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Essay for consideration:

Immunosenescence – can it be reversed?

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Abstract

As we age, the immune system undergoes changes resulting in the progressive deterioration of the ability to respond appropriately to new antigens. These changes include a reduced output of peripheral naïve T cells and elevated level of basal inflammation. This process is termed immunosenescence and is associated with increased morbidity and mortality within the elderly population (over 65 years of age). This review describes the mechanism of immunosenescence and evaluates some of the key methods proposed to restore immune function for the elderly. A number of treatment options being investigated are still in their infancy, such as reprogramming telomeres to extend the lifespan of immune cells, replacing senescent cells with functioning T cells and using biological agents to stimulate the production of peripheral naïve T cells. These approaches are currently labour intensive, risky and expensive so are unlikely to be a management of choice for immunosenescence. The literature suggests that less invasive, more accessible and lower cost options such as exercise and higher quality sleep are more realistic management choices. Our next challenge is to identify ways to alter sleep and exercise routines to best reduce the decline of immunity. In addition to this, improving vaccination outcomes and coverage are priorities. Ongoing research to understand the molecular mechanisms driving immunosenescence, such as the human microbiota composition, offers hope for a future where it may be reversed.

Introduction:

There are over 10 million people in the UK over the age of 65 and it is predicted that by 2050 this number will have nearly doubled to around 19 million. The population on average is living 4 decades longer than 150 years ago (1, 2). Despite this increase in life expectancy, ageing is associated with an increased susceptibility to both persistent and acute infections, impaired wound healing, cancers, autoimmunity and chronic illnesses (2, 3). Additionally, protective interventions, such as vaccines, become less successful with age (4). This is due to the gradual decline in the functioning of the immune system, termed immunosenescence. This weakened immune response significantly reduces the quality of life for the ageing population, as they enter into a phase of 'unhealthy old age' (5). Immunosenescence is becoming of increasing societal importance due to the growing number of elderly people

who are disproportionately consuming our finite healthcare resources (6). In order to improve this situation, it is important to rejuvenate the ageing immune system through medical interventions.

The effect of ageing on the immune system

Ageing affects the ability of the body to fight disease at multiple levels. During ageing, there is a gradual weakening of the body's physical barriers, such as skin. The deterioration of these protective barriers increases the ability of infectious agents to be able to gain entry into the host (7).

The innate immune system is described as being "dysregulated", with the older people having increased levels of proinflammatory cytokines, clotting factors and acute phase reactants, resulting in persistent inflammation (8). It has been hypothesised that the cumulative exposure to environmental factors during a lifetime drives this dysregulation. For example, the metabolic activity of the cell causes cumulative oxidative damage to the DNA, triggering the release of inflammatory cytokines (9-11). This so-called 'inflammageing' process alters the microenvironment for antigen presentation and T cell proliferation (12). This elevated level of basal inflammation is also thought to be associated with the occurrence of various neurodegenerative conditions, such as Alzheimer's disease as well as atherosclerosis (13). Additionally, as shown in Table 1, features of the innate immune cells change with advanced ageing, reducing their functionality.

	Neutrophils	**Monocytes**	**Dendritic cells**	**NK cells**
Number (absolute or at site of infection)	No change	No change	Reduced	Increase
Migratory/ chemotaxis ability	Reduced	Reduced	Reduced	Reduced
Phagocytic ability	Reduced	Reduced	Reduced	N/A
Cytotoxicity/ normal function ability	Reduced	Reduced	No change	Reduced

Table 1: Changes to innate immune cells during advancing age. *Data taken from table 1.1: (8)*

All immune cells are derived from the multipotential haematopoietic stem cell (HSC), as illustrated in Fig. 1. Aged HSCs are less effective at supporting the creation of lymphocytes,

creating a bias towards myeloid progenitor cells (14). This skewing towards the myeloid lineage is thought to be due to the decreased number of progenitor cells and colony stimulating factors as well as increased levels of apoptosis (12). Despite the increase in myeloid progenitor cell number, these cells are often functionally deficient during old age, decreasing their ability to self-renew (15). Additionally, during ageing there are changes to the endocrine system which results in reduced lymphopoiesis, for example a decreased concentration of growth hormone (12).

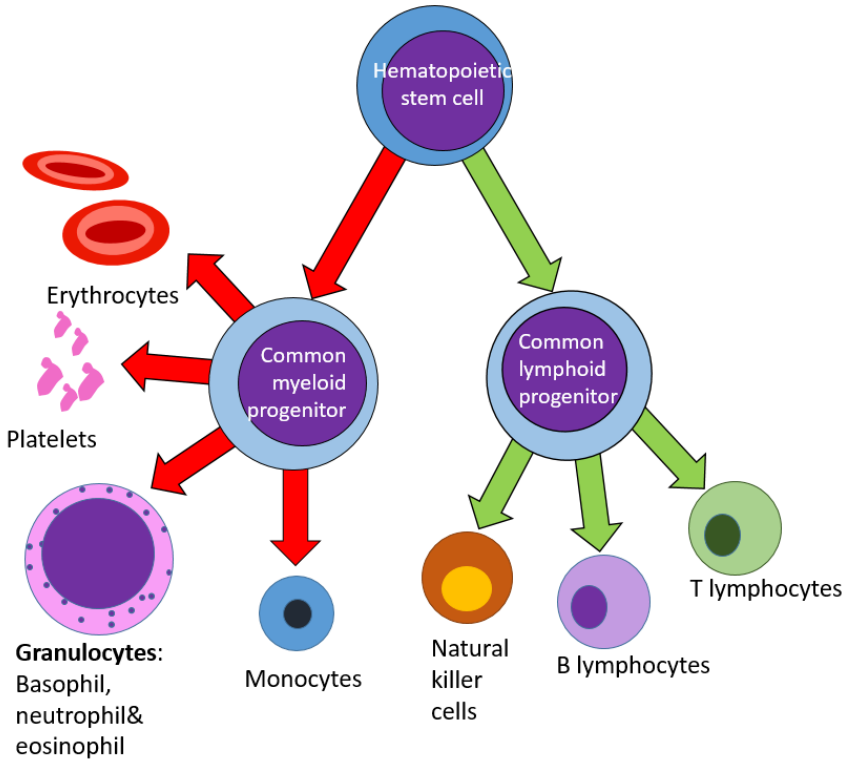

Figure 1: Haematopoiesis from a multipotent stem cell. *In the elderly there is skewing towards the myeloid cell differentiation pathway (red arrows) in comparison to the less favoured common lymphoid cell differentiation pathway (green arrows); this will result in a decreased production of lymphocytes. Additionally, experimental evidence demonstrates aged HCSs are less effective at supporting erythropoiesis, reducing the volume of erythrocytes (16). Information to make figure taken from: (14, 16).*

The thymus is the location where T cells normally mature. The thymus decreases in size during ageing, reducing the number of peripheral naïve T cells entering the secondary lymphoid organs and circulation (12). The pool of naïve T cells is often reduced further because of chronic stimulation by persistent viral infections (such as Epstein- Barr virus) (17). This can result in oligoclonal expansion of memory T cells in niches within the peripheral immune tissues; this means that naïve cells may not be able to take up residence due to lack

of 'space' (17, 18). Furthermore, during ageing these T cell populations have altered expression of co- stimulatory and inhibitory receptors impairing their function (3). Research to treat immunosenescence in the elderly has often focussed on restoring the balance of T cells by increasing the proportion of naïve T cells to memory T cells, as shown in Fig. 2 (19).

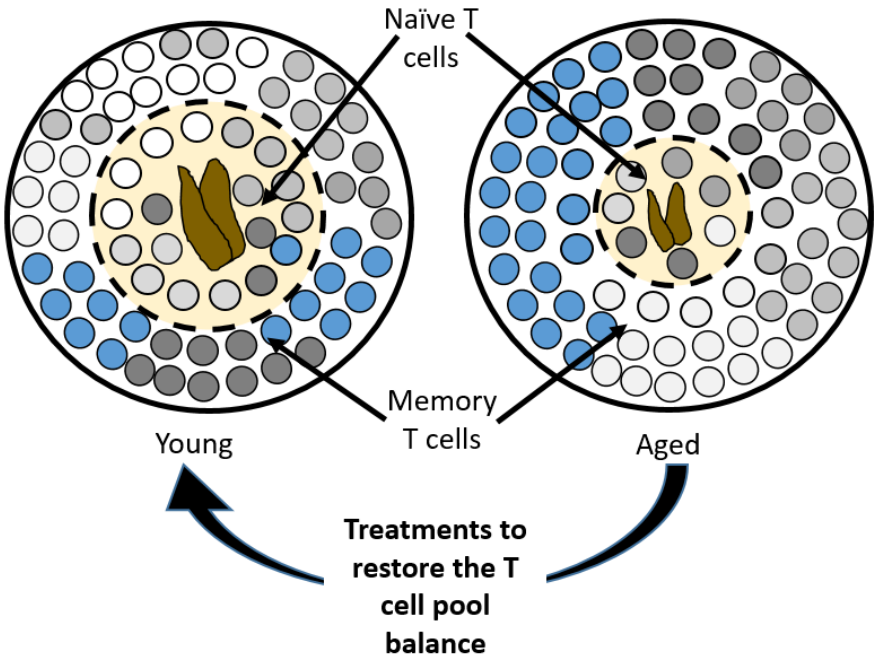

Figure 2: Changes to the T cell pool during ageing. *The centre of the circle (yellow) represents the naïve T cell area, the external white section of the circle represents the memory T cell pool area, the smaller different coloured circles represent the T cells. The thymus is shown in the centre of the circle (getting smaller with age). During the ageing process the number of memory T cells increases, whilst the number of naïve T cells decrease, possible treatments are trying reverse this trend, to increase the proportion of naïve T cells to memory T cells. Figure adapted from figure 1: (19)*

B cells, which secrete antibodies, also develop impaired function during ageing. Despite the number of circulating antibodies not altering dramatically, antibody quality (affinity and specificity) as well as diversity decreases. Furthermore, the risk of autoantibody responses increase with age (12). A summary of these changes that occur to T and B cells are provided in Table 2.

Age related increase/decrease	T lymphocytes	B lymphocytes
Increase	• Number of memory and effector cells • Expanded clones of CD8+ and CD4+ memory T cells (oligoclonal expansion) • Release of pro- inflammatory cytokines • Increased levels of regulatory T cells	• Autoreactive antibodies in the serum
Decrease	• Number of naïve T cells • T cell diversity • Expression of co- stimulatory molecules (CD28, CD27, CD40L)	• B cell precursors • Number of naïve B cells • B cell diversity • Expression of costimulatory molecules (CD27, CD40) • Antibody affinity • Isotype switching

Table 2: Age related changes to T and B-lymphocytes. *Data taken from Table 1:(20) and adapted using information from: (18, 21)*

Costimulatory proteins are normally located on antigen presenting cells, and help activate the adaptive immune response. They become absent in the elderly, reducing their ability to produce appropriate T and B cell response to vaccines and pathogens (22).

Many age-related changes occur to the immune system (as summarised in Fig. 3), the gradual weakening of the immune system begins around the age of 60 and gradually deteriorates with age (5). However, the exact order and precise cellular changes that occur between individuals can be diverse, due to differences in environmental exposures and genetic composition, this will need to be considered when designing therapies to reverse the effect of ageing on immunity (23). The ideal treatment will need to be easy to administer, cost effective and readily available. It does not necessary need to restore the function of the immune system to the levels of 'youth' because even a small increase could be life-changing (18). The remainder of this review will discuss proposed methods to reduce the decline of the ageing immune system.

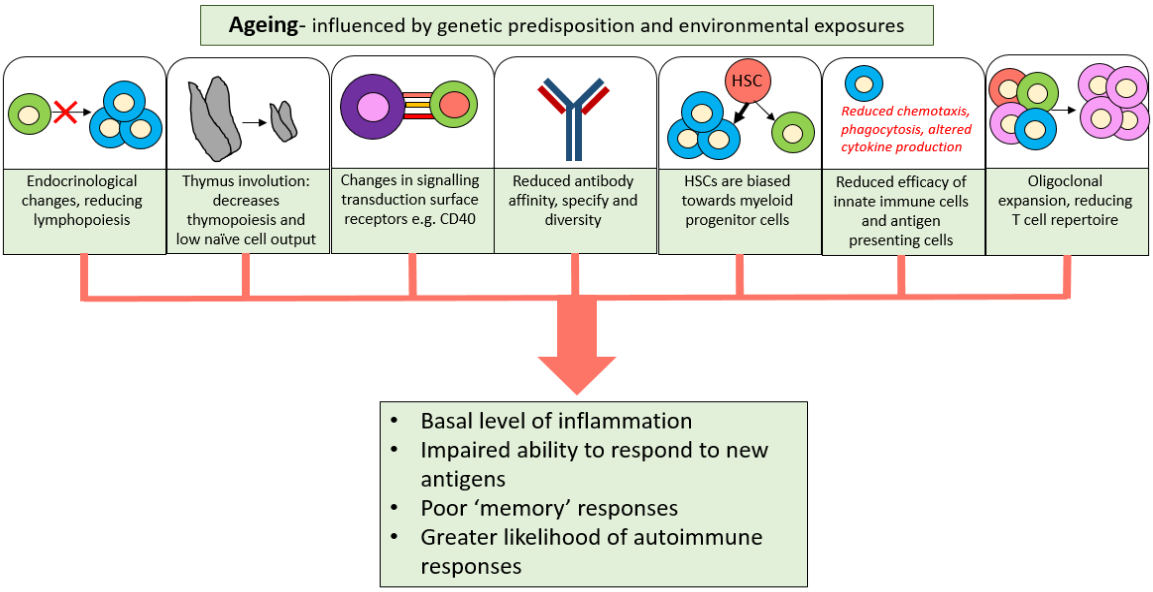

Figure 3: Summary of the major changes to the immune system during advanced ageing. *Figure adapted from (2, 24)*

Results and Discussion:

A number of different ways to reduce the age-related decline of the immune system are currently being investigated, summarised by Table 3, which will be discussed in this review.

Aim	Possible intervention	Sources
Reprogramming telomeres to restore their length in order to increase cellular lifespan	Pharmacological agents or gene therapy to increase telomerase activity	(25)
Replacement of senescent cells with effector or naïve cells	Adoptive therapy with effector cells	(19)
Restoration of the T cell pool balance by stimulating the production of naïve T cells and exporting them to the periphery	Using cytokines and hormones such as growth hormone and interleukin 7	(26)
Lifestyle changes such as increased exercise and sleep. Exercise is thought to mobilise aged T cells into the blood, leading to their degradation, allowing more space for naïve T cells. Regular sleep patterns correlate with better immunological responses.	Increase exercise and sleep in an appropriate way	(27, 28)
Improve the outcome of vaccinations to reduce disease burden	High dose vaccinations or the use of adjuvants	(29)

Table 3: Potential methods proposed to reduce to effect of immunosenescence. *Information taken from: (19, 25-29)*

Reprogramming telomeres to extend cellular lifespan:

Telomeres are located at the termini of human chromosomes. Telomeres are believed to be our inherent biological clocks as there is a correlation between telomere length and division potential (19). Restoration of telomere length using enzyme telomerase could extend cellular lifespan (30).

Studies in mice have shown that premature ageing occurs when there is a low concentration of telomerase, which leads to a reduced adult stem cell proliferation potential (25). Having a reduced proliferation means the number of immune cells created is decreased. However, telomerase overexpression can lead to cancers (19, 25). It is important to consider the level of telomerase activation required, the optimum level should have an observed positive effect on the immune function with minimum adverse effects (19).

Two different studies using blood samples from HIV-1 infected patients increased telomerase activity and resulted in an improved immune response. Patients with chronic HIV-1 infection are an appropriate model for immunological ageing as both groups experience increased proportions of dysfunctional CD8+ T lymphocytes with shortened telomeres (31, 32). One study used gene therapy to consecutively express the telomerase gene (hTERT), whilst the other study used a pharmacological telomerase activator (TAT2) (32, 33). In comparison to gene therapy, the pharmacological approach to increase telomerase activation is likely to be more successful clinically as the dose and duration of the effects can be better controlled, making it less dangerous (33). The pharmacological upregulation of telomerase was demonstrated to be short-term and reversible (33). This suggests that clinically pharmacological telomerase upregulation could be increased short-term to help the patient overcome an infection by temporarily increasing their immune response. As of yet, there appears to be no human clinical trials where the concentration of telomerase has been increased, this will be needed to test whether increasing telomerase activity is a viable treatment option to improve immune response.

Mouse models have been the primary tool utilised to understand the mechanism of immunological ageing and to examine the treatment efficacy (24). This is because the mouse genome is very similar to the human genome, multiple sophisticated molecular and

genetic tools have been developed, and mice are small and relatively cheap, enabling large-scale and high throughput studies to be conducted (34). Despite this, no model organism is ideal, as different species have different characteristics of immunological ageing due to evolutionary differences (24). For example, it is hard to equate mouse years to human years, mice studies are normally conducted in a pathogen free environment and mice have very long telomeres which do not correlate with replicative ageing (24). Because of these differences, immunological processes that occur during human ageing should be identified first by measuring through a relatively non-invasive method (such as a blood test) which could identify changes in immune cell volume and cytokines present in humans. Once identified, if required these immunogenic alterations could be studied further in mouse models, as, if present, the markers of human ageing can be correctly identified and monitored in mice (24, 34, 35).

Replacement of the senescent cells with functioning T cells:

It has been hypothesised that immune function could be restored by the introduction of new immune cells into the body (30). Adoptive therapy is "the infusion of immune effector cells for the treatment or prevention of disease" (19). The introduction of effector T cells through adoptive therapy has been shown in numerous studies to be successful in the removal of latent viral infections, such as cyclomegalovirus (CMV) (36, 37). However, participants in these studies are graft recipients undergoing treatment with immunosuppressive agents to prevent graft versus host reactions (36, 37). Although it is not explicit in the literature, these patients are likely to be younger, meaning the results may not be generalisable to the elderly population. Additionally, immunosuppression may not be an appropriate model for the elderly. This is because, although the elderly patient has a weaker immune system, it may not be weak to the level of immunosuppression and the degree of 'weakening' could be highly variable within the elderly population (23). If this method is successful in the elderly, it is likely to be unfavourable due to complexity in administration and preparation of the T cells as well as high cost (38).

Restoration of the T cell pool balance:

During ageing the expression of hormones, growth factors and neuropeptides changes which causes the accumulation of adipose tissue within the thymus, and a reduced production of

naïve T cells which can be exported to the peripheral tissues (26). Restoration methods aim to maintain the normal thymic environment by utilising hormones and cytokines to stimulate the production of naïve T cells, which can be exported to the periphery to remove 'non-self' substances within the tissues (30, 39). Table 4 shows examples of some agents which can be utilised to aid the restoration of the thymus.

Agent	Mode of action	Sources
Interleukin 7 (IL-7)	Believed to stimulate peripheral T cell survival and expansion through the induction of BcI-2. It is thought to influence T cell progenitors directly	(18, 40)
Interleukin 2 (IL-2)	Aids thymocyte development and peripheral T cell survival and proliferation. IL-2 increases the proportion of thymocytes that differentiate into mature T lymphocytes. Evidence suggests that it reverses thymic atrophy	(30)
Fibroblast growth factor 7 (FGF7)	FGF7 binds to stromal cells, releasing various factors that then act on thymocytes, leading to the downregulation of Ink4a in early T cell progenitors. During ageing there is normally an increase expression of Ink4a, contributing to the ageing process	(23, 41)
Growth hormone (GH) and Insulin-like growth factor 1 (IGF- 1)	GH stimulates rising levels of IGF-1. IGF-1 binds to receptors on the thymic stroma and thymocytes, increasing their differentiation into mature T lymphocytes	(23)

Table 4: Potential hormones and cytokines to aid the restoration of the thymus. *The precise cellular mechanisms are currently not fully understood (18). Data taken from: (18, 23, 30, 40, 41)*

There have been numerous studies demonstrating how specific cytokines and hormones can improve immune activity using mice and rhesus macaques as model organisms as well as human clinical trials (19). However, these cytokine and hormone are potentially hazardous. For example, studies suggest IL-7 increases the levels of circulating naïve T cells and decreases the proportion of CD4+ regulatory T cells (42, 43). Evidence suggests with advanced ageing one has higher numbers of T regulatory cells than the young and this increase could be contributing to the age-associated decline in immune response (21, 44). However, this therapy could potentially be highly risky as if the proportion of regulatory T cells are reduced too much they will be unable to protect the host from potential self-reactive lymphocytes and autoimmune disease may result (21). Additionally, there are multiple studies suggesting the benefits of administering GH for the elderly beyond

improvements in immune function, such as reducing thinning of skin as well as bone and muscle loss (45-47). However, adverse side effects were also found during a clinical trial (39). These side effects are likely to be due to GH having widespread systemic effects as it has receptors expressed on the cells of multiple tissues within the human body (23). An additional concern is that there is considerable evidence that neoplastic cells also express receptors, for example IGF-1 receptors, which could stimulate tumour growth (48). This is a particular concern within the elderly population, as the risk of cancer increases with ageing (49).

The effect of lifestyle changes on immunosenescence:

There is growing evidence that exercise could reverse several characteristics of immunosenescence (28, 50-52). It has been hypothesised that physical exercise mobilizes aged T cells from the peripheral tissues to the blood, where they can be removed from the blood via apoptosis. Once these senescent T cells are removed it is believed that there is 'free space' for functional naïve T cells to occupy these compartments (28). Additionally, exercise is known to benefit the cardiovascular system as well as psychosocial aspects of health, providing the elderly with supplementary health benefits (50).

However, there are contradictions; some studies looking at the immunological effect of exercise for the elderly demonstrated it to have little benefit on improving immune function, as immunological parameters such as white blood cell counts were unaltered (50, 53-55). This conflicting data is likely to be due to differences in the study designs, which include measuring the effect of different types, intensity and lengths of exercise, on different immunological parameters (50). Additionally, studies looking at the effect of marathon training on the immune system suggest that there is a transient reduction in innate immune response lasting up to 72 hours. This is thought to be due to changes in stress hormones, increased blood flow, lymphocyte apoptosis, and dehydration (56). However, the exercise suggested for the ageing population is likely to be of a very different nature; the intensity expected will be less taxing to the body and many of the side effects associated with intensive long duration exercise will not occur. Studies should now focus on optimising the type, frequency and duration of exercise that most improves immune function for the elderly.

Sleep complaints are more common in the elderly. In a large epidemiological study it was shown that more than 80% of over 65-year-olds reported at least one recurrent sleep problem (57). Many of the changes that occur during immunosenescence are also seen in people with sleep deprivation (see Table 5). Despite this correlation, a causal relationship has not yet been shown (27). This is because it is not apparent whether immune changes causes sleep deprivation, or sleep deprivation causes immune changes. Prospective cohort studies are needed in order to understand if poor sleeping habits influence immunosenescence.

Characteristic	**Source**
With sleep deprivation there is an increased expression of DNA damage response genes, increasing senescence, possibly linking sleep loss to the process of cellular ageing	(58)
With lack of sleep the proinflammatory profile is present	(58, 59)
When individuals are sleep deprived the natural killer cell responses and T cell cytokine production are reduced	(60, 61)
People who experience a lack of sleep have an increased risk of inflammatory diseases, similar to immunosenescence	(62-65)

Table 5: Changes to immune characteristics during sleep deprivation. *Data taken from: (58-65)*

Improving vaccinations for the elderly:

The elderly population have a less effective response to vaccination than the young (24). This is due to lower functionality of antigen presenting cells, CD4+ and CD8+ T cells, and B cells (see Fig. 4) (29).

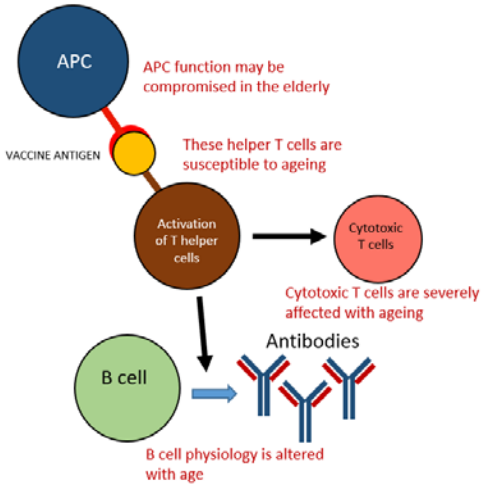

Figure 4: Influence of immunosenescence on vaccination efficacy. *After vaccination, the vaccine antigen is present in the body, the APC (antigen presenting cell) presents this antigen to the T helper cells and this normally leads to cytotoxic T cell and B cell responses. However, in the elderly, as shown by the red font, there are inefficiencies with this process. Data to make this figure is taken from Table 1: (66)*

Techniques previously discussed that restore the T cell balance and improve cellular lifespan could also improve vaccine responses within the elderly population by providing them with a more effective immune response (24). High dose vaccines have also been shown to have higher efficacy in the patients over 65 than standard vaccines. For example, a high dose of inactivated influenza vaccine was demonstrated to be more effective than a standard dose vaccine in clinical trials (67-69). The high dose influenza vaccine was also demonstrated to be safe, with patients experiencing minimal or no associated adverse side effects. However, in these clinical trials patients were excluded if they were medically unstable or in some studies if they experienced a cognitive condition such as dementia (67-69). The potential side effects of the high dose vaccine in these patient groups are therefore unknown.

Alternatively, the humoral and cellular immune response to DNA immunisations can be improved by the use of immunostimulant adjuvant patches (70, 71). Adjuvants added to vaccines can improve their immunogenicity (72). In addition to designing appropriate adjuvants, higher vaccination coverage through public health awareness campaigns should be a priority. This is because a higher vaccination coverage would provide herd immunity with benefits to those who are unresponsive to the vaccination. The elderly should receive regular booster vaccines and shortened vaccination intervals to maximise their immune response, increasing the efficacy of the herd immunity (66, 73).

Conclusions

The methods discussed in this review could reduce the effects of immunosenescence but are unlikely to reverse the decline of immunity completely. This is because they are only targeting a specific aspect of immunosenescence rather than stopping it from occurring or modifying all the molecular changes that occur. Nevertheless, a small increase in immune function is likely to be beneficial to the elderly population.

From the methods discussed, lifestyle changes are likely to be the most promising treatment option for the elderly in comparison to pharmacological and replacement methods. This is because the side effects of lifestyle changes are likely to be less hazardous and they are more cost efficient (50). Lifestyle changes are cheaper as they are more accessible and do not require medical intervention as patients could be educated through health promotion activities within their communities. However, further studies are needed to distinguish the optimum ways to alter sleep and exercise habits to most benefit the ageing immune system. Alongside these lifestyle changes, it is important that public health campaigns ensure the elderly are vaccinated appropriately to reduce the immediate risk of infection. This has been a public health priority since the introduction of a targeted vaccination program for the elderly (74).

In order to successfully reverse immunosenescence, we need to improve our current understanding of the molecular mechanisms which drive it. Future lines of research not discussed in this report that may lead therapeutics include understanding the role of the human microbiota and nutrient dependent signalling pathways in controlling the action of the immune system. It has been found that *bifidobacteria* decreases after 60 years of age, correlating with the occurrence of immunosenescence and may be causally related (75). Restoring the gut flora using probiotics and prebiotics has been associated with reduced chronic inflammation and pathogen colonisation within the host (75, 76). Potentially this restoration of microbiota composition may stop immunosenescence from happening or reverse it once it has arisen. Additionally, studies are beginning to decipher the molecular pathways which demonstrate how nutrition, metabolism and immunity are interconnected

(77, 78). This may lead to exciting preventative treatments as nutrient dependent signalling pathways could be altered by diet to enhance immune activity (78).

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