-Rv2659c	TEDRAPATV-Rv2656c	SEAAEYLAV-Rv2657c	
	ESHGVAAVL-Rv2654c		RRAQRQRDL-Rv2653c	RRRDAYIRR-Rv2656c	VVAPSQFTF-Rv2660c	RSGTRLVRL-Rv2657c		
	AAVELARA L-Rv2654c		ESHGVAAVL-RV2654c	RRDAYIRRV-Rv2656c		SEAAEYLAV-Rv2657c		
	RSGTRLVRL-Rv2657c		AAVELARAL-RV2654c	RRYITISEA-Rv2657c		RPDLRVHDL-Rv2659c		
	ALCLRLSQL-Rv2658c		RRYITISEA-Rv2657c	ALCLRLSQL-Rv2658c				
	RPAGGHIQM-Rv2658c		RSGTRLVRL-Rv2657c	MRYGELTEL-Rv2659c				
	TLAELMQRL-Rv2659c		ALCLRLSQL-Rv2658c	TLAELMQRL-Rv2659c				
	RPDLRVHDL-Rv2659c		RHVIPFSAL-Rv2658c	RPDLRVHDL-Rv2659c				
			MRYGELTEL-Rv2659c					
			RPDLRVHDL-Rv2659c					

4. DISCUSSION

Several studies have highlighted the correlation of HLA genes with susceptibility to tuberculosis (Table 1). The present study was carried out to analyze M. tb specific epitopes in the context of protective and susceptible HLA class I alleles. CTLpred was the bioinformatic tool used for this purpose. CTLpred is based on quantitative matrix (QM), and machine learning techniques like Support Vector Machine (SVM) and Artificial Neural Network (ANN). QM quantifies each amino acid at each position and is simple to use. However since it does not take into account the neighboring residue effects within the peptide it only predicts good binders but does not propose binding motif i.e. it ignores the contribution of overall peptide structure to binding. In contrast ANN can not only generalize from input data and tolerate noise and errors in data but also deal with non-linear problems. Moreover, ANN's are based on structural risk minimization. SVM's can predict epitopes with more accuracy than ANN's, whereas the combined and consensus approaches can predict epitopes with more accuracy than the individual approaches. In case of the combined approach, the sensitivity increases while in consensus approach the specificity increases. We have used the consensus approach to increase the specificity of our analysis [23].

In the present study we have included both protective and susceptible antigens. Ag 85A, Ag 85B, Ag 85C, CFP-10 and ESAT-6 are the secreted antigens that have been studied extensively and are known to produce IFN-γ [19-20]. Among these, antigens like Ag 85A, Ag85B, CFP-10 and ESAT-6 have also been used for vaccine trials [24]. PE/PPE family of proteins includes Rv1818c, Rv3812 and Rv3018c which are also known to increase IFN-γ production and have been selected for analysis [21]. Various RD regions which were studied by Mustafa et al. [6] where it was observed that peptide pools of RD12 region and RD13 region could induce IL-10 production and hence play a role in pathogenesis of the disease have also been included in our study.

Contini et al in their study on in silico selection of Class II specific M. tb epitopes from whole genome observed that lesser number of epitopes bound to susceptible alleles than the protective alleles [25]. In contrast to this in our study we observed that the total number of epitopes that bound to TB susceptible alleles like HLA-B14 (28 epitopes) and HLA-B*2705 (29 epitopes) were significantly higher ($p = 0.0329$) in comparison to the protective alleles like HLA-B*52 (6 epitopes) and –B*4403 (13 epitopes). (Table 2,3,4 and 5). This was in spite of the fact that epitopes were derived from antigens that are both protective (antigen 85A and -85B) as well as TB pathologic (Rv2072c and Rv2645). The contrasting findings may reflect the relative importance of CTLs versus T- helper cells. It is possible that CTLs are more important in TB and hence the increased number of CTL epitopes being associated with susceptible allele in our study also reiterates this fact. In addition, it was also observed that when TB pathologic antigens are queried against all HLA alleles, no or very few peptides of RD12 region (Rv2072c-Rv2075c) and some epitopes of RD13 region (Rv2645-Rv2660c) are predicted to bind to protective HLA's while several epitopes are predicted to bind to susceptible HLA.

3. RESULTS

This was observed in case of HLA-A as well as HLA-B. For example the protective Ag85A, had only one epitope that was predicted for only one protective allele i.e. HLA-A3. Where as in case of susceptible allele all the three epitopes i.e. AMGPTLIGL (for HLA-B7, HLA-B14 and HLA-B*2705) QRNDPLLNV (for HLA-B14 and HLA-B*2705) and HVKPTGSAV (for HLA-B8) were predicted to bind to various susceptible alleles. When protective antigens are queried against all HLA alleles they are shown to bind to protective as well as susceptible HLA alleles (Table 2 and 4). Hence it appears that only peptides that show binding to protective HLA alleles are probably protective and others are not.

Bothamley (1999) observed that there is an increased presence of HLA-B60 in smear positive patients and HLA-B44 in healthy controls [11]. In attempting to validate this, our study showed the same number (six) of epitopes derived from protective antigens predicted for HLA-B60 (susceptible) as well as HLA-B*4403 (protective). Whereas in the case of disease enhancing antigens of RD12 and RD13 region, fifteen epitopes were predicted to bind to HLA-B60 in contrast to seven epitopes for HLA-B*4403. The propensity of some alleles towards disease could be due to their ability to induce IL-10, as seen in other studies where regulatory CTLs have been implicated [26]. Similar association was also seen in the case of HLA-B51 (susceptible allele) and HLA-B52 (protective allele). In this case, seven and four epitopes of protective antigen were predicted to bind to HLA-B51 and HLA-B52 respectively. Whereas in case of disease pathogenesis antigens, five epitopes of RD12 and eight epitopes of RD13 were predicted to bind to HLA-B51. In contrast to this only one epitope of RD12 and RD13 region each were predicted in case of HLA-B52. These observations are in agreement with those made in a study by Vijaya Lakshmi et al. where the increased incidence of HLA-B52 in healthy individuals and HLA-B51 in TB and HIV-TB patients was observed [17].

In case of protective alleles, it was observed that only one epitope, ALRPMFVAL from Rv2073c was found in case of HLA-A2, HLA-A3 and RSWPGCTAV from Rv2072c for HLA-B*5201 and no epitopes were predicted to bind to HLA-A*1101 and HLA-B*4403 when RD12 region was analyzed. Contrasting this, there was an increased number of epitopes of RD12 region being predicted for susceptible alleles like HLA-*B2705 (6 epitopes), HLA-B7 (6 epitopes), HLA-B8 (5 epitopes), HLA-B14 (4 epitopes) and HLA-B61 (3 epitopes) from RD12 region. We also observed the same contrast when RD13 region was analyzed. While only one epitope was predicted for protective alleles like HLA-B*5201, two for HLA-A*1101, six for HLA-A2 as well as HLA-A3 and seven for HLA-B*4403, on the other hand more than one epitope was predicted for all the susceptible alleles with a maximum of seventeen epitopes (HLA-B14) predicted for the same. Increased number of epitopes of RD13 region were predicted for HLA-*B2705 (13 epitopes), HLA-B7 (13 epitopes), HLA-B8 (7 epitopes) and HLA-B61 (9 epitopes).

Though ESAT-6 is considered to be highly immunogenic and is used in vaccine trials, we found that only two epitopes of ESAT-6 were predicted as HLA-binders by CTLpred and none predicted to sceptibility and pathogenesis of the disease.

bind to protective alleles. However, TATELNNAL did bind to susceptible alleles like HLA-B60 and HLA-B8. Hasan et al. observed that ESAT-6 induced IL-10 production was increased in tuberculosis patients [20]. Smith et al. in their study also observed that CTLs from healthy controls as well as tuberculosis patients recognized Ag85A and Ag85B. In contrast to this, ESAT-6 was recognized by CTLs of tuberculosis patients only and not healthy controls [27].

To summarize, the total number of epitopes predicted for both protective and susceptible alleles revealed that the number of epitopes of protective antigens predicted in both the groups are not significantly different (Figure 2). However, the numbers of predicted epitopes of the TB pathologic antigens were found to be significantly high in susceptible alleles as compared to the protective alleles (Figure 1). The association of higher number of TB pathologic antigens with susceptible alleles appears to highlight the role of these HLA alleles in su

5. CONCLUSION

In conclusion we would like to state that while studies indicate the ability of specific antigens or epitopes being able to induce the production of specific cytokines in vitro or ex vivo is informative, however a more comprehensive comparative cytokine analysis needs to be done in the context of specific HLA alleles. As can be correlated from this analysis, one need to know the ability of a specific epitope to induce the protective cytokine, IFN-gamma or the disease enhancing cytokine, IL-10 in the context of a specific HLA allele. Such data would eventually benefit the designing of effective vaccines against tuberculosis.

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