The Use of Artificial Neural Networks in Analysis Cationic Trypsinogen Gene and Hepatitis B Surface Antigen

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Abstract: Problem statement: More and more severe hepatitis cases reported in China are infected with HBV and the immune response of HBV will be reduced if mutations occur in the TCR, therefore it is very important to investigate the relation of T-cellular function and clinical effect by studying the function of T-cell receptor. **Approach:** Artificial Neural Networks (ANN) was applied to analyze basic data (the three structural of HBsAg, the ligand of HBsAg and the clinical immunological characterizations, the laboratory data, the genetypes of cationic trypsinogen gene (PRSS1) derived from 78 patients with pancreatitis and 60 normal controls were also collected. What is more, we used T-cell culture with HBV and flow cytometry to check the result of ANN predict. To examine the characteristics of T-cells capable of coexisting with the secreted HBsAg, T-cell receptor from A121T, C139S, silent mutation and normal PRSS1 gene in the patients with pancreatitis were put into research. To ensure that PRSS1 gene would affect HBsAg-specific T-cells receptor, we compared the rate of multiplication and CD4/CD8 of T-cell after culture with HBV at 0H, 12H, 24H, 36H, 48H and 72H time point. **Results:** The protein's structural predicted by the ANN could specifically explain the phenomenon that the turbulence and different of anti-HBs lever of the patients with pancreatitis. The three-dimensional of the protein that consist with PRSS1 gene would accord with HBsAg. It may be one of the HBsAg-specific T-cell receptor. Result of T-cell culture also showed that different genetypes of PRSS1 had different results. In the T-cell proliferation, the groups of PRSS1 mutation (A121T and C139S) were significant lower than the group of silent mutation and normal controls and it was the same to the result of CD4/CD8. **Conclusion:** The ANN had been integrated into a previously published comprehensive web server to support immunology analysis and the PRSS1 gene may be the unit for HBsAg immune response.

Key words: Cationic trypsinogen gene, HBsAg, T-cell receptor, pancreatitis, artificial neural networks

INTRODUCTION

The T-Cell Receptor (TCR) plays a central role in the human immune system and more than 90% of human T-cells present a receptor that consists of TCRα and TCRβ, especially CD_4 and CD_8 T-cells. Eight tryosinogen genes locus the same to TCR β gene that is 7 q35[1-5]. Of all the 65 Vβ segments and 8 tryosinogen genes, two large insertion-deletion polymorphism affecting three Vβ and two trypsinogen genes (T6 and T7), the polymorphisms or mutations of trypsinogen genes and Vβ can be associated with loss of autoimmune tendencies[6-8] and it was report that high express of TCR β 6 and TCR β 7.2 will be found in the patients with effect of HBV[7-10], but it is not clear why the loss of the functional trypsinogen genes would confer a selective advantage. The mutations of PRSS1 gene (one of the trypsinogen genes, also named T6 or

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cationic trypsinogen gene) have been reported relationship with pancreatitis. We found that the level of anti-HBs and $CD4^+T$/ $CD8^+T$ is very different in the patients with pancreatitis (because of the mutation of PRSS1 gene) in the process of pancreatitis. The characteristics of the mutation of PRSS1 affect TCR in the idiopathic chronic pancreatitis or hereditary pancreatitis can be difficult to accurately characterize from complex clinical data and an Artificial Neural Network (ANN) is a machine learning technique that can effectively process complex and high noise data. Here, ANNs are applied to process the unique subsequence distribution for prediction of the three structural of ligand of HBsAg.

PRSS1 gene is established by the demonstration of its sequence within the sequence of the locus for the T-cell receptor beta chain. Since hereditary pancreatitis had been mapped rather precisely to 7q34-35 and since mutation/polymorphism in the PRSS1 had been identified in pancreatitis, Whitcomb *et al*.[6-8] stated that high degree of DNA sequence homology presents among this cluster of 5 trypsinogen genes. In this study we applied Artificial Neural Networks (ANNs) to analyze the clinical data and the genetypes of PRSS1 in order to identify key immunological characteristics that the mutations of PRSS1 affect the function of TCR response to HBsAg. As we known, ANNs are adaptive, non-linear forms of Artificial Intelligence inspired by the way the human brain learns and processes information in order to solve specific problems, such as pattern recognition and classification problems.

MATERIALS AND METHODS

We made use of the triplets of nucleotides usage frequencies in order to train and evaluate our predictor model. Three specific triplets of nucleotides bring up an amino acid witch is the minimum unit of protein. And the amino acid make up with four possible nucleotides that is Adenine (A), Guanine (G), Cytosine (C) and Thymine (T). The most protein make up with 20 amino acids. All of the 20 amino acids, only two are named by a single codon. One of these is the amino acid methionine, specified by the codon AUG, which also specifies the start of translation and the other is tryptophan, specified by the codon UGG or UGA. The same structural protein often shows particular preferences for specific several codons that consist by the same or similar given amino acid. This is the same to the human TCR (include Vβ, Vα, J$_β$ and Jα) and the antigen of HBV (HBsAg or HBeAg). The codons that a protein prefers to use can predict basic three-dimensional of the protein. Because of HBV is not the

human's organizing, the immunity system will find this disorder and active the immemorial cells to kill or encompass the invertors. Most of the immemorial cells or immunity cells will be T-cells. That is to say, T-cells take charge of recognizing the cells infected with virus. We use Homology Modeling and docking methods, a theoretical study on HBsAg fragment was performed. And the result of the predict will be test by lymphocyte stimulation and list of immunological data. The models developed result in a predictive performance for validation data of 99.0% correct discrimination between the ligand of HBsAg and the three structural of HBsAg. We present a novel and efficient algorithms, named the integration of ANN and BLAST algorithm and establish the database for human PRSS1 gene, Vβ, V$_α$, Jβ and Jα index databases by excel. All of the information of TCRβ segments will be select from the database of NCBI (National Center for Biotechnology Information), using the BLAST program. In order to adapt the three structural of HBsAg, if an ORF (TCR V$_β$, Vα, Jβ and J$_α$) did not have a good matching in the database with HBsAg, then this ORF/segment was filtered out and not included in any downstream analysis. Significant database matches were those having at least 100% identity at the amino acid level while the alignment length should be including one "AUG" codon and one "UGG" or "UGA" codon. Operons in TCR β (like TCR β6) are groups of genes that are simultaneously transcribed. Usually all these genes are located in the same strand and have a small spacing between them. We predict the three-structural of protein basis on the consist of HbeAg-specific T-cell receptor.

The training set is used to train a neural net. The error of this dataset is minimized during training. Finally, codon usage frequencies were extracted from the result. For this latter sequence manipulation we used a locally developed java script. For the training process two online and two batches learning algorithms were used. The Standard Backpropagation algorithm and the Backpropagation with momentum were used for online learning, while for batch learning we have used the Resilient propagation (Rprop) and the Quick Propagation. The validation set consists of VDJ junctional region sequence and 8 tryosinogen genes and it was used to tune the parameters like the network architecture, (number of hidden units) in a neural network. The learning procedure was stopped in the minimum of the validation set error. At this point the net generalizes best. When learning is not stopped, over training occurs and the performance of the net on the whole data decreases, despite the fact that the error on the training data still gets smaller. In our experiment we perform one validation cycle every 100 training cycles.

Since this procedure can itself lead to some over fitting to the validation set, the performance of the selected network should be confirmed by measuring its performance on a third independent set of data called a test set. We use ANN to build the three-dimensional structural of the ligend of HBsAg. As shown in Fig. 1B, there are two Trp acids in the chelate protein structure of HBsAg ligand, that is NO.163 and NO.165. The corresponding ligand pocket was formed because of the different splicing tendency of the Phe, Trp and Tyr amino acid. And the independent PRSS1 gene may just provide the continuous Phe, Tyr, Trp sequences, as the yellow-green regional of (Fig. 1A).

Laboratory test: This study was approved by the clinical gastroenteropathy and each patient provided voluntary informed consent. Including 78 patients with pancreatitis and 60 normal controls were study, other exclusion criteria included chronic HIV or HCV infection, or evidence of any other clinically significant acute or chronic disease. To assess anti-HBsAg responses, blood samples obtained at baseline. Blood was collected from healthy volunteers and from subjects (6 patients with A121T mutation, 3 patients with C139S mutation, 11 patients with D162D silent mutation, 58 patients with normal pancreatitis and 60 normal controls) at the same time in heparin lithium tubes (BD Co. t). Before evaluating HBsAg-specific T-cell responses, the full blood was sent to flow cytometry. To determine the percentage of CD4^{+} and CD8^{+} cells expressing in unstipulated conditions, flow cytometry to secure the validity of small percentages and/or differences. Results following FACS analysis were defined as the difference in response to HBsAg-peptides at H12, H24, H36, H48 or H72 versus H0. The confidence interval depended on the numbers of relevant events (CD4^{+} or CD8^{+} events) collected in each sample, the amount of background stimulation at H0 and difference between H0 and H12, H24, H36, H48 or H72 time points.

RESULTS

The average percentage of T-cell CD4/CD8 of the A121T, C139S, silent mutation and general pancreatitis. At 0H point, the average percentage of T-cell CD4/CD8 of the A121T and C139S groups were significant lower than silent mutation and general pancreatitis groups. All the groups showed that the highest point of CD4/CD8 exist at 48H. The rate of proliferation in the calculate cells for 0H, 12H, 24H, 36H, 48H and 72H. It shows that the group of A121T and C139S mutation were lower than the normal control,
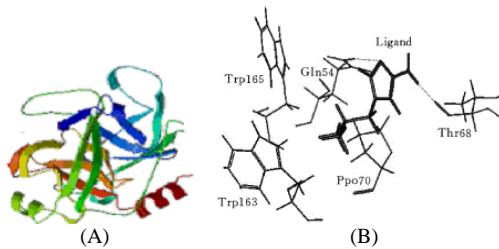

Fig. 1: The relationship between the predict from ANN and the reported ligend of HBsAg. (A): The three structural predict by ANN; (B): The reported ligend of HBsAg[11]
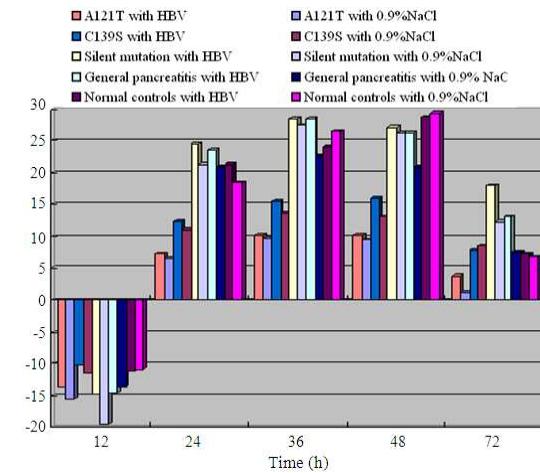

Fig. 2: Percentage of T-cell breeder with HBsAg stimulation

while there are not significant different between the silent mutation group and normal control. All of the 0.9% NaCl groups were lower than the HBV group. This difference was significant with a power of 90% (p<0.05) (Fig. 2).

DISCUSSION

Mutations within these epitopes of HBsAg/ HBeAg will cause a lower affinity of peptide for T-cell antigen receptor (TCR) restricted antigen binding site, then decrease the CTL response and impact upon the elimination of hepatitis B virus[7]. That is to say the immune response of HBV will be reduced if mutations occur in the TCR [11-19]. Some studies have indicated that the priority expression and employment of TCR were related with the specific immune reaction of chronic hepatitis B[20-27]. In our study of the object, we discovered that the growth rates of leukomonocyte were difference among the patients who bring A121T, C139S

mutation, normal controls and common pancreatitis patients and the growth rate of leukomonocyte of the patients who bring A121T and C139S mutation was comparatively low and that the CD4/CD8 proportionality was degraded. In order to decrease the provocation to T leukomonocyte, we deploy the method that take the whole blood with heparin to anticoagulation infuse to the nutrient medium. Then dilution of HBV capsid antigenic to come from blood serum of the patients themselves, besides we even more do the physiological saline to contrast control to offset part the co-intervention of external factor.

The codons that a protein prefers to use can predict basic three-dimensional of the protein. According to this theory, we use ANN to build a protein with different peptides selected from the segments of TCR β. A sequences of the T-cell responses to HBsAg. It is possible to explain approximately 5-10% of healthy vaccine recipients fail to produce protective levels of antibodies to the hepatitis B vaccine after standard immunization[28-32]. Although confirming the conclusion is challenging, there are 3 possible factors link to the phenomenon: the happen of the events by chance (because only 15 patients with PRSS1 gene mutation were researched), the patient's human leukocyte antigen genetype and the incongruent immune response to the vaccine components[33]. Furthermore, the pancreas is a target organ for the hepatitis B virus[34]. There are also suggestions in the literature of several additional mechanisms of immunologic injury such as polyclonal activation (adjuvant reaction) of lymphocytes, "bystander activation" of self-reactive lymphocytes or somatic mutation of immunoglobulin variable genes[35-36]. The HLA genotype of our patient suggests that he may have been genetically predisposed to an autoimmune reaction to the hepatitis B vaccine. HBsAg is a T-cell-dependent immunogen that does not elicit a detectable humoral immune response in 5% of HBsAg vaccine recipients. HBsAg elicit the production of anti-HBs and induce the expansion of HBsAg-specific T lymphocytes. When present at sufficient concentrations (arbitrarily defined as 10 IU L^{-1}), these antibodies convey protection against infection with HBV. The immune responses of humans these response patterns are governed by genes located in the major histocompatibility complex. Poor responses are frequently observed for subjects with HLA haplotypes DR3, DR7, DQ2 and/or DP11 and are seen less frequently for subjects expressing DR1, DR5, DP4, DQ3 and/or DQ5[37-40]. There is a critical requirement for T-cells. There is substantial evidence suggesting that the poor/nonresponsiveness to HBsAg observed in a minority of vaccine recipients could partially be ascribed to a defective T-cellular response to HBsAg.

The mechanism responsible for this remarkable change remains unknown. Since HBsAg sequences are endowed with T-cell immunogenicity, it is conceivable that partial delipidation facilitates the processing and presentation of these important domains.

CONCLUSION

Homologous to construct the three-dimensional model of the HBsAg ligand functional domain, and forecast the homology between the ligand functional domain and the candidate amino acid residues combined constituted by TCR Vβ genes and PRSS1 genes with the molecular-docking methods aided-designed by artificial neural network. The results showed that the structure of the ligand participated by the PRSS1 gene coincided with the today's popular HBsAg ligand basically. Therefore it may well explained why different PRSS1 carrier may respond differently to the HBV vaccine and lay the foundation for further study the pathogenesis of HBV.

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