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Contribution of zinc to reduce $CD4^+$ risk factor for 'severe' infection relapse in aging: parallelism with HIV

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Abstract

Aging and HIV have parallelism in immunodeficiency status because of the appearance of infections or relapse leading to death in both conditions. HIV-RNA is predictor for HIV progression correlated with $CD4^+$ depletion. $CD4^+$ and plasma zinc levels (zincaemia) may be predictors for infections relapse in aging because of zinc relevance for normal immune efficieny against infections and for $CD4^+$ growth. Moreover, zincaemia decreases in aging and infection. A total of 67 elderly subjects affected by infections resistant to antibiotic therapy were recruited. A total of 28 HIV^+ subjects with HAART therapy were also used. $CD4^+$ depletion (507 mm^3) and zincaemia deficiency (76 μg/dl), as compared to $CD4^+$ (700–1100 mm^3) and zincaemia (85–100 μg/dl; age 40–75 years) normal ranges, are possible limits (Cox hazard regression) for severe infections relapse, such as chronic obstructive bronchitis and bronchopneumonia by bacteria or Candida complication, in aging. $CD4^+$ and zincaemia values are within the lower limits of normal range in urinary tract infections. Zincaemia and HIV-RNA or $CD4^+$ are inversely correlated ($r = 0.57$ and $r = 0.72$, respectively) in HIV^+ HAART treated subjects. Consequently there is no appearance of opportunistic infections. Parallelism between aging and HIV may exist because of the resemblance in marked zinc deficiency and $CD4^+$ depletion with high scores in relative risks for severe infections relapse. Supplementing zinc (12 mg Zn^{++}/day) for one month in infected elderly subjects and HAART therapy in HIV^+ subjects reduces risk scores in $CD4^+$ and zincaemia deficiencies for infections relapse, suggesting that the zinc beneficial effect may be independent

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either by HIV-virus or pathogen agents involved. While HAART may reduce the occurrence of opportunistic infections in HIV by means of also major zinc bioavailability, supplementing zinc can be recommended in elderly people as resistence to infections. Since zinc deficiecy is correlated with $CD4^+$ depletion, this latter may also be good diagnostic marker to detect 'clear immunodeficency' in aging, as in HIV condition.

Keywords: Aging; HIV; Zinc; $CD4^+$; HIV-RNA; Infection relapse; Risk factors

1. Introduction

Aging and HIV have parallelism in immunodeficiency status because of the appearance of infections or relapse leading to death in both conditions (Pawelec & Solana, 1997). Proteases inhibitors reduce opportunistc infections (Chaisson & Moore, 1997) because of HIV-RNA relevance for HIV progression as major predictor (Mellors, Rinaldo, Gupta, White, Todd & Kingsley, 1996), other than $CD4^+$ depletion (Levy, 1989). $CD4^+$ count dysregulation causes infections or infections recidive (Pawelec & Solana, 1997). No specific immunological limits under which risk of infection or infection relapse are still available in aging. Together with $CD4^+$, zinc may be a predictor because: (i) zinc is important for immune efficiency against infections (Sugarman, 1983); (ii) zinc is predictor for death in comatose patients for high frequency of infectious episodes (Mocchegiani, Imberti, Testasecca, Zandri, Santarelli & Fabris, 1995a); (iii) zinc deficiency is a cofactor for infections in old age (Chandra, 1989). Finally zinc affects $CD4^+$ maturation and differentiation (Beck, Prasad, Kaplan, Fitzgerald, & Brewer, 1997). Moreover zinc decreases in aging (Fabris, Mocchegiani, Amadio, Zannotti, Licrasto & Franceschi, 1984) and during infections (Mocchegiani Veccia, Ancarani, Scalise, & Fabris, 1995b; Sandstead, 1994; Shankar & Prasad, 1998), whereas $CD4^+$ depletion occurs during infections (Pawelec & Solana, 1997). In turn, infections and infections relapse are constant events in aging (Fabris & Mocchegiani, 1995). Plasma zinc levels (zincaemia) and $CD4^+$ are tested in infected elderly subjects from remission phase as possible limits and, as such, possible risk factors for infections relapse resistant to conventional antibiotic therapy (Lewis & Reeves, 1994). Peripheral T-cells are used to give more complete picture of generalized immunodeficiency in aging, as in canine bronchoalveolar infections (Dirscherl, Beisker, Kremmer, Mihalkov, Voss & Ziesenis, 1995). HIV model with HAART therapy was used to further study the possible parallelism between HIV and aging for infections relapse incidence. Because of zincaemia heterogeneity in aging (Fabris et al., 1984; Prasad, Fitzgerald, Hess, Kaplan, Pelen & Dardenne, 1993) and HIV (Fabris, Mocchegiani, Galli, Irato, Lazzarin & Moroni, 1988; Mocchegiani et al., 1995b), total thymulin (TT) (active zinc-bound ZnFTS + inactive zinc-unbound FTS) and active thymulin (ZnFTS) (AT) ratio, which is the unsaturated fraction of thymulin (FTS) by zinc ions (Fabris et al., 1984) is a good marker to detect real zinc deficiency because of the inverse strict correlation between zincaemia status and the ratio itself (Fabris et al., 1984). Thus, zincaemia was associated with TT/AT ratio. Supplementing zinc from remission phase was carried out in elderly subjects affected by pre-existing chronic

obstructive bronchitis. Relative risk factors were calculated from the remission phase (time 0) of infection to month 4 of observation. An HIV model was used as comparison.

2. Subjects and methods

2.1. Elderly subjects

A total of 48 male and 19 female subjects (63–75 years, mean 69 ± 5.8) admitted to the hospital were recruited in remission phase (time 0) of infection (urinary tract, chronic obstructive bronchitis and bronchopneumonia by bacteria or candida complication). Old patients are divided according to infections. Group A with urinary tract infections (18 subjects, 5 male and 13 female, mean age 70 ± 5 years), group B with chronic obstructive bronchitis (36 subjects, 25 male and 11 female, mean age 67 ± 4 years) and group C with bronchopneumonia by bacteria or candida complication (13 subjects, 10 male and 3 female, mean age 68 ± 3.5 years). Elderly patients with previous infections associated with other pathological conditions were excluded. Conventional antibiotic therapy was used before remission phase. Length of conventional antibiotic treatment was of 12 ± 2.0 and 14 ± 2.7 days before time 0 (Data are the mean number of days of antibiotic treatment) for group A and for groups B and C, respectively, with conventional therapeutic dose dependent by infection severity. No other drugs were administered other than Vitamins B complex in order to maintain a good balance of vitamins B by intestinal bacterial flora, which can be destroyed by antibiotic therapy. Blood withdrawal (time 0) was perfomed in remission phase of infection. The daily diet was similar to healthy old controls. Double-blinded controlled trial with zinc at the dose of 12 mg Zn^{++}/day (USDA, 1976) or placebo (starch containing capsule) was carried out for one month in 15 (13 male and 2 female, mean age 68 ± 3 years) and 14 (11 male and 3 female, mean age 66 ± 2 years) elderly people subjects, respectively, after one week from remission phase of infection (chronic obstructive bronchitis). Relative risk factors as well as possible infection relapse were evaluated from month 0 to 4 of observation. Supplementing zinc was carried out in chronic obstructive bronchitis because of more common infection relapse in aging (Pawelec & Solana, 1997) with consequent major subjects availability.

2.2. HIV^+ subjects

A total of 21 male and 7 female intravenous drug-users HIV^+ subjects (20–36 years, mean 28 ± 5.5) were recruited with $CD4^+$ number between 250 and 400 mm^3 (Stage III of disease) (Murray et al., 1985). Because of HIV-RNA as predictor for HIV progression (Mellors et al., 1996) with HAART to block virus replication (Sepkowitz, 1998), the medical ethic suggested to immediatly perform HAART when $CD4^+ \leqslant 400$–500 mm^3 and HIV-RNA $\geqslant 10{,}000$ copies/ml (Sepkowitz, 1998). No other drugs administered other than HAART [2 nucleoside analogues (AZT + 3TC) and 1 proteases inhibitor (IND or RIT)] at standard doses/day (Sepkowitz, 1998), with compliance of 92%. HIV-RNA, immunological and nutritional parameters were detected at months 0 and 4 after HAART therapy as well as possible opportunistic infections censoring. Relative risk factors were evaluated from month 0 to 4 of observation. The data of

relative risk factors were compared with HIV^+ subjects (18 subjects) with $CD4^+$ between 250 and 400 mm^3 treated with only AZT because of no availability of other antiviral drugs in this retrospective study (Mocchegiani et al., 1995b). The daily diet was similar to young healthy controls. No vegetarian subjects were present. ELISA and Western blot confirmed HIV seropositivity.

2.3. Control groups

Seven men and eight women healthy young subjects (25 ± 3.4 years) admitted to the hospital for minor surgery served as controls. Five men and ten women healthy elderly subjects (67 ± 4.6 years) were recruited in accordance to immunogerontological 'SENIEUR protocol' (Ligthart et al., 1984). Vegetarian subjects were excluded. They were recruited and examined according to the guidelines of Helsinki declaration. The informed consensus for blood withdrawls as well as for zinc trial was obtained.

2.4. Methods

HIV-RNA plasma detection was performed by means of RT-PCR amplification of specific primers (SK38, SK39) (Menzo, Bagnarelli, Giacca, Manzin, Varaldo & Clementi, 1992). TT/AT ratio were tested in the plasma by inhibiting azathioprine-sensitive rosettes assay (Fabris et al., 1984). Zincaemia was tested using A.A.S. (Fabris et al., 1984). $CD4^+$ absolute number was counted with cytofluorimetry (Epics V, Culter, USA) using Leu3a FITC (Becton-Dickinson, USA). Differences were compared using ANOVA (two-way). Cox hazard regression (Matthews & Farewell, 1988) was used to test relative predictors ($CD4^+$ and zincaemia) for infection risks and for possible limits under which rinfections risk may be high. Least-square method and analysis of covariance were used for correlations and to test significance among regression lines, respectively. Differences were significant when $P \leqslant 0.05$.

Table 1
Immunological and nutritional parameters in elderly subjects in remission phase of infection (time 0)[a]

Groups[b]	Ratio T.T./A.T. (log_{-2})	Zincaemia (μg/dl)	CD 4^+ (mm^3)	No of infections/subjects
A	2.0 ± 0.3	83.3 ± 4.0	696 ± 58	18/18
B	$2.5 \pm 0.3^*$	$75.3 \pm 3.2^{**}$	$457 \pm 53^*$	13/13
C	$2.4 \pm 0.2^*$	$76.8 \pm 4.3^{**}$	$460 \pm 66^*$	36/36
D	1.6 ± 0.2	88.5 ± 5.8	877 ± 47	
E	1.1 ± 0.2	120.0 ± 6.5	925 ± 68	

[a] $^*P < 0.01$ and $^{**}P < 0.05$ when compared to group D.

[b] A = Elderly subjects ($n = 18$) with pre-existing infections (urinary tract); B = Elderly subjects ($n = 13$) with pre-existing infections (bronchopneumonia by bacteria or Candida complication); C = Elderly subjects ($n = 36$) with pre-existing infections (Chronic obstructive bronchitis); D = Elderly age-matched healthy controls ($n = 15$) (age 60–73 years); E = Young healthy controls ($n = 15$) (age 20–30 years).

3. Results

3.1. Elderly subjects

TT/AT ratio >2 (Groups B and C) is associated with more marked zinc deficiency as compared to TT/AT ratio (=1.6) and zincaemia values of old healthy controls ($P < 0.01$) (Table 1). The anamnestic analysis at time 0 (remission phase) shows that urinary infections (18/18 patients), bronchopneumonia [by bacteria (8/13 patients) or by Candida complication (5/13 patients)] and chronic obstructive bronchitis (36/36 patients) are pre-existing infections. $CD4^+$ count is reduced (groups B and C) ($P < 0.01$), whereas no difference in group A as compared to old healthy controls (Table 1). $CD4^+$ (437 ± 49 mm^3) and zincaemia (74 ± 3.4 μg/dl) values in old patients affected by bronchopneumonia by Candida complication are not different as compared to those ones by bronchopneumonia by bacteria complication ($CD4^+ = 478 \pm 62$ mm^3; zincaemia $= 76 \pm 3.8$ μg/dl). No differences in $CD4^+$ and zincaemia exist between old patients affected by chronic obstructive bronchitis and by bronchopneumonia (Table 1), as well as between male and female (data not shown). $CD4^+$ and zincaemia values for urinary tract infections are included within the normal range (Table 1). Supplementing zinc for one month from remission phase (time 0) in elderly subjects affected by chronic obstructive bronchitis increases $CD4^+$ cell number (from 462 ± 48 time 0 to 690 ± 41 time 1 month; $P < 0.01$) with significant reduction of infections relapses (2 relapses/15 patients = 13.3%) as compared to placebo group (6 relapses/14 patients = 42.8%) ($P < 0.01$) at month 4 of observation. $CD4^+$ depletion and zinc deficiency in placebo group are relative risk factors for infections relapse with significant scores as compared to old zinc-treated group (Table 2). Moreover, taking into account $CD4^+$ and zincaemia normal ranges (700–1100 mm^3; 20–75 years; 85–100 μg/dl; 40–75 years, respectively) from our laboratory, $CD4^+$ (507 mm^3) (range 454–530) and zincaemia (76 μg/dl) (range 75–91) may be possible limits at borderline for chronic obstrucutive bronchitis infections relapse. When the data of zincaemia from old infected patients before and after zinc supplementation and respective healthy controls are plotted against the corresponding values of $CD4^+$, significant positive inverse correlation is found ($r = 0.81$ $P < 0.01$).

Table 2
Relative risk factors for chronic obstructive bronchitis relapse in elderly subjects treated with zinc and for opportunistic infections in HIV^+ subjects treated with AZT (retrospective study) or HAART

Covariates	Coeff. Regr.	Rel. Risk	*z*-statistic	Coeff. Regr.	Rel. Risk.	*z*-statistic
	Placebo ($n = 14$)			Zinc treatment ($n = 15$)		
$CD4^+$	0.82	2.27	$P < 0.01$	0.36	1.44	$P > 0.05$
zincaemia	0.71	2.03	$P < 0.01$	0.41	1.51	$P > 0.05$
	HIV^+ (AZT treatment) ($n = 18$)			HIV^+ (HAART treatment) ($n = 28$)		
$CD4^+$	0.78	2.18	$P < 0.01$	0.51	1.67	$P > 0.05$
zincaemia	0.85	2.34	$P < 0.01$	0.48	1.62	$P > 0.05$

3.2. HIV^+ subjects

TT/AT ratio > 2 is associated with more marked zinc deficiency at time 0 as compared to day 120 of observation (Table 3). $CD4^+$ and body weight (Δ%) are reduced at time 0 as compared to young healthy controls ($P < 0.001$) (Tables 3 and 1). HAART increases zincaemia, body weight and $CD4^+$ and reduces HIV-RNA (copies/ml) as compared to time 0 ($P < 0.01$) (Table 3). This reduction is also >1 $\log_{10}$. HAART avoids $CD4^+$ depletion and zinc deficiency as relative risk factors for opportunistic infections, as compared to AZT HIV^+ treated subjects (Table 3). HAART induces no appearance of opportunistic infections during the period of observation as compared to AZT treated group (Stage III of disease) of the restrospective study (0 infections vs 13 opportunistic infections among whom, three by Candida) (Mocchegiani et al., 1995b). No difference between male and female and between drug-users and heterosexsuals is found (data not shown). Significant inverse correlations exist between zincaemia and HIV-RNA ($r = 0.57$; $P < 0.05$), between $CD4^+$ and HIV-RNA ($r = 0.72$, $P < 0.01$) and between zincaemia and body weight ($r = 0.76$, $P < 0.01$) in HAART treated HIV^+ subjects.

4. Discussion

$CD4^+$ depletion and marked zinc deficiency are cross-linked in elderly subjects for infections relapse, such as bronchopneumonia and chronic obstructive bronchitis, resistant to conventional antibiotic therapy. HAART induces no appearance of opportunustic infections because of increased $CD4^+$ and zincaemia values, which are both inversely correlated with HIV-RNA. HAART reduces also $CD4^+$ and zincaemia relative risk factors for opportunistic infection incidence. Supplementing zinc in elderly infected subjects restores $CD4^+$ cell number with significant infections relapse reduction, as it occurs in HIV^+ HAART treated subjects. Thus marked zinc deficiency and $CD4^+$ depletion may be considered as probable predictors for infections relapse in aging with HIV parallelism.

$CD4^+$ number is similar in young and old subjects (Cakman Rohwer, Schutz, Kirchner, & Rink, 1996). FasL-apoptosis by bacteria endotoxins may cause $CD4^+$ dysregulation with consequent increased bacteria infections relapse (Pawlec & Solana, 1997; Castro, Bremer,

Table 3
$CD4^+$, zincaemia and HIV-RNA in HIV^+ subjects treated with HAART from day 0–120 of observation[a]

	Ratio TT/AT ($\log_{-2}$)	Zincaemia (μg/dl)	$CD4^+$ (mm^3)	HIV-RNA (copies/ml)	Body weight[b] (Δ%)
t_0	2.33 ± 0.2	78.4 ± 6.4	350 ± 38	28.15 ± 9.36	-1.77 ± 1.23
t_{120}	$0.97 \pm 0.2^*$	$95.6 \pm 5.5^*$	$575 \pm 41^{**}$	$2.49 \pm 1.91^*$	$+0.51 \pm 0.27^*$

[a] $^*P < 0.01$ and $^{**}P < 0.05$ when compared to time 0. No opportunistic infections occur in HIV^+ HAART treated subjects during the period of observation.

[b] The body weight was calculated as individual variance (Δ%) of the body weight from day 0–120 for each patient. The significance of $\Delta\% = \chi^2$ test.

Nobrega, Countinho, & Truffa-Bachi, 1998). Indeed, $CD4^+$ depletion is significant in presence of pre-existing infections (bronchopneumonia and chronic obstructive bronchitis). Such a depletion may be more related to previous infections rather than to conventional antibiotic therapy effect because of the blood withdrawals in remision phase and because of the non-influence of antibiotics (independently by doses used or pathogen agent involved) on altered $CD4^+$ subsets in peripheral blood by adult infected subjects (Kawakami et al., 1997), confirming also, at least, the antibiotic therapy poor efficacy in elderly infected subjects (Lewis & Reeves, 1994). Zincaemia and $CD4^+$ decrease during infections (Pawelec & Solana, 1997; Fabris et al., 1988; Mocchegiani et al., 1995b; Sandstead, 1994), which often are, in turn, age-associated diseases (Fabris & Mocchegiani, 1995). Because of no availability of specific immunological limits under which infection relapse risk may be high in old age, zincaemia and $CD4^+$ may be taken into account because: (i) zinc is important for immune efficiency against infections by virus, fungi and bacteria (Sugarman, 1983); (ii) zinc deficiency is a predictor of death in comatose patients suffering by numerous infectious episodes (Mocchegiani et al., 1995a); (iii) zinc deficiency is a cofactor for infection in aging (Chandra, 1989); (iv) zinc shows an age-related decrease (Fabris et al., 1984) and it is essential for $CD4^+$ maturation and growth (Beck, Prasad, Kaplan, Fitzgerald & Brewer, 1997). Following that, zinc deficiency and $CD4^+$ decrement may be cross-linked with possible limits under which infections relapse risk may be high. Because of no immune differences between chronic obstructive bronchitis and bronchopneumonia, such limits may be the same and both are reduced as compared to $CD4^+$ (Hannet, Erkeller-Yuksel, Lydyard, Deneys, & DeBruyere, 1992) and zincaemia normal ranges (Fabris et al., 1984). $CD4^+$ and zincaemia limits are within the lower limits of normal ranges in urinary tract infections. Thereby, these latter may be considered as 'moderate', whereas the others as 'severe' (Laurence, 1993). Without excluding other T-subsets dysregulations, such as 'memory' and 'naive' T-cells, in aging (Sansoni et al., 1993) and in infections (Pawelec & Solana, 1997), because of significant inverse correlation between $CD4^+$ and zincaemia in remissed elderly infected subjects, the determination of $CD4^+$ may also be good diagnostic marker to detect 'clear immunodeficiency' in aging, because of good $CD4^+$ number maintenance (700 mm^3) for good health (successful of aging) in centenarians (Sansoni et al., 1993).

The parallelism between aging and HIV may exist because, other than similar T-cell dysregulations (Ullum, Lepri, Victor, Skinhoj, Phillips & Pedersen, 1997), strong $CD4^+$ depletion associated with marked zinc-deficiency causes the appearance of 'severe' opportunistic infections, including Candida aesophagea, in HIV^+ subjects (Murray et al., 1985; Mocchegiani et al., 1995b). The pathogen agent involved is quite different between HIV and aging. Because of the lack of difference in $CD4^+$ depletion between bronchopneumonia by Candida and bacteria complication while, one hand possible different immune responses by different pathogen agents may be avoided (Belkaid et al., 1994); on the other hand, the parallelism with HIV in $CD4^+$ depletion for 'severe' infections may be further supported. Indeed supplementing zinc reduces infections relapse with low scores in $CD4^+$ and zincaemia relative risk factors in elderly infected subjects, as it occurs in HIV^+ zinc-treated subiects (Mocchegiani et al., 1995b). This suggests that zinc may induce resistence against infections (Shankar & Prasad, 1998). On the other hand resistence to respiratory and urinary infections in instituzionalized elderly subjects (Girodon et al., 1997) and in Down's syndrome (Licastro et

al., 1994) is observed during supplementing zinc. In addition, benefit of physiological zinc against gram-negative infections has been reported (Wellinghausen et al., 1996). A further evidence of zinc deficiency relevance for infections incidence comes from the AZT poor efficacy, as compared to HAART (Hazura & Kuo, 1997), in HIV condition. Indeed, HAART increases $CD4^+$ cells number and zincaemia values, which are both inversely correlated with HIV-RNA, and, consequently, there is no appearance of opportunistic infections. The disappearance of $CD4^+$ depletion and zinc deficiency as possible relative risk factors for opportunistic infections exclusively during HAART therapy (Table 2), is in line with this interpretation. The HAART effect may also occur by means of major zinc bioavailability due or to decreased acute inflammation (Chandra, 1992) or to better zinc intestinal absorption, as shown by body weight increments. Because of the absence of zinc in HAART drugs (Veccia, unpublished observation), these findings together with the effect of zinc supplementation in elderly subjects (in the present study) and in HIV^+ subjects (Mocchegiani et al., 1995b), while one hand suggest the real efficacy of HAART, on the other hand pin-point zinc as beneficial against 'severe' infections, whose effect may be independent either by HIV-virus or by other pathogen agents involved. Moreover, such an effect may be more addressed to the extracellular matrix because of the disappearance of Candida aesophagea relapse in HIV^+ zinc-treated subjects (Mocchegiani et al., 1995b). While HAART reduces infections occurrence in HIV by means of also major zinc bioavailability, this latter may be acquired by zinc supplementation in aging. Other micronutrients or vitamins deficits have been suggested as infection predictors (Chandra, 1989). Without excluding that, Vitamin A supplementation has been found deleterious on immune response in elderly people, whereas zinc prevents infections (Fortes et al., 1998)—further supporting, this latter, the relevance of zinc deficiency in infected elderly patients during the remission phase. Intestinal malabsorption (Lee, Prasad, Hydrick-Adair, Brewer, & Johnson, 1993) or inflammation (Chandra, 1992) may cause zinc loss in aging. Whatever causes, low zinc bioavailability is associated with $CD4^+$ depletion, despite zinc content into young and old human lymphocytes has been found similar (Bunker, Hinks, Lawson, & Clayton, 1984), whereas it is different for others (Prasad et al., 1993). The free quota of zinc available is more relevant because zinc into lymphocytes (Yurkow & Makhijani, 1998) is more bound with zinc-binding metallothioneins, which are increased in aging (Mocchegiani, Muzzioli, Cipriano & Giacconi, 1998) and in infections (Sobocinski, Canterbury, Mapes, & Dinterman, 1995). Since Atomic Absorption Spectrophotometry (AAS) detects zinc-bound and zinc-unbound, the measure of zinc into lymphocytes may, therefore, result controversial and, at least, also misleading. Other zinc deficiency markers, such as ecto-5 nucleotidase, are useful (Prasad et al., 1993). However, TT/AT ratio purchases peculiar role to test real zinc deficiency because TT/AT ratio detects zinc ions bioavailability due to strict inverse correlation between zincaemia status and ratio itself. Moreover TT/AT ratio > 2 ($\log_{-2}$) is always associated with more marked zinc deficiency, as compared to TT/AT ratio $= 1$ ($\log_{-2}$) in presence of normal plasma zinc values (Fabris et al., 1984). Thus the low zinc ions bioavailability is evident in aging leading to $CD4^+$ depletion and to the appearance of infections relapses. Thereby, the cross-linking between zinc deficiency and $CD4^+$ depletion in aging may be probable relative risk factor avoided by supplementing zinc.

In conclusion, present data, despite obtained in limited number of subjects, may suggest parallelism between aging and HIV because of the resemblance in $CD4^+$ depletion and zinc

deficiecy as relative risk factors for 'severe' infections relapse. Because of $CD4^+$ apoptosis involvement in both conditions (Castro et al., 1998; Algeciras, Dockrell, Lynch, & Paya, 1998), and, in turn, apoptosis may be directly (Sunderman, 1995) or indirectly (Shankar & Prasad, 1998) prevented by zinc, the resemblance may be further supported. New concepts on cause/s of 'immunosenescence' (Pawlec & Solana, 1997) may be thereby added with an emphasis on zinc.

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References

Algeciras, A., Dockrell, D. H., Lynch, D. H., & Paya, C. V. (1998). CD4 regulates susceptibility to Fas-ligand and tumour necrosis factor-mediated apoptosis. *Journal of Experimental Medicine*, *187,* 711–720.

Beck, F. W., Prasad, A. S., Kaplan, J., Fitzgerald, J. T., & Brewer, G. J. (1997). Changes in cytokine production and T-cell subpopulation in experimentally induced zinc-deficient human. *American Journal of Physiology*, *272,* E1002–E1007.

Belkaid, Y., Bouckson, V., Colle, J. H., Goossens, P., Lebastard, M., Leclercq, V., Marchal, G., Montixi, C., & Milon, G. (1994). Transient inducible events in different tissues: in situ studies in the context of development and expression of the immune response to intracellular pathogens. *Immunobiology*, *191,* 413–423.

Bunker, V. W., Hinks, L. J., Lawson, M. S., & Clayton, B. E. (1984). Assessment of zinc and copper status of healthy elderly people using metabolic balance studies and measurements of leucocytes concentrations. *American Journal of Clinical Nutrition*, *40,* 1096–1102.

Cakman, I., Rohwer, J., Schutz, R. M., Kirchner, H., & Rink, L. (1996). Dysregulation of TH1 and TH2 T cell subpopulation in elderly. *Mechanisms of Ageing and Development*, *87,* 197–209.

Castro, A., Bremer, V., Nobrega, A., Coutinho, A., & Truffa-Bachi, P. (1998). Administration to mouse of endotoxin gram negative bacteria leads to activation of apoptosis of T-lymphocytes. *European Journal of Immunology*, *28,* 488–495.

Chaisson, R. E., & Moore, R. D. (1997). Prevention of opportunistic infections in the era of improved antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndrome Retrovirology*, *1,* S14–S22.

Chandra, R. K. (1989). Nutritional regulation of immunity and risk of infection in old age. *Immunology*, *67,* 141–147.

Chandra, R. K. (1992). Nutrition and immunoregulation. Significance for host resistance to tumours and infectious diseases in human and rodents. *Journal of Nutrition*, *122,* 754–757.

Dirscherl, P., Beisker, W., Kremmer, E., Mihalkov, A., Voss, C., & Ziesenis, A. (1995). Immunophenotyping of canine bronchoalveolar and peripheral blood lymphocytes. *Veterinary Immunology and Immunopathology*, *48,* 1–10.

Fabris, N., & Mocchegiani, E. (1995). Zinc, human diseases and aging. A review. *Aging Clinical and Experimental Research*, *7,* 77–93.

Fabris, N., Mocchegiani, E., Amadio, L., Zannotti, M., Licastro, F., & Franceschi, C. (1984). Thymic hormone deficiency in normal aging and Down's syndrome: is there a primary failure of the thymus? *Lancet*, *1,* 983–986.

Fabris, N., Mocchegiani, E., Galli, M., Irato, L., Lazzarin, A., & Moroni, M. (1988). AIDS, zinc deficiency and thymic hormone failure. *Journal of American Medical Association*, *259,* 839–840.

Fortes, C., Forastiere, F., Agabiti, N., Fano, V., Pacifici, R., Virgili, F., Piras, G., Guidi, L., Bartoloni, C., Tricceri, A., Zuccaro, P., Ebrahim, S., & Perucci, G. A. (1998). The effect of zinc and vitamin A supplementation on immune response in an older population. *Journal of American Geriatric Society*, *46,* 19–26.

Girodon, F., Lombard, N., Galan, P., Brunet-Lecomte, P., Monget, A. L., Arnaud, J., Preziosi, P., & Hercberg, S. (1997). Effect of micronutrient supplementation on infection in institutionalized elderly subjects: a controlled trial. *Annals of Nutrition and Metabolism*, *41,* 98–107.

Hannet, I., Erkeller-Yuksel, F., Lydyard, P., Deneys, V., & DeBruyere, M. (1992). Developmental and maturational changes in human blood lymphocytes subpopulation. *Immunology Today*, *131,* 215–218.

Hazura, D., & Kuo, L. (1997). Failure of AZT: a molecular perspective. *Nature Medicine*, *3,* 836–837.

Kawakami, K., Kadota, J., Iida, K., Fujii, T., Shirai, R., Matsubara, Y., & Kohno, S. (1997). Phenotypic characterization of T-cells in bronchoalveolar lavage fluid (BALF) and peripheral blood of patients with diffuse panbronchiolitis: the importance of cytotoxic T-cells. *Clinical and Experimental Immunology*, *107,* 410–416.

Laurence, J. (1993). T-cell subsets in health, infectious diseases and idiophatic $CD4^+$T lymphocytopenia. *Annals of Internal Medicine*, *119,* 55–62.

Lee, D. Y., Prasad, A. S., Hydrick-Adair, C., Brewer, G., & Johnson, P. E. (1993). Homeostasis of zinc in marginal human zinc deficiency: role of absorption and endogenous excretion of zinc. *Journal of Laboratory and Clinical Medicine*, *122,* 549–556.

Levy, J. A. (1989). Human immunodeficiency virus and the pathogenesis of AIDS. *Journal of American Medical Association*, *261,* 2997–3006.

Lewis, D. A., & Reeves, D. S. (1994). Antibiotic at the extremes of age: choices and constraints. *Journal of Antimicrobiology and Chemotherapy*, *34,* 11–18.

Licastro, F., Chiricolo, M., Mocchegiani, E., Fabris, N., Zannotti, M., Beltrandi, E., Mancini, R., Parente, R., Arena, G., & Masi, M. (1994). Oral zinc supplementation in Down's syndrome subjects decreases infections and normalizes some humoral and cellular immune parameters. *Journal of Intellectually and Disability Research*, *38,* 149–162.

Ligthart, G. J., Coberand, J. X., Fournier, C., Galanaud, P., Hijmans, W., Kennes, B., Muller-Hermelink, H. K., & Steinmann, G. G. (1984). Admission criteria for immunogerentological studies in man: the senior protocol. *Mechanisms of Ageing and Development*, *28,* 47–55.

Matthews, D. E., & Farewell, V. T. (1988). *Using and understanding medical statistics*. Basel, Switzerland: Karger.

Mellors, J. W., Rinaldo Jr, C. R., Gupta, P., White, R. M., Todd, J. A., & Kingsley, L. A. (1996). Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science*, *272,* 1167–1170.

Menzo, S., Bagnarelli, P., Giacca, M., Manzin, A., Varaldo, P. E., & Clementi, M. (1992). Absolute quantitation of viremia in human immunodeficiency virus infection by competitive reverse transcription and polymerase chain reaction. *Journal of Clinical Microbiology*, *30,* 1752–1757.

Mocchegiani, E., Imberti, R., Testasecca, D., Zandri, M., Santarelli, L., & Fabris, N. (1995a). Thyroid and thymic endocrine function and survival in severely traumatized patients with or without injury. *Intensive Care Medicine*, *21,* 334–341.

Mocchegiani, E., Veccia, S., Ancarani, F., Scalise, G., & Fabris, N. (1995b). Benefit of oral zinc supplementation as an adjunct to zidovudine (AZT) therapy against some opportunistic infections in AIDS. *International Journal of Immunopharmacology*, *17,* 719–727.

Mocchegiani, E., Muzzioli, M., Cipriano, C., & Giacconi, R. (1998). Zinc, T-cell pathways, aging: role of metallothioneins. *Mechanisms of Aging and Development*, *106,* 183–204.

Murray, H. W., Hillman, J. K., Rubin, B. Y., Kelly, C. D., Jacobs, J. L., Taylor, L. W., Donelly, D. M., Carriero, S. M., Godbold, J. H., & Roberts, R. B. (1985). Patients at risk for AIDS-related opportunistic infections. Clinical and impaired gamma interferon production. *New England Journal of Medicine*, *313,* 1504–1510.

Pawelec, G., & Solana, R. (1997). Immunosenescence. *Immunology Today*, *18,* 514–516.

Prasad, A. S., Fitzgerald, J. T., Hess, J. W., Kaplan, J., Pelen, F., & Dardenne, M. (1993). Zinc deficiency in elderly people. *Nutrition*, *9,* 218–224.

Sandstead, H. H. (1994). Understanding zinc: recent observations and interpretations. *Journal of Laboratory and Clinical Medicine*, *124,* 322–327.

Sansoni, P., Cossarizza, A., Brianti, V., Fagnoni, F., Snelli, G., Monti, D., Marcato, A., Passeri, G., Ortolani, G., Forti, E., Fagiolo, U., Passeri, M., & Franceschi, C. (1993). Lymphocytes subsets and natural killer activity in healthy old people and centenarians. *Blood*, *82,* 2767–2773.

Sepkowitz, K. A. (1998). Effect of HAART on natural history of AIDS-related opportunistic disorders. *Lancet*, *351,* 228–230.

Shankar, A. H., & Prasad, A. S. (1998). Zinc and immune function: the biological basis of altered resistance to infection. *American Journal of Clinical Nutrition*, *68,* 447–463.

Sobocinski, P. Z., Canterbury Jr, W. J., Mapes, C. A., & Dinterman, R. E. (1978). Involvement of hepatic metallothioneins in hypozincaemia associated with bacterial infection. *American Journal of Physiology*, *234,* E399–E406.

Sugarman, B. (1983). Zinc and infection. *Review of Infectious Diseases*, *5,* 137–147.

Sunderman Jr, F. W. (1995). The influence of zinc on apoptosis. *Annals of Clinical and Laboratory Science*, *25,* 134–142.

U.S. Recommended Daily Allowance (USDA) for Dietary Intakes of Minerals (1976). *Federal Register*, *41*, 46172.

Ullum, H., Lepri, A. C., Victor, J., Skinhoj, P., Phillips, A. N., & Pedersen, B. K. (1997). Increased losses of CD^{4+} $CD45RA^{+}$ cells in the late stage of HIV infection is related to increased risk of death: evidence from a cohort of 347 HIV-infected individuals. *AIDS*, *11,* 1479–1485.

Wellinghausen, N., Schromm, A. B., Seydel, U., Branderburg, K., Luhm, J., Kirchner, H., & Rink, L. (1996). Zinc enhances lipopolysaccaride-induced monokine secretion by alteration of fluidity state of lipopolysaccaride. *Journal of Immunology*, *157,* 3139–3145.

Yurkow, E. J., & Makhijani, P. R. (1998). Flow cytometric determination of metallothionein levels in human peripheral blood lymphocytes: utility in environmental exposure assessment. *Journal of Toxicology and Environmental Health*, *54,* 445–457.