

## **Suggested answers-S1**

**Q1a.** A cytosolic tyrosine kinase expressed exclusively in T cells.

**Q1b.** Following TCR ligation, the ITAMs of CD3 (particularly the  $\zeta$  homodimer) become phosphorylated by the tyrosine kinase, Lck (associated with either CD4 or CD8), which recruits ZAP-70 to the phosphorylated ITAMs. ZAP-70 becomes activated and phosphorylates multiple substrates, including additional tyrosine kinases\* that phosphorylate and activate phospholipase C- $\gamma$  (PLC- $\gamma$ ). Ultimately, this leads to secretion of IL-2 and T cell proliferation.

**Q1c.** PLC- $\gamma$  hydrolyzes phosphatidylinositol (4,5) bisphosphate (PIP<sub>2</sub>) to yield inositol trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). Increases in IP<sub>3</sub> trigger cytosolic calcium, activation of the protein phosphatase calcineurin, and translocation of the transcription factor, NF-AT, to the nucleus. Increases in DAG lead to activation of protein kinase C- $\theta$  (PKC- $\theta$ ) and NF- $\kappa$ B activation.

**Q2.** It is not needed for commitment to the CD4 lineage in thymocytes. One potential explanation is that double-positive thymocytes can utilize Syk, a ZAP-70 homolog, to generate TCR-mediated survival signals (Gelfand et al., *J Exp Med* 182:1057, 1995). Interestingly, peripheral blood CD4<sup>+</sup> T-cells cannot signal in these individuals (see Fig. 2), indicating that Syk cannot substitute for ZAP-70 in mediating cytosolic calcium fluxes in peripheral blood lymphocytes.

**Q3.** This atypical fungus is an obligate intracellular pathogen. Immunity to *Pneumocystis carinii*, like viruses, requires an intact cytotoxic lymphocyte response.

**Q4.** These bypass early signaling events following TCR/CD3/ZAP-70 engagement/activation. Ionomycin is a calcium ionophore that fluxes calcium (see Fig. 2) and phorbol ester activates PKC. Both circumvent the early signaling defect to activate NF-AT and NF- $\kappa$ B, respectively, leading to T cell proliferation.

\*For those interested, the kinases in question are members of the Tec family. Students are already familiar with one of these, Btk, which is expressed predominantly in B cells. Loss-of-function mutations in the *btk* gene lead to Bruton's agammaglobulinemia, which results from inadequate calcium signaling and decreased survival in B cell progenitors.