

Calreticulin and T cell regulation

with

Fabio Grassi, M.D., Ph.D. Institute for Research in Biomedicine Via Vincenzo Vela 6 Bellinzona CH-6500 Switzerland
fabio.grassi@irb.unisi.ch



What is known about Calreticulin?

Fabio Grassi: Calreticulin (CRT) is a Ca^{2+} binding chaperone protein predominantly localized in the endoplasmic reticulum (ER) where it assists the folding of glycoproteins. The protein may be divided into three structural and functional domains: the N-terminal N-domain, which is extremely conserved and likely participates in the folding of specific substrates, and together with the extended proline-rich P-domain it has a lectin-like chaperone activity. The acidic C-terminal C-domain with low affinity, high avidity for Ca^{2+} contributes to the Ca^{2+} storage capacity of the ER (1). The amount of Ca^{2+} bound to CRT in the ER may dramatically influence the cell fate, e.g.

overexpression of CRT leads to increased susceptibility to apoptotic stimuli whereas CRT deficient cells are more resistant to apoptosis (2,3). CRT deficiency in mice is embryonically lethal because of altered cardiac development and the majority of *crt*^{-/-} embryos die at day 12/13 of gestation (4). In embryonic *crt*^{-/-} cardiomyocyte defective Ca^{2+} release from the ER upon agonist stimulation results in defective calcineurin-dependent activation of myocyte enhancer factor 2C (MEF2C) and contributes significantly to embryonic lethality (5). Indeed, transgenic expression of a constitutively active isoform of calcineurin in the heart resumed myofibrillogenesis in *crt*^{-/-} embryos (6).

Is there any influence of Calreticulin on T cell activation?

Fabio Grassi: A crucial step in T cell activation following interaction of the T cell receptor (TCR) with cognate antigen is the activation of phospholipase $\text{C}\gamma$ (PLC γ), which generates from phosphatidyl inositol 4,5 bisphosphate (PIP₂) inositol 1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG). IP₃ induces Ca^{2+} release from the endoplasmic reticulum (ER). The depletion of the ER Ca^{2+} stores activates the Ca^{2+} permeable Ca^{2+} release-activated Ca^{2+} (CRAC) channels in the plasma membrane giving rise to a sustained Ca^{2+} influx

from the extracellular space into the cytosol, also termed capacitative Ca^{2+} entry (CCE). This CCE represents an indispensable element for efficient T cell activation as shown by the requirement of a sustained elevation of the intracellular calcium concentration for nuclear translocation of nuclear factor of activated T cell (NFAT) as well as interleukin-2 (IL-2) gene expression (7). We obtained CRT deficient T cell from immunodeficient mice reconstituted with *crt*^{-/-} cells had a reduced Ca^{2+} con-

centration in the ER. Analysis of T cell response to antigen revealed that whereas wild-type T cell clones displayed a sustained calcium entry *crt*^{-/-} T cells displayed pulsatile elevations of cytosolic Ca^{2+} concentration (8). The information encoded by Ca^{2+} probably lies in the spatiotemporal pattern of the signal as well as in its frequency and amplitude. In lymphocytes, it was shown that repetitive cytosolic calcium elevations, termed oscillations, of various frequencies differentially controlled the activation of distinct sets of transcription factors, and therefore the expression of different genes (9). The oscillation frequency may therefore direct cells along specific developmental pathways. Since calcium oscillations trigger a more efficient NFAT dephosphorylation and nuclear translocation than a high sustained cytosolic calcium concentration (10), we investigated the NFAT content in the nuclear fractions of *crt*^{+/+} and *crt*^{-/-} T cell clones following antigen encounter. Of the three calcium-regulated NFAT members expressed in T cells (NFAT1, 2 and 4), NFAT1 is

highly expressed at baseline and its activation by TCR signalling is relatively short-lived. In fact, the bulk of activated NFAT1 reverts to a phosphorylated, cytoplasmic form within few hours of stimulation. At early time points following stimulation we observed no significant differences in NFAT1 nuclear translocation between *crt*^{-/-} and *crt*^{+/+} cells. Strikingly, we found a high amount of NFAT1 in the nucleus of *crt*^{-/-} cells 16 hours following stimulation, whereas in *crt*^{+/+} cells all NFAT1 was present in a hyperphosphorylated cytosolic form. The analysis of calcium signalling in T cell clones stimulated for 16 h with antigen revealed that in *crt*^{+/+} cells the frequency of oscillatory calcium rises was lower and the duration longer compared to *crt*^{-/-} T cells. We hypothesize that these differences in the oscillation frequency of Ca^{2+} might be responsible for aberrant *crt*^{-/-} T cell activation upon antigen encounter with consequent severe T cell dependent immunopathology, which indeed we constantly observe in mice reconstituted with *crt*^{-/-} hemopoietic progenitors.

Do you believe that calreticulin influence on T cell activation could help in understanding T cell dependent immunopathology?

Fabio Grassi: Altered calcium signaling in *crt*^{-/-} T cell with protracted NFAT nuclear translocation and MAPK activation upon antigen encounter causes immunopathology in vivo. Mice reconstituted with hemopoietic progenitors from fetal liver of *crt*^{-/-} embryos provide a unique tool to study the role of calcium homeostasis in regulating T cell activation. The study of *crt*^{-/-} T cells could lead to the identification of genes whose expression is altered by aberrant Ca^{2+} signaling. This could be informative on the

mechanisms leading to tissue damage during T cell dependent inflammatory diseases and lead to the identification of potential pharmacological targets of the calcium signaling machinery in the T cell.

REFERENCES

1. Krause KH et al. Cell 88, 439, 1997
2. Nakamura K et al. J Cell Biol 150, 731, 2000
3. Pinton P et al. Embo J 20, 2690, 2001
4. Mesaeri N et al. J Cell Biol 144, 857, 1999
5. Lynch J et al. J Cell Biol 170, 37, 2005
6. Guo L et al. J Biol Chem 277, 50776, 2002
7. Lewis RS Annu Rev Immunol 19, 497, 2001
8. Porcellini S et al. J Exp Med 203, 461, 2006
9. Dolmetsch RE et al. Nature 392, 933, 1998
10. Tomida T et al. Embo J 22, 3825, 2003