Immunotherapy of Immunosenescence; 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 immunosenescence. 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 demography 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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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 immunosenesence, 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 [36]. 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 (GHSR), 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 FGFR2IIb [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 antioxidative 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 countering 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 x 10^4 and 2 x 10^5 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 Dil-l or Dil-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 naïve 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 implementation 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 immunosenescence, 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...
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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