Supplemental Data

Structural Studies of $V_\gamma^2V_\delta^2$ T Cell Phosphoantigens

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Figure S1. $^1$H and $^{31}$P NMR Spectra of: A, 2 ($^1$H); B, 2 ($^{31}$P); C, 12 ($^1$H); D, 12 ($^{31}$P); E, 15 ($^1$H); F, 15 ($^{31}$P).

* Denotes ethanol peaks in the $^1$H spectra and + denotes inorganic diphosphate (PPI) in the $^{31}$P spectra.
Figure S2. Structures of 6-Phosphogluconate (16), Ribulose-5-Phosphate (17), and 2-Dehydro-3-Deoxy-6-Phosphogluconate (18)

Conversion of 16 to 17 is carried out by 6-phosphogluconate dehydrogenase, decarboxylating, while conversion of 16 to 18 is carried out by 6-phosphogluconate dehydratase.
Figure S3. ESI MS/MS Parent/Daughter Spectra of: (Upper Panel). m/e = 275 Ion Species from *M. smegmatis* Extract (see Ref. 1 for More Details); (Lower Panel). 6-Phosphogluconate (16)
Figure S4. 3-Formyl-1-Butyl Diphosphate (2) and the Hyrate (14) Compound Do Not Antagonize Vγ2Vδ2 T Cell Responses to HMBPP (6)

Since it seemed possible that one of the two compounds could function as an antagonist for the other, we directly tested for inhibitory activity by stimulating Vγ2Vδ2 T cells with HMBPP in the presence of 2 (14). A. 2 (14) does not inhibit HMBPP responses. Varying concentrations of HMBPP and 2 (14) were mixed and used to stimulate the 12G12 Vγ2Vδ2 T cell clone with fixed Va2 cells as APC. Responses to 2 (14) (B) and to HMBPP (C) alone. Note that addition of 2 (14) only slightly inhibits the peak response to HMBPP and does not alter the EC$_{50}$.
Figure S5. Vγ2Vδ2 T Cells Expand in Response to 2 (14) but Not 6-Phosphogluconate (16)

A, Flow cytometric analysis of Vγ2Vδ2 T cell expansion on day 7. B, Expansion of Vγ2Vδ2 T cells at day 7 and day 14. PBMC from 2 normal donors were cultured with either medium, 0.316 µM HMBPP, 50 µM IPP, 50 µM ethyl pyrophosphate (EPP), 50 µM 2 (14) (3-FBPP), and 0.316, 1, 3.16, and 10 mM 6-phosphogluconate (6-PG). On day 3, IL-2 was added to 1 nM. On day 7 and day 14, PBMC were harvested and analyzed by two-color flow cytometry using anti-Va2 and anti-CD3 specific mAbs. The results of the 10 mM 6-phosphogluconate stimulation are shown.