

# Decreased cytosolic free calcium concentration of aged human lymphocytes in resting state

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**Abstract** – Cytosolic free calcium concentration was measured in lymphocytes from individuals over the age of 80, using quin2 and fura-2 calcium indicators. The average intracellular free calcium concentration of the samples was 62 nM, which value is roughly half the adult (age between 35 and 55) level (116 nM). It is supposed that the decline in immune function of aged individuals is connected to the decrease in free calcium concentration in their lymphocytes. We also discuss the consequences and the adaptive character of this decrease.

**Key words:** cytosolic free calcium; human lymphocytes; immunosenescence; calcium indicators; aging mechanism; cellular aging.

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Immunosenescence is one of the most thoroughly studied and most firmly established processes in gerontology. The decline of immune function in ageing is an important factor of the pathogenesis of various disorders characteristic of old age (1). The decrease in the proliferation ability of lymphocytes plays a major role in this process. The possible reasons of the decrease of lymphocyte proliferation can be summarised in the diminished protein synthesis, enzyme induction and availability of various co-factors of enzyme reactions (2). However, the attenuation or defects in signal transduction of lymphocytes can also contribute to the decrease of proliferation.

The intracellular calcium level is a well known regulator of various responses and the overall metabolism of living cells. Though the physiological role of calcium was discovered almost 100 years ago, the importance of intracellular calcium concentration was first emphasized by Hodgkin & Keynes

in 1957 (3). To our present knowledge calcium ions influence the biochemical changes of living cells in two ways. Firstly, as a "second messenger" calcium transfers the extracellular stimuli to the intracellular effector mechanisms. Calcium ions are an important part of this action as calcium ions serve as co-factors of  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase and protein kinase C. Secondly,  $\text{Ca}^{2+}$  is a charge-carrier. The calcium fluxes through the  $\text{Ca}^{2+}$ -channels of the plasma membrane alter the membrane potential and thus regulate the potential-dependent intracellular biochemical machinery (4-6).

The cytosolic free calcium concentration of aged human lymphocytes has hitherto been unknown (6). However, such evidence as a reduction in T lymphocyte cell reactivity (7, 8), decrease in transmembrane  $\text{Ca}^{2+}$  flux (9) and decline in production of interleukin-2 and its receptors (10, 11) suggests that pronounced changes should occur in the intracellular calcium concentration during aging.

In the present report we examine the intracellular calcium concentration of aged human lymphocytes (from individuals over the age of 80) using the new fluorescent techniques developed by Tsien (12, 13).

## Material and methods

### Material

Fura-2\*, fura-2/AM, quin2/AM, A23187 and TPEN were from Calbiochem; chelex 100, DTPA, Hepes and trypan blue were obtained from Sigma Chemicals; digitonin was a Fisher Scientific product. EGTA and Triton X-100 were from Serva; DMSO was a Fluka product.  $\text{CaCl}_2$  and  $\text{MgCl}_2$

\* **Abbreviations:** BSS: balanced salt solution;  $[\text{Ca}^{2+}]_i$ : free cytoplasmic calcium concentration; chelex 100: divalent cation chelating resin; DMSO: dimethyl sulfoxide; DTPA: diethylene-triamine-pentaacetic acid; EBSS: Earle's balanced salt solution; EGTA: ethylene glycol-bis( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid; fura-2 and fura-2/AM: 1-(2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy)-2-(2'-amino-5'-methylphenoxy)-ethane-N,N,N',N'-tetraacetic acid and its pentaacetoxymethyl ester, respectively; Hepes: 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid; PBS: phosphate buffered saline; quin2 and quin2/AM: 2-((2-bis(carboxymethyl)amino-5-methylphenoxy)-methyl)-6-methoxy-8-(bis(carboxymethyl)amino)quinoline and its tetra-acetoxymethyl ester, respectively; TPEN: N,N,N',N'-tetrakis(2-pyridylmethyl)ethylene-diamine; Tris: tris(hydroxymethyl)-aminomethane.