Response to "Interaction between HIV gp41 fusion peptide and T cell receptor: putting the puzzle pieces back together"

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In his letter, Dr. Alexander Sigalov suggests an alternative interpretation to our results mapping the domain of $FP_{1–32}$ that mediates the interaction with the TCR to the $FP_{5–13}$ region (1). He suggests that, in addition to $FP_{5–13}$, the C-terminus domain of $FP_{1–32}$ contains a region capable of inserting itself into the membrane and interacting with the CD3 subunits of the TCR, thereby impairing TCR function. We consider this alternative explanation unlikely for several reasons. First, we have synthesized and investigated $FP_{17–32}$ and found it inactive in all assays (unpublished data). Second, a mutant $FP_{1–32}$ termed V2E shows a diminished ability to interfere with the activation of T cells both *in vivo* and *in vitro* (2), despite the fact that the mutation in V2E is located in the N-terminus and not in the C-terminus of $FP_{1–32}$. Thus, if any inhibitory activity resides on the C-terminus of $FP_{1–32}$, it must be secondary to the activity of the N-terminus. Thirdly, the current working model describing the mechanism of action of $FP_{1–32}$ during membrane fusion in HIV infection suggests that $FP_{1–16}$ inserts into the target T-cell membrane, while the $FP_{17–32}$ region does not insert, but remains positioned parallel to the cell membrane (ref 1, Fig. 1) (3, 4). This positioning of the different domains of $FP_{1–32}$ does not allow the $FP_{17–32}$ region to insert into the membrane and interact with the CD3 subunits of the TCR complex as suggested by Sigalov.

The alternative interpretation suggested by Sigalov is based on the different potency manifested by $FP_{1–32}$, $FP_{1–16}$ and $FP_{5–13}$ in Figs. 1 and 7 of our manuscript (1). However, a careful comparison of Figs. 1 and 7 reveals that $FP_{1–32}$ and $FP_{5–13}$ have similar inhibitory efficiencies; moreover $FP_{1–32}$ and $FP_{1–16}$ (Fig. 2) have similar activities *in vivo*. Thus, we attribute the differences in the efficiency of $FP_{1–16}$ and $FP_{1–32}$ not as an indicator of the existence of another independent inhibitory region, but as the result of the different solubility and state of aggregation of the different peptides; this effect seems to be more important for *in vitro* assays (Fig. 1), but less important for *in vivo* assays (Fig. 2). Indeed, $FP_{1–32}$ is known to form oligomers (2, 4); this multimerization is facilitated by the C-terminus increasing fusogenic activity of $FP_{1–32}$ (3, 4). Whether multimerization plays a role in the immunomodulatory activities of FP and its peptides is still unknown. Nevertheless, we believe that the above-mentioned data rule out the alternative interpretation put forward by Sigalov, that the C terminus region of $FP_{1–32}$ plays a significant role in its immunomodulatory activity.

REFERENCES

1. Bloch, I., Quintana, F. J., Gerber, D., Cohen, T., Cohen, I. R., and Shai, Y. (2007) T-cell inactivation and immunosuppressive activity induced by HIV gp41 via novel interacting motif. *FASEB J.* **21,** 393–401
2. Quintana, F. J., Gerber, D., Kent, S. C., Cohen, I. R., and Shai, Y. (2005) HIV-1 fusion peptide targets the TCR and inhibits antigen-specific T cell activation. *J. Clin. Invest.* **115,** 2149–2158
3. Peisajovich, S. G., and Shai, Y. (2003) Viral fusion proteins: multiple regions contribute to membrane fusion. *Biochim. Biophys. Acta* **1614,** 122–129
4. Peisajovich, S. G., Epand, R. F., Pritsker, M., Shai, Y., and Epand, R. M. (2000) The polar region consecutive to the HIV fusion peptide participates in membrane fusion. *Biochemistry* **39,** 1826–1833

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