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Letter to the Editor

Response to "H1N1 hemagglutinin-specific HLA-DQ6-restricted CD4+ T cells can be readily detected in narcolepsy type 1 patients and healthy controls"

We read with great interest the publication by Schinkelshoek et al. (Schinkelshoek et al., 2019) reporting on T cell reactivity to Hemagglutinin pHA$_{275-287}$ and Hypocretin/orexin HCRT$_{56-68}$ and HCRT$_{87-99}$ peptides. The authors failed to discuss a publication by Luo et al. published a day after submission of their own publication (Luo et al., 2018a). This same work had been published in BioRxiv (Luo et al., 2018b) together with an informative Genome Wide Association (GWAS) paper in 5500 narcolepsy subjects (Ollila et al., 2018), and discussed in Liblau et al. (Liblau, 2018), a companion editorial to the cited Latorre et al. paper (Latorre et al., 2018). In this work, we found that homologous HCRT$_{54-66}$ and HCRT$_{86-97}$ sequences had to be C-amidated (HCRT$_{NH2}$), as in secreted active peptides, to be recognized by CD4$^+$ T cells as an autoantigen. We also found increased reactivity to pHA$_{273-287}$ and HCRT$_{NH2}$ in narcolepsy patients and common CDR3α and CDR3β TCR sequences with tetramers derived from both peptides, suggesting that homology predicts cross reactivity. One of the most common cross-reactive CDR3β sequence was a TRBV4–2 bearing CASSQETQGRNYGYTF CDR3 motif. This is of significance considering that our GWAS data implicate a narcolepsy susceptible variant rs1008599 that is also an eQTL increasing usage of TRBV4–2 bearing CDR3β (Ollila et al., 2018; Sharon et al., 2016). We concluded that there is strong probability of molecular mimicry with this sequence. As the work of Schinkelshoek et al.(Schinkelshoek et al., 2019) is relevant to this publication, but does not cite or agree with our conclusion, we offer a brief comment below.

Schinkelshoek et al. (Schinkelshoek et al., 2019) adds useful pHA$_{275-287}$, HCRT$_{56-68}$ and HCRT$_{87-99}$ crystallographic information confirming projected register for these peptides when bound to DQ602, further pointing out similarities of TCR facing residues across these complexes. The work also adds TCR sequencing data for clones derived from DQ602 pHA$_{275-287}$ tetramers in six subjects, 4 narcolepsy and 2 controls. Among this data, the authors found one TCRα/β CDR3 pair 3 times in one patient and one TCRα CDR3 found 3 times in another but with different TCRβs). The authors also conducted cross cultures of these HA restricted clones with hypocretin peptides, and obtaining nothing, concluded that there no evidence for molecular mimicry.

We believe that such conclusion was premature based on the small number of TCR clones (33 unique CDR3s, 17α and 16β) identified and the low frequency of cross-reacting TCRs we found. In our work, we sequenced thousands (Table 1) of individual DQ602 pHA$_{273-287}$ tetramer positive cells (rather than 33) in more than 14 patients and 11 controls subjects, both narcolepsy and controls and found only a small number of frequently used CDR3s that were common to both pHA$_{273-287}$ and HCRT$_{NH2.}$ As in Schinkelshoek et al. (Schinkelshoek et al., 2019), we found that V17-J34 sequences were common in pHA$_{275-287}$ tetramers notably V17-CATDAYNTDKLIF-J34. Further, the paratope motifs SQR/SQG motifs encompassing the CDR3βs that we reported as a common pHA$_{273-287}$ responsive motif is also found frequently in their CDR3β sequences as predicted(Luo et al., 2018a). Comparing the few CDR3 sequences obtained by the authors with our own data (Table 1 sequences with zero and one mismatch as computed by Levenstein distance metric(Navarro, 2001)), it is clear that sequences retrieved by Schinkelshoek et al. are shared with pHA$_{275-287}$ as expected, although in one case a sequence similar to that we retrieved with HCRT$_{NH2}$ is obtained. In contrast, no sharing is found with nucleoprotein sequence NP$_{17-31}$, a control PR8 sequence without homology with HCRT$_{NH2}$. With our extended data, we could clearly shows that common CDR3α and CDR3β sequences are found in T cell clones recognizing both HCRT$_{NH2}$ and pHA$_{273-287}$ but not control NP$_{17-31}$, something that would not have been possible to see with the

Table 1
Sharing of 33 TCR sequences of Schinkelshoek et al.(Schinkelshoek et al., 2019) with data from Luo et al.(Luo et al., 2018a).

Shared sequences	Zero mismatch				One mismatch			
	CDR3α		CDR3β		CDR3α		CDR3β	
	Unique	Total	Unique	Total	Unique	Total	Unique	Total
HA$_{275-287}$	0.21% (1/344)*	0.07% (3/1452)*	0% (0/349)	0% (0/1343)	1.5% (5/344)	0.67% (10/1452)	0% (0/349)	0% (0/1343)
HCRT$_{NH2}$	0.11% (1/901)**	0.03% (1/3065)**	0% (0/916)	0% (0/2881)	0% (0/901)	0% (0/3065)	0% (0/916)	0% (0/2881)
NP$_{17-31}$	0% (0/399)	0% (0/1706)	0% (0/381)	0% (0/1837)	0% (0/399)	0% (0/1706)	0% (0/381)	0% (0/1837)

Sequence from Schinkelshoek et al.(Schinkelshoek et al., 2019): * = CDR3a TRAV 21*01 CATDAYNTDKLIF TRAJ34*01, **CDR3a = TRAV25*01 CAGDAGNNRKLIW TRAJ38*01.

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very limited depth of TCR sequencing results obtained by Schinkelshoek et al.(Schinkelshoek et al., 2019).

It could be argued that the homology of epitope sequence explains sharing sequences for cognate TCRs independent of mimicry being involved in causing narcolepsy. However, as we argue in our publication, although rare, the Vβ4–2 is the most commonly $HCRT_{NH2}$ ($n = 7$) /$pHA_{273–287}$ ($n = 2$) shared sequence. The other commonly shared cross-reactive sequences use Vα and Vβ chains (TRBV15 and TRAV26–1) where cis QTL SNPs could not be detected (Sharon et al., 2016), which could explain why they are not present in our GWAS. Our genetic association data is therefore suggestive of causality considering that Vβ4–2 is only used on 0.7% of the repertoire. Furthering the validity of our analysis method is the fact the top TCRα chain recognizing $HCRT_{NH2}$ across narcolepsy cases and controls was a Jα24 bearing CDR3α sequence CAVETDSWGKLQF, and that a major SNP rs1154155 predisposing to narcolepsy maps to this segment and is also rarely used (0.8%).

In conclusion, our analysis of the Schinkelshoek et al. publication (Schinkelshoek et al., 2019) is in line with our own data(Luo et al., 2018a). We however argue that mimicry could not be detected by these authors because of the very small number of clones sampled in the study. We also acknowledge that experiments demonstrating actual TCR cross reactivity of $pHA_{273–287}$ and $HCRT_{NH2}$ using transferred Jurkat cell will be useful complement tetramer data, as observing common TCRα and TCRβ sequences after culture does not directly demonstrate functionality and activation. We however argue that mimicry is a low probability event, and that once it has occurred, epitope spreading would lead $HCRT_{NH2}$ specific reactivity (often using J24) taking over the destructive process. HA sequences other than $pHA_{273–287}$ are also involved, considering narcolepsy existed prior to 2009, but was also seasonally regulated.

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Emmanuel Mignot*, Aditya Ambati, Guo Luo
Stanford Center For Sleep Sciences and Medicine, Dept of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, California, USA
E-mail address: mignot@stanford.edu (E. Mignot).

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* Corresponding author.