Chronological List of Reference Articles Contained in this Document:

 Click Link or Scroll Down 

1. Prevalence and Clinical Implications of Hypocalcemia in Acutely Ill Patients in a Medical Intensive Care Setting. *American Journal of Medicine,* 1988 Feb;84(2):209-14.

2. Prevalence and Predictive Value of Ionized Hypocalcemia Among Critically Ill Patients
Acta Anaesthesiologica Scandinavical, 2003; 47: 1264—1269

3. Calcium in Critically Ill Children
J Pediatr. 1989 Jun;114(6):946-51.

4. Roles of Calcium and Annexins in Phagocytosis and Elimination of an Attenuated Strain of *Mycobacterium Tuberculosis* in Human Neutrophils. *Microbial Pathogenesis* Volume 24, Issue 5, May 1998, Pages 309-320.

5. Calcium Spikes in Activated Macrophages During Fc^{γ} Receptor-Mediated Phagocytosis. *Journal of Leukocyte Biology.* 2002;72:677-684.

6. Oxidase Activation in Individual Neutrophils is Dependent on the Onset and Magnitude of the Ca2+ Signal. *Cell Calcium*. 1990 Nov-Dec;11(10):655-63.

7. Calcium Modulation Activates Epstein-Barr Virus Genome in Latently Infected Cells. *Science*. 1986 Jun 20;232(4757):1554-6.

8. How do Cells Signal and Attack Foreign Matter? Univ. of Michigan, Kellogg Eye Center researcher's April 17, 2003.

9. Role of Serum Components in the Binding and Phagocytosis of Oxidatively Damaged Erythrocytes by Autologous Mouse Macrophages. *Cellular and Molecular Life Sciences.* Issue: Volume 58, Number 11/October 2001. Pages: 1727 – 1733.

10. Calcium in Milk Inhibits E. coli-Induced Diarrhea. *Gastroenterology.* 2003;125:469-476.

11. Ideal Drinking Water. Article by Dr. Royal Lee *Let's Live Magazine*, 1958.

12. Protein and Calcium Interplay Important to Bone Health. *United States Dept. of Agricultural Research Service Report.* April 24, 2002.

13. Bone and Nutrition in Elderly Women: Protein, Energy, and Calcium as Main Determinants of Bone Mineral Density. *European Journal of Clinical Nutrition* (2003). 57, 554−565. doi:10.1038/sj.ejcn.1601577.

American Journal of Medicine
1988 Feb;84(2):209-14.

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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Prevalence and predictive value of ionized hypocalcemia among critically ill patients

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Background: Ionized hypocalcemia is common among critically ill patients, and it has been shown to correlate with increased mortality. The purpose of this study was to examine the performance and independence of ionized calcium (Ca2+) in prediction of all-cause day-30 mortality among critically ill adult patients.
Methods: Of 993 critically ill patients treated in the Helsinki University Hospital during a 24-month period, the study comprised 941 patients without calcium supplementation. Patient and laboratory data were obtained retrospectively from an intensive care database. The discriminative powers of admission and lowest Ca2+ values regarding day-30 mortality were evaluated by producing receiver operating curves (ROC). Hazard ratios for death of severe and mild hypocalcemia were calculated by Cox regression model.
Results: The prevalence of ionized hypocalcemia (Ca2+ $<1.16\,mmol\,l^{-1}$) was 85%. Of 941 patients, 45 (4.7%) had ionized calcium $>1.3\,mmol\,l^{-1}$ and were excluded from mortality analysis. Univariate Cox regression model revealed hazard ratios of 5.1 (95% confidence interval, CI 2.9–9.0) for severe ($<0.90\,mmol\,l^{-1}$) and 1.8 (95% CI 1.3–2.4) for mild ionized hypocalcemia ($0.90–1.15\,mmol\,l^{-1}$) on admission, but hypocalcemia was not shown to be independently associated with mortality by multivariate Cox regression model. In prediction of day-30 mortality admission and lowest Ca2+, levels had areas under curves of 0.636 and 0.671, respectively.
Conclusion: Ionized hypocalcemia is common among critically ill adults and it is associated with increased mortality. Although non-survivors and survivors differ significantly in admission Ca2+, hypocalcemia is not independently associated with day-30 mortality.

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Key words: Critical illness; ionized hypocalcemia; mortality; prediction.

IONIZED hypocalcemia is a common phenomenon among critically ill patients. According to different reports its prevalence ranges from 12 to 74% in pediatric population (1, 2) and from 15 to 88% in adults (3, 4). Particularly, septic patients (5–11) are likely to develop ionized hypocalcemia along with those with pancreatitis (12, 13), severe burns (14), and rhabdomyolysis (15, 16). The pathogenesis of critical illness hypocalcemia is probably multifactorial, involving intra- and extracellular redistribution of calcium ion (10). Proposed mechanisms include intracellular accumulation of calcium ions (17, 18), altered sensitivity and impaired secretory function of the parathyroid gland, end-organ resistance to parathyroid hormone, and altered D-vitamin synthesis and action. Proinflammatory cytokines and calcitonin as well as increased chelation of ionized calcium (Ca2+) may contribute to hypocalcemia in the critically ill (19–22).

The consequences of decreased serum ionized calcium are numerous, since calcium has an essential role in different cellular functions, including muscle and myocardial contractility, vascular tonus, enzyme activation and hormone release, neurotransmission, membrane potentials and blood coagulation, and as a critical intracellular messenger (3, 23–25).

Disease severity has been shown to correlate inversely with Ca2+ levels (2, 4, 11, 26, 27). Hypocalcemia as defined by reduced levels of total calcium (28) or Ca2+ (4, 10, 22, 26, 29) or both (1, 27, 30–32) has been associated with increased mortality among both medical and surgical critically ill patients by several authors. While some of these authors have suggested prognostic implications (20, 25, 26, 30) they have not evaluated whether hypocalcemia is an independent risk factor. Even highly statistically significant differences between survivors and non-survivors may not reflect the discriminative power of a single variable when predicting mortality (33, 34). In a recent report by Zivin et al. a negative correlation between Ca2+ levels and disease severity as defined by the Acute Physiology and Chronic Health Evaluation

(APACHE) II score was demonstrated. Hazard ratio for death for Ca2+ decrements of 0.1 mmol l^{-1} was 1.65 (95% confidence interval 0.19–2.28, $P = 0.002$) (4).

However, to the best of our knowledge no study has focused on the assessment of discriminative power of Ca2+ for prediction of mortality by receiver operating characteristic (ROC) curve analysis. Therefore, the aim of the present study was to assess the prevalence of ionized hypocalcemia in a large population of unselected critically ill patients and to evaluate the performance and independence of Ca2+ in prediction of all-cause day-30 mortality.

Methods

All patients admitted to Helsinki University Hospital Intensive Care Unit during the time period between January 1999 and December 2000 were initially included in the study population. Our department is a nine-bed medical-surgical ICU treating adult patients in a tertiary care hospital. The clinical and laboratory data used in this study were attained retrospectively from our computerized ICU database (HP CareVue, Palo Alto, CA). The study protocol was approved by the local Ethics Committee.

Plasma Ca2+ (reference range 1.16–1.30 mmol l^{-1}), creatinine (reference range <100 mmol $^{-1}$ for females and <115 mmol $^{-1}$ for males), albumin (reference range 35–46 g l^{-1}), and arterial blood pH (reference range from 7.35 to 7.45) included in a routine laboratory set taken within 2 h of admission were recorded. All samples were taken from an arterial line. Blood gas analyses taken in a heparinized syringe were routinely accompanied by Ca2+ measurements. To avoid bias from the possibility of more frequent sampling, regarding the hypocalcemic patients, we recorded only the admission, lowest and highest Ca2+ values for each patient.

Ca2+ was analyzed with an ionic-specific electrode (blood gas analyzer Ciba Corning 855, reference range from 1.16 mmol l^{-1} to 1.3 mmol l^{-1}). Interassay variability is 2.5% at level 0.80 mmol l^{-1}. For analysis pH 7.4-corrected values were used.

Patients having received calcium supplementation (n = 52, 5.3% of total) in the ICU were excluded from the analysis regarding prediction of mortality. Due to the small number of these patients the effect of calcium supplementation on mortality could not be analyzed. The total amount of RBC transfusions, platelets and fresh frozen plasma was attained from the database for each patient to be used in the Cox regression model.

The primary outcome measure was day-30 all-cause mortality. The date of death of each patient based on the social security code was provided by the Central Statistical Office of Finland. The APACHE II score and sequential organ failure assessment (SOFA) scores calculated from the first 24-h data were used as a measure of disease severity. The APACHE II and admission and highest SOFA scores were included in the Cox regression model.

Statistical analyses were performed with SPSS 10.1.3 for Windows (SPSS Inc., Chicago, IL). Differences in continuous variables between survivors and non-survivors were compared using the non-parametric Mann–Whitney *U*-test and Fisher's exact test when appropriate. Results with *P*-values less than 0.05 were considered significant.

The discriminative power of admission and lowest Ca2+, regarding day-30 mortality, underwent evaluation by producing receiver operating curves (ROCs) and by calculating areas under the curve (AUCs) (35). Patients with higher than normal Ca2+ (>1.30 mmol l^{-1}) on admission (n = 45, 4.7%) or as the lowest value during the ICU stay (n = 6, 0.6%) were excluded from the ROC analysis and Cox's proportional hazard model. The hazard ratios for death of severe and mild hypocalcemia on admission and during the ICU stay were calculated by Cox's model. For this analysis the patients were first divided into three groups [normal (1.16–1.30 mmol l^{-1}), mild hypocalcemia (0.90–1.15 mmol l^{-1}) and severe hypocalcemia (<0.90 mmol l^{-1})] separately according to the admission and lowest Ca2+ values. Additionally, the admission and lowest Ca2+ values were analyzed as continuous variables.

Results

Of a total number of 993 patients from the database, 52 (5.3%) had received calcium supplementation and were excluded from analysis. Of those 52, only 10 (18.9%) had severe hypocalcemia on admission, and 26 (50%) at any time. The day-30 mortality was 21 of 52 (40.4%). Of the remaining 941, 224 patients (23.8%) died before day 30. The baseline characteristics of the patients are presented in Table 1, and the diagnostic groups in Table 2.

The prevalence of ionized hypocalcemia (Ca2+ <1.16 mmol l^{-1}) and severe hypocalcemia (Ca2+ <0.90 mmol l^{-1}) on admission were 57.8% (575 of 993) and 3.3% (33 of 993), respectively. Of all patients 85.0% (845 of 993) were hypocalcemic and 10.2% (101 of 993) were severely hypocalcemic during

Table 1

Demographic data and Ca^{2+} presented as median (interquartile range).

	Survivors (n = 717)	Non-survivors (n = 224)	*P*-value
Age (years)	55.0 (40.5-67.0)	62.0 (50.0-70.0)	<0.001[a]
Sex (F/M)	265/ 452	77/ 147	0.54[b]
APACHE II	11.0 (7.0-16.0)	19.0 (13.3-24)	<0.001[a]
Admission SOFA	5.0 (3.0-7.0)	10.0 (7.0-12.0)	<0.001[a]
Highest SOFA	6.0 (3.0-8.0)	11.0 (9.0-14.0)	<0.001[a]
Admission Ca2+ (mmol l^{-1})	1.15 (1.09-1.2)	1.11 (1.02-1.18)	<0.001[a]
Lowest Ca 2+ (mmol l^{-1})	1.08 (1.02-1.14)	1.01 (0.94-1.10)	<0.001[a]

[a]Mann-Whitney test.
[b]Fisher's exact test.
SOFA = sequential organ failure assessment.

their ICU treatment. Distribution of the admission (Fig. 1) and lowest Ca2+ levels (Fig. 2) and the corresponding day-30 mortality among patients not having received calcium supplementation are presented in Figures 1 and 2.

When Ca2+ was categorized into three groups, the univariate Cox regression model revealed hazard ratios (HR) (95% confidence interval, CI) of 5.1 (2.9–9.0) for severe (<0.90 mmol l^{-1}) and of 1.8 (1.3–2.4) for mild (0.90–1.15 mmol l^{-1}) ionized hypocalcemia on admission. The R-square for linear regression of admission ionized calcium was 0.05 regarding day-30 mortality.

For lowest Ca2+ values the hazard ratios (95% CI) were 5.2 (2.9–9.2) for severe and 1.6 (1.0–2.8) for mild hypocalcemia, respectively. Multivariate Cox regression model revealed HRs of 2.7 (95% CI 1.1–6.9, $P = 0.039$) for admission severe hypocalcemia, 1.3 (1.2–1.4, $P < 0.001$) for admission SOFA , and 1.1 (1.1–1.1, $P < 0.001$) for APACHE II as independent risk factors. When the maximal SOFA score and lowest Ca2+ (in three groups) were included, the corresponding Cox model revealed HRs of 1.3 (1.2–1.4) for the maximal SOFA score, 1.1 (1.1–1.1) for APACHE II and 2.3 (0.9–5.8, $P = 0.085$) for admission severe hypocalcemia. Additionally, neither admission nor lowest Ca2+ was a statistically significant independent risk factor for day-30 mortality.

Discrimination by ROC analysis revealed AUCs of 0.636 (95% CI 0.591–0.681) and 0.671 (95% CI 0.628–0.714) for the admission Ca2+ and lowest Ca2+ levels in prediction of day-30 mortality. Admission and during-ICU severe ionized hypocalcemia (<0.90 mmol l^{-1}) revealed sensitivities of 7% and 19%, and specificities of 98% and 96% for day-30 mortality, respectively.

Table 2

Diagnostic groups of the patient population.

Diagnostic group	n (%)
Surgical	366 (39)
cardiac	45 (5)
thoracic	63 (7)
trauma	26 (3)
vascular	105 (11)
gastrointestinal	112 (12)
miscellaneous	15 (2)
Medical	575 (61)
cardiac	201 (21)
infection	192 (20)
intoxication	71 (8)
neurologic	37 (4)
respiratory	25 (3)
hematologic	14 (1)
miscellaneous	35 (4)

Discussion

This study confirms that ionized hypocalcemia is a rather common laboratory abnormality among non-selected critically ill adults with no calcium supplementation. An increased risk of death in the severely hypocalcemic patient group was demonstrated and the risk was elevated in the mildly hypocalcemic patient group as well. This is in accordance with a recent study by Zivin and colleagues investigating hospital and ICU hypocalcemia. In their study, mortality was shown to increase progressively with descending Ca2+ values. This increase

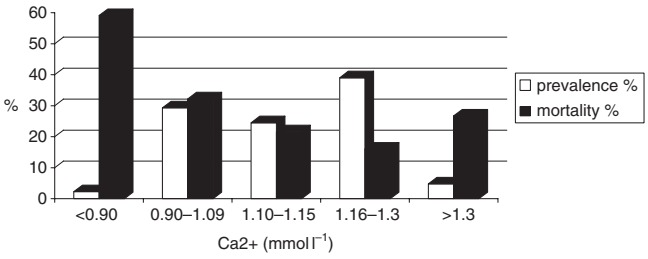

Fig. 1. Percentage of patients (total n = 941) in different admission ionized calcium level groups and mortality within each group.

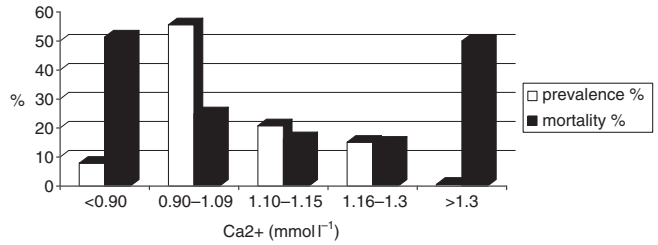

Fig. 2. Percentage of patients (total $n = 941$) in different groups according to the lowest ionized calcium value and mortality within each group.

in mortality was not linear and mortality was higher, 33%, in a group of moderately low Ca2+ levels (Ca2+ 0.90 mmol l^{-1} to 1.1 mmol l^{-1}) compared with the 26% in the group with the lowest Ca2+ levels (Ca2+ <0.90 mmol l^{-1}). Conversely, in our study the increase in mortality was linear between the severely hypocalcemic, mildly hypocalcemic and normocalcemic patient groups. Thus, the hazard ratio for death was highest, 5.1 for admission and 5.2 for lowest Ca2+ values, among those patients with severe hypocalcemia. The differences in the linearity of mortality increase between our study and the Zivin study may be due to the exclusion of patients having received calcium supplementation in our study. Given that the patients with the lowest Ca2+ levels are more likely to receive calcium supplementation, their result (4) may be biased, since they included those patients in analysis. Also, selecting patients for whom Ca2+ values were available may have biased their results. Patients expected to have abnormal values may have been more likely to have Ca2+ measured. In our ICU Ca2+ is routinely analyzed with every blood-gas analysis, rendering it available for all patients up to eight times daily. Furthermore, in the study of Zivin et al. (4) the small total patient group size and the extremely small number of patients in the normocalcemic and the severely hypocalcemic groups reduces the statistic power to be insufficient for predicting mortality.

Another finding of the present study was that ionized hypocalcemia was not an independent predictor of day-30 all-cause mortality. Since hypocalcemia has been reported to correlate with the APACHE II score (4, 11), it is possibly a sign of disease severity. It may reflect dysfunction in acute physiology in critical illness but may not have an impact on mortality.

In our study we also demonstrate that Ca2+ as a single laboratory variable has an inappropriate discriminative power in predicting day-30 mortality. While AUC 0.5 represents a random chance probability, admission and lowest Ca2+ increased only slightly the ability to discriminate between day-30 survivors and non-survivors.

The inadequate performance of Ca2+ in predicting mortality is an interesting finding, since many authors have reported increased mortality among hypocalcemic patients (1, 4, 10, 22, 26, 27, 29–31), which is shown also in the present study. This supports the view previously indicated that even highly statistically significant differences between survivors and non-survivors do not reflect the discriminative power of a single variable when predicting mortality. To the best of our knowledge this study is the first to utilize ROC analysis in evaluation of the predictive value of Ca2+. We found a significant difference in the admission and lowest Ca2+ levels between day-30 survivors and non-survivors. However, fairly low AUCs show that this cannot be interpreted as Ca2+ being a good prognostic indicator. In a recent report by Cusack et al. (36) the performance of various acid-base balance variables in predicting mortality was evaluated in a general adult ICU setting. In a patient population of 100 patients, they discovered AUCs of 0.72 and 0.71 for pH and standard base-excess, respectively. They also found a significant ($P = 0.01$) difference in the admission mean Ca2+ concentration between survivors and non-survivors, but unfortunately they did not report performing ROC analysis to evaluate the discriminative power of Ca2+. They reported an AUCs of 0.76 for the APACHE II score, which was superior to the AUCs of all laboratory variables concerned. Predictive values of other single laboratory variables have been previously investigated among critically ill patients. Procalcitonin, D-dimer and interleukin-6 have undergone assessment by ROC analysis in this setting (37–39). The AUCs of AT III level, platelet count, thromboplastin time, CRP concentration and white blood cell count have been reported to range from 0.53 to 0.71 (40). The predictive value of the admission and lowest Ca2+ is comparable with these variables, none of which was found to be independent predictors of hospital mortality. However, comparison between a single laboratory variable and the APACHE II or SOFA score, the discriminative powers of which are good (41), may be unfair, since these scores comprise various single laboratory variables, the discriminative powers of which have not been assessed separately.

The most important advantages of our study are a large unselected patient population and the consistently available laboratory data. An ideal study protocol to investigate the predictive value of a variable would be a prospective cohort study, and

being retrospective is the most important weakness of our study. However, we believe that due to the large and unselected patient population with routine laboratory data and the computerized data management system, our results can be considered representative for all our patients and a mixed medical–surgical ICU in general. Another weakness of this study is associated with the exclusion of the 52 patients having received calcium supplementation. This group of patients may have been more severely hypocalcemic than the patients included in analysis and this may have influenced the results. However, this patient group was small and only 10 (18.9%) of these patients were severely hypocalcemic on admission, and therefore the influence of excluding this group is probably smaller than the resulting bias had it been included. The effect of calcium substitution on mortality was beyond the scope of this study.

We conclude that ionized hypocalcemia is a common laboratory abnormality among unselected critically ill adults. Furthermore, although non-survivors and survivors differ significantly in both admission and lowest values, ionized calcium is a poor, and not independent, predictor of day-30 all-cause mortality. Thus, even severe hypocalcemia has only limited prognostic implications in the critical care setting

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Hypocalcemia in critically ill children.

Cardenas-Rivero N, Chernow B, Stoiko MA, Nussbaum SR, Todres ID.

Department of Pediatrics, Harvard Medical School, Boston, MA.

To determine the prevalence and clinical consequences of hypocalcemia in pediatric intensive care unit patients, we prospectively studied calcium homeostasis in 145 of these patients. The total serum calcium concentration was measured in all patients. The serum ionized calcium concentration was measured in blood samples collected from those 71 (49%) patients who had low total serum calcium values (less than 8.5 mg/dl (2.12 mmol/L). Of the 71 patients, 26 (36.6%) had ionized hypocalcemia. Therefore the prevalence of ionized hypocalcemia was at least 17.9% (26/145). Death occurred in 8 (31%) of 26 patients with ionized hypocalcemia versus 3 (2.5%) of 119 patients with normocalcemia (p less than 0.0001). However, the severity of illness score was higher (p less than 0.05) in the children with ionized hypocalcemia than in normocalcemic children (mean Therapeutic Intervention Scoring System score 33 +/- 17 vs 22 +/- 11, respectively). More of the children with ionized hypocalcemia had sepsis (p = 0.0299) and they required the administration of vasopressor agents more often (p = 0.0002) than their normocalcemic counterparts. Of the 26 patients with ionized hypocalcemia, 17 (65.4%) had biochemical evidence of either absolute or relative hypoparathyroidism, determined by means of an immunoradiometric assay that measures only biologically active parathyroid hormone. We conclude the following: (1) ionized hypocalcemia is common in severely ill children. (2) Patients with ionized hypocalcemia have a higher mortality rate than those with normocalcemia; however, because the former are more severely ill, no causality is apparent or suggested. (3) Functional hypoparathyroidism may occur in critically ill children.

PMID: 2786063 [PubMed - indexed for MEDLINE]

Microbial Pathogenesis
Volume 24, Issue 5, May 1998, Pages 309-320

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.

JOURNAL OF LEUKOCYTE BIOLOGY

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.

Hallett MB, Davies EV, Campbell AK.

Department of Surgery, University of Wales College of Medicine, Cardiff, UK.

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.**

PMID: 1965710 [PubMed - indexed for MEDLINE]

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.

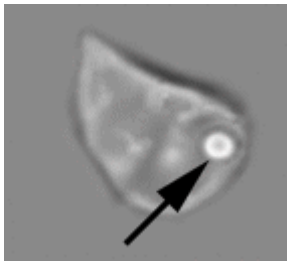
PMID: 3012779 [PubMed - indexed for MEDLINE]

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.

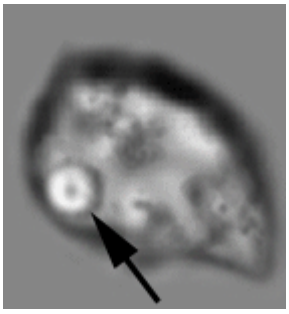
He explains that these high-speed images provide

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

Medscape Medical News

- dietary calcium enhances human resistance to intestinal infection and inhibits enterotoxigenic *Escherichia coli (ETEC)*
- dietary calcium inhibits intestinal colonization and translocation of invasive ***salmonella***
- "To our knowledge, this is the first human infection study showing that dietary calcium supplementation is effective in reducing the severity of ETEC-induced diarrhea. The protective effects of dietary calcium are not restricted to ETEC or *E. coli* infections only," the authors write.

Calcium in Milk Inhibits E. coli-Induced Diarrhea

Gastroenterology. 2003;125:469-476

Laurie Barclay, MD

Sept. 5, 2003 — Calcium in milk enhances human resistance to intestinal infection and inhibits enterotoxigenic *Escherichia coli* (ETEC)-induced diarrhea, according to the results of a rat model *and* parallel, double-blind, placebo-controlled intervention study in humans published in the August issue of *Gastroenterology*.

"In several rat infection experiments, we have shown that dietary calcium inhibits intestinal colonization and translocation of invasive **salmonella**," write Ingeborg M. J. Bovee-Oudenhoven, PhD, from NIZO Food Research in Ede, the Netherlands, and colleagues. "The aim of the present study was to find out whether calcium is also protective against ETEC infection. This was first tested in our rat model and subsequently verified in a human infection study."

Rats received a low- or high-calcium phosphate purified diet and were orally infected with ETEC. In the parallel study, for three weeks, 32 healthy men maintained their usual diet and consumed either regular milk products (calcium supply, 1,100 mg/day) or placebo milk products (calcium supply, 60 mg/day). On day 10, the men ingested a live but attenuated ETEC strain (strain E1392/75-2A) that typically causes mild, transient gastrointestinal symptoms.

In both groups of men, ETEC doubled total fecal output, increased fecal mucin excretion, and reduced mean relative fecal dry weight from 25% to 20%. These fecal parameters were completely normalized on the second day of infection in the high-calcium and on the third day of infection in the placebo group. Similarly, supplemental calcium inhibited ETEC colonization and diarrhea in rats.

"To our knowledge, this is the first human infection study showing that dietary calcium supplementation is effective in reducing the severity of ETEC-induced diarrhea. The protective effects of dietary calcium are not restricted to ETEC or *E. coli* infections only," the authors write. "The combination of a relevant rat infection model for screening purposes and the possibility of verifying subsequently the efficacy in humans might be attractive for functional food development and for supporting health claims."

Reviewed by Gary D. Vogin, MD

High Doses of Calcium May Decrease Severity of E. coli Symptoms

SOURCES: Bovee-Oudenhoven, I. *Gastroenterology*, September 2003; vol 125: pp 469-476.

Sept. 4, 2003 (HealthWire)-- You might want to add a calcium tablet to your next travel kit. A new study shows that calcium -- even in a supplement -- can prevent Montezuma's revenge from ruining your next vacation.

Diarrhea caused by E. coli bacteria is a deadly problem worldwide, particularly in poor countries. People usually contract the bacteria from contaminated drinking water. Tourists traveling to tropical places -- including Mexico, Asia, Africa, and South America -- are among those at high risk.

Now researchers say high does of calcium may prevent the bacteria from multiplying in the intestine, relieving symptoms such as weight loss and diarrhea. The findings are published in the recent issue of Gastroenterology.

During the three-week study, 32 healthy men ate either high-calcium or low-calcium custard. Both desserts tasted the same so volunteers were unaware of who had the higher calcium food. Other dairy products were prohibited during the study.

After 10 days, researchers infected the entire group with a weakened form of toxic E. coli., a kind that causes typical symptoms of Montezuma's revenge -- just less severe. This weakened strain typically causes mild diarrhea for one to three days.

Results showed that both groups had similar diarrhea severity the first day after infection. But the similarities ended there. By day two the high-calcium group recovered completely, whereas the low-calcium group continued to suffer with more diarrhea.

And it looks like a pill might work as well as dairy products to prevent Montezuma's revenge. When researchers gave a calcium supplement to rats and then infected them with E. coli, the calcium largely prevented diarrhea.

Researchers say the findings are promising. They note that the positive effects of dietary calcium are not restricted to Montezuma's revenge from E. coli infections and they are testing calcium for treatment of other forms of bacteria.

Articles by Dr. Royal Lee
***Let's Live Magazine*, 1958**

Ideal Drinking Water

Spring or well water is the best for drinking, preferably a hard water containing calcium ***bic***arbonate (the kind that leaves a calcium deposit in the teakettle.) This kind of calcium is completely assimilated, and builds bone by combining with the organic phosphorus found in cereals and lecithins of natural fats.

Polio, Colds and Fevers

It is this calcium bicarbonate that is essential in the blood stream to prevent our children from becoming susceptible to polio, colds and diseases of childhood which produce fevers. In fact, calcium bicarbonate deficiency alone can cause a child to have recurrent fever, a fever which disappears at once on the administration of calcium lactate or calcium gluconate (which forms calcium bicarbonate after absorption). Such calcium deficiency fevers are common in children during the ages of rapid bone growth, especially where the youngster is getting too much of such cereal foods as oatmeal and processed dry cereals, without enough hard water calcium. The phosphorus of the cereal is out of proportion to the calcium bicarbonate intake.

"Pyrogen" Antibodies

"Good water" is water that has been filtered through the ground to reach the well or spring and has thereby accumulated a load of antigens. These antigens are otherwise known to science as "pyrogens," since they cause fever if injected into the blood stream. They are the residue of disease-producing bacteria, and by drinking them we develop an immunity to the germ or virus that put them into the water. In foreign countries where polio is relatively nonexistent as a known disease, the blood stream of the children has been found loaded with antibodies to polio, which prevented them from contracting the disease. These children were immunized the natural way, not by a shot of Salk vaccine. It is very probable that their diet of unrefined natural foods that promptly supplies the necessary factors to make antibodies was responsible for their freedom from polio.

Boiling of Water

Cooking or boiling water destroys the antigenic effect of the pyrogens, so while boiled water is safe in that it cannot cause infection, it cannot build the real health of the person who needs to accumulate his natural quota for immunities against the prevalent infectious diseases of his community. You may begin to see why the most carefully "protected" children may be the least robust.

Many dentists routinely prescribe calcium lactate tablets for young patients who show soft chalky teeth, are nervous, restless, and unmanageable, as these are all symptoms of the typically calcium-deficient child. These children are worse in summer, for the vitamin D effect of sunlight acts to raise the blood calcium at the expense of the cell fluids, reversing the normal flow of calcium from the blood to the tissues. Natural forms of vitamin D, such as cod liver oil, contain vitamin F as well as the D, the F being the essential partner of the D, causing the diffusion into the cells of the blood calcium. The vitamin D alone acts to load up the blood stream calcium only, and you can realize that a loaded transportation system is no guarantee

of delivery unless some provision exists to unload the commodity at the destination. Butterfat is one good source of vitamin F, but the baby fed on prepared baby foods which have the butterfat removed, and oleo or refined vegetable oil supplied in its place, is the best subject for vitamin F deficiency. Practically all present day baby foods are of this kind.

Fluorides Damage Kidneys

Dr. Clive McCay at Cornell University recently reported that one part per million of sodium fluoride added to the drinking water of rats caused the reversal of the possible evidence of causing a harder tooth enamel, (although probably an abnormal form that is more brittle). He found that in fact it created tooth decay where it otherwise did not exist, and further caused kidney cell breakdown in the older rats. Dr. Alton Oschner, of the celebrated Oschner Clinic of New Orleans, has reported that older persons lose their teeth faster if they get *any* fluorides in their water.

Aluminum Cooking Utensils

You will soon understand how aluminum salts from aluminum cooking utensils may be jeopardizing your health, if you will read *Lee Foundation Report No. 5*, which states in part, "*It is highly probable that a syndrome of symptoms of phosphorus and calcium deficiency can follow a long continued intake of aluminum salts from aluminum cooking utensils, alum baking powders, or aluminum acetate in perspiration deodorants. Aluminum salts appear to rob other food elements of their phosphorus to form insoluble and nutritionally useless compounds, just as mineral oils rob the food of elements and tissues in the intestinal tract of their vitamin A content. Such serious disorders as ulcers of the stomach and duodenum, cardiovascular disease, heart failure, obesity, and varying degrees of paralysis of the sympathetic nervous system appear to be a consequence of aluminum poisoning*."

Check Points for Water

Look to the water you drink and cook with. Does it contain plenty of calcium bicarbonate, with the diet containing the calcium metabolizers, both D and F? Has it been robbed of pyrogens (immunizing mechanisms)? What about fluorides and aluminum salts? There may be other things like copper coils in hot water tanks, a possible source if toxic amounts of copper (when used for cooking purposes) which need to be considered.

Water contains 70% of the weight of the adult body and is one of the most important factors in maintaining equilibrium of the various body systems. The ability to select such food sources as will prevent deficiency disease and poisons is incumbent upon every living being. Any poison added to a food or drink is too much. Like emery powder in a gear box, the damage is proportional to the amount and shortens life accordingly.

Agricultural Research Service
the in-house research arm of the U.S. Department of Agriculture

Protein and Calcium Interplay Important to Bone Health

By Rosalie Marion Bliss
April 24, 2002

It's no secret that the incidence of bone fractures increases among the elderly. **Adding to that concern, some scientists theorize that high-protein diets may leach calcium from bone, itself leading to bone loss. Now scientists funded by the Agricultural Research Service have released a three-year study suggesting that bone mineral density (BMD) may actually benefit from high-protein diets--with one caveat. The high-protein diet must also meet the recommended dietary allowance of calcium and vitamin D.**

The researchers heading the study are with the Calcium and Bone Metabolism Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston, Mass.

The study's design, which looked at 342 men and women older than 65, included prescreening the amount of calcium each consumed. Only those who did not normally consume high amounts of calcium were chosen. In addition, the study included only participants whose baseline BMD measurements proved average for those over 65.

The researchers supplemented half the participants with 500 milligrams (mg) of calcium and 700 International Units of vitamin D daily. The other half were given placebos. Halfway through the study, dietary intakes of protein, calcium, vitamin D and other nutrients were assessed using a self-administered, but staff-monitored, food-frequency questionnaire. The results showed the mean protein intake for all participants was 79 grams per day. For the calcium- and vitamin D-supplemented group, the mean daily calcium intake was 1,346 mg per day. For the placebo group, calcium intake was just 871 mg per day--well below the recommended 1,200 mgs daily for those over 50.

The participants were tested every six months--six times total--for BMD. The study, published in April's *"American Journal of Clinical Nutrition*," showed that a high-protein diet had favorable effects on bone density in the calcium-supplemented group, but not in the unsupplemented group. This suggests that the calcium worked synergistically with the protein to mitigate bone loss. The researchers agree the report proves more research is needed in this area.

ARS is the U.S. Department of Agriculture's chief scientific research agency.

EJCN
European Journal of Clinical Nutrition

European Journal of Clinical Nutrition (2003)
57, 554–565. doi:10.1038/sj.ejcn.1601577

Original Communication

Bone and nutrition in elderly women: protein, energy, and calcium as main determinants of bone mineral density

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[a]*Guarantors:* JZ Ilich and RA Brownbill.

[b]*Contributors:* JZI is a principal investigator and was responsible for analyzing the data and writing the manuscript; RAB was responsible for collecting all data and analyzing nutritional and physical activity; LT was responsible for helping with the study and collecting information on nutritional supplements.

Abstract

Objective: Nutrition is an important factor in the prevention and treatment of osteoporosis. Our goal was to examine the relationship between various nutrients and bone mass of several skeletal sites in elderly women, taking into account possible confounding variables.

Design/methods: A cross-sectional study in 136 healthy Caucasian, postmenopausal women, free of medications known to affect bone was carried out. Bone mineral density (BMD) and body composition (lean and fat tissue) were measured by dual X-ray absorptiometry using specialized software for different skeletal sites. Parathyroid hormone (PTH) and vitamin D, 25(OH)D, as possible confounders, were determined in serum samples. Dietary intake, including all supplements, was assessed by 3-day dietary record and analyzed using Food Processor®.

Past physical activity and present walking were examined as well and accounted for as potential confounders. Simple and multiple regression models were created to assess the relationships between nutrients and BMD. To examine the co-linear variables and their possible independent association with bone, subgroup analyses were performed.

Results: Showed independent influence of calcium, energy, and protein, examined separately and in multiple regression models on BMD of several skeletal sites. Magnesium, zinc and vitamin C were significantly related to BMD of several skeletal sites in multiple regression models (controlled for age, fat and lean tissue, physical activity and energy intake), each contributing more than 1% of variance. Serum PTH and 25(OH)D did not show significant association with bone mass.

Conclusions: Despite the cross-sectional nature of our study we were able to show a significant relationship between BMD and several critical nutrients: energy, protein, calcium, magnesium, zinc and vitamin C. The exact involvement of these nutrients and their clinical significance in bone health need to be further elucidated in humans and conclusions about the effects of a single nutrient on bone mass must be given cautiously, taking into account its interaction and co-linearity with others. Understanding relationships among nutrients, not just limited to calcium and vitamin D, but others that have not been investigated to such extent, is an important step toward identifying preventive measures for bone loss and prevention of osteoporosis.

News-Medical.Net

New and revealing portrait of Yersinia pestis emerges

Posted By: News-Medical in Disease/Infection News

Published: Saturday, 4-Dec-2004

Yersinia pestis, the causative agent of the “Black Death” or “Bubonic” plague, has been a scourge of human civilization. One of the most virulent bacterial pathogens known, killing nearly 90% of those infected, Y. pestis was responsible for three historic pandemics that shattered whole societies. And plague is still with us. In Madagascar, a naturally antibiotic-resistant strain of plague erupted in the 1990s. In western North America, Y. pestis lurks in wild rodent populations. Throughout the world, there is new fear of Y. pestis as a bioterror agent.

Now a new and revealing portrait of this old nemesis is emerging from Lawrence Livermore National Laboratory. Scientists using advanced robotic high-throughput technologies have completed what is believed to be one of the most comprehensive and rapid studies ever of how growth conditions affect the virulence of a deadly bacterium. Ann E. Holtz who works in the laboratory of Sandra McCutchen-Maloney used a battery of phenotype array machines, pre-loaded 96-well plates, and robotic observers to chart the effects of 2,000 nutrients and chemicals, including about 240 different antibiotics, on the viability of Y. pestis under four separate, physiologically relevant growth conditions. Traditional studies of pathogens examined one to two dozen parameters under one or two growth conditions. In contrast the new technologies allowed Livermore researchers, in effect, to conduct 8,000 experiments in about a week.

The picture drawn by Holtz from her high-throughput data shows Y. pestis to be even tougher than suspected under conditions that mimic its life outside the human host, and possibly less vulnerable under human infection conditions to antibiotics currently used to treat plague, such as kanamycin, doxycycline and tetracycline. The researchers caution that these results are preliminary and will require further tests.

High (e.g., human body) temperatures and low calcium levels were known to trigger the virulence of Y. pestis. However an evolutionary challenge for the deadly bacterium is to survive in the hostile biological "climate" of its carrier host, the flea, until it can be transmitted through a fleabite to one of its favorite reproductive hosts, Homo sapiens. Even in the human bloodstream, the bacterium curbs its virulence until it can interact with a target cell where conditions are right to finally unleash its killing powers. Holtz used four conditions as models that mimic the biological conditions when Y. pestis is: (1) located in the flea (low temperature [26º C], high calcium); (2) located in the human bloodstream (high temperature [37º C], high calcium); and (3) interacting with a human cell (high temperature [37º C], low calcium). The fourth condition, low temperature [26º C] and low calcium, was included as a control for full comparison. To see how Y. pestis fared in each condition, microarray wells pre-loaded with each of 2,000 different chemicals were infected with the bacterium and incubated with a marker dye to measure growth. Each well was monitored every 15 minutes for three days by an "Omnilog" robot.

As expected, Y. pestis flourished at the high-temperature, low-calcium growth conditions found inside mammalian cells. The bacterium also had increased resistance to antibiotics in flea-like conditions (low-temperature, high-calcium). Strikingly, at these lower temperatures, the bacterium also resisted many osmotic stressors—salt, phosphates, and urea —that killed it at higher temperatures.

We've always known that the plague-causing bacterium is a formidable enemy, say the Livermore researchers. This new high-throughput format now allows us to study it and other bacterial pathogens in greater depth, under wider conditions and with more speed than ever before. These are encouraging findings, and will help researchers rapidly screen for optimal ways to kill pathogens under human infection conditions.