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Title:

Partial proteolysis of adaptor protein LAT: a novel regulatory mechanism of intracellular T cell signalling

SUMMARY

Linker for Activation of T cell (LAT) is one of the major signal mediators engaged in transduction of environmental signals during T lymphocyte activation. Extensive research on the LAT protein, initiated in the 90s of the twentieth century, also helped to determine its key role in the process of T cell development. Knockout of the LAT gene in mice results in the inhibition of intrathymic development at the stage of double negative CD4-CD8- (DN) thymocytes, which manifests itself by the absence of mature peripheral T cells and leads to a significant impairment of adaptive immune response. The physiological role of LAT adaptor has been well documented, but little is known about two biological events to which it is subjected. (1) the occurrence of an alternative, evolutionarily conserved isoform of LAT and (2) the process of partial proteolytic degradation of LAT in response to environmental cues. This work has been devoted to a better description of these two phenomena.

In the present study, it was determined that human LATi6 isoform is translated into a protein that retains functionality of the canonical LAT molecule. It has been demonstrated that the expression of LATi6 isoform in the peripheral blood leukocytes of selected mammalian species significantly varies from being virtually absent in the mouse to being the only isoform in the cow. By using point mutations that removed the existing or introduced the additional polyguanidine repeats within the human intron 6 sequence of LAT, it was shown that these cis-regulatory elements (poly G repeats) are responsible for the control of LATi6 splicing. LATi6 protein expressed in the JCaM2 cells shows an identical intracellular localization profile and effectively mediates proximal signalling events during T cell activation as the canonical LAT protein. However, in contrast to the canonical LAT, it has a significantly shorter half-life. This may have an impact on the kinetics of T cell response, particularly its enhancement, as observed by the increased secretion of interleukin-2 in JCaM2 cells expressing the LATi6 isoform. This result suggest that the expression of LATi6 isoform from an evolutionary point of view is

justified and its enhanced degradation may lead to an increase in the efficiency of control over effector T-cell response.

The second part of the thesis has been focused on investigating biological function and molecular mechanism of the partial LAT proteolysis in T lymphocytes and their precursors. By using the Western blot, a characteristic pattern of LAT degradation in T cells (and their precursors) exposed to proapoptotic factors was revealed. It was observed that one of the degradation products – the N-terminal fragment of LAT of approximately 17 kDa – remains anchored in the membrane even though the remaining portion of the molecule undergoes a rapid proteolysis. Despite the inability to create a functional signalosome, this 17kDa N-terminal LAT fragment retains a selective capacity of sequestrating TRAF6 and LCK molecules thereby negatively influencing T cell response. It has also been shown that Caspase 8 and/or Granzyme B are responsible for the process of LAT proteolysis. Furthermore, it has been demonstrated that LAT phosphorylation effectively inhibits its degradation. Due to the limitations of the Western blot technique a novel, flow cytometry-based method for assessment of LAT integrity at the single cell level was developed. Using this technique the kinetics of LAT degradation was determined and caspase participation in the process was confirmed. It was also shown that this event precedes phosphatidylserine translocation to the outer leaflet of the plasma membrane during the initiation phase of apoptosis. In addition, it was demonstrated that minute population of FoxP3+CD4+CD8low thymocytes are resistant to LAT proteolysis in response to CD3ε crosslinking. The degradation of LAT, determined by this novel cytometry-based method, can, therefore, be successfully used as an early marker of T-cell apoptosis and resistance for LAT proteolysis an additional marker characterizing the subpopulation of regulatory T-lymphocytes.