

Calcium imaging in a lymphoid organ: Two-photon microscopy of developing thymocytes in a living thymic slice preparation

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An essential outcome of T cell development in the thymus is the survival and maturation of thymocytes bearing T cell receptors (TCR) with moderate avidity for self-MHC (positive selection), and the deletion of strongly self-reactive cells (negative selection). Several studies suggest that intracellular free calcium ($[Ca^{2+}]_i$) plays a necessary role in both positive and negative selection and that the structure of the Ca^{2+} signal may influence the choice between these two developmental outcomes. While it is known that the 3-dimensional stromal environment of the thymus is essential to support efficient selection, Ca^{2+} signals have not been measured within the intact thymus due to the poor light penetration, photobleaching and toxicity that are associated with conventional imaging techniques like confocal microscopy. However, these problems can be overcome with two-photon laser scanning microscopy, which enables live-cell imaging over long periods deep within light-scattering tissues.

We have developed a new preparation for *in situ* imaging of Ca^{2+} signals in developing T cells using two-photon microscopy. 5C.C7 TCR transgenic thymocytes were reintroduced into living thymic slices from mice that either expressed or lacked the selecting MHC class II molecule I-E^k, to create positively and non-selecting environments respectively. Imaging of these slices at physiological temperature revealed vigorous thymocyte movement with velocities of up to $\sim 20 \mu\text{m}/\text{min}$. In a positively selecting environment 30-60% of cells showed $[Ca^{2+}]_i$ elevations that most often were oscillatory (period 50-200 sec) with peaks up to $\sim 1500 \text{ nM}$, and which generally persisted until the cells moved out of the focal plane ($\sim 15-30 \text{ min}$). In contrast, Ca^{2+} signals were seen in only $\sim 10\%$ of cells in the non-selecting environment, and these consisted only of brief and infrequent elevations most often reaching $\sim 500 \text{ nM}$. These results indicate that prolonged $[Ca^{2+}]_i$ oscillations are dependent on MHC recognition and thus are likely to reflect signals delivered during the positive selection process. $[Ca^{2+}]_i$ oscillations were also associated with reduced cellular motility, suggesting that MHC recognition in the intact thymus promotes prolonged interactions with individual stromal cells. Further studies of this type under conditions of negative selection will address the possible diversity of Ca^{2+} signals generated during T cell development. The ability to study signaling events, cell motility and cell-cell interactions within the intact 3-dimensional architecture of the thymus offers a powerful new approach to immune system development and function.