

## T-cell activation and membrane potential

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Initial calcium response in the T-cell activation is an essential for following signal cascade. Major T-cells, as reported, also change their plasma membrane potential ( $\Psi$ ) by activating calcium dependent potassium channels. Its physiological role, however, has not yet been fully clarified. We have approached the question by two different methodologies. First was to analyze T-cell populations which indicate variety characteristics of  $\Psi$  change during activation. By studying it, we are able to understand how the  $\Psi$  would work for those specific T-cell populations and speculate its physiological role. Second approach is genetic cloning of the channel which plays key role for  $\Psi$  behavior.

We used multi-color flow cytometry for the first study that can analyze cytoplasmic calcium ion concentration, membrane potential, and upto three different surface markers, simultaneously of whole thymocytes, lymph nodes and splenocytes. Minor T-cell populations indicated completely opposite behavior, marked depolarization during activation (depol T-cell), while major populations specifically hyperpolarize. One of those cells was CD4/CD44 bright population of spleen or lymph node cells that have been known as a major memory T-cell subset. Since activated T-cells in vitro also behave like a depol T-cell, the finding suggested that T-cell population which depolarize during activation could be previously activated cells in vivo. Since depolarization significantly decreases the driving force of fluxing outside calcium ions into cytoplasm, it could be advantageous for suppressing excessive responses of those concentrated preactivated cells. This regulation could avoid hyperactivation of the cells such in the hypersensitive disorders. However, CD8/CD44 bright cell population did not exclusively concentrate in depolarized T-cells. Depolarization of the  $\Psi$  must possess other role(s) besides negative regulator in the T-cells.

Hyperpolarization is derived by the activation of calcium dependent potassium channel. At least three different channels have been identified. In the lymphocytes, one channel, maxi calcium dependent potassium channel (BK channel) predominantly regulate hyperpolarization. We have cloned the channels gene from random primed mouse thymus cDNA library. The clone (mthyslo) was a common type BK channel gene and distributed wide variety of non-excitabile or excitabile cells, though thymus specific alternative form had been expected. The message was evident even in the cells that possess depolarized characteristics, that is, indication of functionally unexpressed channel activity. Functional expression of the channel, therefore, is suggested to be under the post transcriptional regulation.