The Journal of Immunology

Response to Additional Comment on Three X-ray Crystal Structure Papers

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J Immunol 2016; 196:530; ;
doi: 10.4049/jimmunol.1502468
http://www.jimmunol.org/content/196/2/530.2

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

basic principles of protein structure. We do not believe that any reviewer with access to the PDB validation reports would have accepted these peptide structure models for publication. Presenting this situation as ambiguous and open to interpretation would be a scientific mistake.

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Abbreviations used in this article: CNS, Crystallography and NMR system; PDB, Protein Data Bank; RSR, real space *R*-value; RSRZ, RSR *Z*-score.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1502281

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We did not intend to infer that it is acceptable to project arbitrary structural models into electron density noise. What we emphasized, however, is that it is possible to see meaningful structural information from relatively weak electron density. We provided several examples in support of our argument from published work in our previous response.

Presence of negative density in some region does not necessarily mean that positive density in the ligand binding region is also noise, especially when this positive density is also supported by the presence of densities in simulated annealing 2Fo–Fc composite omit maps. After deletion of Y240 from Protein Data Bank (PDB): 4H0H, the suggestion that electron density for Y240 is not interpretable at contour level 2.5 σ is not correct, as one can clearly make out boundaries of the hydrophobic ring and other features of Y240 in figure 2 of the response by Pozharski et al. to our comments.

The main argument of Pozharski et al. has been concerning the presence of experimental electron density for building the model in the map. In this context, it has to be noted that the Ramachandran parameters are not directly linked to the presence or absence of the electron density. Guidelines to define fit of model to the electron density used by the Uppsala Electron Density Server, on the basis of which the graphs providing real space *R*-value *Z* scores are calculated, are meant only for the relative quality of a PDB entry, but not for its absolute authenticity as indicated by the report of the Task Force of the Worldwide Protein Data Bank (1). It is important to note that a significant number of PDB entries associated with published structures have model fits similar to those in our structures. Incidentally, such data were included in the PDB subsequent to the acceptance of our papers for publication. We disagree with the contention of Pozharski et al. about the interpretations of our electron density maps.

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Abbreviation used in this article: PDB, Protein Data Bank.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1502468

Comment on "Cutting Edge: Inhibiting TBK1 by Compound II Ameliorates Autoimmune Disease in Mice"

With interest, we read the paper by Hasan et al. (1), who showed that inhibition of TANK-binding kinase 1 (TBK1) by Compound II has beneficial effects in a mouse model of autoimmune disease. Similarly, TBK1 inhibition by the small molecule drug amlexanox has been shown to mediate favorable metabolic and anti-inflammatory effects in mouse models of diabetes, obesity (2, 3) and multiple sclerosis (4).

We and others recently showed that haploinsufficiency of *TBK1*, i.e., *TBK1* gene dosage reduction from two to one allele by heterozygous loss-of-function mutations, can cause the genetically related neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (5–7). TBK1 protein levels were reduced to 50% in cell lines derived from patients with heterozygous *TBK1* loss-of-function mutations (5).

Consequently, it is plausible that pharmacological reduction of cellular TBK1 activity to 50% or less might have similar cellular effects as *TBK1* haploinsufficiency. Long-term pharmacological TBK1 inhibition, as proposed for the treatment of autoimmune diseases (1, 4), could thus pose a risk of causing ALS or FTD. Also, in terms of a two-hit-model, chronic TBK1 inhibition may be able to trigger ALS/FTD in individuals exposed to an environmental insult or with a genetic predisposition who would otherwise not have developed ALS or FTD during their lifetime.

Experimental proof that pharmacological TBK1 inhibitors increase the risk for neurodegenerative diseases is currently lacking. Nevertheless, the existing unequivocal genetic evidence that *TBK1* haploinsufficiency causes ALS/FTD with high penetrance, in our opinion, strongly suggests the need to critically reconsider currently ongoing or planned clinical trials that are based on pharmacological TBK1 inhibition.