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The interaction between the T cell receptor (TCR) and peptide-MHC complex (pMHC) target determines whether or not a T cell will respond. However, it is not clear which physical aspects of this interaction are most important in making that determination. Here we explore the biological effects of varying the rates of association and dissociation between TCR and pMHC. To accomplish this, we determined the kinetic rate constants ( $k_{on}$  and  $k_{off}$ ) for the binding of a panel of HLA-A2 variants to the xenoreactive TCR, AHIII 12.2, using surface plasmon resonance. We then assessed the extent to which TCR was stimulated by the A2 variants using hybridomas expressing the  $\alpha$  and  $\beta$  chains of the AHIII 12.2 TCR. In this system,  $\beta$ -galactosidase expression is controlled by NFAT activity, and therefore used as a readout for TCR stimulation. Finally, we tested the ability of AHIII 12.2 T cells to lyse targets expressing the A2 variants, providing insight to the effects of each A2 mutation on T cell function. For both readouts of TCR activity, we observed a stronger correlation with the ratio of the kinetic constants ( $k_{off}/k_{on}$ ) than either  $k_{on}$  or  $k_{off}$  alone. This suggests that the T cell response is driven more by the affinity of TCR for pMHC than either the on or off rates of the TCR-pMHC interaction.

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