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## **Zinc, Immune Plasticity, Aging, and Successful Aging: Role of Metallothionein**

[Strategies for Engineered Negligible Senescence: Why Genuine Control of Aging May Be Foreseeable: Part III. The Immune System]

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### Abstract

The capacity of the remodeling immune responses during stress (immune plasticity) is fundamental to reach successful aging. We herein report two pivotal models to demonstrate the relevance of the immune plasticity in aging and successful aging. One model is represented by the circadian rhythms of immune responses; the other one is the immune responses during partial hepatectomy/liver regeneration (pHx). The latter is suggestive because it mimics the immunosenescence and chronic inflammation 48 hours after partial hepatectomy in the young through the continuous production of IL-6, which is the main cause of immune plasticity lack in aging. The constant production of IL-6 leads to abnormal increments of zinc-bound metallothionein (MT), which is, in turn, unable in zinc release in aging. As a consequence, low zinc ion bioavailability appears for thymic and extrathymic immune efficiency, in particular, of liver NKT cells bearing TCR  $\gamma\delta$ . The remodeling during the circadian cycle and during pHx of zinc-bound MT confers the immune plasticity of liver NKT  $[\gamma][\delta]$  cells and NK cells in young and very old age, not in old age. Therefore, zinc-bound MT homeostasis is crucial in conferring liver immune plasticity with subsequent successful aging.

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## INTRODUCTION

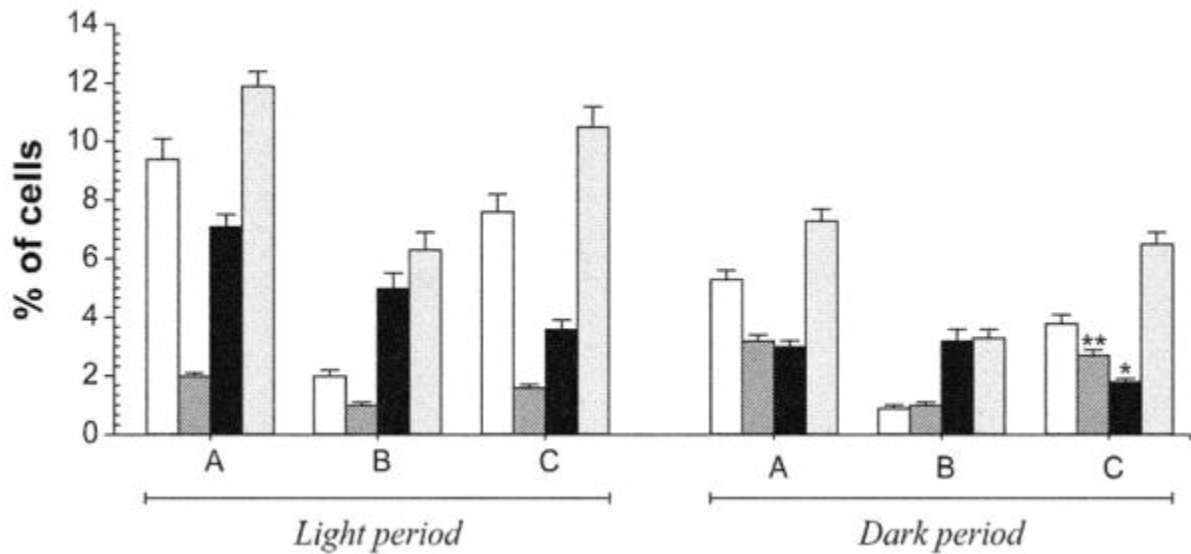
Immune plasticity is a condition "sine qua non" for healthy aging. The absence of the plasticity leads the organism to be a "low responder" to oxidative stress with subsequent appearance of age-related diseases. The remodeling of the immune system to various harmful stimuli allows a prompt immune response and the organism becomes a "high responder." Therefore, the capacity for remodeling can be considered the plasticity of the immune system against oxidative stress. The lack of this capacity leads the cells of the immune system to undergo cell death or necrosis triggered by oxidative stress.<sup>1</sup> Such a plasticity is a common event in young-adult age during transient stress-like conditions. During aging, the capacity of the remodeling of the immune system is very limited because the stress-like condition is chronic.<sup>2</sup> This phenomenon allows reduced immune responses to oxidative stress and a low cellular capacity in DNA repair.<sup>3</sup> As a consequence, the risk of the appearance of age-related diseases, that is, cancer and infections, is high.<sup>3</sup> On the other hand, the "free radical theory," which takes into account the production of free radicals by oxidative stress, is the more common theory of the aging process.<sup>4</sup> The molecular basis of the absence of immune plasticity in aging is poorly understood, and, at the same time, was also poorly studied 10 years ago when the scientific community had seen many centenarians among elderly people. Indeed, healthy centenarians differ from "normal" aged individuals for their optimal metabolic compensation and immune response and for the ability to efficiently counter the alteration of the oxidative status typical of aging. In this context, various hypotheses have been proposed to reach successful aging. Limited inflammation, higher homing of stem cells to substitute the damaged cells, an increased capacity in DNA repair, and, finally, a major genomic integrity are characteristics of oldest individuals.<sup>5</sup> However, the capacity of the remodeling of the immune system also can be pivotal in these exceptional individuals and, as such, an improved immune plasticity. In this context,

the role played by zinc and metallothioneins (MTs) may be crucial for the following reasons. First, zinc is a trace element indispensable for the efficiency of the immune system in both thymic and extrathymic T cell pathways, and this latter is fundamental to compensate thymic failure in aging.<sup>2</sup> Second, MT is relevant in zinc sequestering and in zinc release for the immune efficiency during transient stress. The zinc release by MT does not occur in aging because stress-like condition is chronic, leading to low zinc ion bioavailability for immune efficiency and for zinc-dependent biological functions, such as enzyme antioxidant activity and DNA repair.<sup>6</sup> Third, the gene expression of MT is induced by proinflammatory cytokines (IL-1, IL-6, and TNF-[alpha]) during inflammation.<sup>7</sup> The increment of these cytokines in aging leads to abnormal increase of MT coupled with low zinc ion bioavailability and impaired immune response.<sup>6</sup> Consistent with these findings, zinc and MT homeostasis is crucial in conferring immune plasticity during aging taking also into account that satisfactory zinc ion bioavailability is observed in centenarians.<sup>1</sup> In this article, two relevant models are reported to demonstrate the relevance of the immune plasticity in aging: the variations of the immune functions (1) during the circadian cycle and (2) during the compensatory liver growth after partial hepatectomy. The choice of these two models is based by previous findings showing the impact that thymic circadian variation <sup>8</sup> and the liver extrathymic T cell pathway <sup>1</sup> have in the economy of the immune response in aging and successful aging. In addition, the model of young partial hepatectomy/liver regeneration is very interesting because, other than a good model for the study of acute and chronic inflammation, it mimics the aging process in thymic failure and in impaired peripheral immune efficiency at 48 hours after partial hepatectomy in young pHx mice.<sup>9</sup> Young, old, and very old mice were used in both models. A parallelism with elderly, nonagenarians, and old infected patients is reported.

## **IMMUNE PLASTICITY: MODEL OF THE CIRCADIAN CYCLE.**

Young mice display fluctuating variations in plasma zinc and in thymic endocrine activity during the circadian cycle with nocturnal peaks.<sup>8</sup> In contrast, no significant variations occur in old mice during the circadian cycle with an absence of nocturnal peaks.<sup>8</sup> This absence also is observed in peripheral immune efficiency. In particular, the low natural killer (NK) cell activity observed in old mice during the light period also are maintained during the dark with no significant variations during the whole circadian cycle.<sup>1,8</sup> Such a defect in old mice is closely related to the appearance of age-related diseases (cancer and infection) and subsequent death.<sup>8</sup> Conversely, immune peripheral variations occur in young-adult mice coupled with the capacity of young mice to respond to external antigenic stimuli and, subsequently, in avoiding diseases triggered by the oxidative stress. Indeed, an interleukin, such as IL-2 that is relevant for NK cell activity, displays nocturnal peaks in young mice.<sup>1</sup> Nocturnal peaks of thymic and peripheral immune functions also occur in very old mice.<sup>1</sup> These findings are clear evidence that the immune variations during the circadian cycle are fundamental in maintaining the immune efficiency and plasticity, which are indispensable for health longevity.<sup>1</sup> In this context, an interesting aspect of the immune system, that is, the liver extrathymic T cell pathway deputed to compensate the thymic failure in aging,<sup>2</sup> shows variations during the circadian cycle in young and very old mice, but not in old ones.<sup>1</sup> The liver NKT cells bearing TCR [alpha][beta] or [gamma][delta] play an intriguing role. These cells are the first sentinels for the host defense against viruses and bacteria because secreting IL-2 and IFN-[gamma], which, in turn, affect the activity of classic NK cells.<sup>10</sup> These particular liver NKT cells display a circadian rhythm in young and very old mice with significant modifications between the light and dark period. In particular, the number of NKT [gamma][delta] cells increases in young and very old mice during the dark, whereas it

remains unmodified in old mice. The number of NKT  $\alpha\beta$  cells displays an opposite trend with a decrement in young, old, and very old mice during the dark as compared with the light period ([Fig. 1](#)). These findings suggest that NKT  $\gamma\delta$  cells may be more involved in the maintenance of liver extrathymic immune plasticity during aging leading to successful aging. This maintenance may be because of a better preservation by cell death of NKT  $\gamma\delta$  cells than  $\alpha\beta$  because of low Fas expression (CD95) in NKT  $\gamma\delta$  cells in oldest individuals.<sup>12</sup> On the other hand, a significant decrement in liver NKT cells expressing Fas (CD95) occurs in very old mice in the dark as compared with old mice during the same period ([Fig. 1](#)). In contrast, old mice display low numbers of NKT  $\gamma\delta$  cells for the whole circadian cycle with no fluctuations ([Fig. 1](#)), impaired NKT  $\gamma\delta$  cell cytotoxicity, or decreased production of IL-2 and IFN- $\gamma$  in comparison with very old mice.<sup>1</sup> Similar phenomena occur in centenarians. Although no data are available during the dark in centenarians but exclusively in the light period, the major preservation of NKT  $\gamma\delta$  cells in centenarians <sup>12</sup> is also coupled with satisfactory NKT cell cytotoxicity <sup>13</sup> and IL-2 production.<sup>11</sup> Thus, the functionality and the number of these cells, in particular, of liver origin, are pivotal to reach successful aging because some age-related diseases, such as infections, might be avoided. Indeed, old infected patients display a still lower number of NKT  $\gamma\delta$  cells than elderly,<sup>11</sup> giving further support to the relevance of liver T $\gamma\delta$  cells for host defense against viruses and bacteria.<sup>10</sup>

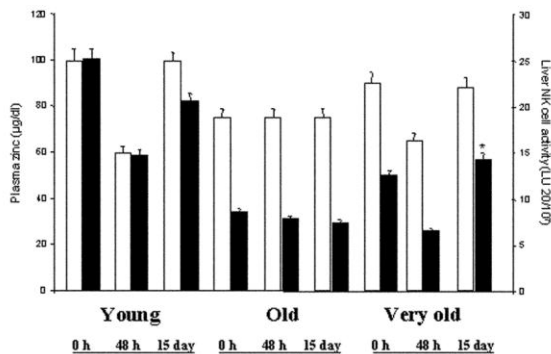


**FIGURE 1.** Percentage of NKT cells (NK 1.1+ CD3+) (gray bars), NKT [alpha][beta] (white bars), and [gamma][delta] (striped bars) cells and NKT cells expressing Fas (CD95) (black bars) in young (A), old (B), and very old mice (C) during the circadian cycle. \* $P < .01$  in comparison with B mice in the dark; \*\* $P < .05$  in comparison with B mice in the dark. (E. Mocchegiani, unpublished results.)

## IMMUNE PLASTICITY: MODEL OF THE PARTIAL HEPATECTOMY/LIVER REGENERATION<sup>9</sup>

Partial hepatectomy/liver regeneration (pHx) is a good model for the study, other than the liver regeneration, of acute and chronic inflammation in aging because of the likeness with aging in impaired thymic endocrine activity, low zinc ion bioavailability, and peripheral immune efficiency (NK cell activity and IL-2 production) in young pHx mice at 48 hours after pHx.<sup>2,9</sup> A complete remodeling of zinc ion bioavailability and immune efficiency, however, occurs in the late period of compensatory liver growth (7th and 15th day) in young pHx mice. In contrast, no remodeling occurs in old mice displaying the same low zinc ion bioavailability and impaired immune functions for the whole period of the compensatory liver growth (time 0, 48 hours, 7th and 15th day).<sup>9</sup> These findings are intriguing because they suggest that pHx is also a good model to show the immune plasticity and, at the same time, the relevance of this plasticity in liver extrathymic T cell pathway during aging. This assumption is supported by

the fact that very old pHx mice show the same pattern in zinc ion bioavailability and liver NKT cell activity observed in young pHx mice (Fig. 2). In other words, both zinc ion bioavailability and liver NKT cell activity are not lost during the compensatory liver growth in very old mice, but a remodeling occurs in the late period of the liver regeneration (15th day), as occurring in young pHx mice (Fig. 2). These findings in very old mice, whereas on one hand demonstrate the presence of the immune plasticity in very old age, on the other hand they pinpoint that very old mice are still capable of responding to a great inflammation, such as partial hepatectomy, with a remodeling of the liver immune efficiency.



**FIGURE 2.** Plasma zinc ( $\mu\text{g}/\text{dL}$ ) (white bars) and liver NKT cell activity (L.U.  $20/10^7$ ) (black bars) during the compensatory liver growth after partial hepatectomy in young, old, and very old mice. (0 h = sham controls). \* $P < .01$  as compared with old (15th day). (Redrawn from Cipriano *et al.*<sup>14</sup> with permission.)

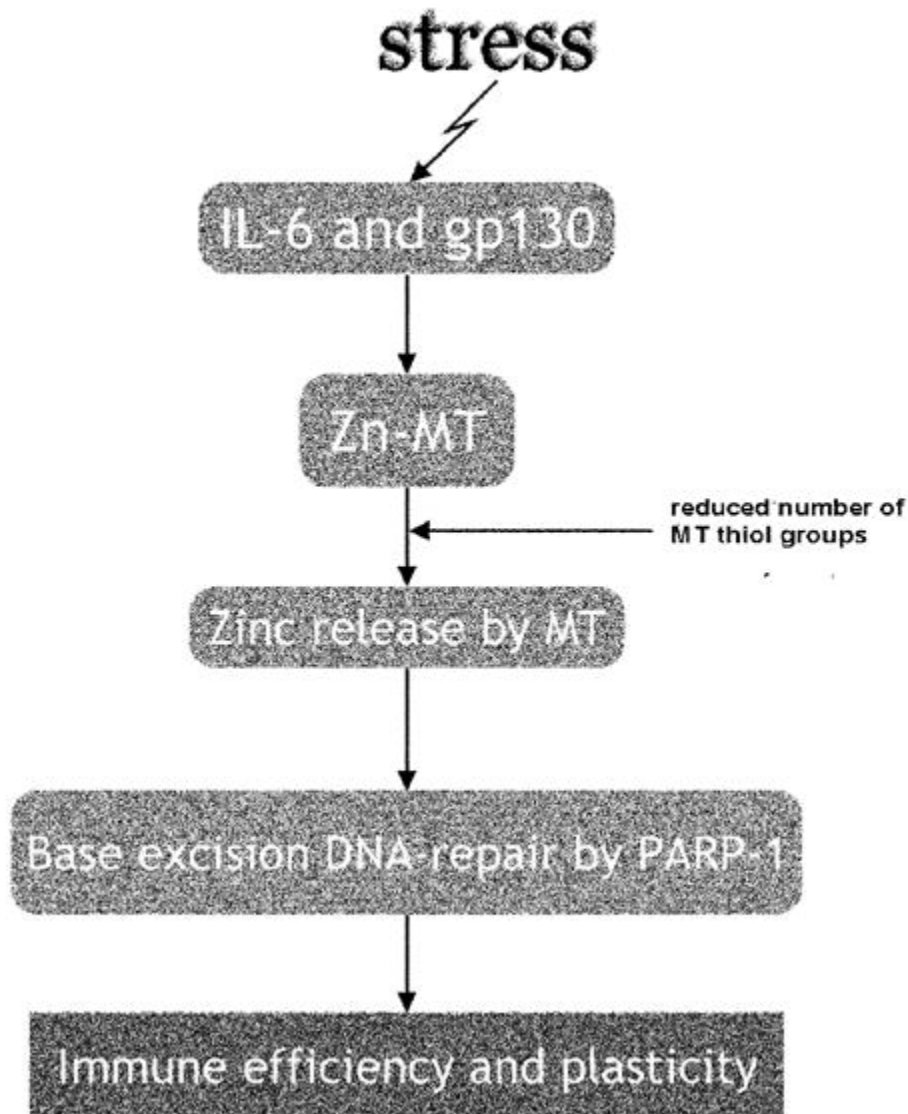
This fact is very important in the oldest individuals because it means that many age-related diseases may be avoided. As a consequence, very old individuals become "high responders" to oxidative stress, as occurring in the young.<sup>1</sup> Indeed, the lack of response to a great inflammation (such as partial hepatectomy) in old age provokes a shorter survival in old pHx mice in comparison with old sham controls.<sup>14</sup> Indeed, old pHx mice display a greater incidence of cancer and infections.<sup>14</sup> Thus, a good functioning of liver immune plasticity is pivotal to reach successful aging.

## MECHANISMS OF ACTION IN THE MAINTENANCE OF THE IMMUNE PLASTICITY

It has been demonstrated that the zinc ion bioavailability is fundamental for the efficiency of the immune system.<sup>2</sup> The loss of zinc ions by intestinal malabsorption or by reduced food intake provokes a zinc deficiency with damage in cell-mediated immunity, including thymic efficiency, NK cell activity, and cytokine production. In particular, anti-inflammatory cytokines, such as IL-2, IL-12, IFN-[alpha], IFN-[gamma], decrease whereas proinflammatory cytokines, such as TNF-[alpha], IL-1, IL-6, increase.<sup>2</sup> In this context, zinc more affects the cytokine production by Th1 than Th2 cells.<sup>2</sup> That zinc has a beneficial effect on IFN-[alpha] production by Th1 cells is supported by the recent discovery in virus transfected cells showing a protein Staf-50 involved in a new family of IFN-[alpha] production that contains two zinc finger motifs.<sup>15</sup> This fact suggests an unbalance of Th1/Th2 paradigm during zinc deficiency toward Th2 cytokine production,<sup>2</sup> which leads to the induction of some proteins deputed in fighting the oxidative stress. In this context, MT plays a pivotal role because it sequesters and dispenses zinc. MT acts as antioxidant against wide spectrum of stressor agents, because the zinc-sulfur cluster is sensitive to changes of cellular redox state and oxidizing sites in MT (reduced number of thiol groups) induce the transfer of zinc from its binding sites in MT to those of lower affinity in other proteins.<sup>16</sup> This transfer occurs in conferring biological activity to antioxidant metalloenzymes, such as superoxide dismutase,<sup>6</sup> in the base excision DNA repair by PARP-1, in the genomic stability by telomerases, and, finally, in conferring directly or indirectly, via zinc finger motifs, the immune efficiency <sup>6</sup> (Fig. 3). Therefore, the redox properties of MT and their effect on zinc in the clusters are crucial for the biological functions of MT. Indeed, MT is peculiar in cellular proliferation and in protecting cells against cytotoxic effects of reactive oxygen species, ionizing radiations, electrophilic anticancer drugs, and mutagens and heavy metals.<sup>2</sup> A peculiar role of MT is played during partial hepatectomy/liver regeneration,

with a strong MT induction that is useful, other than in facilitating the liver regeneration by various hepatocyte growth factors, in protecting the cells by the inflammation after partial hepatectomy. It has been shown that high MT, either as gene expression or protein, is present in young pHx mice at 48 h from pHx coupled with low zinc ion bioavailability, high IL-6, and impaired thymic and extrathymic T cell pathways.<sup>9,14</sup> A complete downregulation of MT and IL-6 followed by a restoration of the immune efficiency occurs in the late period of the compensatory liver growth (7th and 15th day from pHx).<sup>14</sup> In contrast, the high MT and IL-6 gene expressions as well as the low zinc ion bioavailability and the impaired immune functions, already present in old mice, are not modified during the liver regeneration in old pHx mice.<sup>14</sup> An intriguing aspect is the complete remodeling of MT, zinc ion bioavailability, and immune function in very old pHx mice at 7th and 15th day from partial hepatectomy.<sup>14</sup> These findings further demonstrate that MT is not protective against chronic inflammation, like in aging, because it is unable to release zinc, whereas its protective role occurs during young-adult age.<sup>1</sup> Therefore, MT turns from role of protection in young age to harmful one in aging because of its inability in zinc release.<sup>1,6</sup> This phenomenon in aging provokes low zinc ion bioavailability for zinc-dependent enzyme antioxidant activity, for base excision DNA repair by PARP-1, and for thymic and extrathymic T cell pathways. Therefore, MT plays a pivotal role in zinc turnover in aging and consequently in conferring the immune plasticity.<sup>6</sup> Such an assumption is supported during the circadian cycle in which the high nocturnal peaks of zinc and immune efficiency observed in young and very old mice are related to low MT either as gene expression or as protein.<sup>1</sup> No circadian variation of MT occurs in old mice.<sup>1</sup> In addition, low MT gene expression and good zinc ion bioavailability also are observed in lymphocytes from centenarians.<sup>1</sup> This phenomenon of MT in regulating zinc turnover is closely dependent by the inflammatory status, in particular, by the gene

expression and induction of proinflammatory cytokines, such as IL-6, and of its subunit receptor gp130. As IL-6 and gp130 are constantly high in aging,<sup>2</sup> this fact leads to continuous increase of MT followed by the stealing of intracellular zinc ions and no subsequent zinc release by MT. As a consequence, low zinc ion bioavailability appears in the maintenance of the immune plasticity in aging.<sup>1,2,6</sup> It is not a simple coincidence that both very old mice and centenarians display low gp130 despite IL-6 being high.<sup>11</sup> This fact leads to low MT induction, good zinc ion bioavailability, satisfactory immune efficiency, and an increased capacity in base excision DNA repair by PARP-1 in very old age.<sup>1,14</sup> In contrast, high MT, IL-6, and gp130 coupled with reduced capacity in base excision DNA repair are present in elderly and in old infected patients.<sup>11</sup> In these latter, alterations in DNA repair and in MT are still more severe. Indeed, abnormal high expression of MT is an index of unfavorable prognosis in cancer and infections.<sup>2,11</sup> Therefore, zinc-bound MT homeostasis, via IL-6 and gp130, is a fundamental mechanism in conferring immune plasticity to reach successful aging. In conclusion, MT can be considered a potential biological and genetic marker of immunosenescence upstream affecting functional biochemical cascade involved in the maintenance of the immune plasticity, in particular, liver NKT gd cells, with subsequent successful aging.



**FIGURE 3.** Schematic mechanism of action in conferring immune efficiency and plasticity in young and very old age involving the interrelationships among stress, IL-6, and MT (for explanations, see text).

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Keywords: NKT cells; NKT [alpha][beta] and [gamma][delta] cells; zinc; metallothionein; IL-6; gp130; PARP-1; immune plasticity; liver extrathymic T cell pathway; circadian cycle; partial hepatectomy; liver regeneration; aging; successful aging

### **Section Description**

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