Selective deletion of CD8+ T cells by saporin-coupled MHC class I tetramers *(continued from page 1)*

We and others have shown that, after binding to the TCR, tetramers are endocytosed by the T cell.[2, 3] These two characteristics – specific binding and rapid internalization – suggested that tetramers might be a useful way to selectively deliver an intracellularly-active toxin to pathogenic T cells. To investigate this hypothesis, we used TCR-transgenic P14 mice as a source of CD8+ T cells, which recognize a viral glycoprotein-derived peptide, gp33, presented by the class I MHC molecule, H2-Db, and bind to the tetramer, gpC9M. To confirm our observations with a second epitope, we employed TCR-transgenic HY mice, whose CD8+ T cells bind to the H2-Db tetramer, hyC2A. Toxic tetramers were generated from gpC9M and hyC2A pMHC monomers using streptavidin-saporin (SA-SAP; Cat. #IT-27). After assembly, these tetramers retained the TCR-binding specificity of their fluorophore-labeled counterparts, and inhibited translation in a cell-free assay as potently as parent SA-SAP alone.[3] To determine whether T cells would efficiently internalize SAP-coupled tetramers, we briefly cultured quiescent P14 T cells with the gpC9M-SAP tetramer, or as negative controls, with non-toxic gpC9M (not shown) or toxic hyC2A-SAP tetramers. Following the addition of FITC-labeled SAP antibody (Cat. #FL-02), T cells were subsequently incubated at either 37°C or 4°C, which permitted or prohibited endocytosis, respectively. To discriminate internal and external fluorescence, tetramer-antibody-fluorophore complexes on the surface were either removed with an acetic acid solution ("stripped"), or allowed to remain intact ("washed"), prior to analysis. As shown in Fig. 1, acid-resistant fluorescence (gray line), corresponding to endocytosed SAP, was found in all metabolically-active P14 T cells incubated with the cognate gpC9M-SAP, but not with control tetramers. We next evaluated the ability of the SAP-coupled tetramers to kill T cells *in vitro*. Purified P14 and HY T cells were incubated with either non-toxic tetramers alone; non-toxic tetramers plus free (unbound) SAP; or SAP-coupled relevant or irrelevant tetramers. Surviving cells were identified by exclusion of a membrane-impermeant dye, 7-aminoactinomycin-D, at the time points shown in Fig. 2A. Some cell loss was observed over time with all treatments, characteristic of stimulated, cultured T cells; however, incubation of HY T cells with hyC2A-SAP resulted in the death of 98% of cells within a 3-day period. Free SAP was not toxic to T cells. *In vitro* killing of these CD8+ T cells by the cognate SAP-coupled tetramers depended on the tetramer dose (Fig. 2B), and the avidity of the tetramer-TCR interaction (not shown).

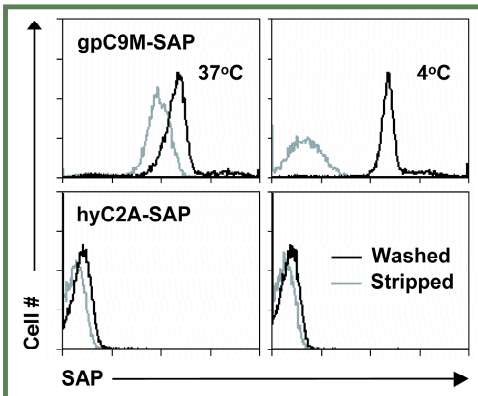

Fig. 1. All metabolically-active (37°C) P14 T cells incubated for 1 hour with the cognate tetramer gpC9M-SAP (5 nM) have endocytosed SAP. Stripped cells show only internal SAP; washed cells show total (internal & external) SAP.

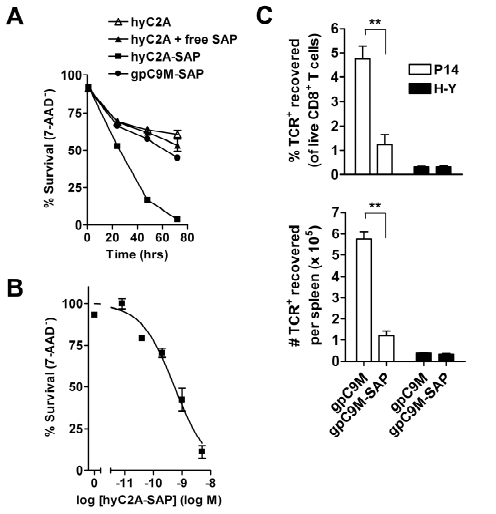

Fig. 2. A. Virtually all HY T cells incubated with 5 nM hyC2A-SAP tetramer are killed by 72 h. Neither free SAP nor SAP coupled to the gpC9M-SAP caused cell death above the background seen with non-toxic tetramer. **B.** HY T cells incubated for 72 h with varying concentrations (5 – 0.008 nM) of the SAP-coupled cognate tetramer are killed in a dose-dependent fashion. **C.** Intravenous injection of gpC9M-SAP tetramer (22 pmol) resulted in significant loss of adoptively-transferred P14 T cells in the spleens of recipient C57BL/6 mice 3 d after administration; recovery of co-transferred control HY T cells did not differ between treatments. The top and bottom panels show results as cell % and cell #, respectively.
**P<0.005 by 2-tailed Student's t test.

We then sought to determine if our toxic tetramers could delete specific CD8+ T cells *in vivo*. Fluorophore-labeled tetramers, when injected intravenously, rapidly bind to cognate T cells in lymph nodes, spleen, and bone marrow, suggesting that SAP-coupled tetramers similarly should be able to reach their targets. To test this hypothesis, we transferred P14 T cells into recipient mice, and after 24 h, administered either gpC9M or gpC9M-SAP. Fig. 2C shows that, after 3 d, >75% of P14 cells were deleted from the spleen in gpC9M-SAP-treated mice; the recovery of control HY cells was not different between treatment groups. At this dosage, injection of the SAP-coupled tetramers caused an acute, mild liver injury, but mice showed no clinical signs of illness.[3]

These studies showed that tetrameric pMHC complexes can be used to deliver a potent toxin, SAP, to epitope-specific CD8+ T cells *in vitro* and *in vivo*, leading to deletion of the target population. Such toxic tetramers could represent a novel and effective means for eradicating pathogenic T cell responses in selected immune-mediated diseases.

References:

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