Artificial neural networks for immunological recognition

Jin Xu$^{1,2}$, and Junghyo Jo$^{3}$

$^1$Asia Pacific Center for Theoretical Physics, Pohang, Republic of Korea

$^2$Department of Physics, Pohang University of Science and Technology, Pohang, Republic of Korea

$^3$Korea Institute for Advanced Study, Seoul, Republic of Korea

* Corresponding author

E-mail: jojunghyo@kias.re.kr (JJ)
Abstract

Adaptive immunity is analogous to machine learning as the immune system learns about dangerous pathogens and discriminates them from safe self-molecules. Each immune cell has a unique receptor for specific immunological recognition. The binding affinity between the receptors and antigenic peptides mainly determines immunological recognition followed by subsequent responses. Is the integrated binding affinity sufficient to probe the digital information of peptide sequences? To answer this fundamental question, we examined whether the affinity-based discrimination of peptide sequences is learnable and generalizable by artificial neural networks (ANNs) that process the digital information of receptors and peptides. We made use of large scale high-throughput data of T-cell receptors (TCRs) and open-source peptides amino acid sequences as the input layer data, and computationally examined the success and failure of immune recognition based on the pairwise binding energy of their amino acid sequences as output layer data. Machine learning successfully captured the relevant information of molecular interactions and discriminated between strong and weak affinity pairs. This suggests the potential applicability of artificial neural networks to predict the immune responsiveness of certain antigenic peptides once their sequence information is provided. In addition, we used two methods to describe amino acids: with and without considering the amino acid strength. The sequences with heterogeneous strengths of amino acids diminished TCR “reading” of the exact information of each amino acid site on the peptides for the immune response. This may be why natural TCRs have moderate amino acid compositions after thymic selection.
Introduction

Adaptive immunity (AI) and artificial intelligence (AI) are analogous learning processes. The immune system generates distinct T cells that express unique receptors through stochastic gene recombination, and select part of them in the thymus to avoid recognizing self-molecules [1]. Once the selected T cells are activated by certain antigenic molecules, the immune system memorizes the experience by keeping the specific T cells as memory cells to quickly recognize the experienced antigenic molecules in the future. The evolution and adaptation of immunological receptors have indeed inspired the development of a computational algorithm for pattern recognition [2, 3]. In general, machine learning optimizes artificial neural networks (ANNs) to successfully classify samples in a training set with the hope that the optimized feature extraction for the training set also works for classifying unseen samples in a test set. The learnability and generalizability of the supervised learning are also critical issues in adaptive immunity that are important in optimizing an effective repertoire of immune cells for discriminating seen and unseen antigenic molecules.

Despite the close analogy between the two AIs, a fundamental difference exists. Machine learning reads digital information of samples as the input layer. However, the immune system cannot directly read the sequence information of antigenic molecules, and instead mainly relies on the overall binding affinity between immunological receptors and antigenic molecules. Some experiments have shown the important variety of the different amino acid sites on the sequences of T-cell receptors (TCRs) and peptides [4-6], as has also recent theoretical work [7]. The binding energy based model cannot catch the information of each amino acid site. Andrej Košmrlj has written that mutations in antigenic peptide amino acids contacting strong amino acid sites on TCRs are supposed to abrogate the original recognition [8]. Is the integrated binding affinity sufficient to probe the digital information of peptide sequences? With this question in mind, we have examined whether the affinity-based discrimination of peptide sequences can be learnable and generalizable by ANNs.
In addition to the recent revolution of machine learning, high-throughput sequencing technologies are able to provide sequence data from TCRs and antigenic peptides [9-12]. However, it is challenging for experimentalists to measure the interactions between TCRs and peptides due to their complexity and large scale. So far, there are just hundred TCR-pMHC (peptide Major Histocompatibility Complex) data available in the Protein Data Bank. T-cell reactivity prediction is greatly helpful for vaccine designs and understanding immune response. And this issue is able to be generalized to interactions via receptors and even protein-protein interactions. Computationally, there is some pioneer work that applies machine learning methods to predict T-cell reactivity [13-15] considering part of the physicochemical properties. To simplify the problem, theoretical immunologists have developed string models to understand molecular recognition. They describe TCR and peptide sequences by alphabet strings and define molecular recognition based on matching between the pairwise TCRs and peptides. The string model has been used to explain diverse immune questions, including self-nonself discrimination [16], alloreactivity [17], etc.

To solve our particular question, we adopted the string model to define immune recognition with binding affinity from TCRs to antigenic peptides [8]. Based on the sequence data and the defined recognition, we examined whether ANN can learn affinity-based molecular recognition. Our results reveal that the ANN classification of immune activation can be learnable with affinity-based discrimination of peptide sequences. This means the ANN can represent the immune response effectively and categorize immune activation based on the information of amino acid sequences from TCR and pMHC to predict immune recognition. In other words, the integrated binding affinity between TCR and peptide is sufficient to probe the digital information of peptide sequences to determine immune activation.

This quantitative study on the binding affinity between TCR and pMHC can shed light on its biological mechanisms due to the amino acid strengths. People have been studying the immune system through the interactions of variable regions from lymphocytes and molecules [1], called
immune network theory [18]. One work has modeled the whole immune process by considering the receptors and antigens as binary strings [3]. Here, we understand the immune response by the interface between TCR and pMHC, and zoomed into a smaller scale, i.e. amino acids. We used two types of binary strings to describe amino acid with and without considering the amino acid strengths. The heterogeneous amino acid strengths diminish the test accuracy, although it still makes ANNs learnable. This means that the heterogeneous amino acids increase the degeneracy of each amino acid site. The binding affinity cannot catch the information of each amino acid site, but sequences with homogenous amino acid strengths can supplement this weakness. This can be one reason why in nature, TCRs have moderate amino acids instead of significant heterogeneous compositions after thymic selection [8].
Methods

Model

We use a feedforward neural network to represent the immune response, and consider a three-layer neural network first (Fig 1A). The green nodes represent TCR amino acids, and the orange nodes represent peptide amino acids. Fig 1A shows the network structure schematically. The blue nodes represent the hidden layer and black nodes represent the output layer.

Fig 1. Feedforward neural networks schematically represent immune responses. First, we prepare input layer data as TCR and peptide amino acid sequences, and output layer data as immune recognition or not, as judged by the binding energy between certain pairs of TCRs and peptides.
One event is defined as one set of input and output layer data. Then, we put $10^4$ events into the ANNs to see the training process and test accuracy. (A) Fully connected neural network. In the input layer, the green nodes represent the amino acids of TCRs (length $L=12$), and the orange nodes represent the amino acids of the peptides (length $L=15$). The blue nodes are the hidden layer. The black node is the output layer, representing successful immune recognition or its absence. (B) Partially connected neural network excluding the self-interactions between amino acids of TCRs or peptides. Either the amino acids of TCRs (B1) or peptides (B2) are correspondingly connected with the hidden layer nodes, while peptides (B1) or TCRs (B2) are fully connected to the blue nodes. (C) Binary codes represent one amino acid. One link in the schematic (C1) is actually 20 links for Dataset 1 (equivalent amino acids) considering each amino acid as a 20-bit “one-hot” code. In addition, one link in (C2) is 5 links for Dataset 2 (considering the amino acid strengths), representing each amino acid as a 5-bit binary code from the weakest to the strongest, i.e., K: 00000, D: 00001, E: 00010, …, I: 10001, F: 10010, L: 10011.

However, the fully connected network cannot exclude the self-interactions between amino acids of the receptors and the amino acids of peptides. This is because the fully connected network structure cannot distinguish the nodes representing the amino acids from TCRs or peptides. To exclude self-interactions, a partially connected network between the input and the hidden layer is designed, as shown in Fig 1B. The basic idea is that, in B1 for instance, the TCR amino acids in green are connected to the hidden layer node one by one, while the peptide amino acids in orange are fully connected to the blue nodes.

**Data**

The ANNs described above initially has three layers. This subsection will introduce the details of the prepared input and output layer data.

**Input layer Data**
The input layer data are TCR and antigenic peptide amino acid sequences. We used published naïve coding/in-frame CD4+ T cell beta chains [9], which were abstracted from the blood samples of nine human individuals [19, 20]. The database contains $10^4$-$10^5$ sequences of TCRs per person. Humans have approximately $5 \times 10^6$ distinct T cells, although larger T-cell diversity is likely to exist [11]. We are able to translate the nucleotide sequences of DNA into corresponding amino-acid sequences, based on the DNA codon table. The sequence lengths are from 3 to 17 amino acids, with the most frequent length being $L=12$. For the antigenic peptide sequences, we obtained human linear infectious/autoimmune disease peptides with major histocompatibility complex (MHC) restriction from the open source “Immune epitope database and analysis resource” (IEDB) [10]. Their sequence lengths are from 5 to 45 amino acids, with most frequent length of $L=15$. To avoid the effects of the sequencing length and for technical reasons due to the fixed structures of the ANNs, we used TCRs with the most frequent length being $L=12$ ($\sim 10^4$ sequences per subject) and peptides with its most frequent length being $L=15$ ($\sim 10^4$ sequences).

If we consider each type of amino acid equivalent, one amino acid can be represented by a 20-bit “one-hot” code (Dataset 1: equivalent amino acids). We setup the ANN with 540 nodes (TCR with an amino acid length of 12, and peptide with an amino acid length of 15) in the input layer. However, amino acids have heterogeneous amino acid strengths in reality, which we need to consider. The weak to the strong amino acids, i.e. K, D, E, …, I, F, L, can be encoded by 5-bit binary codes (Dataset 2: considering the amino acid strengths), i.e., 00000, 00001, 00010, …, 10001, 10010, 10011. There are only 135 nodes with 5 bits for each amino acid (TCR with 12 amino acid length, and peptide with 15 amino acid length) in the input layer. To assess the amino acids with binary bits, Fig 1C schematically shows how the networks corresponds.

**Output layer Data**

We set 2 nodes (10 for not recognized, and 01 for recognized) in the output. For the dataset, we considered immune activation and TCR specificities for the particular peptides, based on binding
affinity as a judgment (Fig 1). Basically, once there is a pair of TCR and peptide amino acid sequences in the data, we can calculate the binding energy between them using a Miyazawa-Jernigan (M-J) Matrix. Based on this method, to be explained in detail below, if the binding energy is larger than a certain threshold a successful immune recognition process happens. We made use of “real data” of TCR and peptide sequences for binding affinity calculations based on the M-J matrix, which is the main novelty beyond previous work [8].

Complementary-determining region 3 (CDR3) is a specific region of TCRs and is critical in recognizing pMHC. Due to the short lengths, CDR3 and pMHC have been approximately considered to be linear sequences neglecting their three-dimensions [8, 21]. Many studies have made use of a simple string model to describe TCR-pMHC interactions, including thymic selection [22-24]. The binding energy between a pair of TCR and pMHC molecules can be calculated as follows [8]

\[ E(\vec{r}, \vec{p}) = E_c + \sum_{i=1}^{L} J(t_i, p_i). \]  

(1)

Here, the first term represents the interaction between MHC and the part of TCR other than CDR3. The second term describes the interaction between the amino acid sequence

\( \vec{r} = (t_1, t_2, \ldots, t_L) \) of one TCR CDR3 and \( \vec{p} = (p_1, p_2, \ldots, p_L) \) of one peptide. If \( E_c \) is assigned to a random value chosen uniformly from the interval \((E_{c,\text{min}}, E_{c,\text{max}})\), it can present different MHCs [25]. \( J \) is defined by the statistical potentials between each pair of amino acids, i.e., the \( 20 \times 20 \) Miyazawa-Jernigan matrix [26]. Since the statistical potential values can reasonably describe protein-protein interactions, the Miyazawa-Jernigan matrix has been used a lot in the simulation of protein design and folding [27]. The constant binding energy part has been estimated as \( E_c = 33k_B T \) [8, 21], with \( k_B \) as the Boltzmann constant and \( T \) as the temperature. At normal body temperatures, the thermal energy approximately equals
$k_B T = 0.6 \text{ kcal/mol. Therefore, the immunological recognition can be defined when the binding energy between } \tilde{t} \text{ and } \tilde{p} \text{ is larger than an activation threshold, } E(\tilde{t}, \tilde{p}) > E_R .\]

Here, the value $E_R$ satisfies the relation $(E_R - E^*) / L = 4k_B T$, which has reasonably explained the thymic selection process [8]. To define the binding between a 12-mer TCR and a 15-mer peptide, we considered the random starting positions of binding at each TCR and peptide encounter. In addition, we considered the case of a TCR binding sequence of a peptide reversely.

One set of input and output layer data is called one event. The event data size prepared is $10^4$, and each event includes information on the TCR, peptide sequences and presence or absence of immune activation. In other words, one event includes a single encounter of one pair of TCR and peptide with the resulting successful recognition or not. The output layer data prepared includes 50% (5000) successfully recognized and 50% (5000) not recognized ones. The reason why we chose our event size to be $10^4$ with a 50% split, will be discussed in detail later based on results with different parameters. We split the events ($10^4$) into a training set (2/3) and a test set (1/3) and trained the ANNs with the supervised learning and back propagation algorithm [28]. Online learning was performed from the 1st event to the $10^4$th. The test accuracy shown in the Results later occurs after 100 epochs with a learning rate of 0.01; if there is no specific instruction. For each result, 5 ensembles of prepared events, usually $10^4$, were considered.
Results

We have observed that the affinity-based discrimination of peptide sequences can be learnable by ANNs, which is applicable to different infectious and autoimmune diseases. Our method is able to either neglect the different strengths of amino acid by Dataset 1 (equivalent amino acids) or consider the strength of amino acids by Dataset 2 (considering the amino acid strengths). Both the cases of Dataset 1 and Dataset 2 reveal that ANNs representing immune responses based on binding affinity can be learnable by the supervised learning and back propagation algorithm. However, heterogeneous amino acid strengths with Dataset 2 diminishes the test accuracy, compared to Dataset 1.

Affinity-based discrimination of peptide sequences can be learnable by ANNs

Dataset 1 (equivalent amino acids)

Fully connected ANNs (Fig 1A), do not exclude self-interactions between receptors and peptides. We set the node number in the hidden layer as 300. This hidden layer node number is the same as the generalized case of B2 in Fig 1, which enables comparison. Partially connected ANNs (Fig 1B) exclude the self-interactions between receptors and peptides. In B1, for instance, the TCR amino acids in green are connected to a hidden layer node one by one correspondingly, while the peptide amino acids in orange are fully connected to the blue nodes. Therefore, the number of hidden layer nodes are 12 the same as the TCR length for case B1, and 15 for the case of B2. Because there are 20 realized nodes for a single amino acid, the actual schematic network is supposed to be like that shown in Fig 1C1.

The results of the partially connected ANNs show a lower test accuracy than the fully connected ANN in Fig 2A due to the limited number of hidden layer nodes. To improve this, we have tested another partially connected ANN design with more hidden layer nodes. In B1, for instance, the
TCR binary codes ($12 \times 20 = 240$ nodes) instead of amino acids, are connected to the hidden layer node one by one, while the peptide binary codes are fully connected to the blue nodes. Therefore, the number of hidden layer nodes is 240 for B1 and 300 for B2. However, more nodes will make the computation more expensive. We have also tested the double hidden layers, which are unable to improve the test accuracy significantly, as shown in Fig 2A, with a lower learning rate of 0.001 to avoid overfitting. Importantly, it will slow the learning process shown in Fig 2C. The number of nodes in the second hidden layer is 300, which is the same as that in the first hidden for the fully connected ANN for comparison.

**Fig 2. Test and training accuracy from infectious and autoimmune diseases.** (A) and (B) show the test accuracy vs. the (first) hidden layer node number from different structures of ANNs and datasets. (A) shows Dataset 1 (equivalent amino acids), and (B) shows Dataset 2 (considering the
amino acid strengths). Different shapes represent different structures of ANNs, i.e., diamonds represent fully connected ANNs, circles represent partially connected ANNs (solid B1 and hollow B2), triangles represent more hidden layer nodes, and squares represent cases with two hidden layers. Different colors represent different diseases, i.e., red represents infectious diseases, green represents autoimmune disease and blue represents random input-output mapping, with 50% recognizable cases in the output layer as a control. The error bars show the standard errors from five different ensembles of $10^4$ events prepared. (C) and (D) show the learning process by the training accuracy vs. epochs. Only the first 50 epochs of the learning processes are shown, as there are no significant changes from the 50th to the 100th epoch with an event size of $10^4$.

**Dataset 2 (considering the amino acid strengths)**

For fully connected ANNs (Fig 1A), we also set the hidden layer node number as 300. For partially connected ANNs (Fig 1B), there are 12 (15) nodes in the hidden layer for B1 (B2).

Again, to improve the test accuracy shown in Fig 2B, we tested the ANN with more hidden layer nodes. There are 60 (75) nodes in the hidden layer for B1 (B2). Double hidden layers cannot improve the test accuracy for this dataset, as shown in Fig 2B, with a learning rate of 0.001. Indeed, they will slow down the learning process, as shown in Fig 2D. Compared to Dataset 1, Dataset 2 is computationally less expensive due to its use of fewer nodes.

Both Dataset 1 and Dataset 2 (Figs 2A and 2B) show ANNs representing immune response based on binding affinity, which can be learned by the supervised learning and back propagation algorithm. In principle, ANN can map any arbitrary input-output mapping. We have also designed a control experiment, and prepared a random input-output mapping by generating 50% successful recognitions as the output layer data. The results in Figs 2A and 2B (blue) have shown that the test accuracies are approximately 50%. The learning rate here was set to 0.001 to avoid overfitting, although it was set to 0.0001 for the two hidden layer cases. This learning rate for random mapping
is smaller than that of the original. The original cases showed higher test accuracy in Figs 2A and 2B compared to that of a random control. Furthermore, the results in Figs 2A and 2B show that larger numbers of hidden layer nodes increase the test accuracy in the common sense, while the partially connected networks sometimes disturb this.

**Peptide amino acid compositions**

To understand why some peptides are recognizable and some are not, we calculated the amino acid composition frequency of recognizable and nonrecognizable peptides (Fig 3A). Our results show that the recognizable peptides have stronger amino acids.

**Fig 3 Amino acid compositions of different peptides.** (A) shows the amino acid composition differences between recognizable and nonrecognizable peptides. Recognizable peptides have stronger amino acids. Errors are from 5 different ensembles of $10^4$ events. (B) – (D) show how the amino acid compositions differ between infectious vs. autoimmune disease, Hepatitis C virus vs
infectious disease and Mycobacterium tuberculosis vs infectious disease. Amino acids are sorted from the strongest to weakest according to the maximum binding energy interacting with all other amino acids.

**Reasonable parameters and repertoire data**

The event data size prepared as $10^4$ is enough to complete the machine-learning task. We also tested with event sizes 2000 and $3 \times 10^4$ (S1 Fig). With an event size of 2000, the ANNs are already mostly learnable. Increasing the event size can significantly speed up the learning process, but cannot change the test accuracy significantly. Actually, a smaller event size (2000) is able to show the learning process in detail. Referring to S1 Fig, with a smaller event data size (2000), it does not reach a high accuracy at the beginning of the limited epochs compared to a large size ($10^4$, $3 \times 10^4$). Furthermore, it does not converge quickly during the learning process. This was most significant with two hidden layers.

We split the events into 50% recognizable and 50% nonrecognizable ones for the output layer data. We also tested a lower successful recognition rate (20%). A lower recognition split rate will not decrease the test accuracy (S2 Fig). We also prepared a control, for which random input-output maps were 20% recognizable ones as the output layer data. The results in S2 Fig (blue) show that the test accuracy is approximately 80%, which is too high to judge whether ANNs are learnable or not. It is also difficult to observe the differences among the diverse designed network structures and between two types of amino acid datasets (equivalent amino acids and considering the amino acid strengths).

Additionally, we tested some other T-cell pools, with coding/in-frame CD8+ T cell alpha and beta chains [12]. This dataset is from six individuals. The database contains $10^4$ sequences of TCRs per individual with a certain length $L=12$ of either the alpha or the beta chain, which is dominated by a naïve T-cell repertoire. The results do not show significant differences from the original test data (S3 Fig).
Heterogeneous amino acid strengths diminish the test accuracy

The case considering the heterogeneous strengths of amino acids (Dataset 2: considering the amino acid strengths) usually does not have a test accuracy as high as is the case of considering each amino acid equivalently (Dataset 1: equivalent amino acids), as shown in Figs 2A and 2B. For Dataset 2, which is the more realistic case, the binding affinity cannot “read” as much information for each amino acid site. This means that the heterogeneous amino acids will increase the degeneracy of each amino acid site. For Dataset 1, the binding affinity can be used to investigate more information for each amino acid site from peptide sequences by the TCRs. The binding affinity cannot catch the information of each amino acid site, but sequences with homogenous amino acid strength can supplement this weakness. The comparison of the learning process between two datasets (Figs 2C and 2D) confirms that the heterogeneous amino acid strengths will diminish the information “reading” from peptides by TCRs. This shows that the learning process differences among various diseases in Fig 2C are more dramatic than those Fig 2D. In other words, heterogeneous amino acid strengths diminish the differences between infectious and autoimmune diseases for immune recognition.

Applicable to different diseases

We are motivated to test data from different diseases and check whether our method is valid for use in various antigens/diseases. This may help us to understand disease differences. For this reason, we selected the Hepatitis C virus as one example of a virus infectious disease and selected Mycobacterium tuberculosis as another example of a bacterial infectious disease. The results in Figs 2 and 4 show that, the ANNs are able to learn autoimmune diseases and different infectious diseases similarly. The peptides from autoimmune and different viral/bacterial diseases are picked from IEDB with specific antigen organisms and diseases.
Fig 4. Test and training accuracy from different infectious diseases. (A) and (B) show the test accuracy vs. the (first) hidden layer node number from different structures of ANNs and datasets. (A) shows Dataset 1 (equivalent amino acids) and (B) shows Dataset 2 (considering the amino acid strengths). Different shapes represent different structures of ANNs, i.e., diamonds represent fully connected ANNs, circles represent partially connected ANNs (solid B1 and hollow B2), triangles represent ANNs with more hidden layer nodes, and squares represent ANNs with two hidden layers. Different colors represent different diseases, i.e., red represents different infectious diseases, green represents Hepatitis C virus and blue represents Mycobacterium tuberculosis. The error bars show standard errors from five different ensembles of $10^4$ events prepared. (C) and (D) show the learning process by the training accuracy vs. epochs. Only the first 50 epochs of the learning process are shown, due to a lack of significant changes from the 50th to the 100th epoch with an event size of $10^4$. 
One reason why different diseases show similar learning and test accuracies maybe because their peptide amino acid compositions are not very differently distributed. We have tested the amino acid compositions of these different infectious disease peptides (Fig 3B-D).
Discussion

Some experiments have shown the importance of the different amino acid sites on the sequences of TCRs and peptides [4-6] for immune response, and also recent theoretical work [7]. Are TCRs able to “read” enough information from the peptides to determine the immune response based on the binding affinity? What affects the information “reading” at the scale of the amino acid site? Measuring the large-scale scanning of immunological recognition between TCRs and peptides is experimentally challenging. To date, there are only a hundred TCR-pMHC complex data available in the Protein Data Bank. Therefore, we tried to make use of computational methods with real data to answer these questions.

The main functions of the immune system are recognition and categorization of pathogens [2], which are similar to ANNs. We made use of large scale high-throughput data of TCRs [9, 12] and open-source peptide amino acid sequence data [10] as the input layer data. Furthermore, we computationally examined the success and failure of immune recognition based on the pairwise binding energy of their amino acid sequences [8] as the output layer data. Then, we put the prepared data into a feedforward neural networks describing the immune response. The results demonstrate that ANNs representing the immune response can be trained by the supervised learning and back propagation algorithm with a higher test accuracy compared to that of random input-output mapping. That is, ANN classification can categorize immune activation or not based on information from amino acid sequences from TCR and pMHC. Therefore, the integrated binding affinity between the TCR and peptide is sufficient to probe the digital information of peptide sequences to determine immune activation. This method can describe immune response for different infectious diseases and autoimmune disease effectively.

People have considered immunology as an information process [29] with binary strings presenting TCRs and peptides. Our study considered immune receptors and pMHCs at a more detailed coarse-grained scale and transformed each amino acid into information by binary bits. We
used two methods to describe amino acids: with and without considering the amino acid strengths. The findings suggest that the ANNs considering the amino acid strengths can be trained to determine an immune response. However, it cannot reach as high as the test accuracy of the case without considering the amino acid strengths. This difference investigates that the heterogeneous amino acid strengths of TCRs will diminish their abilities to obtain precise information on each amino acid site on the peptide sequences. Previous work suggests that mutations in antigenic peptide amino acids contacting strong amino acid sites on TCR are supposed to abrogate original recognition [8]. In other words, the heterogeneous amino acid strengths will increase the degeneracy of each amino acid site. The binding affinity cannot catch the information of each amino acid site, but sequences with homogenous amino acid strength can somehow make up this weakness. This can be one reason why natural TCRs have moderate amino acids after thymic positive and negative selections [8], which may occur via deleting excessively strong amino acids.

In summary, ANNs can catch a smaller scale, i.e. the strength of one amino acid site, than the binding affinity-based model.

Our ANN method requires large-scale TCRs and infectious peptides. For instance, to prepare 1000 recognizable events with input and output layer data, approximately $10^6$ total pairings between TCRs and peptides are needed. Therefore, 200 peptides need at least 5000 TCRs. In addition, our method requires fixed lengths of TCR and peptide sequences, which is due to the fixed ANN structures for calculations. Therefore, for some infectious diseases carried by most humans (i.e. influenza), cannot be tested with reasonable method accuracy due to their data limitation currently. However, our method can highlight how the strength of each amino acid is able to affect the immunological recognition. In addition, our designed network for immune response with partially connected neural networks, may represent the interface between two molecules/individuals as cell-cell interactions through their receptor/individual information, considering coarse-grained details of each receptor/individual.
Acknowledgments

We thank the reviewers and the editor for their valuable comments and suggestions to improve the quality of this paper.

References

1. Jerne NK. The generative grammar of the immune system. Science. 1985;229(4718):1057-9. Epub 1985/09/13. PubMed PMID: 4035345.

2. Dasgupta D. Artificial neural networks and artificial immune systems: Similarities and differences. Ieee Sys Man Cybern. 1997:873-8. PubMed PMID: WOS:A1997BJ80Y00151.

3. Farmer JD, Packard NH, Perelson AS. The immune system, adaptation, and machine learning. Physica D: Nonlinear Phenomena. 1986;22(1):187-204. doi: https://doi.org/10.1016/0167-2789(86)90240-X.

4. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glanville J, Dobbins J, et al. Deconstructing the peptide-MHC specificity of T cell recognition. Cell. 2014;157(5):1073-87. Epub 2014/05/27. doi: 10.1016/j.cell.2014.03.047. PubMed PMID: 24855945; PubMed Central PMCID: PMCPMC4071348.

5. Calis JJ, de Boer RJ, Kesmir C. Degenerate T-cell recognition of peptides on MHC molecules creates large holes in the T-cell repertoire. PLoS Comput Biol. 2012;8(3):e1002412. Epub 2012/03/08. doi: 10.1371/journal.pcbi.1002412. PubMed PMID: 22396638; PubMed Central PMCID: PMCPMC3291541.

6. Stadinski BD, Shekhar K, Gomez-Tourino I, Jung J, Sasaki K, Sewell AK, et al. Hydrophobic CDR3 residues promote the development of self-reactive T cells. Nat Immunol. 2016;17(8):946-55. Epub 2016/06/28. doi: 10.1038/ni.3491. PubMed PMID: 27348411; PubMed Central PMCID: PMCPMC4955740.

7. Chen HR, Chakraborty AK, Kardar M. How nonuniform contact profiles of T cell
receptors modulate thymic selection outcomes. Phys Rev E. 2018;97(3). doi: ARTN 032413
10.1103/PhysRevE.97.032413. PubMed PMID: WOS:000428013000002.
8. Kosmrlj A, Jha AK, Huseby ES, Kardar M, Chakraborty AK. How the thymus designs antigen-specific and self-tolerant T cell receptor sequences. Proc Natl Acad Sci U S A. 2008;105(43):16671-6. doi: 10.1073/pnas.0808081105. PubMed PMID: WOS:000260913500049.
9. Murugan A, Mora T, Walczak AM, Callan CG, Jr. Statistical inference of the generation probability of T-cell receptors from sequence repertoires. Proc Natl Acad Sci U S A. 2012;109(40):16161-6. doi: 10.1073/pnas.1212755109. PubMed PMID: 22988065; PubMed Central PMCID: PMCPMC3479580.
10. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, et al. The immune epitope database (IEDB) 3.0. Nucleic Acids Res. 2015;43(Database issue):D405-12. doi: 10.1093/nar/gku938. PubMed PMID: 25300482; PubMed Central PMCID: PMCPMC4384014.
11. Warren RL, Freeman D, Zeng T, Choe G, Munro S, Moore R, et al. Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes. Genome Res. 2011;21(5):790-7. doi: 10.1101/gr.115428.110. PubMed PMID: WOS:000290088000017.
12. Zvyagin IV, Pogorelyy MV, Ivanova ME, Komech EA, Shugay M, Bolotin DA, et al. Distinctive properties of identical twins' TCR repertoires revealed by high-throughput sequencing. Proc Natl Acad Sci U S A. 2014;111(16):5980-5. Epub 2014/04/09. doi: 10.1073/pnas.1319389111. PubMed PMID: 24711416; PubMed Central PMCID: PMCPMC4000852.
13. Saethang T, Hirose O, Kimkong I, Tran VA, Dang XT, Nguyen LA, et al. PAAQD: Predicting immunogenicity of MHC class I binding peptides using amino acid pairwise contact potentials and quantum topological molecular similarity descriptors. J Immunol Methods. 2013;387(1-2):293-302. Epub 2012/10/13. doi: 10.1016/j.jim.2012.09.016. PubMed PMID: 23058674.
14. Tung CW, Ho SY. POPI: predicting immunogenicity of MHC class I binding peptides by
mining informative physicochemical properties. Bioinformatics. 2007;23(8):942-9. Epub 2007/03/27. doi: 10.1093/bioinformatics/btm061. PubMed PMID: 17384427.

15. Tung CW, Ziehm M, Kamper A, Kohlbacher O, Ho SY. POPISK: T-cell reactivity prediction using support vector machines and string kernels. BMC Bioinformatics. 2011;12:446. Epub 2011/11/17. doi: 10.1186/1471-2105-12-446. PubMed PMID: 22085524; PubMed Central PMCID: PMCPMC3228774.

16. Percus JK, Percus OE, Perelson AS. Predicting the size of the T-cell receptor and antibody combining region from consideration of efficient self-nonself discrimination. Proc Natl Acad Sci U S A. 1993;90(5):1691-5. Epub 1993/03/01. PubMed PMID: 7680474; PubMed Central PMCID: PMCPMC45945.

17. Detours V, Mehr R, Perelson AS. Deriving quantitative constraints on T cell selection from data on the mature T cell repertoire. J Immunol. 2000;164(1):121-8. doi: DOI 10.4049/jimmunol.164.1.121. PubMed PMID: WOS:000084321200020.

18. Jerne NK. Towards a network theory of the immune system. Ann Immunol (Paris). 1974;125C(1-2):373-89. Epub 1974/01/01. PubMed PMID: 4142565.

19. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009;114(19):4099-107. doi: 10.1182/blood-2009-04-217604. PubMed PMID: 19706884; PubMed Central PMCID: PMCPMC2774550.

20. Robins HS, Srivastava SK, Campregher PV, Turtle CJ, Andriesen J, Riddell SR, et al. Overlap and effective size of the human CD8+ T cell receptor repertoire. Sci Transl Med. 2010;2(47):47ra64. doi: 10.1126/scitranslmed.3001442. PubMed PMID: 20811043; PubMed Central PMCID: PMCPMC3212437.

21. Kosmrlj A, Chakraborty AK, Kardar M, Shakhnovich EI. Thymic Selection of T-Cell Receptors as an Extreme Value Problem. Phys Rev Lett. 2009;103(6). doi: ARTN 068103 10.1103/PhysRevLett.103.068103. PubMed PMID: WOS:000268809300075.
22. Chao DL, Davenport MP, Forrest S, Perelson AS. The effects of thymic selection on the range of T cell cross-reactivity. Eur J Immunol. 2005;35(12):3452-9. Epub 2005/11/15. doi: 10.1002/eji.200535098. PubMed PMID: 16285012; PubMed Central PMCID: PMCPMC1857316.

23. Detours V, Mehr R, Perelson AS. A quantitative theory of affinity-driven T cell repertoire selection. J Theor Biol. 1999;200(4):389-403. Epub 1999/10/20. doi: 10.1006/jtbi.1999.1003. PubMed PMID: 10525398.

24. Detours V, Perelson AS. Explaining high alloreactivity as a quantitative consequence of affinity-driven thymocyte selection. Proc Natl Acad Sci U S A. 1999;96(9):5153-8. Epub 1999/04/29. PubMed PMID: 10220434; PubMed Central PMCID: PMCPMC21832.

25. Kosmrlj A, Kardar M, Chakraborty AK. The Influence of T Cell Development on Pathogen Specificity and Autoreactivity. J Stat Phys. 2012;149(2):203-19. doi: 10.1007/s10955-011-0403-8. PubMed PMID: WOS:000309875000001.

26. Miyazawa S, Jernigan RL. Residue-residue potentials with a favorable contact pair term and an unfavorable high packing density term, for simulation and threading. J Mol Biol. 1996;256(3):623-44. doi: 10.1006/jmbi.1996.0114. PubMed PMID: 8604144.

27. Li H, Tang C, Wingreen NS. Nature of driving force for protein folding: A result from analyzing the statistical potential. Phys Rev Lett. 1997;79(4):765-8. doi: DOI 10.1103/PhysRevLett.79.765. PubMed PMID: WOS:A1997XM975000057.

28. Rumelhart DE, Hinton GE, Williams RJ. Learning representations by back-propagating errors. Nature. 1986;323:533. doi: 10.1038/323533a0.

29. Segel LA, Cohen IR. Design principles for the immune system and other distributed autonomous system. New York: Oxford University Press; 2001.
Supporting information

S1 Fig. How ANN test and training accuracy depend on event data size. (A) and (B) show the test accuracy vs. the (first) hidden layer node number from different structures of ANNs and datasets. (A) shows for Dataset 1 (equivalent amino acids) and (B) is for Dataset 2 (considering the amino acid strengths). Different shapes represent different structures of ANNs, i.e., diamonds represent fully connected ANNs, circles represent partially connected ANNs (solid B1 and hollow B2), triangles represent cases with more hidden layer nodes, and squares represent cases with two hidden layers. Different colors represent different diseases, i.e., red represents an event data size of 2000, green represents a size of $10^4$ and blue represents a size of $3 \times 10^4$. The error bars are standard errors from five different ensembles of the $10^4$ events prepared. (C) and (D) show the learning process through the training accuracy vs. the epochs. Only the first 50 epochs of the learning processes are shown, as no significant changes occur from the 50th to 100th epoch with an event
S2 Fig. ANN test and training accuracy depend on different percentages of successful recognition as the output layer data. (A) and (B) show the test accuracy vs. the (first) hidden layer node number from different structures of ANNs and datasets. (A) shows Dataset 1 (equivalent amino acids), and (B) shows Dataset 2 (considering the amino acid strengths). Different shapes represent different structures of ANNs, i.e., diamonds represent fully connected ANNs, circles represent partially connected ANNs (solid B1 and hollow B2), triangles represent the cases with more hidden layer nodes, and squares represent cases with two hidden layers. Different colors represent different diseases, i.e., red represents infectious diseases with 50% recognizable cases in the output layer, green represents infectious diseases with 20% recognizable cases in the output layer, and blue represents random input-output mapping with 20% recognizable cases in the output layer as a control. The error bars show standard errors from five different ensembles of $10^4$ events.
prepared. (C) and (D) show the learning process by the training accuracy vs. the epochs. Only the first 50 epochs of the learning processes are shown, as no significant changes occur from the 50th to 100th epoch with an event size of $10^4$.

S3 Fig. Different T-cell repertoire pools do not affect the ANN test and training accuracy significantly. (A) and (B) show the test accuracy vs. the (first) hidden layer node number from the different structures of the ANNs and datasets. (A) shows Dataset 1 (equivalent amino acids) and (B) shows Dataset 2 (considering the amino acid strengths). Different shapes represent different structures of ANNs, i.e., diamonds represent fully connected ANNs, circles represent partially connected ANNs (solid B1 and hollow B2), triangles represent cases with more hidden layer nodes, and squares represent cases with two hidden layers. Different colors represent different diseases, i.e. red represents CD4+ T cell beta chain data, green represents CD8+ T cell alpha chain data and blue represents CD8+ T cell beta chain data. The error bars show standard errors from five different
ensembles of $10^4$ events prepared. (C) and (D) show the learning process by the training accuracy vs. epochs. Only the first 50 epochs of the learning process are shown, as no significant changes occurred from 50th to 100th with an event size of $10^4$. 