Changes in Intracellular Calcium Metabolism in T Lymphocytes After Transient and Persistent Psychosocial Stress of Mice of Various Ages

Péter Csermely and Sándor Tóth

Abstract. Intracellular calcium level is a sensitive marker of the homeostasis of living cells and its changes are essential steps of T lymphocyte activation. Environmental stress provokes an adaptive response of the organism. In recent studies the authors have investigated the effect of transient and persistent psychosocial (overcrowd) stress on resting and lectin-stimulated cytoplasmic free Calcium (Ca) concentration of splenic T lymphocytes from young and aged mice (106 animals total). The animals were kept under "normal" (68 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 or 90 days respectively. Young animals showed no change in resting Ca after overcrowd stress. The lectin-induced rise in intracellular Ca level, however, was three times higher ($p < 0.01$) in transiently stressed young mice compared to the control group. Changes were returned to the control level after persistent overcrowd stress. T cells from aged mice displayed significantly smaller levels of resting, and lectin-stimulated intracellular calcium concentration ($p < 0.01$ each), as compared to those of the non-stressed, aged animals. This age-induced inadequate adaptation in the calcium metabolism of T lymphocytes may significantly contribute to the diminished immune response of the aged in stress.

Introduction

Intracellular calcium metabolism plays a prominent role in the homeostasis of living cells. Calcium acts as a second messenger, inducing a vast variety of changes in the cell. Elevation of intracellular and intranuclear calcium concentration accompanies activation and apoptosis of many cells, e.g. thymic lymphocytes (1-6).

Various forms of psychosocial stress, such as overcrowding, were shown to modulate the immune response in mice and rats (7-9). Huie *et al* (10) demonstrated a decrease of serum ionized calcium in stressed rats. Besides this finding, knowledge about the changes of intracellular calcium concentration after psychosocial stress is very limited.

The authors' earlier studies indicated that ageing induces a decrease in resting levels of intracellular free calcium of human T lymphocytes (11,12). Transient (10 days) "overcrowd-stress" induced an elevated Ca-response of T lymphocytes from young mice (13). In recent studies the authors also investigated the effect of persistent (90 days) "overcrowd-stress" on Ca-metabolism of T lymphocytes of young and aged mice, finding that T cells from aged mice displayed significantly smaller levels of resting, and lectin-stimulated intracellular calcium concentration, as compared to those of the non-stressed, aged animals[1].

Materials and methods

Reagents and cells

Concanavalin-A (type IV), digitonin, dimethyl-sulfoxide (DMSO), EGTA, foetal calf serum (FCS), Hepes, phytohaemagglutinin, RPMI 1640 medium and succinyl-Concanavalin-A were from Sigma. Fura-2 acetoxymethyl ester (fura-2 AM) was from Calbiochem. Young (20 weeks) and old (24 months) CBA/CA mice were kept under "control" (68 cm^2/animal) or "overcrowded" (22 cm^2/animal) conditions for 10 or 90 days respectively. Splenic T lymphocytes were separated from erythrocytes by hypotonic lysis in ice-cold distilled water and from granulocytes, monocytes and B lymphocytes by plastic adherence in RPMI 1640 medium with 10% FCS.

Measurement of intracellular calcium concentration

Intracellular calcium concentration was measured as described earlier (11,12). Splenic T lymphocytes (5 x 106 cells/ml) were incubated with fura-2 AM at a final concentration of 2 uM in RPMI 1640 medium for 30 minutes at 37°C. After a 5-fold dilution, the incubation was continued for an additional 15 minutes. Cells were washed twice in a modified Hank's medium (143 mM NaCl, 1 mM Na2SO4, 5 mM KCl, 1 mM NaH2PO4, 0.5 mM MgCl2, 1 mM CaCl2, 5 mM glucose, and 10 mM Hepes, pH 7.45). The experiment was completed within 45 minutes. Fluorescence measurements were performed

at a cell density of 5 x 106 cells/ml with gentle stirring using a PTI Deltascan V-1048 D101 type interfaced fluorimeter at 37°C. Excitation and emission wavelengths were 340 (380) and 520 nm, respectively with 5 nm slits. After recording the initial fluorescence, Concanavalin-A (Con-A), succinyl-Con-A, or phytohemagglutinin were added at final concentrations of 6, 6, and 4 ug/ml respectively. The 1 mg/ml stock solution of Con-A was contained in 0.5 mM MnCl2 to obtain maximal efficiency. Samples were calibrated with the digitonin-(10 uM)-EGTA (5 mM) method and the intracellular calcium concentration was calculated as described earlier (11). Data were corrected for the aspecific lysis of fura-2 by the addition of extracellular Mn^{2+} or EGTA (11).

Results and Discussion

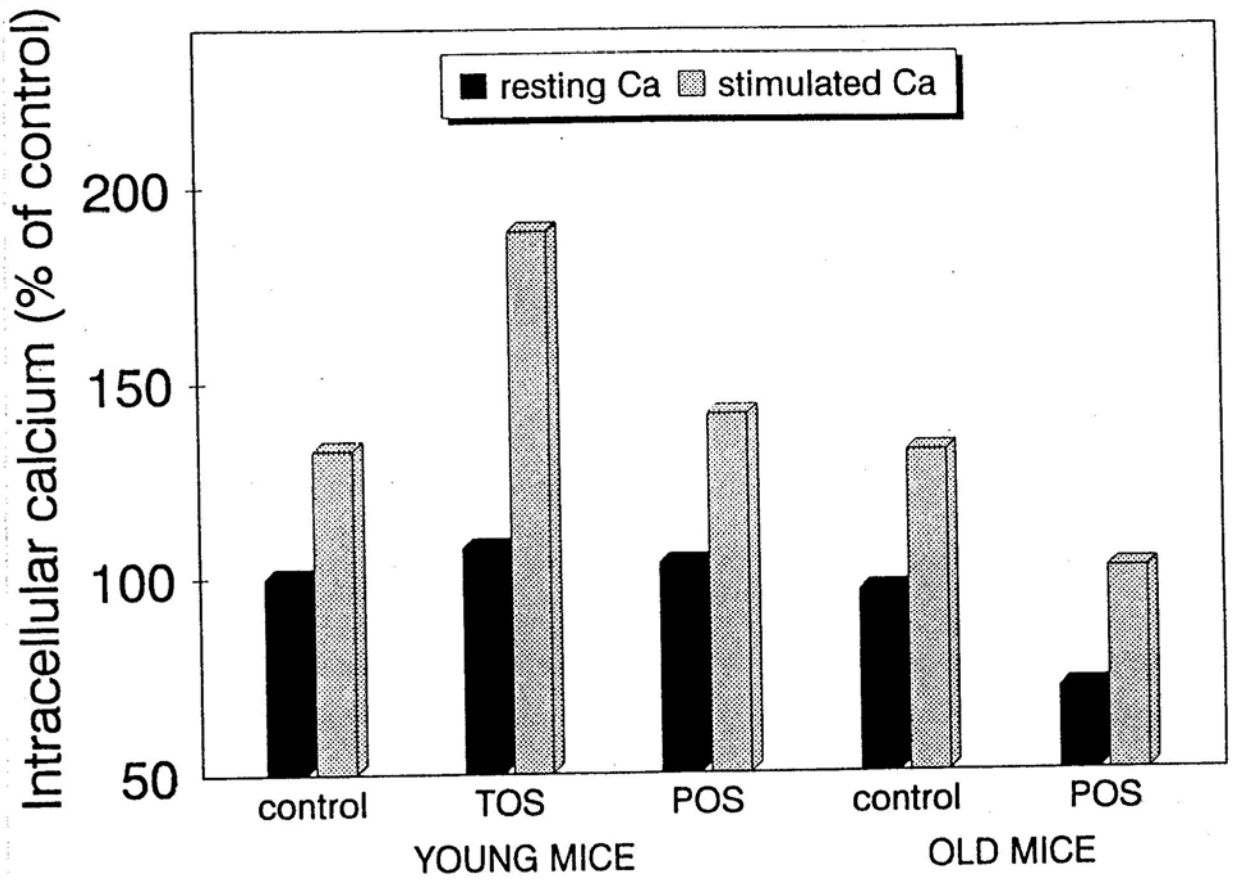
Resting intracellular calcium concentration (Cai) was not significantly different in *control* and in *stressed* young animals (cf. groups 1 through 4 in Table I.). Splenic T lymphocytes of old mice, however, showed a significant ($p < 0.01$, using the Student t-test) decrease in Cai after persistent overcrowd stress (POS) of 3 months (groups 5 and 6 in Table I.). There is quite a big variety of data on changes of resting intracellular calcium levels in lymphocytes of aged mice and humans. Decrease (11,12,14), increase (15) and no significant change (16,17) in resting Cai were all reported. Various levels of psychosocial stress may cause the appearance of these differences.

Table 1: Resting and Concanavalin A-stimulated intracellular calcium concentration of splenic T lymphocytes in young (5 months) and aged (24 months) mice with or without overcrowd stress.

Group No.	Age	Treatment	Resting [Ca] (nM)	Stimulated [Ca] (nM)	Difference (nM)
1	young	control	93.0 ± 13.8 (9)	125.8 ± 11.2[a] (8)	32.8[a]
2	young	TOS	104.8 ± 6.0 (23)	197.5 ± 12.9[a] (16)	92.7[a]
3	young	control	100.2 ± 23.7 (9)	130.0 ± 24.4 (5)	29.8
4	young	POS	100.7 ± 31.1 (15)	137.9 ± 28.2 (10)	37.2
5	old	control	93.8 ± 19.7[b] (10)	128.4 ± 20.7[c] (9)	34.6
6	old	POS	69.0 ± 21.4[b] (16)	98.7 ± 26.5[c] (12)	29.7

Isolated splenic T lymphocytes were treated with Concanavalin A ("stimulated" cells) and intracellular calcium concentration was measured as described in Materials and Methods. From the final calculations, Ca_i values from those animals which showed significant pathological changes (e.g. visible tumors of liver and spleen; 1, 3, 1 and 6 mice from groups 3 through 6, respectively) were omitted. (Data were similar, but if these omissions were not taken, the level of significance would have decreased to a value of $p < 0.05$.) Data are means ±S.D. (standard deviation). The numbers in parentheses denote the number of animals of each experimental group. [Ca] refers to intracellular calcium concentration; TOS refers to transient overcrowd stress (10 days); POS refers to persistent overcrowd stress (90 days); [a,b,c]level of significance at $p < 0.01$ (calculated by the Student's t test).

Figure 1: Percent changes of resting and Concanavalin A-stimulated intracellular calcium concentration of T lymphocytes from young and aged mice, with our without overcrowd stress



Data are calculated from the Cai values of Table I. and expressed as percentages of the resting intracellular calcium levels of *control* young mice (97.5 nM). TOS refers to transient overcrowd stress (10 days); POS refers to persistent overcrowd stress (90 days).

If T lymphocytes were stimulated with Concanavalin-A (Con-A) at a final concentration of 6 ug/ml, cells from young animals showed a significantly (approx. 3 times, $p < 0.01$) higher increase in cytoplasmic calcium after transient overcrowd stress, which leveled off after persistent stress treatment (POS; cf. groups 1 through 4 in Table I). Lymphocytes from aged animals treated with persistent overcrowd stress reached a significantly ($p < 0.01$) smaller Cai level after Con-A stimulation, than those from non-stressed mice (groups 5 and 6 in Table I). Con-A-stimulated intracellular calcium concentration of T cells from aged mice was commensurate with resting calcium levels of splenocytes from young animals. Despite the marked differences in the level of stimulated Cai, Con-A-induced differences in intracellular calcium concentration were similar in control and persistently stressed old animals (34.6 and 29.7 nM, respectively, see groups 5 and 6 in Table I and Figure 1).

The time course of Concanavalin-A-induced changes in Cai was similar in control and stressed aged animals. Similar effects were observed if we used succinyl-Concanavalin-A (6 ug/ml), or phytohemagglutinin (PHA-L, 4 ug/ml) as stimulant. Changes in calcium level of T lymphocytes from adult (12 months old) mice were similar to young ones.

Chelation of extracellular calcium (by the addition of extracellular EGTA at a final concentration of 5 mM) abolished the lectin-induced increase in intracellular calcium levels in T lymphocytes, from both control and stressed young and old animals. Thus, the observed differences in changes of intracellular calcium concentration, reflect stress-induced changes in lectin-induced calcium influx to splenic T lymphocytes.

Acute stress, such as heat shock, induces an elevated intracellular calcium concentration and inositol trisphosphate levels, as well as significant increases in both phospholipase A2 and phospholipase C activities (18,19). The authors' results, showing a 3-fold increase in the Ca-response of T lymphocytes of young mice to lectin stimulation after transient overcrowd stress, demonstrate that increased levels of intracellular calcium may participate in other forms of transient stress as well.

On the contrary of the increase in Ca-response after transient (10 days) overcrowd stress, persistent (90 days) overcrowd stress does not cause any significant changes in the difference between resting and stimulated Cai levels in young mice. Similarly to these observations, Monjan and Collector (7) also demonstrated that stress-induced mitogenic stimulation of mouse splenic T lymphocytes levels off after 1.5 months. Chronic stress may evoke different levels of adaptation.

Old animals are much more sensitive to the suppressive effect of stress than that of young subjects (9,20,21). This age-dependent sensitization, which particularly affects the immune system (20,21) is generally thought to be mediated by various changes in the endocrine status of old subjects (20). The authors' results may provide an alternative / extension of this explanation. The intracellular calcium concentration must reach a certain threshold level around 120-150 nM to be able to induce the activation of T lymphocytes (5,6,11). Our results indicate, that T lymphocytes of old (but not young) animals barely reach this Cai level after persistent overcrowd stress. Since the subjective stress level ("felt-stress") of an aging subject is generally higher, than that of a young one, this inadequate adaptation in the calcium metabolism of T lymphocytes may significantly contribute to the diminished immune response of the aged subject.

The mechanism of the observed changes in the calcium responsiveness of T lymphocytes from aged animals after overcrowd stress is not clear. Huie *et al* (10), demonstrated a decrease of 0.1 mM in the concentration of serum ionized calcium after persistent (90 days) overcrowd stress in rats. Since the intracellular calcium concentration of T lymphocytes shows parallel changes with the extracellular ionized calcium (11,22), one may "calculate" the "required" difference in serum ionized calcium concentration to cause the 25 nM drop in intracellular calcium concentration after overcrowd stress in aged animals. The result of this calculation is 0.3 mM which is close to the observed 0.1 mM change after persistent overcrowd stress (10). Stress-induced decrease in serum ionized calcium may significantly contribute to the decrease of Cai in old stressed animals. Ghoneum *et al* (21) and Hoffman-Goetz *et al* (23) described various changes in the subpopulations of splenic T lymphocytes after psychosocial stress. Changes in the composition of the splenic T lymphocyte pool may also influence both the resting and stimulated intracellular calcium levels after stress.

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Footnotes

[1]P. Csermely, I. Pénzes and S. Tóth, submitted for publication

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Bio-Data

Peter Csermely, Ph.D.

Nationality: Hungarian

Address: Semmelweis University, School of Medicine, Institute of Biochemistry I., H-1444 Budapest, P.O. Box 260, Hungary

Education:

Master of Science (honours degree) 1982 Eotvos Lorand Univ. Budapest (chemistry, pharmaceutical chemistry)
Ph.D. (summa cum laude) 1983 Eotvos Lorand Univ. Budapest (colloidal chemistry, cell biology)
Ph.D. (summa cum laude) 1988 Hungarian Academy of Sciences (biochemistry, immunochemistry)
Habilitation 1994 Semmelweis University (medicine, biochemistry)
D.Sc. 1994 Hungarian Academy of Sciences (cell biology, medicine)

Sandor Toth, Ph.D.

Place and date of birth: Budapest, 1927

Education: Budapest, Fridrichshafen, Tuebingen, St. Petersburg and Moscow
Qualification: Research Biologist
Ph.D. degree: 1963, Hungarian Academy of Sciences

Organization Data
Semmelweis University
Budapest, Hungary

The Semmelweis University (named after Ignac Semmelweis, the "saviour of mothers", a great Hungarian physician of the last century who discovered the reason of puerperal (fever) was founded 230 years ago and is the largest among the four Medical Universities of Hungary. The University hosts Schools for Medicine, Dentistry and Pharmacy while Schools of Nursery and Public Health will be open soon. Recently, a separate School of postgraduate (Ph.D.) studies has been also established. The University has more than 400 students, who study Hungarian, German and English languages. The Hungarian National Center of Gerontology accommodates basic geriatric research (as one of the largest projects of recent years it concluded a nationwide study of all the centenarians in Hungary) and hosts and organizes various projects for the aged.

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