American Journal of Medicine
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Prevalence and clinical implications of hypocalcemia in acutely ill patients in a medical intensive care setting.

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The incidence and the clinical implications of hypocalcemia were evaluated in acutely ill patients admitted to the Medical Intensive Care Unit of the Detroit Receiving Hospital. Total and ionized calcium levels were prospectively evaluated upon admission for all patients over a three-month interval. **A high proportion of patients (62 of 88, 70 percent) were found to have decreased levels of both total and ionized calcium.** Known causes of hypocalcemia could be identified in only 28 patients (45 percent). These included hypomagnesemia (17, 28 percent), renal insufficiency (five, 8 percent), alkalosis (four, 6 percent), and acute pancreatitis (two, 3 percent). In the remaining 34 patients (55 percent), no readily identifiable cause could be found. These 34 patients had a lower mean albumin level than did the 23 normocalcemic patients (p less than 0.01), but there were no differences in age, pH, serum creatinine, magnesium, or phosphate between the two groups. Serum albumin correlated directly with ionized calcium levels (n = 82, r = 0.33, p less than 0.01), as well as with total calcium levels (n = 76, r = 0.70, p less than 0.01). **There was a strong association between sepsis and hypocalcemia.** Patients who survived the hospitalization had higher mean ionized calcium, total calcium, and albumin values than did nonsurvivors, but there were no differences in age, serum creatinine, magnesium, and phosphate between the two groups. **The mortality of the hypocalcemic patients (44 percent) was significantly greater (p less than 0.05) than the mortality of the normocalcemic patients (17 percent). These findings suggest that hypocalcemia is a very common abnormality in acutely ill patients and is associated with a poor prognosis.**

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Roles of calcium and annexins in phagocytosis and elimination of an attenuated strain of *Mycobacterium tuberculosis*in human neutrophils

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Abstract

The phagocytic function of neutrophils is a crucial element in the host defense against invading microorganisms. We investigated phagocytosis and intracellular killing of an attenuated strain of *Mycobacterium tuberculosis* (H37Ra) by human neutrophils focusing on the role of the cytosolic free calcium concentration $[Ca^{2+}]_I$ and certain cytosolic calcium-dependent membrane-binding proteins annexins. Phagocytic uptake did not trigger a calcium rise and occurred independently of different calcium conditions, and in a serum-dependent manner. Changes in the viability of H37Ra were determined by agar plate colony count and a radiometric assay. Neutrophils showed a capacity to kill ingested mycobacteria and this occurred without a rise in $[Ca^{2+}]_i$. **The ability to kill H37Ra [***Mycobacterium tuberculosis]* **decreased in the absence of extracellular calcium and when intra-extracellular calcium was reduced.** Immunofluorescence staining revealed that during phagocytosis of H37Ra, annexins III, IV and VI translocated from cytoplasm to the proximity of the H37Ra-containing phagosomes, whereas the localization of annexin I and V remained unchanged. The translocation of annexin IV occurred even when Ca^{2+}-depleted neutrophils ingested H37Ra in the absence of extracellular calcium**. We concluded that neutrophil-mediated killing of mycobacteria is a Ca^{2+}-dependent process.** The fact that the association of certain annexins to the membrane vesicle containing H37Ra differ from other phagosomes suggests a selective regulatory mechanism during phagocytosis of mycobacteria by neutrophils.

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Calcium spikes in activated macrophages during Fcγ receptor-mediated phagocytosis

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Rises in intracellular-free calcium ($[Ca^{2+}]_i$) have been variously associated with Fcγ receptor (FcR)-mediated phagocytosis in macrophages. We show here that activation of murine bone marrow-derived macrophages increases calcium spiking after FcR ligation. Ratiometric fluorescence microscopy was used to measure $[Ca^{2+}]_i$ during phagocytosis of immunoglobulin G (IgG)-opsonized erythrocytes. Whereas 13% of nonactivated macrophages increased $[Ca^{2+}]_i$ in the form of one or more spikes, 56% of those activated with lipopolysaccharides (LPS; 18 h at 100 ng/ml) and interferon-γ (IFN-γ ; 100 U/ml) and 73% of macrophages activated with LPS, IFN-γ, interleukin (IL)-6 (5 ng/ml), and anti-IL-10 IgG (5 μg/ml) spiked calcium during phagocytosis. Calcium spikes were inhibited by thapsigargin (Tg), indicating that they originated from endoplasmic reticulum. **The fact that activated macrophages showed a more dramatic response suggested that calcium spikes during phagocytosis mediate or regulate biochemical mechanisms for microbicidal activities.** However, lowering $[Ca^{2+}]_i$ with ethyleneglycol-bis(ß-aminoethylether)-*N,N*'-tetraacetic acid or inhibiting calcium spikes with Tg did not inhibit phagosome-lysosome fusion or the generation of reactive oxygen or nitrogen species. Thus, the increased calcium spiking in activated macrophages was not directly associated with the mechanism of phagocytosis or the increased antimicrobial activities of activated macrophages.

Cell Calcium. 1990 Nov-Dec;11(10):655-63.

Oxidase activation in individual neutrophils is dependent on the onset and magnitude of the Ca2+ signal.

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Using single-cell ratio imaging of Fura-2-loaded neutrophils, we demonstrate that the heterogeneity and asynchrony of the oxidase response originates from variability in the timing and magnitude of the cytosolic free Ca2+ signal. The Ca2+ signals from individual cells could be classified into four types: (a) type 1, a transient rise in Ca2+ occurring within 6 s; (b) type 2, an oscillating cytosolic free Ca2+; (c) type 3, a latent Ca2+ transient significantly delayed (21-56 s); and (d) type 4, no significant Ca2+ rise. These response types accounted for approximately 41%, 15%, 26% and 18% of the population respectively for stimulation with 1 microM f-met-leu-phe peptide (n = 27) and 52.5%, 15%, 11.5% and 21% respectively for 0.1 microM f-met-leu-phe peptide (n = 52). **The oxidase in neutrophils in which the cytosolic free Ca2+ concentration rose to greater than 250 nM always became activated. In the presence of extracellular Ca2+, cytosolic Ca2+ rose uniformly throughout the cell, whereas in the absence of extracellular Ca2+, a localized Ca2+ 'cloud' was observed in approximately 30% of cells. A localized activation of the oxidase accompanied the presence of the Ca2+ 'cloud' when the 250 nM Ca2+ threshold was exceeded. The data presented here therefore demonstrate a tight coupling in individual neutrophils between an elevation in cytosolic free Ca2+ above a threshold of 250 nM and activation of the oxidase.**

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Science. 1986 Jun 20;232(4757):1554-6.

Calcium modulation activates Epstein-Barr virus genome in latently infected cells.

Faggioni A, Zompetta C, Grimaldi S, Barile G, Frati L, Lazdins J.

In many viral infections the host cell carries the viral genome without producing viral particles, a phenomenon known as viral latency. **The cellular mechanisms by which viral latency is maintained or viral replication is induced are not known. The modulation of intracellular calcium concentrations by calcium ionophores induced Epstein-Barr viral antigens in lymphoblastoid cell lines that carry the virus. When calcium ionophores were used in conjunction with direct activators of protein kinase C (12-O-tetradecanoyl phorbol-13-acetate and a synthetic diacylglycerol), a greater induction of viral antigens was observed than with either agent alone.** Activation of protein kinase C may be required for the expression of the viral genome.

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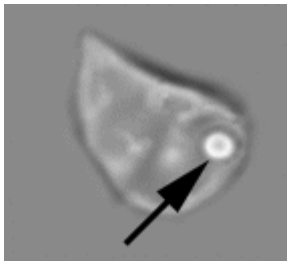
April 17, 2003

How do cells signal and attack foreign matter?

U-M Kellogg Eye Center researcher's high-speed images show how cells mobilize for immune response

ANN ARBOR, MI - New high-speed imaging techniques are allowing scientists to show how a single cell mobilizes its resources to activate its immune response, a news research study shows.



In phagocytosis, a wave traveling around the cell's perimeter splits in two, with the second wave encircling the phagosome or sac-like compartment. This second wave allows the digestive enzymes to enter the phagosome and destroy the target.

Howard R. Petty, Ph.D., professor and biophysicist at the University of Michigan Health System's Kellogg Eye Center, has dazzled his colleagues with movies of fluorescent-lit calcium waves that pulse through the cell, issuing an intracellular call-to-arms to attack the pathogens within.

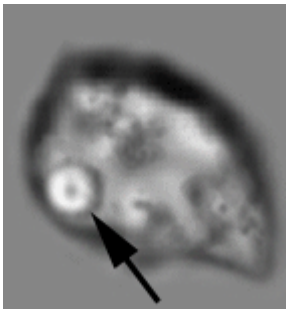
He explains that these high-speed images provide

a level of detail about cell signaling that simply wasn't possible just a few years ago.

In the April 15 issue of the *Proceedings of the National Academy of Sciences*, Petty provides more detail on cell signaling, depicting what he calls the "molecular machinery" underlying the immune response. He has identified a sequence of amino acids (LTL) that controls the calcium wave pathway and, crucially, the ability of immune cells to destroy targets.

The findings are important because they could eventually lead scientists to design drugs based on the amino acid motif.

"Our clinical goal," explains Petty, "is to characterize the immune cell's signaling function so that we can interrupt it or somehow intervene when it begins to misfire." The process has implications for treating autoimmune diseases such as arthritis, multiple sclerosis, and the eye disorder uveitis.



When a mutation is introduced, phagocytosis is not completed because the calcium wave circles the cell and bypasses the phagosome altogether.

Through images of phagocytosis, the process by which a cell engulfs and then destroys its target, Petty is able to track the movement of calcium waves as they send signals to key players in the immune response. The "calcium wave" is a stream of calcium ions coming into the cell, which is detected by the fluorescence emission of a calcium-sensing dye.

As a cell membrane begins to surround its target, two calcium waves begin to circulate. When the target is completely surrounded, one wave traveling

around the cell's perimeter splits in two, with the second wave encircling the phagosome or sac-like compartment. This second wave allows the digestive enzymes to enter the phagosome and finally destroy the target.

When Petty introduced a mutation in the gene (FcyRIIA) that controls phagocytosis, he found that the calcium wave simply circled the cell and bypassed the phagosome altogether. As a result, the immune cell could engulf, but could not carry out the destruction of its target. This led him to conclude that the LTL sequence orchestrates the cell signaling process.

The sequence may also have a role in directing other cell activities, for example signaling the endoplasmic reticulum to form a spindle that connects the phagosome and the outer cell membrane. "The spindle seems to act as an extension cord that signals the calcium wave into the phagosome to finish the attack," suggests Petty.

Petty explains that many of these findings are possible thanks to high-speed imaging techniques that enable him to merge knowledge of physics with cell and molecular biology. He uses high sensitivity fluorescence imaging with shutter speeds 600,000 times faster than video frames.

"Before the advent of high-speed imaging, you could not ask many of these questions because we had no way to see the movement of calcium waves," he says. "With conventional imaging you ended up with a blur of calcium." By contrast, Petty's images resemble the movement of a comet across the night sky.

In the study reported in PNAS, Petty used leucocytes as a model for the process. The amino acid sequence is in the region of the gene FcyRIIA. He is currently studying the same phenomena in the eye, where phagocytosis disposes of the regularly-shed remnants of photoreceptor cells.

The paper, *Signal sequence within FcRIIA controls calcium wave propagation patterns: Apparent role in phagolysosome fusion, also appears on the PNAS* internet site at www.pnas.org.

In addition to Petty, authors on the paper include Randall G. Worth, Moo-Kyung Kim, Andrei L. Kindzelskii, and Alan D. Schreiber.

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Role of serum components in the binding and phagocytosis of oxidatively damaged erythrocytes by autologous mouse macrophages

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Abstract:

Abstract. To investigate the role of autologous serum components in the recognition of damaged cells by macrophages, we examined the binding and phagocytosis of damage oxidatively damaged red blood cells with Cu^{2+} and ascorbate (oxRBCs -- oxidatively damaged red blood cells) by autologous resident mouse peritoneal macrophages. The binding of oxRBCs by macrophages was independent of the presence of serum. However, phagocytosis by macrophages increased with serum concentration, and macrophages showed little ingestion of oxRBCs in a serum-free medium. Macrophages neither bound nor appreciably ingested native RBCs (before oxidation) in either the absence or presence of autologous serum. Mouse macrophages ingested significantly more native as well as oxRBCs in the presence of heat-inactivated fetal calf serum than in the presence of heat-inactivated mouse serum. Pretreated oxRBCs with normal serum were rarely ingested by macrophages in a serum-free medium. **Phagocytosis of oxRBCs was significantly inhibited by depletion of IgG* or calcium from serum, by heat inactivation of complement, or by antiserum against mouse C3. These results demonstrate that serum components such as IgG, C3, and calcium are involved in phagocytosis of oxRBCs by autologous macrophages.**

* IgG : A class of immunoglobulins that include the most common antibodies circulating in the blood, that facilitate the phagocytic destruction of microorganisms foreign to the body, that bind to and activate complement, and that are the only immunoglobulins to cross over the placenta from