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Immunotherapy of Immunosenesence; Who, How and When?

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Abstract: Major changes in social welfare, economic conditions and medical knowledge over the last 60 years have produced a demographic shift in the population. More individuals are living longer, and in a decade there will be more people over 65 than infants under 5 for the first time in history. Taking the analysis beyond mere numbers reveals that older individuals are now physically more active than their forebears and travel more widely. This provides a greater opportunity for encountering infectious agents which could present a considerable problem. Older individuals are more susceptible to infection and do not respond as well as younger people to vaccination because of an age related decline in immunity, a state which has been termed immunosenesence. This decline is not uniform and some older individuals show a greater decline in their immune response than others. In this review we have sought to consider who are the 'at risk' individuals, how they might best be treated and when.

Keywords: Immunosenescence, rejuvenation, reversion, healthy ageing, frailty.

INTRODUCTION

The world is going grey. In less than ten years there will be more individuals over the age of 65 than children under the age of 5, for the first time in history. A recent report revealed that the estimated increase in the population over 65 between July 2007 and July 2008 was more than 10.4 million people, an average of 870,000 each month [1]. If we restrict our view to Europe the shift in the demographic profile of the population in the last 60 years has been considerable. In 1950, young people outnumbered older individuals by a ratio of more than 3:1, with about 143 million people under the age of 14 and almost 45 million people over the age of 65. Medical advances and the improvement in social conditions in Europe have resulted in a steady increase in lifespan which has almost doubled in the last 160 years [2]. This, in conjunction with the decline in the birth rate, has produced a population whose demographic profile is skewed towards older individuals. Within the next 40 years the European Rural Development Project [3] suggests that the ratio of young: old (under 14:over 65) will have altered to 1:2 in 2050 (compared to 3:1 in 1950), and those over the age of 65 will constitute almost 28% of the overall population in Europe.

The optimism created by an increased life expectancy is countered by the reality of the health care burden produced by an ageing population. Human ageing is not only inextricably linked with a plethora of co-morbid conditions but is also associated with diminished resistance to common infections, as a consequence of immunosenescence. Immunosenescence has been defined as "the age-associated decrease in immune competence that renders individuals more susceptible to diseases and increases morbidity and mortality due to infections and a variety of other age-related pathologies" [4]. If we want to couple a longer lifespan with healthy ageing, it is imperative to be able to identify the degree of susceptibility, and also to develop intervention strategies to reduce the effects of immunosenescence. The focus of this review, is to explore the means by which we could identify those individuals "Who" are at risk of accelerated immunosenescence, "How" intervention could be administered and "When" would be an appropriate time of intervention.

WHO?

Epidemiological surveys and clinical observations reveal an increase in the prevalence and severity of infection with advancing age [5,6], whilst laboratory tests confirm a decline in immune function. Clinicians recognise that older individuals often have difficulty in dealing with previously encountered pathogens resulting in the reactivation of otherwise latent pathogens such as the herpes viruses, herpes zoster [7] and cytomegalovirus [8]. Furthermore, increases in the prevalence of infection in the elderly range from three-fold for community acquired pneumonia to twenty-fold for urinary tract infections. There is also an increased frequency of nosocomial bacterial infections, in part due to greater institutionalisation rates [9]. The increased susceptibility of older individuals to infection is coupled with progression to more severe illness rather than recovery. For example, in the young infection with influenza is typically followed by a contagious illness of 1-2 weeks duration with full recovery, but in the elderly infection is often associated with considerable morbidity and mortality [5]. For influenza, vaccination with inactivated virus is the most common approach to pre-

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vention, with an annual review of the antigenic composition of the vaccine to match the most prevalent strains [6,10]. However the efficacy of the vaccine, defined as the percentage reduction in attack rates between vaccinated and unvaccinated populations, is affected by age. Efficacy of the influenza vaccine is expected to be between 70-90% in young individuals but is considerably reduced to between 30-40% in those over the age of 65 [6, 11-13]. A similar story exists with Hepatitis B where an antibody titre of anti-HBs level $\geqslant$10 IU/L following vaccination provides protection [14]. Comparing efficacy of hepatitis B vaccination between healthy elderly individuals (average age 74) versus young control group (average age 28), all of the young individuals developed a protective response, compared with only 42% of the elderly cohort [15]. Failure of the immune system to provide protection to the body is not only related to factors such as co-morbid conditions, nutritional status (nutrients and/or protein-energy malnutrition and obesity) and hormonal pathway dysregulation, but also age itself.

As described above, vaccination studies reveal that older individuals, even healthy ones, are more prone to develop vaccine preventable diseases such as influenza, hepatitis B and pneumonia, and this cannot be ascribed to reduced vaccination coverage [9,16] or vaccine inadequacy. The most likely, and currently most recognised cause of vaccine failure, is dysfunctional immunity in the elderly. The lifelong reshaping and adaptation of the immune system in response to a plethora of pathogenic challenges, as well as cellular and molecular changes, are believed to result in this dysfunctional immunity. A further factor may be chronic low-grade inflammation in the elderly due to co-morbid conditions. The degree to which these changes occur and when they occur within an individual's lifetime is poorly understood. Therefore the manifestation of dysfunctional immunity or immunosenescence between individuals will vary considerably, as will the response to immune intervention. Hence measurements are needed to provide an estimation of an individual's degree of immune competency or incompetency. Owing to the complexity of factors giving rise to immunosenescence it is unlikely that a single predictor will be adequate. Instead, a selection of parameters could provide an evaluation of an individual's immunosenescence status, against which the efficacy of immune intervention therapies could be tested.

Assessing Immunosenescence

A number of studies have described methods for identifying and measuring immune competency in older individuals. The earliest arose following the need to classify elderly subjects "Senior Europeans" or "SENIEUR Protocol" for strict admission criteria, in order to make meaningful laboratory cross-comparisons in the evaluation of healthy aged people and immune parameters. Consideration of patient medical conditions and on-going prophylactic intervention resulted in a further classification of so-called "non-SENIEURs" individuals or frail elderly [17]. As the vast majority of elderly fall into the latter group, and these are the individuals that would benefit most from immune intervention therapies, further characterization of these individuals could help identify at-risk groups and intervention strategies.

Immune Risk Phenotype and Inflamm-aging

The pioneering OCTO and NONA studies were aimed at identifying factors predicting 2, 4 and 6 year mortality rates and have resulted in the emerging concept of an Immune Risk Profile (IRP) [18-20]. The IRP defined from healthy octogenarians and nonagenarians, characteristically display (i) high levels of $CD8^+$ and low level of $CD4^+$ T-cells (an inverted $CD4^+$:$CD8^+$ ratio), (ii) an increase in the number of dysfunctional terminally differentiated memory T-cells ($CD8^+CD28^-$) and (iii) the depletion in the number of naïve T and B-cells, as well as (iv) cytomegalovirus (CMV) seropositivity and large increases in the number of $CD8^+CD28^-CD57^+$ T-cells, known to be associated with CMV status [21].

The complex remodeling of the immune system during ageing includes a profound modification within the cytokine network. The typical features of this phenomenon are a general increase in plasma cytokine levels and a chronic low-grade pro-inflammatory condition named inflamm-aging [22,23]. This results due to the shift from a $CD4^+$ T-helper cell TH1-like cytokine response to a TH2-like response, and furthermore from an increase in levels of pro-inflammatory cytokines (i.e. interleukin-6 (IL-6), Tumor necrosis factor (TNFα), as well as IL-1β, IL-18, and IL-12). A wide range of other factors have also been claimed to contribute to this low-grade inflammatory state, including increased amount of fat tissue, decreased production of sex steroid and chronic co-morbid conditions (e.g. diabetes and chronic heart disease) [4]. In addition, this altered inflammatory response has also been attributed to continuous exposure to external stressors such as chronic CMV antigen stimulation, and/or reactive oxygen species, leading to a progressive activation of macrophages and related cells in most organs and tissues of the body [21]. This is likely to be the most important cause of inflamm-aging that, in association with the genetic background of the individual, potentially triggers the onset of the most important age-related diseases, such as atherosclerosis, metabolic syndrome, insulin-resistance and diabetes mellitus, Alzheimer's disease, arthritis, osteoporosis, and two complex age-associated syndromes that are sarcopenia and frailty [16,24].

Thymic Output

One of the major features of human immunosenescence is involution of the thymus which is the key site for T cell development and maturation [25]. Thymic atrophy is characterized by a progressive, age-related size reduction in and profound changes in its anatomy caused by the loss of thymic epithelial cells and replacement of thymocytes with adipocytes. The resulting impairment in thymopoiesis is a key contributory factor in the reduction of naïve T-cells in aged individuals [4,26]. Studies tracking changes in thymic output have attempted to establish the number of circulating naïve cells and thereby provide an assessment of immune status. This was first achieved in immunocompromised HIV patients undergoing highly active antiretroviral therapy (HAART) [27], using an excisional by-product of T cell receptor (TCR) gene rearrangement, known as TREC (T-cell receptor excision circle) [28]. TREC estimation has subsequently been utilized not only as a measure of biological

ageing but also as a tool for predicting immune senescence status within the ageing population [26, 29].

The immunological parameters identified from the pioneering Swedish OCTO and NONA longitudinal studies provide a robust set of criteria for the determination of an individual's immunological status. However, these criteria may require fine tuning, since the increased mortality risk profile exhibited in individuals 65 and over does not necessarily translate through to individuals assessed in their mid 50's [18]. Therefore further studies are required with a broader demographic and a wider geographical net in order to discover biomarkers that are identifiable earlier in life, so that intervention strategies can be administered sooner rather than later.

HOW?

Since thymic atrophy is a preceding event in all cases of immunosenescence, different ways have been investigated to rejuvenate the peripheral T cell pool, to delay or even reverse the senescence of the immune system caused by thymic atrophy and immunosenescence. The vast majority of these studies have been performed in mouse models, and interpretation of those studies that were performed with human subjects are generally complicated by the pathology of cancer or transplantation. Whilst these examples are invaluable in providing a proof of concept, in many instances they are far from being directly applicable to rejuvenation of the waning immune system in ageing humans. Nevertheless, the following is an exploratory overview of several reported strategies which are categorized into the three R's of Rejuvenation: Restoration, Replacement and Reprogramming.

Restoration

This approach is based on the use of cytokines, hormones, and growth factors known to play a role in the maintenance of a normal thymic microenvironment with the aim of restoring a functional cellular milieu. Some of these agents have already been evaluated in clinical trials, even though it is not yet clear whether they can restore thymopoiesis to healthy and durable levels. In fact, the minimum degree of 'rejuvenation' of the thymus required in order to obtain a significant clinical response has yet to be determined.

One of the first approaches developed was sex steroid ablation, as these hormones are known to negatively regulate T cell development by inducing thymic involution. In mice, prepubescent castration has been shown to prevent thymic atrophy, while post-pubescent castration is thought to reverse thymic atrophy and to augment thymic output [30]. Sex steroid ablation has been successfully tested in a clinical trial with the LHRH (lutenising hormone releasing hormone) agonist goserelin after autologous and allogeneic haemopoietic stem cell transplant (HSCT). This treatment was reported to induce strong CD4 T cell regeneration, along with a more varied TCR repertoire and enhanced peripheral T cell activity [30, 31].

Growth hormones are also known to play a role in T cell development. There are data showing that aged humans have lower serum levels of Growth hormone (GH) and Insulin-like growth factor (IGF-1) [32]. GH is produced by the pituitary gland and may also be generated in a variety of haemopoietic cells [33]. GH receptors are expressed on thymocytes and peripheral T cells [34]. Concordantly, it has been demonstrated that the administration of GH or IGF-1 can enhance T cell recovery in different experimental conditions such as syngeneic and allogeneic HSCT recipients, ageing mice, and ageing bone marrow transplantation (BMT) murine recipients [35]. Interestingly, although GH effects are mainly mediated by IGF-1, it has been observed that these effects would be orchestrated in part by the direct action of GH [32]. However IGF-1 seems to directly modulate thymocyte development and it is believed to induce IL-7 and stem cell factor (SCF) production by thymic epithelial cells (TECs) [32]. Along with GH and IGF-1, the peptide hormone ghrelin, principally known to induce GH by binding through the growth hormone secretagogue receptor (GHS-R), has been reported to enhance thymopoiesis in aged mice [36]. In addition, ghrelin is able to repress inflamm-aging, that in turn would contribute to the enhanced T cell reconstitution observed in older mice [37].

Keratinocyte growth factor (KGF), also called fibroblast growth factor 7 (FGF-7), regulates the proliferation and differentiation of epithelial cells, including TECs, by acting through the KGF receptor FGFR2IIIb [38] within the thymus where it is normally released by thymic fibroblasts and developing thymocytes [39]. KGF is able to protect the thymus from damage and to boost thymopoiesis in young and old mice. In the latter case, it has been found to improve thymocyte numbers, thymic architecture, and T cell function [40], and its administration in combination with sex steroid blockade has also been successful [39, 41]. Similar results have been obtained with the administration of nerve growth factor (NGF) in aged mice [42]. Presently, clinical trials are in progress to evaluate the ability of KGF administration to promote the reconstitution of thymopoiesis after allogeneic HSCT.

IL-7 is a γ-chain cytokine produced by stromal cells in the thymus, and plays a pivotal role in supporting thymocyte development, as well as peripheral T cell survival and proliferation [43]. It is believed that reduction in the intra-thymic IL-7 level is critical in the process of age associated thymic atrophy [44]. Several studies in both animal models and humans have been carried out. The reversion of age-associated thymic atrophy and concomitant enhanced thymopoiesis and thymic output has been demonstrated in old mice treated with exogenous IL-7 [45]. Moreover, a study in old female rhesus macaques treated with IL-7 prior to vaccination with influenza A/PR/8/34 (H1N1) virus would endorse IL-7 administration as an adjuvant to enhance vaccine responsiveness in the elderly [46]. These results have been extended to clinical trials. Recombinant IL-7 administered to humans with metastatic cancer has proven to be safe and effective, where an IL-7 dependent expansion of CD4 and CD8 T cells with a concomitant reduction in the percentage of regulatory T cells was observed [47]. In another clinical study with patients with non-hematologic cancers who received IL-7 administration, the expanded circulating T cells were found to exhibit a broad TCR repertoire [48]. Finally, although IL-12 levels are not decreased in ageing animals, *in vitro* stimulation of thymocytes with IL-7 togeth-

er with IL-12 induced a synergistic enhancement of cell proliferation [49], suggesting that IL-12 administration may contribute toward the retardation or even reversal of thymic involution in elderly individuals.

An adequate intake of both vitamins and trace elements are required for the immune system to function optimally [50]. In the elderly, a deficiency of these nutrients may lead to suppressed immunity, which in turn could predispose individuals to infections and further progress immunosenescence. In fact, compounds that possess antioxidant properties could be useful for the rejuvenation of the thymus, such as those contained in the leaves of Ginkgo biloba [51]. In many studies zinc supplementation has been proposed to be potentially useful in counteracting thymic ageing. Zinc deficiency induces age-independent thymic atrophy [52] while its supplementation increases thymic cellularity and possibly thymopoiesis [53], responsiveness to vaccines [54] and thymulin secretion [55] in aged individuals. In spite of all these data, overall results regarding zinc supplementation are still controversial [56]. A comparison of a number of existing clinical studies by El Kadiki *et al* concluded that there is no definitive evidence to recommend the routine use of multivitamin and/or mineral dietary supplementation to reduce infections in the elderly [57]. However this contradiction may be explained by the variable degree of micronutrient deficiency between individuals. Whilst the healthy ageing population, who generally have no nutritional deficit, exhibit changes in T cell subsets because of the higher amount of pathogen recognition over the years, the elderly population with signs of under nutrition or even protein energy malnutrition (PEM) display eroded immune functions of both the adaptive and innate arm. This condition frequent in the institutionalized elderly population [58], is the cause of recurrent infections, and can be partially reversed by increasing food intake unless an inflammatory process is present [59, 60].

Replacement

The strategies of immune system "replacement" developed to date encompass the adoptive transfer and the ex vivo generation of T cells.

Adoptive transfer procedures have been used in the HSCT setting to reduce the incidence of viral reactivation in immunosuppressed post-transplantation and cancer patients. Cobbold and colleagues described the infusion of antigen specific lymphocytes directed against viruses which cause chronic infections, such as CMV. Direct donor isolation of CMV-specific $CD8^+$ T cells is achieved using magnetic beads conjugated to HLA-peptide tetramers directed to one epitope of the CMV viral envelope protein, pp65. Tests in patients following HSCT were non-toxic and, importantly, relatively low doses (of between 1.2×10^4 and 2×10^6 lymphocytes) were sufficient to control and prevent viral reactivation [61].

Another example of adoptive transfer which has been used in the treatment of cancer involves the use of artificial antigen presenting cells (aAPCs). The *in vitro* induction and expansion of these cells over successive rounds of stimulation results in the generation of therapeutically high levels of antigen-specific cytotoxic T lymphocytes (CTL) [62]. In parallel with HSCT, the ageing immune system has a reduced ability to effectively combat recurrent and newly acquired infections [63, 64]. Lifelong latent infection with CMV has been reported to drive the exhaustion of the immune system through the reduction of the naïve T cell repertoire and the disproportionate expansion of CMV-specific $CD8^+$ memory T cell pool, which results in a reduction in memory pool diversity [21]. CMV infection has been associated with increased mortality in the very elderly [8], and frailty [65], therefore therapies for the treatment of chronic CMV infection are of great interest. It remains to be seen if the encouraging results obtained in the HSCT setting could be replicated in elderly individuals to prolong their immunological competency at the point where immunosurveillance against CMV reactivation is defective. Progress in the development of artificial antigen presenting cells may herald a means of rejuvenating components of the innate immune system.

An alternative and possibly more valid approach for replacing immune function lost with ageing is *in vitro* T cell development. Xenosystems such as fetal thymic organ cultures (FTOC); reaggregate thymic organ cultures (RTOC) and thymic stromal cell monolayer cultures (TSMC), have provided knowledge about T cell differentiation and positive and negative selection processes [66]. Importantly the TSMC system consists of OP9 mouse bone marrow stromal cells transduced to overexpress either the Delta Notch ligand Dll-1 or Dll-4 [67, 68]. This system has definitively demonstrated that the induction of T cells, and a simultaneous block in B cell lineage potential, depends on Delta ligand Notch signaling [69]. However, systems based on the use of cells cultured as a monolayer fail to push T cell differentiation over the double positive stage [70]. A very promising approach to produce terminally differentiated T cells relies on the use of 3-dimensional matrix structures to recreate the cellular microenvironment of the thymus. Using autologous stem cells, this method would be able to generate T cells which have successfully undergone both positive and negative selection *in vitro*. The resultant naive T cell population would have a diverse repertoire and should be able to be transferred into patients with defective thymopoiesis, such as aged individuals. An initial study based on this technique has shown that T cells can be generated from bone marrow stem cells *in vitro* using a 3-dimensional scaffold seeded with skin fibroblasts and keratinocytes. The authors were able to reproduce a thymic microenvironment equivalent, and the mature T cells produced exhibited rearrangement of the TCRβ and TCRα genes, with expression of a diverse antigen receptor repertoire [71]. This discovery opens up the possibility of generating new T cells *in vitro* from host skin and stem cells, with the benefit of being efficiently and safely injected back into the host in the absence of graft versus host disease, whilst responses to antigen would also be self restricted.

Reprogramming

Finally and probably the most "revolutionary" treatment of immunosenescence could be based on reprogramming the ageing immune system. It has been demonstrated that there exists a correlation between telomere length and limited division potential. To date, there is general consensus that

telomeres represent an inherent biological clock [72]. Therefore, restoring telomere length could significantly extend cellular lifespan [73], and this would be possible by regulating telomeres through the activity of telomerases, enzymes required in order to maintain telomere length and stability. Some recent papers have described the successful extension of the replicative lifespan of T cells through the ectopic expression of telomerase catalytic subunit (hTERT) [74]. With the implemetation of such a method it would be possible to collect, rejuvenate and re-introduce antigen specific lymphocytes from older individuals, with the aim of prolonging and enhancing a specific immune response in aged individuals.

Once an elderly individual has been identified as exhibiting signs of immunosenscence, a difficult problem in itself, the choice of how to improve their immunity has then to be made. Immune restoration therapies are unlikely to be a "one-off" treatment, but will have to be repeated. A critical factor in any treatment regime is compliance, which in turn is dependent on dose efficacy, safety, frequency, and route of delivery. Clearly any treatment administered either orally or by inhalation will be much preferred to one which depends on multiple injections.

WHEN?

The problem of defining immunosenescence and deciding whether to implement therapeutic intervention is extremely complex. One current hypothesis is that it is similar to defining frailty in older individuals [75]. One broad definition of frailty was through equating frailty to the degree of functional dependence in daily living [76]. While it is easy to identify individuals who are dependent on others for issues of daily living, this is not useful as a single clear identifying factor. The approach used by Rockwood *et al* over a number of years has been to associate frailty with an accumulation of deficits [77, 78]. In this approach an index of frailty was constructed by counting the deficits that people accumulated. Although the deficits could be individually weighted, the authors chose to treat all characteristics equally even though some, for example cancer, carry a higher risk of mortality than others, such as skin disorders. The results revealed that the probability of an adverse event was related in a dose dependent fashion to the accumulation of deficits [79]. We propose that a similar approach could be used to define immunosenescence. Based on this assumption, we could use an accumulation of immune deficits to predict immune status and decide whether to implement therapeutic intervention (Fig. **1**). Before we discuss this further, let us consider the justification of this approach.

In complex systems such as the immune system, reliability is dependent in part on the quality of the components and also on any functional overlap. Thus reliability in the face of possible component failure can be achieved by having redundancy within the system, which should ensure that whilst some components fail, the system as a whole remains functional. In any system where there is redundancy one would not expect there to be a single component whose failure leads to complete system failure. Thus it is with the immune system.

Immunosenescence is a multifaceted phenomenon encompassing several complex changes affecting both the innate and adaptive branches of the immune system [2-4]. Whereas the exact mechanisms leading to immunosenescence are still poorly understood, immune system failure is unlikely to come from a single deficit but probably arises from an accumulation of defects in different components within the system. The question is which components and the magnitude of the deficits. To answer this we could produce a larger number of parameters all known to alter with age. Such a list could include: thymic involution measured through assessing thymic output; the degree of chronic antigenic stimulation; signal transduction changes in the immune cells; protein-energy malnutrition; reduction in the repertoire; changes in cell phenotype profiles; alteration in the number of regulatory T cells; increases in the number of cells with a senescent phenotype; reduction in the numbers of naïve B or T cells, poor *in vitro* proliferative responses; the presence of large clones of B cells (BMGUS) or enlarged clones of T cells; and infection either with persistent

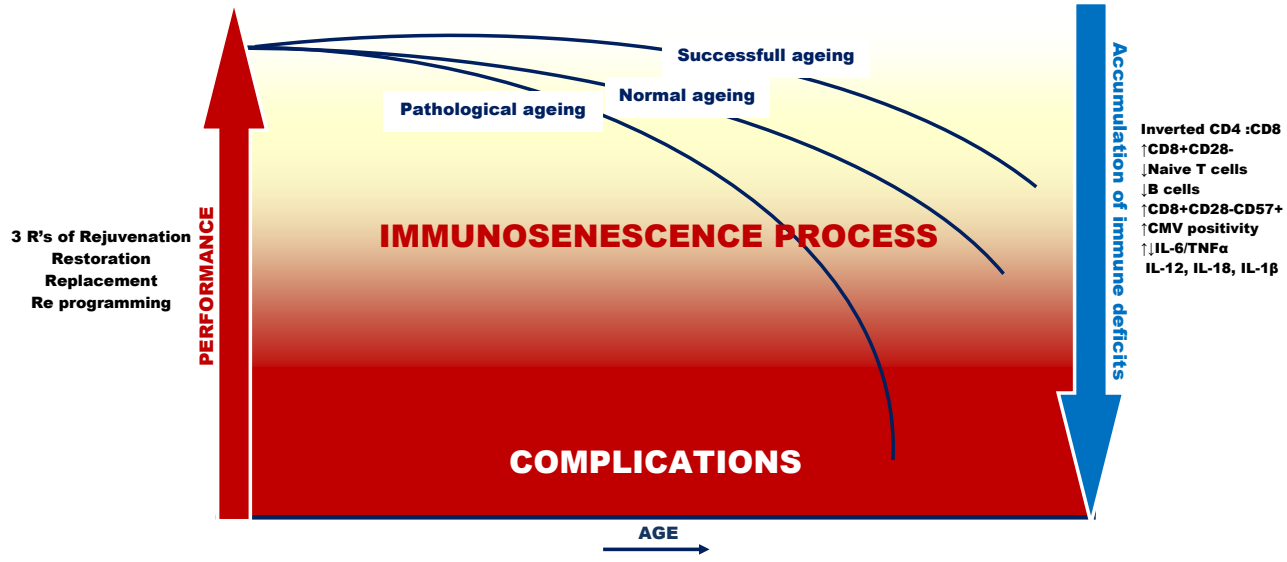

Fig. (1). The accumulation deficits concept applied to immunosenescence.

viruses such as herpes or bacteria such as Mycobacterium tuberculosis, Helicobacter pylori or members of Salmonella species. Our idea would be that when an individual is positive for a number of these parameters, they may be considered to be showing an immunosenescent phenotype, and as a consequence, for example, be unable to respond adequately to vaccination. Such a model proves a testable hypothesis to approach the difficult clinical definition of immunosenescence.

CONCLUSION

Despite growing evidence that changes in the responsiveness of the immune system with age lead to an increased susceptibility to various age-related diseases, poorer response to vaccination and increased incidence of infectious diseases, the problem of defining immunosenescence and how to implement therapeutic intervention still remains an unresolved challenge. This review demonstrates that the achievement of an accurate assessment of an immunosenescent phenotype remains fraught with clinical and biological difficulties. Immunosenescence, although complex, is undoubtedly a real and important condition affecting healthy ageing. Hence methods for identifying and measuring immunosenescence and understanding its implications justify further evaluation and development. Strategies for enhancing immune capacity either through the prevention, retardation or rejuvenation of the immune system are currently still largely addressed in animal models, which may or may not be pertinent to humans. Thus, complementary clinical and translational studies at the epidemiological, clinical, cellular, molecular and genetic levels are needed to elucidate the complexity of immunosenescence in the ageing human population. In view of the functional interplay between different components of the immune system, future approaches designed for human beings should probably combine two or more immune targets in studies to impart greater insight into the best way of promoting immune rejuvenation. Further studies are essential in order to achieve the restoration of immunity in the elderly, with the aim of increasing the capacity of older individuals to protect against infections and delay the onset and severity of age-related diseases.

CONFLICT OF INTEREST

None declared.

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REFERENCES

[1] Kinsella K, He W. An Aging World: 2008. International Population Reports. Washington DC: U.S. Census Bureau June 2009.

[2] Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science 2002; 296(5570): 1029-31.

[3] Heilig GK. IIASA European Rural Development (ERD) project. April 2002.

[4] Ostan R, Bucci L, Capri M, *et al*. Immunosenescence and immunogenetics of human longevity. Neuroimmunomodulation 2008; 15(4-6): 224-40.

[5] McElhaney JE. The unmet need in the elderly: designing new influenza vaccines for older adults. Vaccine 2005; 23 (Suppl. 1): S10-25.

[6] Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res 2004; 103(1-2): 133-8.

[7] Schmader K. Herpes zoster in older adults. Clin Infect Dis 2001; 32(10): 1481-6.

[8] Hadrup SR, Strindhall J, Kollgaard T, *et al*. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. J Immunol 2006; 176(4): 2645-53.

[9] Gavazzi G, Krause KH. Ageing and infection. Lancet Infect Dis 2002; 2(11): 659-66.

[10] Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. Scand J Immunol 2004; 59(1): 1-15.

[11] Lang PO, Govind S, Mitchell WA, Siegrist CA, Aspinall R. Vaccine effectiveness in older individuals: What has been learned from the influenza-vaccine experience. Ageing Res Rev 2011; 10(3): 389-95.

[12] Siegrist CA, Aspinall R. B-cell responses to vaccination at the extremes of age. Nat Rev Immunol 2009; 9(3): 185-94.

[13] Monto AS, Ansaldi F, Aspinall R, *et al*. Influenza control in the 21st century: Optimizing protection of older adults. Vaccine 2009; 27(37): 5043-53.

[14] Van Herck K, Leuridan E, Van Damme P. Schedules for hepatitis B vaccination of risk groups: balancing immunogenicity and compliance. Sex Transm Infect 2007; 83(6): 426-32.

[15] Looney RJ, Hasan MS, Coffin D, *et al*. Hepatitis B immunization of healthy elderly adults: relationship between naive CD4+ T cells and primary immune response and evaluation of GM-CSF as an adjuvant. J Clin Immunol 2001; 21(1): 30-6.

[16] Lang PO, Mitchell WA, Lapenna A, Pitts D, Aspinall R. Immunological pathogenesis of main age-related diseases and frailty: Role of immunosenescence. Eur Geriatr Med 2010; 1(2): 112-21.

[17] Ligthart GJ, Corberand JX, Fournier C, *et al*. Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech Ageing Dev 1984; 28(1): 47-55.

[18] Wikby A, Mansson IA, Johansson B, Strindhall J, Nilsson SE. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20-100 years of age. Biogerontology 2008; 9(5): 299-308.

[19] Strindhall J, Nilsson BO, Lofgren S, *et al*. No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. Exp Gerontol 2007; 42(8): 753-61.

[20] Wikby A, Ferguson F, Forsey R, *et al*. An immune risk phenotype, cognitive impairment, and survival in very late life: impact of allostatic load in Swedish octogenarian and nonagenarian humans. J Gerontol A Biol Sci Med Sci 2005; 60(5): 556-65.

[21] Pawelec G, Derhovanessian E, Larbi A, Strindhall J, Wikby A. Cytomegalovirus and human immunosenescence. Rev Med Virol 2009; 19(1): 47-56.

[22] Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev 2007; 65(12 Pt 2): S173-6.

[23] Franceschi C, Capri M, Monti D, *et al*. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev 2007; 128(1): 92-105.

[24] Fulop T, Larbi A, Witkowski JM, *et al*. Aging, frailty and age-related diseases. Biogerontology 2010; 11(5): 547-63.

[25] Aspinall R, Pitts D, Lapenna A, Mitchell W. Immunity in the elderly: the role of the thymus. J Comp Pathol 2010; 142 (Suppl. 1): S111-5.

[26] Mitchell WA, Lang PO, Aspinall R. Tracing thymic output in older individuals. Clin Exp Immunol 2010; 161(3): 497-503.

[27] Douek DC, McFarland RD, Keiser PH, *et al*. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998; 396(6712): 690-5.

[28] Aspinall R, Pido J, Andrew D. A simple method for the measurement of sjTREC levels in blood. Mech Ageing Dev 2000; 121(1-3): 59-67.

[29] Lang PO, Mitchell WA, Govind S, Aspinall R. Real time-PCR assay estimating the naive T cellpool in whole blood and dried blood spot samples: Pilot study in young adults. J Immunol Methods 2011;369 (1-2): 133-40.
[30] Sutherland JS, Goldberg GL, Hammett MV, *et al*. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol 2005; 175(4): 2741-53.
[31] Sutherland JS, Spyroglou L, Muirhead JL, *et al*. Enhanced immune system regeneration in humans following allogeneic or autologous hemopoietic stem cell transplantation by temporary sex steroid blockade. Clin Cancer Res 2008; 14(4): 1138-49.
[32] Welniak LA, Sun R, Murphy WJ. The role of growth hormone in T cell development and reconstitution. J Leukoc Biol 2002; 71(3): 381-7.
[33] Weigent DA, Baxter JB, Wear WE, Smith LR, Bost KL, Blalock JE. Production of immunoreactive growth hormone by mononuclear leukocytes. FASEB J 1988; 2(12): 2812-8.
[34] Dardenne M, Mello-Coelho V, Gagnerault MC, Postel-Vinay MC. Growth hormone receptors and immunocompetent cells. Ann N Y Acad Sci 1998; 840: 510-7.
[35] Chen BJ, Cui X, Sempowski GD, Chao NJ. Growth hormone accelerates immune recovery following allogeneic T-cell-depleted bone marrow transplantation in mice. Exp Hematol 2003; 31(10): 953-8.
[36] Dixit VD, Yang H, Sun Y, *et al*. Ghrelin promotes thymopoiesis during aging. J Clin Invest 2007; 117(10): 2778-90.
[37] Dixit VD, Yang H, Cooper-Jenkins A, Giri BB, Patel K, Taub DD. Reduction of T cell-derived ghrelin enhances proinflammatory cytokine expression: implications for age-associated increases in inflammation. Blood 2009; 113(21): 5202-5.
[38] Finch PW, Rubin JS. Keratinocyte growth factor/fibroblast growth factor 7, a homeostatic factor with therapeutic potential for epithelial protection and repair. Adv Cancer Res 2004; 91: 69-136.
[39] Erickson M, Morkowski S, Lehar S, *et al*. Regulation of thymic epithelium by keratinocyte growth factor. Blood 2002; 100(9): 3269-78.
[40] Alpdogan O, Hubbard VM, Smith OM, *et al*. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. Blood 2006; 107(6): 2453-60.
[41] Kelly RM, Highfill SL, Panoskaltsis-Mortari A, *et al*. Keratinocyte growth factor and androgen blockade work in concert to protect against conditioning regimen-induced thymic epithelial damage and enhance T cellreconstitution after murine bone marrow transplantation. Blood 2008; 111(12): 5734-44.
[42] Turrini P, Zaccaria ML, Aloe L. Presence and possible functional role of nerve growth factor in the thymus. Cell Mol Biol (Noisy-legrand) 2001; 47(1): 55-64.
[43] Jiang Q, Li WQ, Aiello FB, *et al*. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev 2005; 16(4-5): 513-33.
[44] Andrew D, Aspinall R. Age-associated thymic atrophy is linked to a decline in IL-7 production. Exp Gerontol 2002; 37(2-3): 455-63.
[45] Andrew D, Aspinall R. Il-7 and not stem cell factor reverses both the increase in apoptosis and the decline in thymopoiesis seen in aged mice. J Immunol 2001; 166(3): 1524-30.
[46] Aspinall R, Pido-Lopez J, Imami N, *et al*. Old rhesus macaques treated with interleukin-7 show increased TREC levels and respond well to influenza vaccination. Rejuvenation Res 2007; 10(1): 5-17.
[47] Rosenberg SA, Sportes C, Ahmadzadeh M, *et al*. IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. J Immunother 2006; 29(3): 313-9.
[48] Sportes C, Hakim FT, Memon SA, *et al*. Administration of rhIL-7 in humans increases *in vivo* TCR repertoire diversity by preferential expansion of naive T cell subsets. J Exp Med 2008; 205(7): 1701-14.
[49] Li L, Hsu HC, Stockard CR, *et al*. IL-12 inhibits thymic involution by enhancing IL-7- and IL-2-induced thymocyte proliferation. J Immunol 2004; 172(5): 2909-16.
[50] Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 2007; 51(4): 301-23.
[51] Tian YM, Tian HJ, Zhang GY, Dai YR. Effects of Ginkgo biloba extract (EGb 761) on hydroxyl radical-induced thymocyte apoptosis and on age-related thymic atrophy and peripheral immune dysfunctions in mice. Mech Ageing Dev 2003; 124(8-9): 977-83.
[52] Prasad AS. Clinical, endocrinological and biochemical effects of zinc deficiency. Clin Endocrinol Metab 1985; 14(3): 567-89.
[53] Fraker PJ, King LE. Reprogramming of the immune system during zinc deficiency. Annu Rev Nutr 2004; 24: 277-98.
[54] Duchateau J, Delepesse G, Vrijens R, Collet H. Beneficial effects of oral zinc supplementation on the immune response of old people. Am J Med 1981; 70(5): 1001-4.
[55] Prasad AS, Meftah S, Abdallah J, *et al*. Serum thymulin in human zinc deficiency. J Clin Invest 1988; 82(4): 1202-10.
[56] Haase H, Rink L. The immune system and the impact of zinc during aging. Immun Ageing 2009; 6: 9.
[57] El-Kadiki A, Sutton AJ. Role of multivitamins and mineral supplements in preventing infections in elderly people: systematic review and meta-analysis of randomised controlled trials. BMJ 2005; 330(7496): 871.
[58] Muhlethaler R, Stuck AE, Minder CE, Frey BM. The prognostic significance of protein-energy malnutrition in geriatric patients. Age Ageing 1995; 24(3): 193-7.
[59] Lesourd B. Nutrition: a major factor influencing immunity in the elderly. J Nutr Health Aging 2004; 8(1): 28-37.
[60] Lesourd B. Nutritional factors and immunological ageing. Proc Nutr Soc 2006; 65(3): 319-25.
[61] Cobbold M, Khan N, Pourgheysari B, *et al*. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. J Exp Med 2005; 202(3): 379-86.
[62] Oelke M, Maus MV, Didiano D, June CH, Mackensen A, Schneck JP. *Ex vivo* induction and expansion of antigen-specific cytotoxic T cells by HLA-Ig-coated artificial antigen-presenting cells. Nat Med 2003; 9(5): 619-24.
[63] Khan N, Hislop A, Gudgeon N, *et al*. Herpesvirus-specific CD8 T cell immunity in old age: cytomegalovirus impairs the response to a coresident EBV infection. J Immunol 2004; 173: 7481-9.
[64] Trzonkowski P, Mysliwska J, Szmit E, *et al*. Association between cytomegalovirus infection, enhanced proinflammatory response and low level of antihemagglutinins during the anti-influenza vaccination–an impact of immunosenescence.Vaccine 2003; 21: 3826-36.
[65] Wang GC, Kao WHL, Murakami P, *et al*. Cytomegalovirus Infection and the Risk of Mortality and Frailty in Older Women: A Prospective Observational Cohort Study. Am J Epidemiol 2010; 171(10): 1144-52.
[66] Hare KJ, Jenkinson EJ, Anderson G. *In vitro* models of T cell development. Semin Immunol 1999; 11(1): 3-12.
[67] de Pooter RF, Cho SK, Carlyle JR, Zuniga-Pflucker JC. *In vitro* generation of T lymphocytes from embryonic stem cell-derived prehematopoietic progenitors. Blood 2003; 102(5): 1649-53.
[68] Lefort N, Benne C, Lelievre JD, *et al*. Short exposure to Notch ligand Delta-4 is sufficient to induce T cell differentiation program and to increase the T cell potential of primary human CD34+ cells. Exp Hematol 2006; 34(12): 1720-9.
[69] Deftos ML, Bevan MJ. Notch signaling in T cell development. Curr Opin Immunol 2000; 12(2): 166-72.
[70] La Motte-Mohs RN, Herer E, Zuniga-Pflucker JC. Induction of T celldevelopment from human cord blood hematopoietic stem cells by Delta-like 1 *in vitro*. Blood 2005; 105(4): 1431-9.
[71] Clark RA, Yamanaka K, Bai M, Dowgiert R, Kupper TS. Human skin cells support thymus-independent T cell development. J Clin Invest 2005; 115(11): 3239-49.
[72] Mera SL. The role of telomeres in ageing and cancer. Br J Biomed Sci 1998; 55: 221-5.
[73] Westin ER, Chavez E, Lee KM, *et al*. Telomere restoration and extension of proliferative lifespan in dyskeratosis congenita fibroblasts. Aging Cell 2007; 6: 383-94.
[74] Rufer N, Migliaccio M, Antonchuk J, Humphries RK, Roosnek E, Lansdorp PM. Transfer of the human telomerase reverse transcriptase (TERT) gene into T lymphocytes results in extension of replicative potential. Blood 2001; 98: 597-603.
[75] Lang PO, Michel JP, Zekry D. Frailty syndrome: a transitional state in a dynamic process. Gerontology 2009; 55(5): 539-49.

[76] Rockwood K, Stolee P, McDowell I. Factors associated with institutionalization of older people in Canada: testing a multifactorial definition of frailty. J Am Geriatr Soc 1996; 44(5): 578-82.
[77] Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. J Gerontol A Biol Sci Med Sci 2007; 62(7): 722-7.
[78] Rockwood K, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin Geriatr Med 2011; 27(1): 17-26.
[79] Rockwood K, Mitnitski A. Limits to deficit accumulation in elderly people. Mech Ageing Dev 2006; 127(5): 494-6.

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