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The intracellular Ca\textsuperscript{2+} concentration optimal for T cell activation is quite different after ionomycin or CD3 stimulation

Abstract The relationship between the initial increase of intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) (measured at the single-cell level with an imaging system) and the ensuing proliferation was examined in a human T cell clone stimulated by a phorbol ester in combination with ionomycin, thapsigargin or an anti-CD3 mAb (monoclonal antibody against the CD3 molecule, UCHT1). From the responses to various ionomycin concentrations, one can define a range of [Ca\textsuperscript{2+}]\textsubscript{i} values (400-900 nM) which appears optimal for T cell proliferation; lower [Ca\textsuperscript{2+}]\textsubscript{i} values are suboptimal, higher values are cytotoxic. It was then examined if the [Ca\textsuperscript{2+}]\textsubscript{i} requirements were similar following anti-CD3 stimulation. [Ca\textsuperscript{2+}]\textsubscript{i} oscillations elicited by a concentration of UCHT1 (1/1,000) optimal for mitogenicity fall precisely within the 400-900 nM range. However, very low concentrations of UCHT1 (1/100,000) which evoke barely detectable [Ca\textsuperscript{2+}]\textsubscript{i} responses still cause the cells to proliferate. The possibility that the lower [Ca\textsuperscript{2+}]\textsubscript{i} requirements observed following anti-CD3 stimulation was due to [Ca\textsuperscript{2+}]\textsubscript{i} oscillations was tested under conditions which prevented the appearance of these oscillations. It turns out that an oscillatory Ca\textsuperscript{2+} signal is not more mitogenic than a sustained augmentation of [Ca\textsuperscript{2+}]\textsubscript{i}. Finally, it was examined if overstimulation via CD3 could have toxic consequences similar to those elicited after ionomycin overstimulation. Large transient [Ca\textsuperscript{2+}]\textsubscript{i} responses can be observed following anti-CD3 stimulation in appropriate conditions, and namely in T cells pretreated with interleukin-2. These [Ca\textsuperscript{2+}]\textsubscript{i} augmentations are not cytotoxic. A role for the plasmalemmal Ca\textsuperscript{2+} pump in the prevention of cytotoxicity can be demonstrated. In conclusion, the correspondence between the [Ca\textsuperscript{2+}]\textsubscript{i} response and cell proliferation is entirely different following stimulation by ionomycin and by anti-CD3. In addition, cell proliferation evoked by very low UCHT1 concentration might reveal the existence of a yet unidentified activation pathway.

Key words Ca\textsuperscript{2+} · Ca\textsuperscript{2+} oscillations · T cell proliferation · Ca\textsuperscript{2+} imaging

Introduction

The activation of T cells results from a cascade of intracellular events triggered by the stimulation of the T cell receptor (TCR). Antigen recognition by the variable part of the TCR is immediately transmitted intracellularly through the transducing component of the TCR, i.e., CD3. After the initial activation of tyrosine kinases, the intracellular cascade diverges first (namely with the activation of a phospholipase C on one hand and of ras on the other), and later converges on transcription factors responsible for the expression of interleukin-2 (IL-2) and of its receptor (see [38] for a review). One of the key pathways for T cell activation is the phosphatidylinositol cascade, which leads to the release (mediated by inositol 1,4,5-trisphosphate) of Ca\textsuperscript{2+} from intracellular stores. The initial Ca\textsuperscript{2+} release is accompanied by the appearance of a second messenger, the nature of which is still under discussion [1, 11, 28]. This leads to a prolonged and oscillatory influx of Ca\textsuperscript{2+} flowing through very low conductance channels in the plasma membrane [41].
The functional importance of an augmentation of the intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) in T cell activation stems from a series of facts.

1. All stimuli leading to T cell proliferation (antigen recognition, anti-CD3 antibodies, mitogenic lectins) also elicit a rise in [Ca\textsuperscript{2+}]. [26, 35, 39].

2. Early steps of T cell activation can be bypassed by synergy between phorbol esters and either ionomycin [34], or activation of the phosphatidylinositol pathway [5], i.e. by synergy between a [Ca\textsuperscript{2+}]\textsuperscript{2+} rise and the activation of protein kinase C (PKC).

3. Suppression of extracellular Ca\textsuperscript{2+} during a 1-h stimulation with phytohaemagglutinin (PHA) prevents IL-2 expression and cell proliferation [23].

4. A number of blockers of K\textsuperscript{+}-channels impair PHA-induced T cell proliferation [4]. The most probable mode of action of these molecules is to reduce Ca\textsuperscript{2+} influx as a consequence of the depolarization of the cell induced by these drugs ([3] see also [29]).

5. Similarly, K\textsuperscript{+}-induced depolarization decreases in parallel both Ca\textsuperscript{2+} influx and PHA-induced T cell proliferation [12].

6. In a recent and elegant study, the Ca\textsuperscript{2+} dependence of IL-2 gene expression was established in a murine T cell hybridoma stimulated with thapsigargin or ionomycin [25]. However, the Ca\textsuperscript{2+} dependence following CD3 stimulation could not be established in this study.

The main goal of this paper was to examine if a [Ca\textsuperscript{2+}] value optimal for T cell activation could be determined. To this end were measured, in parallel and under various stimulating conditions, the initial [Ca\textsuperscript{2+}] response (for the first 10-30 min) and the subsequent T cell proliferation 72 h later. Particular attention was paid to the comparison of those results obtained following ionomycin or anti-CD3 stimulation. Following anti-CD3 stimulation, the [Ca\textsuperscript{2+}] response was oscillatory in individual cells, and therefore the [Ca\textsuperscript{2+}] value averaged in a large number of cells was quite different from the value found in individual cells. The same observation can be made after stimulation with antigen-presenting cells [9]. Several experiments were devised to test if the functional consequences of an oscillatory Ca\textsuperscript{2+} response were different from those of a steady Ca\textsuperscript{2+} response.

From this work, it can be concluded that the [Ca\textsuperscript{2+}] values optimal for T cell activation are markedly different depending upon the stimulus. In particular, anti-CD3 antibodies induce T cell proliferation at very low concentrations, which cause small [Ca\textsuperscript{2+}] augmentations, measured at the single-cell level, and no augmentation at all on the averaged signal. This suggests that, besides the classical Ca\textsuperscript{2+}- and PKC-dependent pathways, additional pathways can be turned on in parallel in TCR-mediated signalling.