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High level of cross-reactivity in influenza virus hemagglutinin-specific CD4+ T-cell response: Implications for the initiation of autoimmune response in multiple sclerosis

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Abstract

Viral infections play a role in shaping and maintaining the peripheral T-cell repertoire, as well as in the initiation of autoimmune response via mechanisms of molecular mimicry. In this study, we addressed the flexibility of T-cell receptor (TCR) recognition and the degree of structural and sequence homology required for cross-reactive immune response in the induction of autoimmune response. We studied the extent of cross-reactivity of a CD4+ T-cell clone (TCC) specific for the immunodominant influenza virus hemagglutinin (Flu-HA) peptide derived from a patient with multiple sclerosis (MS) using positional scanning synthetic peptide combinatorial libraries (PS-SCL). We documented cross-reactivity against 14 Flu-HA variants, 11 viral, 15 human, and 3 myelin-derived peptides. Moreover, we identified six naturally occurring peptides with higher stimulatory potency than the native ligand, implicating high potential for cross-reactivity even for a virus-specific memory TCC. Our study demonstrates that flexibility of TCR recognition is present even in a clone with a high degree of TCR specificity for an infectious agent. The results have implications for vaccine design and for antigen-specific treatment strategies for autoimmune diseases.

Keywords: Multiple sclerosis; Influenza haemagglutinin; Immunodominant epitope; Molecular mimicry; T-cell receptor repertoire

1. Introduction

TCR cross-reactivity plays an important role in shaping T-cell repertoire through thymic selection and in protecting the host against a spectrum of potential pathogen-derived antigens that are much broader than the limited number of memory T-cells (Casrouge et al., 2000). In addition to the described physiological role, TCR degeneracy raises the potential for cross-reactivity between pathogen-derived and self-antigens. Molecular mimicry, whereby autoreactive T-lymphocytes are activated by cross-reactive foreign antigens, is a relatively frequent phenomenon (Martin et al., 2000). However, its pathogenic role in the initiation of autoimmune response in MS remains to be determined. In order to study the requirements for the molecular mimicry, we examined the flexibility of TCR recognition and the degree of sequence homology required for a cross-reactive immune response. The structural basis for TCR flexibility was

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elucidated by studies of crystal structure of TCR-major histocompatibility complex (MHC)/peptide complexes showing that most of the complex binding energy is determined by the affinity of TCR for a given MHC molecule, while peptides only modulate this affinity (Engelhard, 1994). In addition, recent report indicated that complementarity-determining region 3alpha undergoes rearrangements to adapt to structurally different peptides (Reiser et al., 2003). TCR recognition is degenerate: single T-cell can be activated by different antigenic peptides in the context of the same MHC class II molecule. The half-maximal stimulatory concentrations of these peptides differ over several orders of magnitude from sub-picomolar to micromolar ranges (Hemmer et al., 1997; Wilson et al., 1999). Our previous observations have shown that autoantigens are recognized only at intermediate to high concentrations, while foreign antigens are recognized with much higher sensitivity (Hemmer et al., 1999).

The immunological response to myelin basic protein (MBP), a putative autoantigen in MS, has been extensively studied. We will use it here to outline advances in our understanding of molecular mimicry. Based on the structural requirements for MHC class II and TCR binding of an immunodominant MBP 83-99 epitope (Wucherpfennig et al., 1994), molecular mimic motifs were designed. Database search detected 129 peptides and eight of the identified peptides were stimulatory for the MBP-specific clones. Only one of these peptides could have been predicted by a sequence homology search (Wucherpfennig and Strominger, 1995).

Further probing the TCR degeneracy revealed that multiple substitutions are tolerated, even at the primary MHC and TCR contact positions (Vergelli et al., 1996) and peptides with no sequence homology were predicted and tested stimulatory for individual TCC (Hemmer et al., 1998).

While stringent antigen recognition by virus-specific TCCs ensures viral elimination, the in vivo activation, expansion, and maintenance of TCCs are facilitated by their capacity to recognize a range of different peptides (Selin et al., 1998). Within the memory pool, T-cells specific for cross-reactive epitopes are preferentially maintained, while those specific for non-cross-reactive epitopes are selectively lost.

In addition to influences on repertoire dynamics at the T-cell population level, we hypothesize that the extent of cross-reactivity of a single TCR will also impact on the risk of developing autoimmune response triggered by anti-viral immune responses. In order to characterize the requirements for structural and sequence homology in an individual TCR cross-reactive response, we identified a hierarchy of antigen specificities for a clone generated against immunodominant Flu-HA epitope in the setting of acute viral infection. By using positional scanning PS-SCL and biometric data analysis (Hemmer et al., 1999), we accurately predicted a range of cross-reactive epitopes that may play a role in the development of autoimmune response in multiple sclerosis (MS).

2. Materials and methods

2.1. T-cell clones

T-cell clone GP5F11 was established from peripheral blood mononuclear cells (PBMC) of a MS patient during acute respiratory influenza-A virus infection by stimulation with the immunodominant Flu-HA 306-318 (PKYVKQNTKLAT) peptide as described (Lamb et al., 1982; Zhao et al., 2001). TCC clonality was confirmed by staining with TCR Vβ-fluorescein conjugated antibody panel (Beckman Coulter, Somerset, NJ). Cytokine secretion was measured using ELISA according to manufacturer's recommendations (Beckton Dickinson, San Diego, CA).

TCCs GP34 and GP3G6 were generated from the same MS patient, whereas the clone GDBP was derived from his identical twin concordant for MS. All clones were generated against immunodominant MBP epitope 83–99 as described above.

2.2. Decapeptide combinatorial peptide libraries

Individual peptides were synthesized by the simultaneous multiple peptide synthesis method (Houghten, 1985) and arranged in synthetic *N*-acetylated, C-amide L-amino acid combinatorial decamer peptide library in a positional scanning format (Pinilla et al., 1994). The PS-SCL consists of 200 mixtures of the OX_9 format, where O represents one of the 20 L-amino acids and X represents all of the natural L-amino acids except cysteine. Each OX_9 mixture consists of 3.2×10^{11} (19^9) different decamer peptides in equimolar concentration, resulting in 0.26 fM individual peptide concentration.

2.3. Proliferation assays

3×10^4 TCC cells were plated with 10^5 irradiated autologous PBMCs in duplicate wells in the absence or presence of PS-SCL mixtures at the final concentration of 100 μg/ml. Proliferation was measured after 72 h incubation by standard [^{3}H]thymidine incorporation assay. Results were expressed as stimulation index (SI), a ratio between counts per minute (cpm) for proliferation induced by peptide mixtures and background proliferation. Individual peptides with the highest predicted stimulatory scores were synthesized and tested in six independent experiments in a concentration range from 0.001 to 100 μg/ml. Results were expressed as SI (cpm with antigen/cpm without antigen) at 1 μg/ml and the half-maximal stimulatory concentration (EC_{50}), which serves as a measure of actual stimulatory potency of predicted stimulatory peptide. A peptide was considered stimulatory if it induced SI >3 at 1 μg/ml and EC_{50} <5.5 μg/ml.

2.4. Scoring matrix and database searches

A scoring matrix was generated by assigning a numerical value for the stimulatory potency of each amino acid at each position of decapeptide PS-SCL, as described (Hemmer et al., 1999; Zhao et al., 2001). Calculations were based on the results of PS-SCL experiment. The numerical score for each amino acid at each position of the peptide was calculated by subtracting proliferation results in counts per minute (cpm) in the absence, from the cpm in the presence of peptide mixtures. The results were divided by a smoothed estimate of the standard error of background and peptide-induced proliferation. Assuming independent and additive contribution of each amino acid at each position to the stimulatory potency of the whole peptide (Hemmer et al., 1999), the PS-SCL biometrical analysis scores each peptide by adding values of the individual amino acids in a 10-mer peptide. We performed a GenPept database analysis of human, viral, and bacterial proteins by scoring all 10-mer peptides using the scoring matrix for this TCC. A 10-mer peptide window was moved over all available protein-coding sequences in the database. Scored peptides were ranked according to their predicted stimulatory potency. The peptides predicted to be stimulatory were synthesized and tested in vitro in proliferation assays (Wilson et al., 1999).

Receiver operator characteristic (ROC) (Swets, 1988) analysis was applied to the data set of 80 synthesized peptides tested in dose titration experiments for TCC GP5F11 by computing the sensitivity and specificity using different threshold of Z score for predicting the stimulatory potency of synthesized peptides. Peptide was considered stimulatory if SI was >3 at 1 μg/ml and EC_{50} were below 5.5 μg/ml (Fig. 1.).

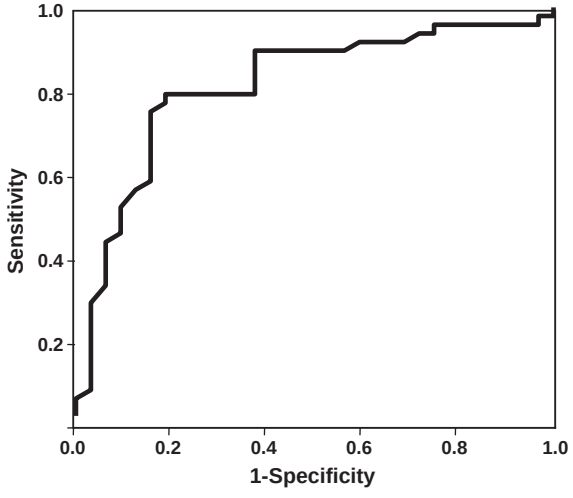

Fig. 1. The ROC curve shows the performance of the Z score matrix based classification model. The x-axis is 1-specificity and the y-axis is sensitivity. The area under the curve is 0.814.

3. Results

3.1. Combinatorial peptide libraries and biometric data analysis accurately predict stimulatory peptides for a Flu-HA specific TCC

TCC GP5F11 has been established with the immunodominant Flu-HA epitope 306-318 (YVKQNTLKLA) from PBMCs of a MS patient with acute influenza virus infection. The clone expresses a Th1 cytokine profile (IFN-γ576 pg/ml; IL-4 122 pg/ml) and is restricted by DRB1*0404. Dose titration of the Flu-HA peptide documents sensitivity of GP5F11 at the nanomolar concentrations indicating that it is representative of high avidity pathogen-specific TCCs. In order to detect all stimulatory epitopes for this clone, we tested its proliferative response to a decapeptide PS-SCL composed of complex peptide mixtures, in which individual peptides are present at 0.26 fM concentration. Since proliferative responses to peptide mixtures are induced by simultaneous TCR binding to millions of peptides, they reflect T-cell activation induced by the simultaneous recognition of multiple peptides represented in PS-SCL.

Our results indicate that K3 is the primary TCR contact, as it is the only amino acid tolerated at this position, with the highest SI among all amino acids tested for this TCR. Similarly, our data suggest that N5 is a secondary TCR anchor, where conservative Q substitution is tolerated, as reported by Wedderburn et al. (1995).

Positional scanning libraries results showed that only few mixtures at each position of the PS-SCL elicit a clear response by GP5F11 clone (Table 1). The amino acids corresponding to Flu-HA 306-318 are among the defined amino acids of the mixtures with high stimulatory potency. However, at positions P 1, 2, 7, and 10, mixtures with amino acids different from those in Flu-HA 306-318 had greater stimulatory capacity indicating that the Flu-HA peptide may not be optimal stimulatory ligand for this clone. While the complete scoring matrix data were used in the database search for cross-reactive peptides, Table 1 shows only the defined amino acids in the mixtures with SI >3.0.

3.2. Superagonist peptides were identified even for a TCC with high sensitivity for an immunodominant influenza virus epitope

Assuming that the clone is highly specific for this particular foreign peptide that led to in vivo expansion of a TCC, we investigated whether cross-reactivity still includes peptides that surpass the native peptide in terms of stimulatory potency. We therefore synthesized peptides that incorporate the optimal amino acids in each position of the decamer peptide. These were more stimulatory than the native peptide, and the hierarchy of proliferative responses correlated in most cases with the peptides' predicted stimulatory potencies. Overall, peptides composed of residues with higher predicted scores than the native

Table 1
Proliferative response of GP5F11 clone to decapeptide PS-SLC

Position	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	(SI score)
FLU HA 306-318	Y	V	K	Q	N	T	L	K	L	A	(256)
Amino acid (SI score)	W (57)	M (28)	K (72)	Q (59)	N (20)	T (18)	G (30)	R (39)	F (11)	L (14)	
	Y (31)	L (19)		V (20)	H (5)	S (17)	P (15)	K (31)	R (11)	M (12)	
	F (19)	I (16)		I (10)	Q (3)	N (12)	A (11)	G (8)	M (10)	I (11)	
	R (3)	V (16)		L (9)		I (11)	H (8)	P (4)	Y (9)	F (9)	
		A (5)		Y (9)		Q (9)	F (5)	M (3)	K (7)	V (4)	
		D (3)		H (4)		G (5)	S (5)		L (6)	Y (4)	
		F (3)		K (4)		V (4)	T (5)		V (5)		
				P (3)		A (4)	Y (4)		H (5)		
				T (3)		H (3)	V (3)		Q (4)		
							N (3)		P (4)		
							Q (3)		N (3)		
							L (3)		I (3)		
							I (3)		S (3)		
									W (3)		
									G (3)		

Proliferative responses of Flu-HA 308-316-specific clone were tested against peptide mixtures arranged in a positional screening format. Each mixture consisted of decamer peptides with single amino acid fixed at designated position, while other positions contained randomly assigned amino acids. Results are presented as stimulation indices (SI) for most stimulatory amino acids at defined positions. At each position of the decamer peptide (P1 to P10) all 20 amino acids were tested. Here we show the most stimulatory amino acids with SI >3 at each position.

peptide, induced higher proliferative responses than FLU-HA 308-316 and served as superagonist peptides for TCC GP5F11. Based on the scoring matrix that assigned a numerical value for stimulatory potency of every amino acid at each position of a decapeptide mixture, we also predicted a control non-stimulatory peptide EPASAKEWDR consisting of the defined amino acids having low SIs in the PS-SCL mixtures. When tested, the peptide had a low stimulatory potency with EC_{50} value of 9.34 μg/ml.

Next, serial substitutions of the most stimulatory amino acids introduced into the native Flu-HA 308-316 epitope were designed to test the contribution of each position to the peptide's stimulatory potency. Results confirmed predicted higher stimulatory potency for substitutions W1, M2, V4, N6, I6, G7, P7, F9, and L10 in comparison to the native peptide. Our results show that peptide stimulatory potency represents the sum of single amino acid contributions at each position of the peptide. Each amino acid with higher predicted stimulatory potency that was substituted to the native FLU-HA peptide induced a superagonist response and contributes independently to the peptide's stimulatory potency.

3.3. Identification of biologically relevant peptide mimics for TCC highly specific for influenza HA

Naturally occurring cross-reactive antigens for TCC GP5F11 were identified by systematic search of the viral, bacterial, and human GenPept database. The results provide a quantitative prediction of stimulatory potencies for individual peptides. Among the eighty highest scoring peptides that were synthesized, we confirmed stimulatory potency of 14 influenza-A virus variants, 11 viral, 15 human, and 3 peptides derived from human myelin proteins. Table 2A presents viral peptides, and Table 2B presents human peptides identified in the GenPept database search and confirmed as stimulatory ligands for TCC GP5F11. Their stimulatory potency was tested in dose titration experiments in Fig. 2a and b, respectively. Results are representative of six independent experiments. The peptides were ranked according to their actual stimulatory potencies confirmed in proliferation assays. EC_{50} and the SI value at a peptide concentration of 1 μg/ml served as most discriminating measures of stimulatory potencies. Protein source and function are listed for each tested peptide. Fourteen out of sixteen (87.5 %) of predicted influenza-A variants were stimulatory for this TCC, with six peptides having even higher stimulatory potencies than the native Flu peptide. This is remarkable in view of the high stimulatory potency of the Flu-306-318 peptide.

Among viral mimics, 11 out of 22 synthesized peptides (50%) proved stimulatory for this TCC. Equine influenza-A HA peptide had more than seventy times higher stimulatory potency than the Flu-HA peptide.

Fifty percent (15 out of 30) of predicted human mimic peptides were confirmed stimulatory, demonstrating the potential of this highly specific clone to cross-react with autoantigens and to initiate an autoimmune response. Several stimulatory peptides were derived from CNS proteins and proteins involved in inflammatory response. Since TCC GP5F11 was derived from a patient with MS, we were particularly interested in cross-reactivity with CNS- or myelin-derived antigens. For this purpose, we extended the search for stimulatory peptides into lower ranges of predicted scores, and identified two peptides derived from myelin oligodendrocyte glycoprotein (MOG), and one from

Table 2
Recognition of naturally occurring peptides with highest predicted stimulatory scores retrieved by GenPept database searches

Peptide order	Sequence	Predicted scores	Actual scores		Function
			EC_{50}	SI at 1 μg/ml	
A					
Influenza-A virus mimics					
1	Y I K Q N T L K L A	257	0.001	21	Equine/New Market/76 HA
2	Y I K Q N T L K L A	257	0.005	31	Equine influenza virus HA
3	Y I K Q N T L K L S	256	0.006	27	New York/94 HA
4	Y V K Q N T L R L A	264	0.006	28	Ohio/95 HA
5	Y V K Q N S L K L A	255	0.007	27	Swine/Hong Kong HA
6	Y V K Q N T L K V A	255	0.007	24	Sydney/97-like HA
	Y V K Q N T L K L A	199	0.074	28	Texas/77 HA
7	Y V K Q S T L K L A	238	0.566	27	France/97 HA
8	Y I K Q D T L K L A	238	0.691	29	New York/94 HA
9	Y V K Q H T L K L A	242	1.007	13	Nangchang/93 HA
10	Y F K I H T G K S S	202	1.139	11	USSR/26 HA
11	Y G K Q N T L K L A	241	1.187	11	Christ Church/88 HA
12	Y V K E N T L K L A	198	2.666	6	Argentina/94 HA
13	Y V K Q D T L K L A	237	5.115	16	Shangdong/94 HA
14	Y V K Q T T L K L A	237	5.316	3	Cordoba/96 HA
Viral mimics					
	Y V K Q N T L K L A	199	0.074	28	Texas/77 HA
1	Y L K G N N G R E T	226	0.127	3	Human papillomavirus type 29
2	Y L D A N I T R L L	146	1.040	11	HIV-2 strain VI1011 Ghana
3	F L D A N I T K L L	126	1.120	16	HIV type 2
4	F K K Y N V P G P M	164	1.305	11	Saimiriine herpesvirus 2
5	Y F D V N S G G G L	146	1.776	9	Human herpes virus 6 alkaline exonuclease
6	Y M D V N A S R A L	164	1.869	8	Heron hepatitis B virus
7	Y V D V N S H G L I	137	2.146	9	Little cherry clostero virus
8	F I D V H I P K F K	131	2.412	7	Cowpox virus white-pockvariant
9	V M K Q V T G K L K	247	5.147	11	HIV type 1 gene for envelope glycoprotein gp120
10	W E K Q Y A F R S F	250	5.204	3	Vesicular stomatitis virus L protein
11	W Q K Q E L Q R K C	242	5.315	6	HIV type 2 gag
B					
Human mimics					
1	Y L K Q H L P K R L	258	0.038	26	Autotaxin-t
	Y V K Q N T L K L A	199	0.074	28	Texas/77 HA
2	F M K H N T S R Q N	210	1.015	20	Myosin heavy chain12
3	Y L K P N W Q K L L	199	1.107	15	Serotonin transporter
4	F V D L N N G K F Y	152	1.478	12	DNA damage repair and recombination protein RAD5
5	F Y D N N T G K L I	138	1.700	9	Striatin
6	F G K L N S L K S I	184	1.733	8	Toll/interleukin-1 receptor-like protein
7	W L K L N L H K K Y	227	1.852	9	Ras GAP-related protein
8	F I D L N S S R N L	143	1.927	9	KIAA0238 gene
9	Y L D L N I A K K L	152	1.998	4	Microsomal glutathione *S*-transferase 2
10	F L K K N R K K K L	188	2.024	3	RNA helicase
11	F V K R N R G G K Y	178	2.040	7	Neural cell adhesion molecule (CALL)
12	Y I D D N S K K V F	132	2.155	11	1-phosphatidyl inositol-4-phosphate 5-kinase
13	F L K G N I K K E L	189	4.847	4	Hemeoxygenase-2
14	Y F K V N S D G G L	189	4.898	4	H-cadherin
15	Y K K N H S G R K L	218	5.065	3	Vasopressin activated calcium
Human myelin mimics					
	Y V K Q N T L K L A	199	0.074	28	Texas/77 HA
1	V L I K N T L R S L	121	0.158	5	Oligodendrocyte myelin glycoprotein
2	S A A N N N I K L L	94	0.211	6	Oligodendrocyte myelin glycoprotein
3	L Y S L G N G R W M	111	0.908	18	2′, 3′-cyclic nucleotide 3′-phosphodiesterase

Peptide sequences, predicted scores, EC_{50} and SI, peptide source and function were listed in A for influenza-HA, and other viral-derived peptides. B presents human, and human myelin protein-derived mimics. Results were ranked according to actual stimulatory potencies (EC_{50}) in each group.

2′3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) that activated TCC GP5F11. In all database searches, the positive predictive value for high scoring peptides was 72%, confirming that our method efficiently predicted the range of cross-reactive epitopes for TCC with known specificity. Moreover, we identified six naturally occurring

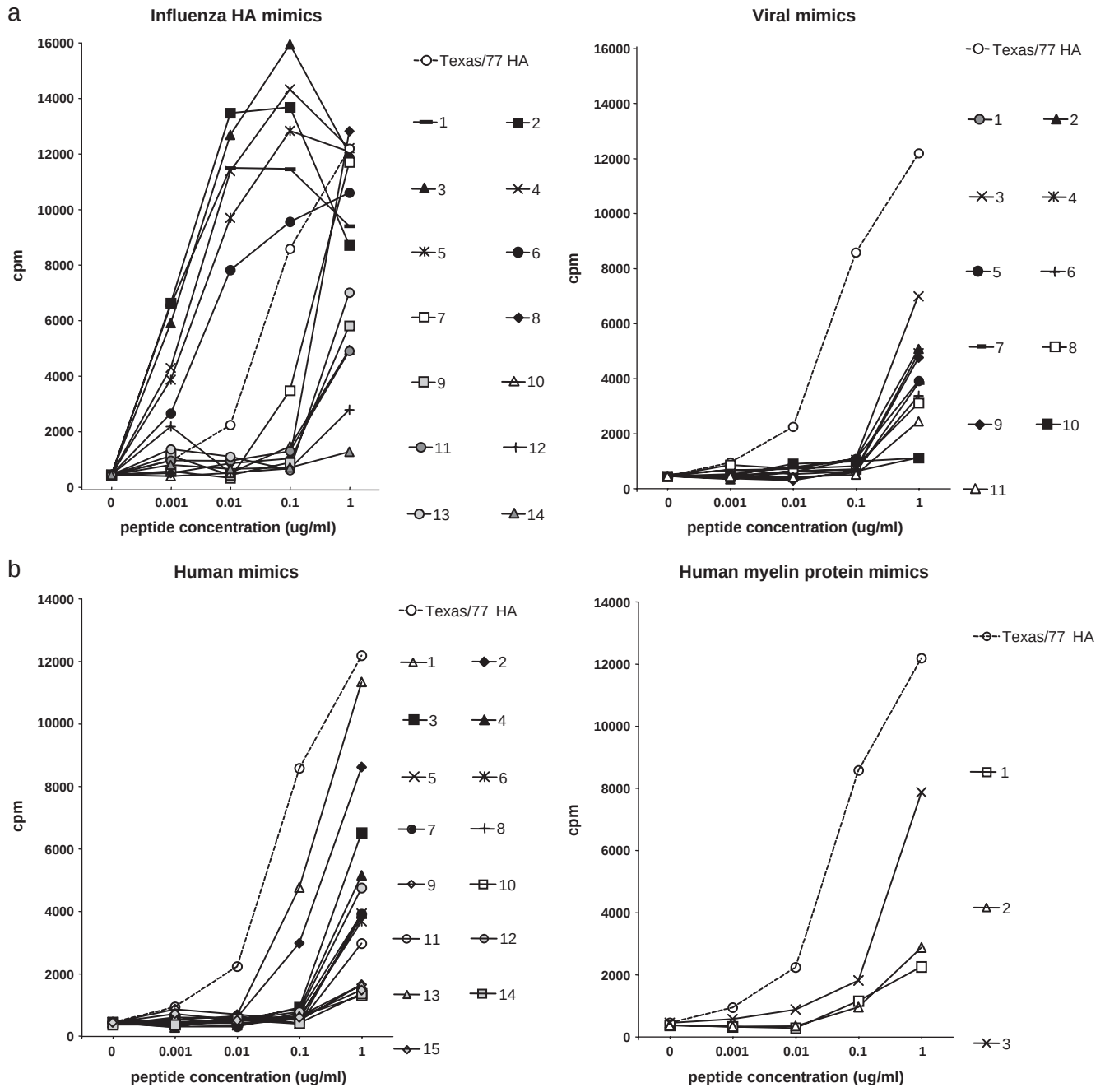

Fig. 2. Predicted stimulatory peptides were tested in dose titration experiments. (a) Presents proliferative response to actually stimulatory influenza-HA and viral-derived peptides. (b) Shows response to stimulatory human and myelin protein-derived peptides. Presented results are representative of six independent experiments performed in duplicate wells.

peptides with higher stimulatory potency than the native ligand, implicating high potential for cross-reactivity even for a TCC with a stringent TCR response.

The ROC curve measuring accuracy of *Z* score matrix based prediction system for 80 synthesized decamer peptides shows that the area under the curve is 81.4% (Fig. 1). Under an optimal threshold, the specificity and sensitivity are balanced around 80%.

In order to further assess overlap between deduced peptides for Flu-HA- and MBP-reactive clones, stimulatory decapeptides for GP5F11 were scored using matrix for GDBP, MBP-reactive clone derived from identical twin concordant for MS. The median score of the twenty-one stimulatory human database search-derived peptides from GP5F11 experiments ranked within the top 25 percentile of all human 10-mers stimulatory for TCC GDBP. The highest score of the 21 stimulatory peptides is among the top 0.1% of all human decamers stimulatory for TCC GDBP. Fig. 3 shows distribution of new scores for the 21 Flu-HA-reactive TCC stimulatory peptides in the all human 10-mer spectrum

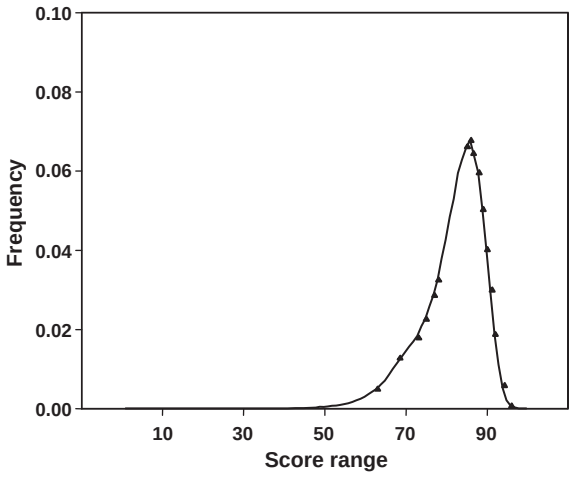

Fig. 3. Score distribution of stimulatory peptides for Flu-HA-reactive clone in all human decamers was ranked using MBP-reactive clone scoring matrix. Scores for GP5F11 TCC stimulatory peptides were generated by using scoring matrix of the GDBP TCC. Vertical axis, percentage of the amount of 10-mer peptides in all human decamers; horizontal axis, score range, calculated as described in Hemmer et al. (1999).

for GDBP MBP-reactive TCC. The results are further supporting the potential of Flu-HA-reactive clone to initiate an autoimmune response against self-antigens.

4. Discussion

The present study documents that flexibility of TCR recognition also applies to highly sensitive foreign antigen-specific TCC that recognizes peptide with high avidity at nanomolar concentrations. Using PS-SCL, we identified a range of epitopes including a series of highly stimulatory Flu-HA variants documenting that cross-reactivity at the level of a single TCR likely assures protection against many more than the originally stimulating viral variant.

We also identified a range of stimulatory self-antigens indicative of this clone's potential to initiate an autoimmune response. Our study demonstrated that two high-ranking peptides were derived from CNS proteins: serotonin transporter and neural cell adhesion molecule, suggesting a potential for cross-reactivity with CNS proteins, which may play a role in the development of inflammatory/autoimmune response in MS. One stimulatory peptide was derived from toll/IL-1 receptor-like protein. Further database searches for CNS myelin-derived proteins extended to peptides with lower biometric scores and identified 12 potentially stimulatory myelin-derived peptides that are stimulatory for GP5F11. Although this selective database search introduces a bias, myelin proteins are clearly implicated in the pathogenesis of demyelinating lesions in MS and therefore are of interest in the current study (Bielekova et al., 2000). We have confirmed high stimulatory potency of two MOG-, and one CNPase-derived peptide as antigens capable of inducing cross-reactive responses in TCC GP5F11. Moreover, the CNPase-derived peptide had no homology with the native Flu-HA epitope, and hence would not be detected by methods based on MHC or TCR binding motif searches (Wucherpfennig and Strominger, 1995). However, while the molecular mimicry is a frequent event, it only leads to autoimmune disease when it takes place in the context of chronic local inflammation (Goverman et al., 1993), presentation of self antigens (Evans et al., 1996), and the sufficient number of autoreactive T-cells.

Our strategy for identification of cross-reactive antigens for individual TCC has multiple advantages over previously used methods. Most importantly, it does not require any presumptions about the sequence of cross-reactive antigens, and it provides quantitative predictions for stimulatory potential of every peptide based on the data from complex peptide libraries (Borras et al., 2002; Rubio-Godoy et al., 2002). Database searches use information for all amino acids tested at every position of the peptide, and are more comprehensive than searches based on sequence homology. A limiting factor of this strategy is the incompleteness of databases, which are however, rapidly expanding (Jacobsen et al., 2001). Further, once multiple stimulatory ligands are identified, it is not possible to determine which ones are likely to be processed and presented by antigen presenting cells in vivo. While future studies of TCR degeneracy should involve testing of whole proteins to confirm the biological relevance of identified cross-reactive peptides, limited availability of purified proteins and their complexity hampers advances in the identification of immunodominant epitopes recognized by the immune system. Biological relevance of stimulatory peptides identified by database searches in this study is supported by their recognition by PBMCs (data not shown). These results indicate that immune response against identified peptides may contribute to inflammatory tissue damage in this patient.

The present study identifies an unexpectedly high degree of TCR degeneracy even in a TCC with a very stringent TCR response to an immunodominant viral antigen. We anticipate that our strategy may improve vaccine design by including mixtures of peptide antigens representative of viral variants. This will provide a longer-lasting protection against viral infections and possibly decrease their effect on the initiation of relapses of MS.

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