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Somatotrope GHRH/GH/IGF-1 axis at the crossroads between immunosenescence and frailty

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Immunosenescence, characterized by complex modifications of immunity with age, could be related to frailty syndrome in elderly individuals, leading to an inadequate response to minimal aggression. Functional decline (i.e., the loss of ability to perform activities of daily living) is related to frailty and decreased physiological reserves and is a frequent outcome of hospitalization in older patients. Links between immunosenescence and frailty have been explored and 20 immunological parameters, including insulin-like growth factor-1 (IGF-1), thymopoeisis, and telomere length, were shown to be affected in elderly patients with functional decline. A strong relationship between IGF-1 and thymic ouput was evidenced. IGF-1, a mediator of growth hormone (GH), was subsequently shown to induce interleukin-7 secretion in cultured primary human thymic epithelial cells. We are exploring the stress hypothesis in which an acute stressor is used as the discriminator of frailty susceptibility. GH can counteract the deleterious immunosuppressive effects of stress-induced steroids. Under nonstress conditions, the immunosenescent system preserves physiological responses, while under stress conditions, the combination of immunosenescence and a defect in the somatotrope axis might lead to functional decline.

Keywords: immunosenescence; aging; frailty; growth-hormone-releasing hormone; GHRH; growth hormone; insulin-like growth factor-1; IGF-1

Immunosenescence

Immunosenescence is characterized by a panel of changes in immune function associated with aging and has been shown to contribute to the higher incidence of infectious diseases, inflammatory disorders, cancer, and poorer responses to vaccines in older compared to younger adults.[1–4] Most studies on human immunosenescence are cross-sectional and face the challenge of comparing different young and aged populations, and there are very few longitudinal observations. Moreover, investigation of the specificity of immune alterations with age in humans is susceptible to several biases, such as the selection bias of healthy elderly individuals in the SENIEUR protocol and supercentenarians, who reflect a survivor selection, or biases related to the changes in life conditions between the mid-20th century and the present day.[5,6] However, some consensual observations have emerged about aging immunity that are especially relevant for the T cell compartment. Cell-mediated immunity is predominantly affected by aging and a decline in cell-mediated immunity has been attributed to thymic involution.[3,7] While thymopoiesis persists until 60 years of age, its progressive decline and the decreased generation of T cell receptor (TCR) diversity are not sufficient to renew the total percentage of naive T cells in the periphery.[8] Hallmarks of immunosenescence include expansion of the T cells bearing a memory phenotype,[9] together

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with an attrition of TCR diversity.[10] These two events have been attributed to nonexclusive phenomena of recurrent and consecutive stimulation of T cell clones filling the immune space and a loss of T cell renewal from the shrinking aged thymus. Common features include a decrease in the CD4:CD8 ratio, with CD4 lymphopenia describing the immune risk profile.[11] Immunosenescence is not only characterized by changes in immune cell proportion, but also by functional alterations, such as, notably, the increase in $CD28^-$ cells; CD28 is the ligand of CD80/86 expressed by antigen presenting cells, and the loss of CD28 makes T cells unable to respond to antigen presentation.[12] In very old age, there is a shift in the T helper (T_H)1/T_H2 cytokine profile toward a T_H2 dominance.[13,14] Nevertheless, a proinflammatory cytokine profile in old age has also been reported, which initiates the inflammaging paradigm.[15–17] Since the concept emerged in the late 20th century, at least five causes for this low-grade chronic inflammation have been identified: (1) self-debris, (2) harmful products from gut microbiota and mitochondria, (3) senescent cells producing proinflammatory cytokines, (4) increased activation of the coagulation system, and (5) an increase in innate immunity.[18] Interestingly, the inflammatory cytokine interleukin (IL)-6—used as a biomarker of inflammation, found at high levels in the elderly, and associated with risk of morbidity—has been shown to decrease circulating insulin-like growth factor-1 (IGF-1) levels, which has anti-inflammatory effects.[19,20]

Immunological parameters as biomarkers of frailty

Functional decline (FD) frequently occurs in older patients after hospitalization and is associated with not only illness severity but also the patient's premorbid frailty status.[21] FD is defined as a loss of autonomy in carrying out activities of daily living (ADL; e.g., walking, dressing). Several authors have suggested that the extent of FD after acute stress reflects the level of frailty, defined as "the inability to withstand acute illness without loss of function."[22,23] Identification of patients at risk for FD is important as geriatric intervention may prevent or limit these losses. Several clinical tools have therefore been developed to assess this risk,[24–26] but their predictive performance is limited.[27] Combining clinical and biological markers has been suggested as a way to improve this prediction.[28] Nevertheless, little is known about the precise biological mechanisms underlying frailty and FD. Immunosenescence and telomere shortening have been proposed as significant candidates.[29]

On the basis of the assumption that immunological parameters delineating immunosenescence could be reliable biomarkers to diagnose and predict frailty status, a previous research program investigated over 600 potential immune-related markers in four well-defined elderly cohorts: community dwelling (as an example of robust subjects) and three acutely stressed clinic populations with hip fracture, acute heart failure, or documented infection.[30–34] These studies examined the relationship between immune parameters measured at emergency admission and FD, defined as a loss of at least one point on the ADL scale[35] between baseline level (2 weeks before admission) and 3-month post-discharge functional status. Among 20 statistically significant associations identified were high plasma IL-6, low plasma IGF-1, shorter peripheral blood mononuclear cell (PBMC) telomere length, and lower levels of PBMC TCR rearrangement excision circles (TRECs) levels.

Immunological biomarkers of frailty

Ongoing thymopoiesis provides new T cells with stochastic TCR gene segment rearrangements. During the rearrangement process, episomal DNA fragments (TRECs) are produced.[36] TRECs are stable molecules that persist in peripheral T cells until mitosis, but they are not replicated and therefore dilute with cell proliferation. It has been demonstrated that TREC frequency decreases with age, while the ratio of late-to-early TRECs (sj/Dβ TRECs) reflects intrathymic pre-T cell proliferation.[36–39]

Antigen encounter in the periphery leads to massive T cell proliferation, and the number of possible T cell divisions is tightly correlated to the length of telomeric DNA. Given the repeated stimulation of antigen-specific T cells throughout life, there is risk of telomere erosion affecting global immune function.[40,41] An association between telomere length and functional level has been suggested,[42] since telomere attrition is associated with various age-related diseases,[43,44] as well as with physical and emotional burdens[45] that predispose to frailty.

IGF-1 is a growth-promoting factor regulating cellular survival, proliferation, and differentiation.[46] Mainly produced by the liver under the control of growth hormone (GH), IGF-1 and GH are involved in several immune functions, especially T cell proliferation and thymic function.[47] Interestingly, GH has been shown to increase IL-6 production in aging animals,[48] while high levels of IL-6 and low levels of IGF-1 both appear in the frail population of subjects reported in previous studies.

GH and thymic function in adults with GH deficiency

The concomitant low levels of TRECs and IGF-1 in frail subjects strongly support the hypothesis that the somatotrope axis could be involved in decreased thymic function, thereby leading to T cell replacement under a threshold where adaptive immune system homeostasis is compromised. As a consequence, physiological stress encounters insufficient immune responses and the subject is unable to deliver an optimal response, which is a definition of frailty. To examine this issue, a study in patients with adult growth hormone deficiency (AGHD) assessed plasmatic sjTREC frequency, sj/b TREC ratio, and IGF-1 concentrations.[37] All subjects were asked to stop GH treatment for 1 month before resuming it, and samples were collected before the arrest, 1 month after withdrawal, and 1 month after resumption. It was found that plasma sjTREC frequency decreased after 1 month without GH and was restored after resumption. Moreover, plasma sjTREC frequency was highly correlated with IGF-1 concentration (Fig. 1). Intrathymic T cell proliferation was also reduced after GH withdrawal, as indicated by the reduced sj/Dβ TREC ratio. From this study, we concluded that the somatotrope GH/IGF-1 axis is involved in the maintenance of normal thymus function in human adults.

Somatotrope axis and thymic function

Hypophysectomy was already shown in 1930 to induce thymus involution.[49] Forty years later, GH antiserum was shown to induce thymus atrophy.[50] If somatotrope axis impairment seemed to drive thymic dysfunction, GH supplementation also appeared to restore normal thymus function, as implantation of GH-secreting cells reverses thymic aging in rats,[51] and GH administration improves thymic cellularity and thymic T cell proliferation in dwarf DW/J mice that lack GH and prolactin.[52] IGF-1, the main mediator of GH, intimately modulates the thymic homing of T cell precursors, thymopoiesis, and trafficking of thymocytes in the thymus microenvironment, and is also implicated in several peripheral immune functions.[53–56] More recently, ghrelin, a GH secretagogue, was shown to significantly improve thymopoiesis in old mice, as revealed by the increased number of recent thymic emigrants and TCR diversity of the peripheral T cell repertoire.[57]

Among others, these important observations promoted clinical studies using GH supplementation to improve thymopoeisis in immunodeficient

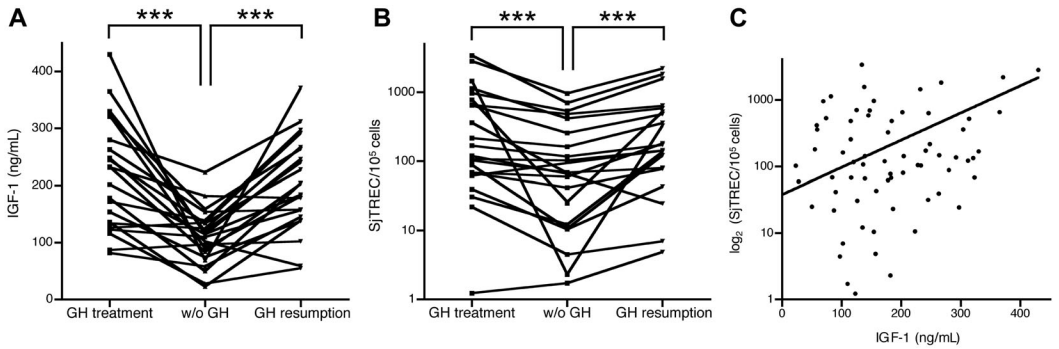

Figure 1. Plasma IGF-1 concentration and sjTREC frequency in PBMCs from patients with GH deficiency and following GH treatment. (A) The interruption of GH treatment for 1 month induced a significant decrease in blood IGF-1 and sjTREC levels. (B) Both parameters were restored to initial levels 1 month after GH resumption. $^{***}P < 0.001$ (by Wilcoxon's signed rank test, $N = 22$). As shown in C, there is a significant positive correlation between blood IGF-1 levels and sjTREC frequencies ($R = 0.61$, $P < 0.01$ by Spearman's analysis). Adapted, with permission, from Morrhaye *et al.*[37]

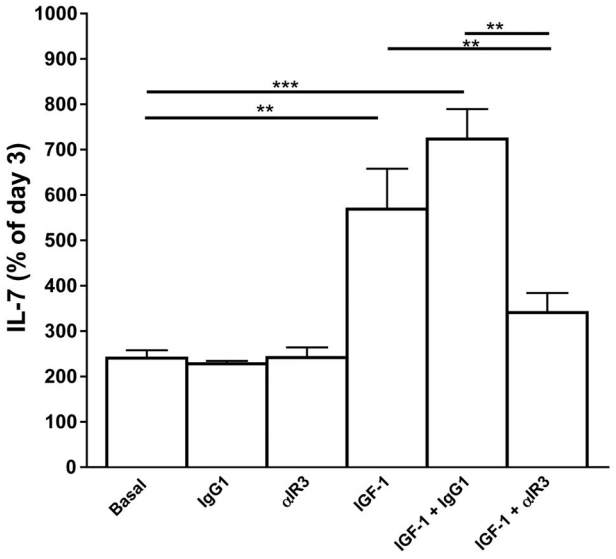

Figure 2. IL-7 secretion by human TECs under IGF-1 stimulation. Results are expressed as percentage of day-3 culture IL-7 supernatant concentration considered as basal levels. IGF-1 (10 nM) treatment significantly increased IL-7 secretion by cultured human TECs on day 8 of culture versus control medium, IgG1 (650 ng/mL) and αIR3 (650 ng/mL). This effect was inhibited by anti-IGF-1R αIR3 (650 ng/mL). $^{**}P < 0.01$; $^{***}P < 0.001$ (by Wilcoxon's signed rank test, $N = 6$). Adapted, with permission, from Goffinet *et al.*[60]

subjects. For example, GH treatment was evaluated to enhance immune reconstitution in HIV patients under highly active antiretroviral therapy (HAART). A first pilot study showed that GH supplementation reverses thymic atrophy of HAART-treated HIV-infected patients and enhances circulating naive CD4 T cells.[58] A prospective randomized study confirmed these data and further evidenced that GH strongly increases the number of circulating sjTRECs in PBMCs.[59]

Given that TREC formation is due to recombinase-activating gene (RAG) activity and that RAG is strongly induced by IL-7, the relationship between IGF-1 and IL-7 production by thymic epithelial cells (TECs) was explored *in vitro*.[60] IGF-1 induces strong expression and release of IL-7, which can be completely counteracted by blocking antibody to IGF-1R (Fig. 2). Interestingly, physiological concentrations of GH do not induce any significant production of IGF-1 by TECs, suggesting that the increase of IL-7 production by human TECs is essentially mediated by peripheral IGF-1.[61]

Limits of immunological biomarkers in the prognosis of frailty and FD

Using a combination of proinflammatory and hormonal biomarkers (IL-6 and IGF-1) with a clinical screening tool has been shown to improve the accuracy of FD prediction 3 months after hospitalization.[34] The association of IL-6, IGF-1, TREC frequency, and telomere length with clinical frailty scoring using the predictive tool SHERPA[26] first led to an improvement in the area under the receiver operating characteristic (ROC) curve from 70% to more than 84%. However, relaxing the inclusion criteria to include more individuals in larger cohorts (i.e., hospitalized instead of emergency patients) did not confirm that the selected biomarkers improved the clinical SHERPA score. Several biomarkers still correlated with the SHERPA score without improving evaluation of the prognosis.

In reexamining the difference between old and new cohorts, a hypothesis emerged: an acute stress might be the crucial factor for immune biomarkers of frailty to show their significance. Indeed, the first cohort of patients in earlier studies[30–34] was recruited in the emergency department at admission, while the second cohort stayed in the more comfortable geriatric hospital department. This hypothesis is closely related to the stress hypothesis (Fig. 3) proposed by Dorshkind and Horseman,[62] who suggest that GH exerts a minimal effect on thymic function and the T cell system under basal conditions, but is able to successfully counteract the

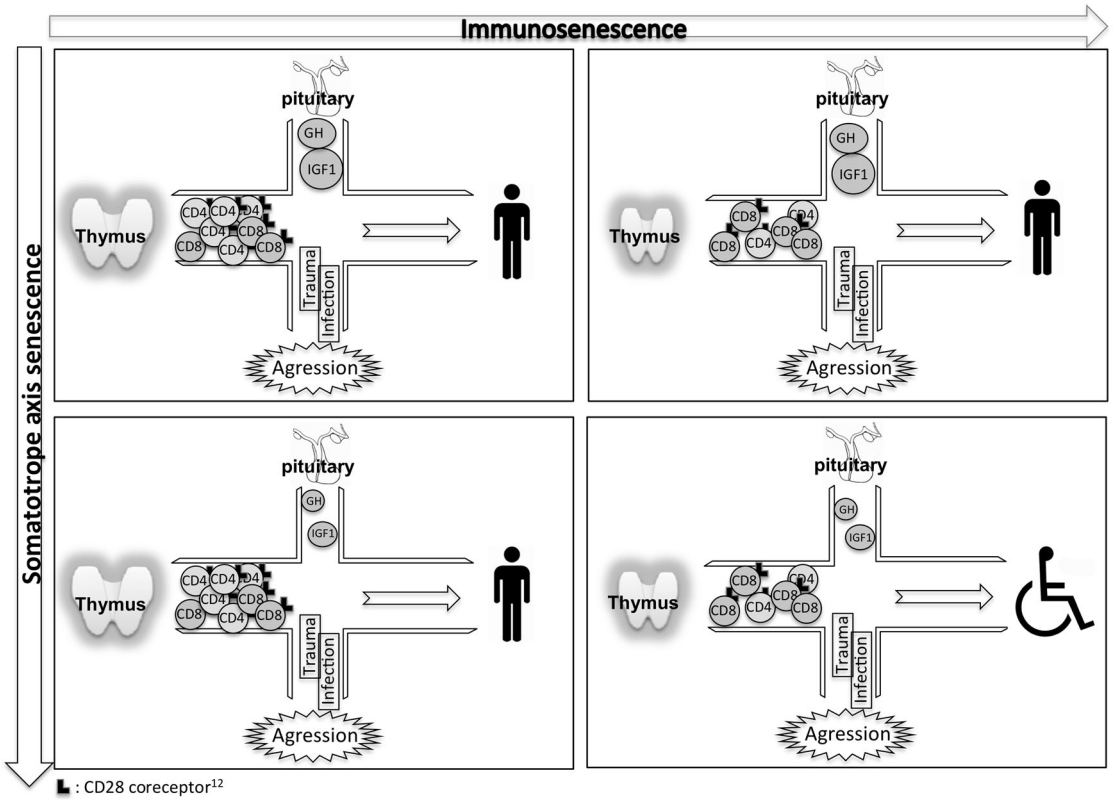

Figure 3. Combined senescence of the immune system and somatotrope axis causes frailty. Upper left: young and mature subjects have functional thymuses producing abundant new $CD28^+$ T cells and normal levels of GH and its mediator IGF-1. External aggression, such as infection and trauma, is overthrown and subjects fully recover. Upper right and lower left: either thymus or somatotrope axis is senescent. Loss of $CD28^+$ T cells and a decreased CD4:CD8 ratio result from an involutive thymus (upper right) or low GH and IGF-1 concentrations (lower left). Nevertheless, aggression is still defeated without harmful consequences. Lower right: both the somatotrope axis and immune responses are deficient. Stress from aggression leads to a functional decline in these frail individuals.

deleterious effects of stress mediated by corticoids on thymic and effective T cells.[63]

Ongoing studies

We are currently exploring this frailty-related stress hypothesis both in animals and humans. In these ongoing studies, a model of transgenic mice deficient in hypothalamic growth hormone–releasing hormone (GHRH)[64] is used to assess the effect of somatotrope axis hormones on immune function and responses. Preliminary results showed no evident defects in parameters of thymus and immune system function under basal conditions, except for a limited B cell lymphopenia.[65] Moreover, the dwarf phenotype of this model has been shown to be partially reverted by GH supplementation,[66] which can be tested for the effect of induced stress on immune system and thymic function. Recently, this model of $Ghr^{-/-}$ mice was shown to be resistant to experimental allergic encephalomyelitis while becoming sensible after GH supplementation.[67]

We are also beginning new studies in a population of aged family caregivers intended to explore the immunological biomarkers identified in previous studies. The subjects are potentially stressed or nonstressed, depending on, for example, the social situation, and will be tested for psychological and physiological parameters. This specific population has already been noted for showing telomere erosion and compromised immunity,[45] and telomere shortening was one of our potential frailty biomarkers. Examining aged caregivers for physiological status and selected biomarkers with regard to their stress situation might help to solve

the contradiction related to the pertinent frailty markers seen in previous studies without extension to a larger elderly population.

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Conflict of interest

The authors declare no conflicts of interest.

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