Figures and figure supplements

Gamma delta T cells recognize haptens and mount a hapten-specific response

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Figure 1. Cy3 is a γδ T cell antigen. (A) Chemical structure of Cyanine 3 (Cy3). FACS analysis of (B) Cy3 tetramer (Cy3-SA v) staining of splenic γδ T cells in the presence of 10-fold molar excess of moth cytochrome c peptide coupled SAv (MCC-SA v); (C) NX6/58α-β- cells stained with Cy3-MCC-SA v or PE-MCC-SA v; (D) NX6/58α-β- cells stained with Cy3-MCC-SA v in the absence (left), or presence of anti-Cy3 Fab (right). (E) IL-2 production by NX6/58α-β- cells activated by the indicated amount of plate-bound Cy3-OVA, OVA, PE, anti-CD3 for 16 hr. (F) The saturating binding curves of Cy3- SAv and un-conjugated SAv to a soluble form ofNX6 as determined by surface plasmon resonance. No detectable binding was observed for 1 mM applications of PE or BSA (not shown). (G) Kinetics of Cy3-SA v binding to NX6/58α-β- cells. $t_{1/2}$ was determined using real time flow cytometry in the presence of anti-Cy3 antibody Fab fragments (left). $K_d$ was determined from Scatchard analysis (right). All results are representative of at least three independent experiments.

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Figure 1—figure supplement 1. NX6/58αβ- cells stained with different fluorescently labeled ovalbumin preparations. Flow cytometry analysis of NX6/58αβ− cells stained with Cy3-OVA, FITC-OVA and APC-OVA. DOI: 10.7554/eLife.03609.004

Figure 1—figure supplement 2. Correlation between the mean fluorescence intensities of PE-SA v and Cy3-SA v on red blood cells. DOI: 10.7554/eLife.03609.005
Figure 2  Cy3-specific γδ T cell response after immunization. (A) CD44 expression on Cy3-OVA+ (red) and Cy3-OVA− γδ T cells in the draining lymph nodes of mice immunized with Cy3-CGG-alum or CGG-alum 24 hr prior. (B) BioMark analysis of CD62L+CD44+ Cy3+ and CD62L+CD44− Cy3− γδ T cells isolated from the draining lymph nodes of C57BL/6 mice immunized with Cy3-CGG 60 hr prior (5 cells/sample). The heatmap, where rows are individual genes and columns are individual samples, indicates the expression or non-expression of a gene/sample pair (relative to the β2m expression). Upper panel shows genes expressing higher (p < 0.001) in Cy3+ cells than in Cy3− cells. Middle panel shows non-varying genes. Bottom panel shows genes expressing lower (p < 0.001) in Cy3+ cells than that in Cy3− cells. (C) Thy1.1 expression on γδ T cells from IL-17Fγ1.1/Thy1.1 mice immunized with Cy3-CGG-alum 60 hr prior, representative of three independent experiments.

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Figure 3. NP is a γδ T cell antigen. (A) Chemical structure of 4-hydroxy-3-nitrophenyl acetyl (NP). Flow cytometry analysis of (B) NP-PE staining of γδ T cells from C57BL/6 or G8/Rag2−/− mouse splenocytes and PE staining of γδ T cells

Figure 3. Continued on next page
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from B6 splenocytes; (C) staining of 58α-β- cells expressing an NP-specific γδ TCR, 1G9, with NP$_{43}$-CGG-Cy5 or CGG-Cy5, showing staining in relation to γδ TCR expression (left) or as a histogram (right); (D) staining of 58α-β- cells expressing an NP-specific γδ TCR, 1E3, with NP$_{43}$-CGG-Cy5, NP$_{26}$-BSA-Cy5, or BSA-Cy5 (left) and NP$_{67}$-PE alone, NP$_{67}$-PE with a 20-fold molar excess of anti-NP Fab, or PE (right). (E) IL-2 production by 1E3/58α-β- cells activated by the indicated amount of plate-bound NP$_{25}$-KLH, KLH (light gray bars), or 0.1 μg/ml anti-CD3. (F) Sensorgram and steady state analysis of NP$_{43}$-CGG (0–7 μM) binding to soluble 1G9 TCR measured by surface plasmon resonance. Apparent K$_D$ was determined by steady state analysis of SPR measurements (circles). Equal concentrations of un-modified CGG were tested (squares), as well as NP$_{43}$-CGG with a PE-specific γδ TCR, MA2 (triangles).

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