SCIENCE & SOCIETY

The ageing immune system: is it ever too old to become young again?

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Abstract | Ageing is accompanied by a decline in the function of the immune system, which increases susceptibility to infections and can decrease the quality of life. The ability to rejuvenate the ageing immune system would therefore be beneficial for elderly individuals and would decrease health-care costs for society. But is the immune system ever too old to become young again? We review here the promise of various approaches to rejuvenate the function of the immune system in the rapidly growing ageing population.

As a result of advances in medicine, public-health policies and socioeconomic development, we are living longer than ever before. The proportion of individuals aged 60 years and older, which accounted for approximately 10% of the total world population in 2000, will increase to approximately 22% of the population by 2050 (FIG. 1). The US Census Bureau predicts that this age group will comprise 25% of the total population of the United States by 2050, and similar demographic trends apply to other developed and developing countries (see the World Health Organization website).

Although the news that we are living longer is positive, this fact presents new challenges both to individuals and to society. Advanced age is often accompanied by chronic disease and an increased susceptibility to infections that negatively impact an individual's quality of life. This, in turn, has societal implications, as the costs incurred in caring for an increasing number of elderly people can have a significant impact on health-care systems. Therefore, interventions that can either slow or reverse the negative effects of ageing and thereby increase health-span — that is, the number of years of healthy, active life — would have major benefits for individuals and for our society.

Ageing can affect multiple organ systems and processes, so which of these should be targeted to best increase the quality of life for individuals as they age? It is now clear that the function of the immune system declines with age, which leads to an impaired ability to respond to vaccinations and to fight infections[1,2]. For example, elderly people are particularly susceptible to influenza, with 80–90% of mortalities from infection with influenza virus occurring in individuals aged 65 years and older[3]. Elderly individuals also suffer from autoimmunity more frequently, which further indicates the dysregulation of immune-system function that can occur with age[4]. Therefore, the ability to delay or reverse the effects of ageing on the immune system would have significant beneficial effects on increasing health-span in the ageing population.

The American actress Mae West once quipped, "You're never too old to become younger", but is this true for the immune system? Recent research indicates that it might be. Our understanding of the cellular and molecular changes that underlie the decline in immune function with age has increased significantly in recent years, and clinical trials to evaluate various methods by which to augment immunity in elderly individuals are now underway. In this Perspective article, we discuss the promise and the limitations of these and other potential therapeutic interventions. To set the stage for this discussion, we begin with a brief overview of how ageing affects the immune system.

Effects of ageing on immune function

Effective immunity to the multitude of pathogens that are encountered over the lifetime of an individual depends on the coordinated responses of the innate and adaptive immune systems. Age-related changes in innate immune function have been reported, but many of the findings are contradictory. Some studies have shown an age-related decline in the function of neutrophils, macrophages and natural killer cells, whereas other studies have not observed such changes[5]. There is evidence that ageing compromises the function of dendritic cells (DCs)[6], particularly the capacity of these cells to migrate to sites of infection and capture antigen[6]. However, it is not clear whether the number of DCs decreases with age. Therefore, additional research is required to clarify the effects of ageing on the innate immune system. By contrast, the results of studies in mice and humans — which are, in general, complementary — have provided important insights into how ageing affects the adaptive immune system. Here, we focus our discussion on the effects of ageing on adaptive immunity.

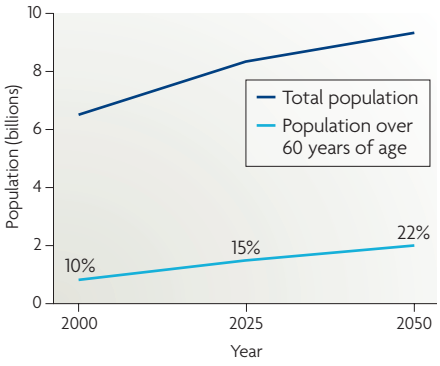


Figure 1 | **The proportion of individuals aged 60 years or older is projected to increase.** The total world population and the number of people aged 60 years or older in 2000, and the projected values for 2025 and 2050, are shown. The figure also shows that individuals aged 60 years or older currently constitute 10% of the world population and that this value is projected to increase to 22% in 2050. The figure is based on statistics from the World Health Organization website.

Lymphocyte development. T-cell development occurs in the thymus, which involutes with age in both mice and humans due to age-related changes that affect both T-cell progenitors and the thymic microenvironment. It was generally thought that this process of involution, which results in a decrease in thymic epithelial volume, begins at puberty and that the thymus is non-functional in elderly individuals. However, there is evidence that thymic involution begins during early childhood in humans[7]. Although the production of new T cells declines significantly with age, the thymus still has limited activity even in individuals of almost 100 years of age[8].

Studies in mice indicate that B-cell production in the bone marrow also declines significantly with age[9–12]. However, the extent to which a similar decline occurs in humans is still unclear as a result of conflicting reports on B-cell production in elderly individuals[13,14]. Nevertheless, as there is a decrease in the volume of haematopoietic tissue in the bone marrow in humans with increasing age[15], it is probable that primary B-cell lymphopoiesis also decreases with age.

Lymphocyte function. Ageing also compromises many aspects of lymphocyte function. For example, B cells from aged humans produce antibodies with decreased affinity for antigen and have an impaired ability to undergo class-switch recombination compared with B cells from younger individuals[16]. Some of these effects, which result in impaired humoral immune responses in elderly individuals, might be B-cell intrinsic. However, an age-related decline in the function of $CD4^+$ T helper cells might also be a contributing factor[17].

Alterations in the $CD8^+$ T-cell compartment are some of the best characterized age-related changes in the immune system[18]. These cells have a particular propensity for oligoclonal expansion with age (FIG. 2) such that the $CD8^+$ T-cell repertoire becomes increasingly skewed towards previously encountered antigens, particularly those derived from cytomegalovirus[19,20]. This, in turn, can limit the ability of the $CD8^+$ T-cell population to respond to newly encountered viruses, such as emerging strains of influenza virus. It is probable that $CD4^+$ T cells also undergo oligoclonal expansion in elderly individuals, but to a lesser extent than $CD8^+$ T cells[18].

In addition to restricting the repertoire of antigens to which T cells can respond, another consequence of T-cell oligoclonal expansion is that an increasing proportion of the niches in peripheral immune tissues become occupied by terminally differentiated cells[21]. Therefore, the few naive T cells that are produced in the thymus of elderly individuals might not be able to take up residence in peripheral tissues because of a lack of 'space', resulting in a further inability to renew the immune repertoire (FIG. 2). This might also be the case for B cells. Studies in mice indicate that B cells from aged animals have increased sensitivity to growth and survival factors and might thereby compete more effectively than nascent B cells for space in peripheral niches[22]. It remains to be determined whether this is also true for human B cells.

Perspectives on lymphocyte ageing. There is a consensus view that the changes in immune function that we have described occur with age, but there is controversy regarding how to refer to them. One school of thought is that age-related impairments in the function of the immune system are 'defects' that can be 'cured'. An alternative view is that ageing is a normal, physiological process that should not be referred to in a disease context. However, it is not clear if or how the resolution of this issue might affect the development of strategies to increase immune function in the elderly population. We leave it to the reader to decide from which of these perspectives the age-related effects on the immune system are best considered.

Why do we age?

A detailed discussion of the underlying molecular mechanisms that occur in ageing cells — which include the accumulation of DNA damage, oxidative stress, shortening of telomeres and the activation of tumour-suppressor genes[23,24] (BOX 1) — is beyond the scope of this article. But why does ageing occur in the first place? That is, does a decline in the function of the immune system offer any benefits to an individual or to a species?

In the case of primary lymphocyte development, there might indeed be benefits to immune-system ageing. Most lymphoid progenitors fail to productively recombine antigen-receptor genes[25] and undergo apoptosis. Therefore, from an evolutionary perspective, it might be advantageous to slow lymphopoiesis to conserve energy for other biological processes. In addition, the recombination of antigen-receptor genes depends on the cutting and ligation of DNA, which is thought to make lymphoid progenitors particularly susceptible to transforming events such as chromosomal translocations[26]. Indeed, B-cell acute lymphoblastic leukaemia is the most common type of paediatric cancer[27]. It is tempting to speculate that the decrease in B-cell production that occurs with age is initiated in early childhood, analogous to the decline in thymopoiesis[7]. This decline

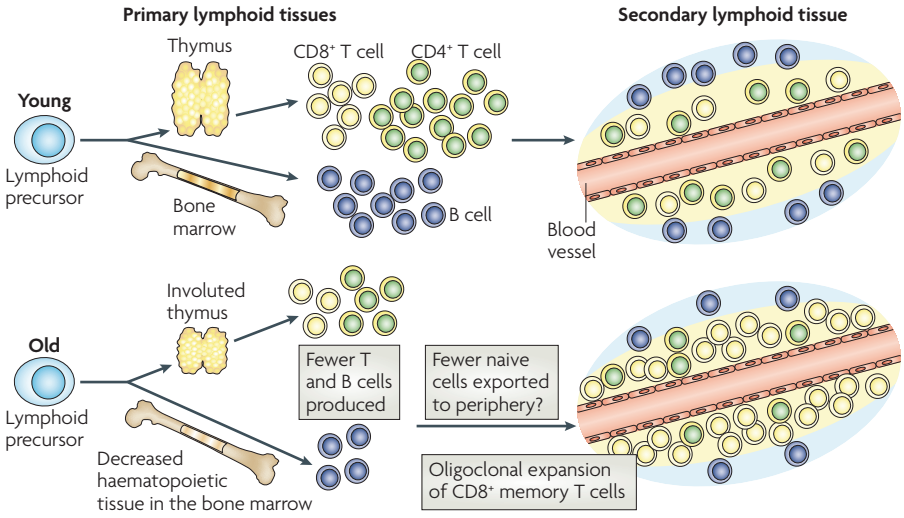


Figure 2 | **Effects of ageing on lymphocyte production and the distribution of cells in secondary lymphoid tissues.** Several events contribute to the decrease in the number and function of adaptive immune cells that occurs with age. T-cell generation is decreased as a result of thymic involution (which involves a decrease in thymic cortical and medullary mass and an increase in fat content). B-cell production probably also decreases with age due to a decrease in the amount of haematopoietic bone marrow. Therefore, fewer naive T, and possibly B, cells are exported to the periphery. Oligoclonal expansion of the $CD8^+$ T-cell population starts around the sixth decade of life, which results in skewing of the T-cell repertoire and an increased number of terminally differentiated memory $CD8^+$ T cells in peripheral niches.

in production, in turn, might decrease the probability of developing a fatal lymphoid malignancy and thereby increase the probability that an individual will reach reproductive age.

It is more difficult to determine what benefits might be conferred by an age-related decrease in the number or function of mature lymphocytes. It seems unlikely that such changes are due to evolutionary pressures, as most individuals are well past reproductive age by the time that age-related changes in the immune system become apparent. In fact, when declines in the function of the immune system do occur, individuals do not become severely immunodeficient, even in the eighth decade of life.

Ageing and the environment

Many of the studies that have defined the effects of ageing on the immune system have used syngeneic strains of mice. Interestingly, there is considerable variation in the extent of immune-system ageing between these animals[12], even between those that are housed together[9]. Epigenetic regulation — which can be defined as changes in gene expression that occur in the absence of alterations in DNA sequence[28] — is one mechanism that could explain the differences in immune-cell ageing that are observed between genetically identical mice. Epigenetic changes in gene expression might also underlie aspects of human ageing; for example, a recent study showed that the epigenomes (based on the content and distribution of methylated and acetylated DNA) of monozygotic twins were indistinguishable as infants but differed later in life[29].

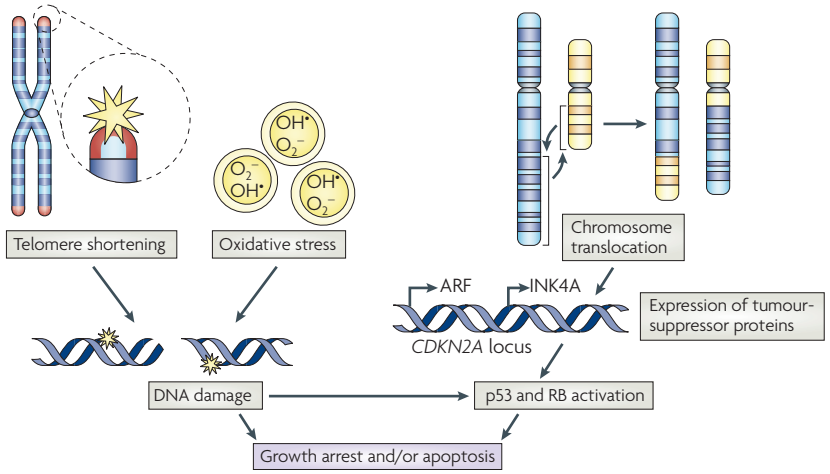
An important question raised by these studies is what triggers these epigenetic changes? One possibility is that they are stochastically determined[30]. Alternatively, various environmental stimuli might be responsible for their induction. For example, mice housed together establish a social order[31] and being in a submissive or dominant social position could result in stress that, in turn, could affect patterns of gene expression or the activity of some proteins. In support of this idea, reports have shown that life stress in humans can decrease the activity of telomerase, the enzyme that is responsible for maintaining the protective sequences of DNA at the ends of chromosomes[32,33].

Much remains to be learned regarding the interplay between gene expression, the environment and ageing. Nevertheless, the apparent links between these factors indicate that physical, behavioural and emotional considerations all influence the process of ageing.

Box 1 | **Molecular basis of ageing**

Several molecular changes occur in ageing cells[24,53,54]. Over time, the build-up of free radicals (generated through normal metabolic activity) can cause DNA crosslinking, strand breaks and base lesions through oxidative stress. With successive cell divisions, DNA can become increasingly susceptible to degradation due to the shortening of telomeres, which are regions of repetitive DNA sequence at the ends of chromosomes. Human $CD8^+$ T cells, in particular, show progressive telomere loss with antigen-driven proliferation that can limit their further proliferation[52].

Chromosome translocations can make immune cells susceptible to transformation, particularly during their development in the bone marrow. To prevent the occurrence of transformation events, cells can undergo senescence (a state of cell-cycle arrest in which cells remain metabolically active) as a result of the activation of various tumour-suppressor genes. In this regard, particular attention has been focused on the tumour-suppressor proteins INK4A (also known as p16) and ARF (alternate reading frame; also known as p14 in humans and p19 in mice), which are both encoded by the *CDKN2A* locus[23,55–57]. Expression of INK4A and ARF results in activation of retinoblastoma protein (RB) and p53, respectively. We recently showed that the expression of INK4A and ARF is increased in aged B-cell progenitors, which accounts for their decreased proliferation and increased apoptosis. In addition, INK4A- and ARF-expressing B-cell progenitors are more resistant to transformation[58]. These findings provide a molecular basis for the effects of ageing on lymphopoiesis, and they also link the decreased incidence of lymphoblastic leukaemia that is observed in elderly individuals with mechanisms of ageing in lymphoid cells.



To identify additional events that underlie the effects of ageing, sophisticated genomic, proteomic and systems-biology approaches are being used to compare cells isolated from young and old mice or humans. For example, haematopoietic stem cells (HSCs) from old animals have downregulated expression of genes that control immune-cell development compared with HSCs from young animals[59]. A challenge will be to identify those genes that act at crucial checkpoints at which multiple intracellular pathways converge, as these are likely to be the most promising targets for preventing or reversing immune-cell ageing.

Implications for clinical practice

The effects of ageing on the immune system have implications for clinical medicine. An obvious example is the case of vaccination against influenza virus, which has an efficacy of only 30–40% in protecting elderly patients from disease[34]. There are also consequences for transplantation, as bone marrow donated by older individuals has a decreased capacity to reconstitute the immune system in recipients[35]. Such examples highlight the need to increase the number of physicians with knowledge of how ageing affects the immune system as the proportion of aged individuals in the population increases[36]. It is probable that specialists in geriatric medicine, particularly those in academic settings, will be increasingly called on to translate the most recent research developments to the clinic.

Therapies that aim to rejuvenate the immune system in elderly individuals need not necessarily restore immune function to the levels that are found in younger individuals, as even a modest increase in immune function might be sufficient for clinical benefit. The ideal therapies will be easy to administer, cost effective and readily available to a large number of individuals. The following sections review some of these developments.

Caloric restriction. Caloric restriction, which is defined as a decrease in dietary intake of calories of 30–50%, meets these criteria for an ideal therapy. Caloric restriction has been shown to increase longevity in many species, but the precise mechanisms by which it acts remain to be defined. It has been reported that the expression of sirtuin (silent mating type information regulation 2 homologue) proteins, a family of histone deacetylases that regulate gene expression, is upregulated by caloric restriction, and that these proteins mediate some of the beneficial effects associated with decreased caloric intake[37]. Interestingly, resveratrol, a compound found in red wine that has been associated with increased lifespan in many species, induces the activity of sirtuin proteins[37].

We know little about the effects of caloric restriction on the immune system. Studies in non-human primates indicate that caloric restriction increases the number of naive T cells and the diversity of the T-cell repertoire[38,39]. However, although caloric restriction might inhibit the age-related decline in immune function, it has been reported that aged mice on a regimen of caloric restriction have increased mortality in response to influenza virus because they lack the energy reserves that are required to respond to the infection[40]. Therefore, controlled studies in humans are needed to determine whether caloric restriction will be of benefit in delaying or reversing the age-related decline in immune function. It will also be important to determine if a short period of caloric restriction is sufficient for a beneficial effect on the immune system and, if not, whether individuals would be willing to adopt a regimen of caloric restriction for a prolonged period of time.

Cytokine and hormone treatment. Considerable attention has been focused on rejuvenation of the involuted thymus. Numerous pharmacological interventions that involve manipulating the concentration of interleukin-7 (IL-7)[41], sex steroids[42], growth hormone[43] or keratinocyte growth factor (KGF; also known as FGF7)[44,45] *in vivo* have been reported to inhibit age-related declines in T-cell development and/or function in preclinical and/or clinical trials[46]. For example, a Phase I clinical trial with a non-glycosylated form of human IL-7 resulted in the expansion of both naive and memory $CD4^+$ and $CD8^+$ T-cell populations[41]. In addition, a recent trial showed that treatment with growth hormone increased thymus size and stimulated peripheral immune responses in humans[43]. KGF is also a candidate for rejuvenation of the involuted thymus; preclinical studies showed that systemic administration of KGF to 15-month-old mice restored thymus cellularity to levels equivalent to those found in young animals[44,45].

Although the possibility of using various hormones and/or cytokines to stimulate thymopoiesis seems promising, it will be important to identify any negative side-effects associated with the use of these agents and to define the cellular and molecular bases for their actions (BOX 2). In particular, it will be essential to confirm whether the naive T cells that are generated after the administration of these agents can displace memory T cells and take up residence in peripheral niches. If not, then cytoablative conditioning regimens that eliminate some or all of the senescent lymphocytes might be required before the use of therapies that restore immune-cell number and/or function. These issues aside, cytokines and hormones meet the criteria for widespread use as many are already being manufactured at clinical-grade quality and they can be administered to patients in a relatively noninvasive manner.

Future perspectives

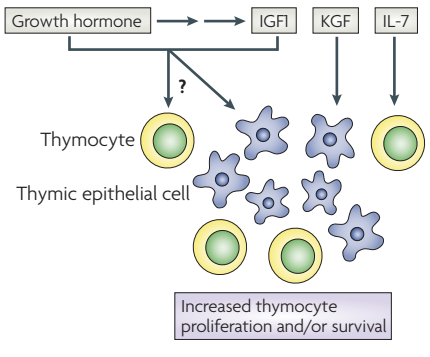
As we learn more about the cellular and molecular changes that underlie ageing of the immune system, it is probable that new approaches by which to reverse this process will be uncovered. For example, it will be interesting to see if advances in the field of stem-cell biology will be applicable to immune-system ageing[47]. It is now possible to generate most haematopoietic cell types, including B cells[48] and T cells[49], from human embryonic stem cells. If it is found that B and T cells can also be derived from induced pluripotent stem cells, which are generated by the reprogramming of adult somatic cells through the expression of a defined set of transcription factors[50], it might be possible to generate nascent, autologous lymphocytes for the purpose of repopulating the immune system of elderly patients. However, as lymphocytes can currently be generated from human embryonic stem cells with only limited efficiency, this remains only a possibility for the future. It is also not clear whether it will be possible to adapt such experimental approaches for widespread, cost-effective use in the clinic.

As discussed above, a promising strategy for rejuvenation of the aged immune system is to target lymphocyte production to increase the number of naive B and T cells that migrate to secondary lymphoid organs. The fact that

Box 2 | Effects of IL-7, growth hormone and KGF on the thymus

Manipulating the concentration of various cytokines and hormones — including interleukin-7 (IL-7), sex steroids, growth hormone and keratinocyte growth factor (KGF; also known as FGF7) — has been shown to be a promising strategy for rejuvenation of the involuted thymus. The precise mechanisms by which these agents act have not been fully elucidated. Some, such as IL-7, probably act through direct effects on T-cell progenitors, which are known to express the IL-7 receptor and which depend on this cytokine for their development. By contrast, the actions of growth hormone are more complex. Many of the effects of growth hormone are mediated through the induction of expression of insulin-like growth factor 1 (IGF1) in local tissues, and both of these hormones could have direct or indirect effects on developing thymocytes. In support of this is the finding that $CD4^-CD8^-$ thymocytes express the receptor for IGF1 (REF. 60). However, it is also possible that growth hormone and/or IGF1 affect thymic epithelial cells, which are an important part of the thymic microenvironment. These hormones could enhance the growth, survival and/or function of these cells, which in turn would increase the level of thymopoiesis. Recent clinical trials have shown that growth hormone increased the thymic mass of immunocompromised patients[43].

It has recently been shown that systemic administration of KGF to 15-month-old mice restores the cellularity of the thymus to levels that are found in young animals[44,45]. Interestingly, the KGF receptor (FGFR2) is not expressed by thymocytes in mice; current evidence indicates that KGF increases the number of T cells through stimulating thymic epithelial cells to secrete various cytokines that then act on developing thymocytes[45].



$CD4^+$ T cells derived from aged progenitors have normal function provides support for the potential effectiveness of this strategy[51]. An alternative approach would be to attempt to reverse the effects of ageing on the function of mature immune cells in peripheral lymphoid tissues. However, as these cells consist mainly of memory cells that might have a skewed repertoire, it remains unclear whether rejuvenation of these populations will significantly increase overall immune function in elderly individuals.

Because the ageing process varies widely between individuals, it will be important to develop ways by which to identify those patients who would benefit most from immunomodulatory treatments. It would therefore be useful to have simple biomarkers with which to accurately measure the effects of ageing on the immune system. Progress in this area is already apparent; for example, the loss of CD28 expression by senescent $CD8^+$ T cells correlates with a decreased response to vaccination[52]. It is also interesting that ageing is associated with an increased level of circulating, pro-inflammatory cytokines, such as IL-6. Studies carried out under the auspices of the Senior Europeans (SENIEUR) protocol have found that elderly individuals with low serum levels of IL-6 generated a more robust response to vaccination, whereas those with high levels of IL-6 responded poorly to vaccination[3].

Developing a means to mitigate the effects of ageing is a subject of great interest to the general public. Indeed, we have been bombarded by a wealth of information suggesting that ageing can be reversed with quick and easy interventions. For example, a general internet search for articles on this topic leads to more than two million information sources that range from the scientifically accurate to the truly bizarre. As we have summarized here, we have reason to be optimistic that the immune system is never too old to be young again. However, the scientific and medical communities have a responsibility to refute claims that are not based on scientific evidence whenever possible, to provide sound information to the public and to temper the increasing tendency to publicize basic research results or preliminary clinical data that could be years away from widespread therapeutic application.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD28 | *CDKN2A* | FGFR2 | growth hormone | IGF1 | IL-6 | IL-7 | KGF

FURTHER INFORMATION
Kenneth Dorshkind's homepage: http://faculty.uclaaccess.ucla.edu/institution/personnel?personnel_id=45615
US Census Bureau: http://www.cdc.gov/nchs/data/hus/hus07.pdf#fig01
World Health Organization: http://www.who.int/en/
SENIEUR Protocol: http://www.medizin.uni-tuebingen.de/eucambis/home/senieur.html

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OPINION

Annexin A1 and glucocorticoids as effectors of the resolution of inflammation

Mauro Perretti and Fulvio D'Acquisto

Abstract | Glucocorticoids are widely used for the management of inflammatory diseases. Their clinical application stems from our understanding of the inhibitory effect of the corticosteroid hormone cortisol on several components of the immune system. Endogenous and exogenous glucocorticoids mediate their multiple anti-inflammatory effects through many effector molecules. In this Opinion article, we focus on the role of one such effector molecule, annexin A1, and summarize the recent studies that provide insight into its molecular and pharmacological functions in immune responses. In addition, we propose a model in which glucocorticoids regulate the expression and function of annexin A1 in opposing ways in innate and adaptive immune cells to mediate the resolution of inflammation.

Inflammation is a primordial response that functions to protect the host against invasion by pathogens or exposure to xenobiotics. However, overly aggressive or prolonged inflammatory responses can be detrimental to the host. Therefore, higher organisms have evolved mechanisms to ensure that the inflammatory response is limited in time and space. Many endogenous anti-inflammatory and pro-resolving mediators function to counteract the properties of pro-inflammatory factors and to ensure a prompt resolution of inflammation. The concept that inflammation is resolved in a time- and space-specific manner is emerging, such that different outcomes can be produced according to the stage or site at which a given pathway or mediator becomes operative[1,2]. The action of anti-inflammatory and pro-resolving mediators and pathways on several components of the inflammatory response is crucial for restoring tissue structure and homeostasis. Elucidation of their effects on immune cells could lead to the identification of the molecular target (or targets) of a given mediator, thereby prompting innovative drug discovery for the treatment of chronic inflammatory conditions[2,3].

Glucocorticoids are the first class of endogenous anti-inflammatory mediators that have been successfully used for therapeutic purposes; budesonide and beclomethasone are widely used for the treatment of asthma, prednisolone is used for rheumatoid arthritis and other autoimmune diseases, and mometasone and hydrocortisone are used for eczema and psoriasis. In healthy individuals, the circadian release of glucocorticoids (such as cortisol and corticosterone) from the adrenal glands facilitates the control of several homeostatic processes that are crucial for health. During inflammation, increased levels of cortisol dampen local and systemic inflammatory events, thereby favouring proper resolution of the inflammatory response[4,5]. These fundamental pathophysiological functions of glucocorticoids are achieved through many molecular mechanisms, which can be broadly divided into genomic mechanisms (involving transactivation or transrepression of gene transcription) and non-genomic mechanisms (that are rapid and independent of *de novo* protein synthesis) (BOX 1). In this Opinion article, we focus on one downstream mediator of glucocorticoids, the 37 kDa protein annexin A1 (also known as lipocortin 1; encoded by *ANXA1*). We review the recent evidence indicating that glucocorticoids regulate the synthesis and function of annexin A1 (REFS 4,6), possibly through a combination of both genomic and non-genomic processes, depending on the cell type and the time of induction. In addition, we describe the emerging data showing that glucocorticoids can differentially affect the annexin A1 pathway in cells of the innate and adaptive immune system, in which this pathway can have opposing effects. We propose that the annexin A1 pathway is an important mediator of the anti-inflammatory effects of glucocorticoids.

Effects in innate immunity

Annexin A1 is a member of a superfamily of annexin proteins that bind acidic phospholipids with high affinity in the presence of Ca^{2+} (REF. 7). There are 13 mammalian annexin proteins, each of which has specific biological functions. Similarly to other annexin proteins, annexin A1 is expressed in resting cells and binds to acidic phospholipids in the presence of Ca^{2+}; original studies of annexin A1 therefore focused on its role in granule fusion and exocytosis, in which it was shown to promote the fusion of vesicle membranes with plasma membranes in reconstituted systems[7].

The actions of annexin A1. In resting conditions, human and mouse neutrophils, monocytes and macrophages constitutively contain high levels of annexin A1 in their cytoplasm[8–10]. Following cell activation (for example, by neutrophil adhesion to endothelial-cell monolayers), annexin A1 is promptly mobilized to the cell surface and secreted[11]. The molecular mechanisms that are responsible for this rapid secretion are cell specific. In macrophages, the