LETTER

Reply to van de Sandt and Rimmelzwaan: Matching epitope display with functional avidity

We appreciate the comments of van de Sandt and Rimmelzwaan (1) on our paper (2), as well as the opportunity to respond.

First, we agree that in Berkhoff et al. (3) single alanine replacements resulted in reduced kinetics of viral replication. Nonetheless, to claim "a reduction of progeny virus of >90%" (1) for all substitutions is misleading. Although figure 1*A* of Berkhoff et al. shows this at one unspecified time after Madin-Darby canine kidney infection, figure 1 *B* and *D* of Berkhoff et al., plotting viral titers as a function of time, reveals far less reduction (3). The latter may be more relevant for physiologic infection, where immune mechanisms limit viral replication. The conclusion in the Berkhoff et al. abstract that "alanine replacements for each of the nine amino acids of the $M1_{58–66}$ were tolerated to various extents, except for the anchor residue at the second position" (3) is reasonable and reflected in our report (2). Cao et al. (4) demonstrate that the nuclear export signal overlaps the $M1_{58–66}$ epitope and reveals how the nuclear export signal motif tolerates substantial sequence variability (3). Most interestingly, all mutant viral epitopes tested by chromium release cytolysis assay were no longer targeted by $M1_{58–66}$ cytotoxic T lymphocyte (CTLs) (figure 4*M* in ref. 3), implying that viral escape from immune recognition could be achieved by mutation within this segment without abrogated M1 nuclear export function. That a high host-cell surface copy number of invariant $M1_{58–66}$ persists during influenza A virus (IAV) infection implies an absence of immune selection pressure against this epitope.

Second, the suggestion that low functional avidity of $M1_{58–66}$-responding T cells was an artifact of peptide stimulation is excluded by the HLA-A2 transgenic mouse study. There, we show (2) that after a primary infection, the bulk polyclonal $M1_{58–66}$ CD8 memory T cells have a functional avidity 1,000-times poorer than that of the protective $NP_{366–374}/D^b$ specificity T-cell population present in the same animal. Such high-frequency $M1_{58–66}$ responses are consistent with an independent study as well (5).

Third, as correctly noted, we have not examined CTL against the conserved epitopes uncovered by our LC-DIAMS (data independent acquisition) analyses. $M1_{58–66}$ epitope immunodominance precludes responsiveness to those conserved epitopes. Hence, responses subsequent to in vitro stimulation will be primary in nature and of low avidity. Future development of candidate T-cell epitope-based universal IAV vaccines will allow us to elicit such responses upon in vivo priming, exploring avidity directly or following in vitro secondary stimulation.

Fourth, although Boon et al. (6) nicely show that the magnitude of IAV response is linked to HLA, with HLA-A2 affording the greatest response to specific epitopes using 5-μM peptide concentrations, these data do not speak to the quality of such responses and their relevance to protective T-cell immunity.

In sum, we agree that careful investigation of T-cell responses requires exhaustive analyses, of which physical detection of epitopes is but one part. That said, we caution that emphasis needs to be placed not just on elucidating the frequency of T-cell responses but their quality and, in particular, the match between quantitative epitope display on infected lung epithelium and the avidity of the responding T cells.

Derin B. Keskin[a,b,c], Bruce R. Reinhold[a,b,c], Guang Lan Zhang[b,d], Alexander R. Ivanov[e], Barry L. Karger[e], and Ellis L. Reinherz[a,b,c,1]

[a]Department of Medical Oncology, Laboratory of Immunobiology, [b]Cancer Vaccine Center, Dana-Farber Cancer Institute, and [c]Department of Medicine, Harvard Medical School, Boston, MA 02115; [d]Computer Science Department, Metropolitan College, Boston University, Boston, MA 02115; and [e]Barnett Institute, Northeastern University, Boston, MA 02115

1 van de Sandt C, Rimmelzwaan GF (2015) Immunodominant responses to the influenza virus $M1_{58–66}$ epitope: Stealth or protection? *Proc Natl Acad Sci USA* 112:E2417.

2 Keskin DB, et al. (2015) Physical detection of influenza A epitopes identifies a stealth subset on human lung epithelium evading natural CD8 immunity. *Proc Natl Acad Sci USA* 112(7): 2151–2156.

3 Berkhoff EG, et al. (2005) Functional constraints of influenza A virus epitopes limit escape from cytotoxic T lymphocytes. *J Virol* 79(17):11239–11246.

4 Cao S, et al. (2012) A nuclear export signal in the matrix protein of influenza A virus is required for efficient virus replication. *J Virol* 86(9): 4883–4891.

5 Tan AC, La Gruta NL, Zeng W, Jackson DC (2011) Precursor frequency and competition dictate the HLA-A2-restricted CD8+ T cell responses to influenza A infection and vaccination in HLA-A2.1 transgenic mice. *J Immunol* 187(4):1895–1902.

6 Boon AC, et al. (2002) The magnitude and specificity of influenza A virus-specific cytotoxic T-lymphocyte responses in humans is related to HLA-A and -B phenotype. *J Virol* 76(2):582–590.

Author contributions: D.B.K., B.R.R., G.L.Z., A.R.I., B.L.K., and E.L.R. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: ellis_reinherz@dfci.harvard.edu.