NIH Public Access
Author Manuscript
*Semin Immunol*. Author manuscript; available in PMC 2008 October 1.

Published in final edited form as:
*Semin Immunol*. 2007 October ; 19(5): 310–317.

# Non-human Primate Models of T-cell Reconstitution

**Janko Nikolich-Žugich**
*Vaccine and Gene Therapy Institute and the Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, 97006*

## Introduction

Non-human primates (NHP) have become an indispensable model in studying the common and dangerous human chronic infections, including HIV/SIV, Hepatitis C virus, and tuberculosis. More recently, we and others have used aged NHP to model human immune aging. Chronic infections and aging are both characterized by a significant depletion of defined lymphocyte subsets and the compensatory attempts to regenerate the immune system. As efficacious antiviral drugs and novel methods to improve and boost the immune system emerge, therapeutic immune regeneration has become a realistic goal in both the physiologic and pathologic settings. This article will summarize our current knowledge on this topic and will discuss future research directions as well as the potential and power of translational studies in non-human primate models of infection, aging and bone marrow transplantation.

## Model discussion

Most of the data describing lymphocyte development, lymphocyte homeostasis and the age-related changes in homeostasis and function of the immune system, come from studies of the immune system in specific pathogen-free (SPF) housed rodents. This model is popular among immunologists for obvious reasons – defined immunogenetics, versatile genetic manipulation, a plethora of reagents and tools and the ability to expand and house large animal numbers. These advantages, along with a number of established infectious disease models make incisive mechanistic studies realistically achievable in inbred laboratory mice. Consequently, laboratory rodents handily outcompete any other model by a margin of nearly 10:1 with regard to the number of scientists using them, the number of the NIH awards employing the model, and, on the average, the number of speakers presenting the data from a given model at any major meeting.

Therefore, it is not surprising that rodent models have been invaluable in elucidating various fundamental facets of the structure and function of the immune system. When it comes to translating the findings into human health and clinical medicine, however, rodent models are less informative than large animal models. There are reminders that the results from rodent models do not automatically translate to humans [1], including failure in humans of vaccine approaches that were validated in rodents [2]. Therefore, the vaccination efficacy in primates is frequently used as a stringent, “real-life”, validation of results obtained in rodents, and pharmaceuticals must be tested in both rodents and non-rodents for safety and toxicity prior to

Correspondence : Dr. Janko Nikolich-Zugich, Vaccine and Gene Therapy Institute, Oregon Health & Science University, West Campus, 505 NW 185th Avenue, Beaverton, OR, 97006, USA; tel. 503/418-2752, e-mail: nikolich@ohsu.edu.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

human trials [XXX}. Old world monkey species (mangabeys, macaques, baboons, mandrills, patas monkeys etc.) have the closest evolutionary relationship to humans among the approachable animal models. This makes them the most appropriate, and often the only, model for a variety of infectious diseases, including AIDS, Hepatitis C virus (HCV), emerging diseases and diseases caused by select (bioterrorism) agents[3-7]. The organization and function of the immune system in these animals closely resembles that of humans [8-12]. Thus, NHP provide an invaluable in vivo model of pathogenesis and immunity to, infection, but also a key tool to develop vaccines and therapies to protect against such agents. Furthermore, this model is essential to investigate fundamental immunological questions relevant to the primate immune system, which cannot be ethically studied in humans.

In case of immunity to infection and of the aging of the immune system, there are additional inherent differences between rodent models and humans. First, the span of chronological aging is widely different between rodents and humans (differences in median and maximal lifespan between mice/rats and humans are ~30-fold). Second, inbred laboratory rodents, different from genetically diverse humans, are maintained under SPF) conditions which constitutes an enormous difference in the microbial environment that shapes the state of activation of the immune system. Finally, rodents and humans diverged from common ancestor ~210 million years ago. The non-human primate (NHP) model scores much better in all of these points (difference in lifespans only 3-fold; outbred genetics and exposure to environmental pathogens, unless deliberately manipulated, are comparable to humans; extremely close evolutionary relationship with ~30 million year divergence between the old world monkeys and humans [13]; shared markers of T-cell phenotype, differentiation and function [8-10]), and thereby represents a highly desirable and relevant model of infection, immunity and immunogerontological conditions observed in humans.

Despite this potential, the scientific yield of immunological and, even more so, immunogerontological studies in NHP has historically been severely hampered by the lack of the necessary tools and expertise to perform and correctly interpret incisive studies of NHP immunobiology. Although human-specific reagents frequently cross-react with NHP cells, such cross-reactivity is often incomplete and/or inadequate, and many of these reagents have been applied without adequate optimization and validation in various NHP species. Moreover, procedures and protocols for longitudinal, systemic analysis of immunity were poorly developed in NHP, negating a key advantage of these animals as models of immune function and vaccine efficacy. This picture is rapidly changing with the latest phase of AIDS/SIV research, which brought state-of-the-art tools and their verification to the NHP model [8,14, 15}. Similarly, there has been a recent effort by our and other laboratories to characterize aging of the immune system in NHP and to popularize NHP as a model to study immune aging [10, 16-18]. More importantly from the standpoint of this review, induced and acquired immunodeficiencies and aging are all accompanied by depletion of certain lymphocyte subsets and are therefore ideal to study immune reconstitution in cases where the primary cause for such depletion can be retarded or eliminated.

## A brief overview of T-cell homeostasis

For the purposes of this review, we shall define T-cell homeostasis as the maintenance of naïve and memory T-cell pool numbers and diversity and the ability to rebound to prior state after acute Ag challenge. Most of our knowledge on how homeostasis is maintained comes from rodent studies, with occasional contributions from larger animal or human models (rev. in [19-22]. Maintenance of the peripheral T-cell pools is an active and dynamic process [23,24]. Naïve and memory T-cells are believed to inhabit distinct, but somewhat overlapping, niches, which are ill-defined anatomically, but are likely to be discrete parts of T-cell microenvironment that can provide them with survival and trophic signals. Naive cells are

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

predominant in secondary lymphoid organs (lymph nodes and spleen), while memory cells patrol the tissues. Moreover, experiments in young mice indicated that naïve and memory T-cells may be largely independently regulated [25], although these results may be subject to alternative interpretations as well.

Life cycle of a peripheral T-cell begins at the time of its emigration from the thymus. Recent thymic emigrants (RTE) seed the periphery at a relatively constant rate (~1-2× $10^6$/day in mice, or ~1% of the total thymic cell content/day) throughout much of adulthood [26]. However, thymic involution begins almost immediately after birth in humans [27] and a second decline in thymic production in humans may occur between the fourth and the fifth decade of life [28]. Parallel situation has been observed in mice, where the number of RTE drops by at least tenfold between young adult to 22-mo-old mice, paralleling the reduction of thymic cellularity and mass [29,30]. Despite that decline, in both mice and humans the thymus produces naïve T-cells longer than previously believed [29,31]. Which factors regulate this production? It appears that thymic output is regulated independently from the size of the peripheral pool[32,33], and it is likely that the output is regulated by the level of seeding by bone marrow precursors and the availability of thymic stroma. Upon emigration, RTE contribute to the pool of naïve peripheral T-cells, which have no pre-set life spans[34,35]. Rather, naive CD8 and many of the naïve CD4 T-cells are maintained by trophic signals from interaction of their T-cell receptor TCR with self peptide-MHC (pMHC) complexes. Naïve T-cells also need the cytokine IL-7, and can survive indefinitely in serial transplantation experiments if these two contacts are provided[23,36]. However, these cells are also regularly replaced by RTE in a random fashion, ensuring diversity of the naïve pool [32]. Murine RTE proliferate faster than naïve peripheral T-cells in the first three weeks after export [33], perhaps in order to maximize naïve T-cell diversity. After that, they equilibrate with other naïve T-cells. *In vivo*, naïve T-cells display very low levels of spontaneous (dubbed “homeostatic”) cycling. Homeostatic cycling is greatly increased in lymphopenia, where T-cells sense the “space” (most likely by sensing an excess of unused cytokines IL-7 and IL-15) and try to fill it by responding to signals that drive them into antigen (Ag)-independent homeostatic proliferative expansion (HPE) [21,23]. TCR:pMHC contact and the common γ chain cytokines IL-2, IL-7 and IL-15 have the potential to critically influence T-cell survival, maintenance and expansion. In mice, TCR:pMHC contact and IL-7 and IL-15 are believed to molecularly define “niches” or “space”, respectively, for conventional TCR αβ T-cells, and to allow HPE. It is unclear whether these signals are provided by a particular cell type (e.g. dendritic cells). It is also not clear whether the competition between T-cells for survival may be confined in the anatomic or histologic sense so that only certain cell types (e.g. dendritic cells) would be able to provide survival and trophic signals. IL-2 opposes some of the actions of IL-7 and IL-15[37] and its primary role appears to be in immune regulation and production and expansion of T regulatory cells [38]. While it is accepted that IL-2 production plummets with aging [39], the evidence for age-related alterations in IL-7 or IL-15 levels is somewhat contradictory [40-43], in part because it is extremely difficult to measure relevant IL-15 concentrations due to its highly localized mechanism of action. It is also controversial whether exogenously administered IL-7 exerts its main effects at the level of thymopoiesis [40,44] or the level of peripheral T-cell maintenance [42,45,46].

The memory compartment arises from the naive T-cells following Ag encounters, and is broadly divided into central memory cells, which self renew but lack acute activation markers and need a short lag phase to perform the optimal spectrum of effector functions, and effector/effector memory cells that are acutely activated and able to perform immediate effector function[47]. Neither central memory nor effector/effector memory T-cells require specific pMHC contact for survival; they cycle and self-renew *in vivo* three to four-fold faster than naïve T-cells and are capable of vigorous proliferation during lymphopenia [23]. In mice, this proliferation, as well as memory T-cell maintenance, is dependent on IL-15, or, in its absence, on IL-7[48]. Therefore, in the face of repeated pathogen challenges, homeostatic forces must

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

balance Ag-driven expansion of naïve Ag-specific T-cells, generation of effector population, their subsequent contraction, and the recruitment of the surviving cells into the memory compartment. This simultaneously preserves T cell repertoire diversity to combat new pathogens and enables vigorous recall response to reinfection. In young and adult mice, numbers and diversity of peripheral T-cells are remarkably similar in different individuals [23,24,49], suggesting that clonal expansion and contraction must be tightly regulated in adulthood (rev. in[24]). However, this balance likely depends upon the stable naïve T-cell pool, which, in youth, critically depends upon production of new T-cells by the thymus [29,33], and also on the absence of manifest pathogen challenge in SPF animals.

## T-cell reconstitution in adult NHP in the presence or the absence of SIV infection and antiviral therapy

HIV, and its simian homologue, SIV, are known to cause one of the most devastating immunodeficiencies, characterized by massive and acute depletion of memory CD4 T-cells from the gut [50-52], from which the immune system usually cannot recover [53-55]. While the naïve CD4 T-cells and the entire CD8 compartment remain largely unaffected by the virus in the course of the acute HIV/SIV infection, they both undergo excessive activation (perhaps due to microbial translocation[56]) and turnover, followed by eventual exhaustion. Recent work has linked the onset of chronic AIDS with the inability of central memory CD4+ T cells to maintain themselves and to produce sufficient effector memory CD4+ T cells to replace those lost by ongoing infection and immune activation Most recent results suggest that this exhaustion occurs due to progressive conversion of central memory CD4 T-cells into effector memory, rather than due to activation of naïve CD4 T-cells that then become infected by the virus and killed [57]. The advent of highly active antiretroviral therapy (HAART) has rendered the prognosis of HIV/SIV less bleak, and has provided impetus to find ways to reconstitute the wounded T-cell compartment. NHP represent the best experimental model for HIV infection, and much of the knowledge on T-cell reconstitution was gained using this model. Given the importance of the thymus and the key role of T-cell maintaining cytokines, it is not surprising that the first therapeutic agents used were the common γ chain cytokines aimed to improve and/or protect thymic function and to improve maintenance of different T-cell subsets. Biology of these molecules was reviewed elsewhere in this volume, and we will therefore only briefly mention their key characteristics.

### IL-2 and T-cell reconstitution

IL-2[58] was mostly used in NHP as a vaccine adjuvant. In one study IL-2 was administered in the form of an IL-2/Ig fusion protein to test its effect on peripheral T-cells in uninfected and SIV-infected monkeys [59]. In healthy young adult animals, a significant increase in CD4 T-cells was observed, and these cells were found to express CD25. Similar induction of CD25 expression and a transient increase in CD4 T-cells was also found in SIV-infected animals [59]. Importantly, these results closely paralleled the findings from HIV patients, where intermittent treatment with IL-2 led to an increase in CD4 T-cells [60,61]. However, most of the NHP cells expressing CD25, also expressed FoxP3 [62], suggesting strongly that IL-2 induces expansion of T regulatory cells (Treg) in primates. According to this evidence and the propensity of IL-2 to expand Treg cells in several models [38], it is unlikely that this cytokine will be useful for immune reconstitution in situations other than treatment of autoimmune diseases.

### IL-7 and T-cell reconstitution

IL-7 is the key differentiation factor for the very immature thymocytes (DN2-DN3 stage of the $CD4^{-}CD8^{-}$ double-negative thymocyte subsets) [63-65] and is secreted by resting epithelial cells, including the thymic stroma (rev in. [21]. This tempted researches to postulate that IL-7 defects

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

are at the heart of thymic involution [40] and to attempt IL-7 therapy of thymic involution. Evidence that IL-7 improved thymic function was scant [44,46]. However, IL-7 is also critically involved in the maintenance of naïve (rev in. [23,66] and, in some cases, memory, T-cell in mice [48,67], and it can exert profound effects upon T-cell expansion and homeostasis in humans. This led to experimental administration of IL-7 to NHP [42,45,68]. Nugeyre et al. administered the cytokine twice a day over three weeks to two young uninfected and two young SIV-infected rhesus macaques, and noted an increase in both CD4 and CD8 T-cells that persisted over several weeks in both infected and uninfected animals, but returned to baseline by 11 weeks [68]. Naïve as well as memory T-cells increased within days of administration, and the number of recent thymic emigrants, as judged by the TCR recombination excision circle (TREC) values, decreased (particularly in infected animals), arguing against the authors' interpretation that central (thymic) as well as peripheral effects are at play. In this and other studies [42,68,69], IL-7 did not increase viral loads in infected animals and did not seem to promote B-cell expansion or tumorigenesis. This not only suggests that IL-7 is unlikely to induce enhanced viral replication, but also indicates that in primates (unlike in mice) IL-7 may not act on B-cells.

In a complementary experiment with cynomolgus macaques, IL-7 led to expansion of all T-cell subsets, and this too led to dilution of newly generated T-cells, suggesting that homeostatic proliferation, rather than thymic effects, are responsible for the IL-7 effects [42]. Still, it is unclear whether the observation period was long enough to observe possible thymic effects. Similar effects were seen in both control, T-cell replete, and SIV-infected, moderately T-cell depleted animals – cell cycling was increased and T-cell numbers, both naïve and memory, went up [42]. In another study using uninfected young adult macaques, IL-7 induced T-cell proliferation in a dose-dependent manner, and led to a marked phenotypic conversion of naïve T-cells to memory phenotype and function (the latter as assessed by TNFα and IFNγ secretion [45]. Remarkably, these T-cells reverted back to naïve phenotype after IL-7 therapy withdrawal [45], suggesting that transient treatment did not convert these cells to the memory fate.

IL-7 was also used in a model of monkey reconstitution after autologous $CD34^+$ cell transplantation [69]. Baboons were subjected to total body irradiation and autologous $CD34^+$ cell transplantation and IL-7 was administered from weeks 6-10 post transplantation (twice/day). A remarkable increase in T-cell numbers was observed, which appeared to originate from peripheral expansion, rather than from an increase in thymic production [69], as judged by TREC levels, and by the imaging and direct analysis of the thymic tissue.

## IL-15 and T-cell reconstitution

The other critical cytokine in T-cell maintenance is IL-15. This cytokine is critically involved in the maintenance of memory T-cells and NK cells [70,71] in mice. Likewise, it was shown that IL-15 is the most potent and perhaps critical cytokine for the maintenance of NK and memory T-cells in NHP [72,73] and humans [74]. A recent study has examined the effects of IL-15 administration to SIV-infected or control rhesus macaques[75]. The authors found a surprising and dramatic expansion of the effector memory cells, including the effector memory CD4 T-cells, which are precisely the population hit the hardest by the SIV and HIV in their respective hosts [75]. Effects of IL-15 were severely blunted in SIV-infected RM with uncontrolled SIV replication, but were robust in SIV-infected animals following HAART [75]. Furthermore, even in the uninfected rhesus macaques, the initial robust expansion was followed by waning of the response upon further continuous treatment, suggesting a feedback inhibition or exhaustion of the responsive cells [75].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

### Summary of the effects of common γ chain cytokines on NHP T-cell reconstitution

Overall, among the three main common γ chain cytokines involved in T-cell maintenance, IL-2 is unlikely to help in general T-cell reconstitution protocols due to its confined action on Treg cells. With regard to population maintenance, IL-7 and IL-15 appear to dominantly target non-overlapping T-cell differentiation stages (although some biological overlap may exist, particularly at higher cytokine doses). Thus, IL-7 dominantly acts on T-cells recirculating between secondary lymphoid organs (naïve and central memory), whereas IL-15 operates on cells differentiating towards the effector memory stage, which are likely not to express the IL-7R nor to react to IL-7. While this provides hope in rejuvenation of each of the three subsets, a cautionary note is in order. Based upon the NHP studies, IL-7, at doses most effective for reconstitution, seems to convert many naïve T-cells into memory-phenotype T-cells[42], which would be an undesirable effect in any model of reconstitution. By contrast, IL-15 seems to quickly lose its effect after the initial dramatic increase in effector memory cells. Therefore, both refinement and optimization of treatment protocols will be necessary to achieve optimal effects of these cytokines. In that regard, it would be interesting to test the above cytokines administered in complex with antibodies, which may significantly prolong their half-life [76].

### Keratinocyte growth factor (KGF) and T-cell reconstitution

KGF was discovered as a factor that induces proliferation of epithelial, but not endothelial or fibroblastoid cells [77], with a particularly potent effect on keratinocytes. This molecule, formerly also known as the fibroblast growth factor 7 (FGF-7), acts on the early stages of T-cell development, allowing differentiation up to the point of action of IL-7 (rev in [78]. That prompted its use in various models of thymic regeneration. In the mouse model of bone marrow transplantation following total body irradiation, KGF was shown to protect thymic epithelial cells, reduce inflammation and enhance T-cell reconstitution [79-81]. Moreover, it was shown to increase thymic reconstitution after cyclophosphamide and dexamethasone treatments and to improve thymic output in old mice [82]. Based upon these studies, KGF was tested for the ability to enhance T-cell reconstitution in a rhesus macaque model of autologous bone marrow transplantation [83]. Young adult monkeys were treated before, or before and after, total body irradiation and autologous $CD34^+$ cell transplant. This treatment led to preservation of thymic architecture and an increase in naïve cells, which was significant in the lymph nodes [69]. A decline in the expression of the proliferation-associated marker Ki-67 in peripheral T-cells (characteristic of successful reconstitution of the T-cell compartment), correlated with the preservation of thymic stroma in monkeys receiving multiple KGF injections [69]. Thus, KGF provides hope as a therapeutic agent capable of affecting thymopoiesis, at least in young adult individuals.

## Age-related disturbances in T-cell homeostasis in NHP

Aging of the immune system leads to the common but poorly understood state of immunodeficiency. Unlike the severe congenital or acquired immunodeficiencies, aging is not associated with a decrease in lymphocyte numbers, but rather with gradual shifts in lymphocyte responsiveness, population ratios and repertoire (rev. in [84-87]). Specific changes include: (i) decreased or impaired lymphocyte responsiveness, including impaired or blunted signaling along several branches of the BCR/TCR and co-stimulatory signaling pathways; (ii) an increase in memory and a decrease in naive lymphocyte representation, likely due to combined effects of decreased bone marrow and thymic output, inadequate maintenance of naïve lymphocytes in the periphery and life-long exposure to pathogens; and (iii) a decrease in lymphocyte diversity as exemplified by the appearance and persistence of B-and T-cell clonal expansions (BCE and TCE). Importantly, it has long been known that old animals and humans experience greater morbidity and mortality from infectious diseases and respond poorly to immunization with new Ag, including vaccination against the most frequent and severe pathogens of the

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

elderly: influenza virus, pneumococcus and varicella-zoster virus [88-91]. Although this is certainly compounded by alterations in other organ systems, the above immunological decline is likely a primary underlying cause.

While defects were reported in several branches of adaptive and innate immunity, the most remarkable changes so far were detected in T and B lymphocyte adaptive responses. Often, reconstitution of the T-cell numbers and function can restore the overall immune competence in old animals [18,92,93]. Therefore, much of the ensuing discussion will focus upon T-cell numbers, phenotype and function. Age-related changes in the T-cell compartment of rhesus macaques follow the pattern observed in rodents and humans, with a possible exception that the changes may be occurring sooner and to a more dramatic extent in the outdoor-housed animals[10,16]. By contrast, changes are more gradual, but also strongly significant, in the indoor-housed animals (ref. 18 and L. Cicin-Sain et al. in preparation), probably due to differences in pathogen exposure between the two housing situations. There is an early conversion, starting at birth, of naïve T-cells into memory phenotype T-cells [10,16] and that is accompanied by an accumulation of memory cells that readily secrete proinflammatory cytokines [16]. CD4/CD8 ratios also decrease, and there is evidence that the dominance of CD8 T-cells in the old age occurs due to their prolonged cycling and, perhaps, preferential survival, compared to the CD4 T-cells [16]. Moreover, just like their human counterparts and unlike the laboratory rodents, macaque T-cells lose CD28 expression in the course of activation [16], and show a pronounced accumulation of the $CD28^-$ subset with aging [10,16]. As aging progresses and the thymic output further declines, one would expect that paucity of naïve T-cells may prompt the remaining naïve T-cells to homeostatically proliferate in an attempt to maintain their numbers. We were recently able to document this phenomenon in aged rhesus macaques. We observed a strongly statistically significant inverse correlation between the naïve T-cell proliferation and the number of remaining naïve T-cells, particularly for the $CD8^+$ subset (L. Cicin-Sain et al., in preparation). Therefore, aging in rhesus macaques exhibits all the expected characteristics seen in human T-cell aging to date.

## Retardation of T-cell aging in NHP by caloric restriction

Caloric restriction (CR) has long been known to increase median and maximal life spans and to decrease mortality and morbidity in short-lived animal models, likely by altering fundamental biological processes that regulate aging and longevity (rev. in [94]. In rodents, CR was reported to delay the aging of the immune system [95], and possibly even the retardation of thymic involution [96], although results reported in that study could have alternative explanations. We investigated the effect of long-term caloric restriction upon T-cell subset distribution and function of old NHP [18] and J.N-Z et al., unpublished results). We noted a marked preservation of naïve T-cells, accompanied by an increase in TREC content and TCR repertoire diversity [18]. It remained unclear whether CR delays thymic involution in primates, and experiments that are currently in progress, will hopefully resolve this issue.

Moreover, T-cells from CR old animals, but not form age-matched controls, proliferated better in response to polyclonal stimulation, and fewer of their memory cells were found to secrete pro-inflammatory cytokines (TNFα and IFNγ) in response to brief stimulation [18]. Therefore, long-term CR was sufficient to retard the symptoms of T-cell aging in macaques, and has also improved the response of adult monkeys to vaccination (J. N-Z, et al., unpublished results).

## Concluding comments

The above studies illustrate the power of the NHP model in validating findings from rodents that can be translated to primates, and in outlining findings where the divergent evolution limits the relevance of rodent models. More importantly, the studies above highlight a clear path

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

towards the future studies in T-cell (and immune) reconstitution. To the best of our knowledge, KGF was the only treatment attempted so far in NHP to substantially affect thymic function; IL-7 and CR affected naïve T-cell turnover (albeit in a different manner), whereas IL-15 affected the effector memory T-cell subset. It remains to be determined whether the above treatments will bear their described effects in different models of T-cell depletion and whether their effects can be optimized by combining them. For example, it is particularly provoking to try to use KGF in aged animals and test whether the thymus can truly be rejuvenated. Likewise, it would be of utmost interest to test whether androgen blockade [97] can affect thymus production in old NHP. Overall, development of treatments that can significantly increase T-cell reconstitution is coming of age, and one can look at the years in front of us with realistic optimism and excitement.

**Acknowledgements**

The author would like to thank Dr. L. Picker for sharing the results prior to publication, and Drs. L.Picker, L. Cicin-Sain, and A. Wertheimer and Ms. A. Lang for critically perusing the manuscript.

Supported by the USPHS Awards AG21384 and AG23664 from the National Institute on Aging (to J. N-Z.) and RR0163 (to the ONPRC) from the National Center for Research Resources, National Institutes of Health.

## References

1. Hein WR, Griebel PJ. A road less travelled: large animal models in immunological research. 2003;3:79–84.
2. MacGregor RR, Boyer JD, Ugen KE, Lacy KE, Gluckman SJ, Bagarazzi ML, Chattergoon MA, Baine Y, Higgins TJ, Ciccarelli RB, Coney LR, Ginsberg RS, Weiner DB. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. 1998;178:92–100.
3. Soike K, Rangan S, Gerone P. Viral disease models in primates. Adv Vet Sci Comp Med 1984;28:151–199. [PubMed: 6098169]
4. Enria D, Barrera Oro J. Junin virus vaccines. 2002;263:239–261.
5. Lacy M, Smego R. Viral hemorhagic fevers. Adv Pediatr Infect Dis 1996;12:21–53. [PubMed: 9033974]
6. McClure H. Nonhuman primate models for human disease. Adv Vet Sci Comp Med 1984;28:267–304. [PubMed: 6395673]
7. JR M, N GJ. Vaccines for the prevention of HIV-1 disease. Curr Opin Immunol 2001;3:489–495.
8. Watkins DI, Kannagi M, Stone ME, Letvin NL. Major histocompatibility complex class I molecules of nonhuman primates. European Journal of Immunology 1988;18:1425–1432. [PubMed: 3169121]
9. Levinson G, Hughes AL, Letvin NL. Sequence and diversity of rhesus monkey T cell receptor beta chain genes. Immunogenetics 1992;35:75–88. [PubMed: 1531206]
10. Pitcher C, Hagen S, Walker J, Lum R, Mitchell B, Maino V, Axthelm M, Picker LJ. Development and homeostasis of T cell memory in rhesus macaque. Journal of Immunology 2001:167.
11. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA, Kozyr NL, Earl PL, Smith JM, Ma HL, Grimm BD, Hulsey ML, Miller J, McClure HM, McNicholl JM, Moss B, Robinson HL. Control of mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science 2001;292:69–74. [PubMed: 11393868]
12. Chakrabarti L, Lewin S, Zhang L, Gettie A, Luckay A, Martin L, Skulsky E, Ho D, Cheng-Mayer C, Marx P. Normal T-Cell Turnover in Sooty Mangabeys Harboring Active Simian Immunodeficiency Virus Infection. J Virol 2000;74:1209–1223. [PubMed: 10627531]
13. Groves, C. Primate taxonomy. Washington, DC: Smithsonian Institution Press; 2001.
14. Bontrop RE, Otting N, Slierendregt B, Lanchbury JS. Evolution of Major histocompatability complex polymorphisms and t cell receptor diversity in primates. Immunological Reviews 1995;143:33–62. [PubMed: 7558081]
15. Cannon JP, Haire RN, Rast JP, Litman GW. The phylogenetic origins of the antigen-binding receptors and somatic diversification mechanisms. Immunol Rev 2004;200:12–22. [PubMed: 15242392]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

16. Jankovic V, Messaoudi I, Nikolich-Zugich J. Phenotypic and functional T-cell aging in Rhesus Macaques (Macaca mulatta): differential behavior of CD8 and CD4 subsets. Blood 2003;102:3244–3251. [PubMed: 12869504]
17. Nikolich-Zugich J, Messaoudi I. Mice and flies and monkeys too: caloric restriction rejuvenates the aging immune system of non-human primates. Exp Gerontol 2005;40:884–893. [PubMed: 16087306]
18. Messaoudi I, Warner J, Fischer M, Park B, Hill B, Mattison J, Lane MA, Roth GS, Ingram DK, Picker LJ, Douek DC, Mori M, Nikolich-Zugich J. Delay of T cell senescence by caloric restriction in aged long-lived nonhuman primates. Proc Natl Acad Sci U S A 2006;103:19448–19453. [PubMed: 17159149]
19. Douek DC. Disrupting T-cell homeostasis: how HIV-1 infection causes disease. AIDS Rev 2003;5:172–177. [PubMed: 14598566]
20. Fry TJ, Mackall CL. Current concepts of thymic aging. Springer Semin Immunopathol 2002;24:7–22. [PubMed: 11974583]
21. Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol 2005;174:6571–6576. [PubMed: 15905493]
22. van den Brink MR, Alpdogan O, Boyd RL. Strategies to enhance T-cell reconstitution in immunocompromised patients. Nat Rev Immunol 2004;4:856–867. [PubMed: 15516965]
23. Surh CD, Sprent J. Regulation of naive and memory T-cell homeostasis. Microbes Infect 2002;4:51–56. [PubMed: 11825775]
24. Freitas AA, Rocha B. Population biology of lymphocytes: the fight for survival. Annu Rev Immunol 2000;18:83–111. [PubMed: 10837053]
25. Tanchot C, Rocha B. The peripheral T cell repertoire: independent homeostatic regulation of virgin and activated CD8+ T cell pools. European Journal of Immunology 1995;25:2127–2136. [PubMed: 7545110]
26. Scollay R, Butcher E, Weissman I. Thymus migration: quantitative studies on the rate of migration of cells from the thymus to the periphery in mice. European Journal of Immunology 1980;10:210. [PubMed: 7379836]
27. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. Nat Immunol 2004;5:133–139. [PubMed: 14749784]
28. Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, Bryl E, Witkowski J, Fulbright J, Weyand CM, Goronzy JJ. The influence of age on T cell generation and TCR diversity. J Immunol 2005;174:7446–7452. [PubMed: 15905594]
29. Hale JS, Boursalian TE, Turk GL, Fink PJ. Thymic output in aged mice. Proc Natl Acad Sci U S A 2006;103:8447–8452. [PubMed: 16717190]
30. Heng TS, Goldberg GL, Gray DH, Sutherland JS, Chidgey AP, Boyd RL. Effects of castration on thymocyte development in two different models of thymic involution. J Immunol 2005;175:2982–2993. [PubMed: 16116185]
31. Douek DC, Koup RA. Evidence for thymic function in the elderly. Vaccine 2000;18:1638–1641. [PubMed: 10689141]
32. Berzins SP, Boyd RL, Miller JFAP. The role of the thymus and recent thymic migrants in the maintenance of the adult peripheral lymphocyte pool. Journal of Experimental Medicine 1998;187:1839–1848. [PubMed: 9607924]
33. Berzins SP, Godfrey DI, Miller JFAP, Boyd RL. A central role for thymic emigrants in peripheral T cell homeostasis. Proceedings of the National Academy of Sciences USA 1999;96:9787–9791.
34. Sprent J, Schaefer M, Hurd M, Surh CD, Ron Y. Mature murine B and T cells transferred to SCID mice can survive indefinitely and many maintain a virgin phenotype. Journal of Experimental Medicine 1991;174:717–728. [PubMed: 1875169]
35. Tough DF, Sprent J. Life span of naive and memory T cells. Stem Cells 1995;13:242–249. [PubMed: 7613491]
36. Surh CD, Sprent J. Homeostatic T cell proliferation: how far can T cells be activated to self-ligands? J Exp Med 2000;192:F9–F14. [PubMed: 10952731]
37. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 2000;288:675–678. [PubMed: 10784451]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript


38. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol 2004;4:665–674. [PubMed: 15343366]
39. Ginaldi L, De Martinis M, D'Ostilio A, Marini L, Loreto MF, Martorelli V, Quaglino D. The immune system in the elderly: II. Specific cellular immunity. Immunological Research 1999;20:109–115.
40. Andrew D, Aspinall R. Age-associated thymic atrophy is linked to a decline in IL-7 production. Exp Gerontol 2002;37:455–463. [PubMed: 11772533]
41. Kim HR, Hong MS, Dan JM, Kang I. Altered IL-7R{alpha} expression with aging and the potential implications of IL-7 therapy on CD8+ T cell immune responses. Blood 2005:2855–2862. [PubMed: 16357322]
42. Fry TJ, Moniuszko M, Creekmore S, Donohue SJ, Douek DC, Giardina S, Hecht TT, Hill BJ, Komschlies K, Tomaszewski J, Franchini G, Mackall CL. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Blood 2003;101:2294–2299. [PubMed: 12411295]
43. El Kassar N, Lucas PJ, Klug DB, Zamisch M, Merchant M, Bare CV, Choudhury B, Sharrow SO, Richie E, Mackall CL, Gress RE. A dose effect of IL-7 on thymocyte development. Blood 2004;104:1419–1427. [PubMed: 15155461]
44. Henson SM, Snelgrove R, Hussell T, Wells DJ, Aspinall R. An IL-7 fusion protein that shows increased thymopoietic ability. J Immunol 2005;175:4112–4118. [PubMed: 16148161]
45. Moniuszko M, Fry T, Tsai WP, Morre M, Assouline B, Cortez P, Lewis MG, Cairns S, Mackall C, Franchini G. Recombinant interleukin-7 induces proliferation of naive macaque CD4+ and CD8+ T cells in vivo. J Virol 2004;78:9740–9749. [PubMed: 15331707]
46. Phillips JA, Brondstetter TI, English CA, Lee HE, Virts EL, Thoman ML. IL-7 gene therapy in aging restores early thymopoiesis without reversing involution. J Immunol 2004;173:4867–4874. [PubMed: 15470027]
47. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–712. [PubMed: 10537110]
48. Tan J, Ernst B, Kieper W, LeRoy E, Sprent J, Surh C. Interleukin (IL)-15 and IL-7 Jointly Regulate Homeostatic Proliferation of Memory Phenotype CD8+ Cells but Are Not Required for Memory Phenotype CD4+ Cells. Journal of Experimental Medicine 2002;195:1523–1532. [PubMed: 12070280]
49. Callahan JE, Kappler JW, Marrack P. Unexpected expansions of CD8-bearing cells in old mice. Journal of Immunology 1993;151:6657–6669.
50. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science 1998;280:427–431. [PubMed: 9545219]
51. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004;200:749–759. [PubMed: 15365096]
52. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, Boden D, Racz P, Markowitz M. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 2004;200:761–770. [PubMed: 15365095]
53. Veazey RS, Lackner AA. HIV swiftly guts the immune system. Nat Med 2005;11:469–470. [PubMed: 15875046]
54. Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? Nat Immunol 2006;7:235–239. [PubMed: 16482171]
55. Picker LJ, Watkins DI. HIV pathogenesis: the first cut is the deepest. Nat Immunol 2005;6:430–432. [PubMed: 15843796]
56. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365–1371. [PubMed: 17115046]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript


57. Okoye AM-S M, Brenchley JM, Hagen SI, Walker JM, Rohandekar M, Lum R, Edgar JB, Planner SL, Legasse A, Sylwester AW, Piatak M Jr, Lifson JD, Maino VC, Sodora DL, Douek DC, Axthlem MK, Grossman Z, Picker LJ. Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection. 2007submitted
58. Smith KA, Baker PE, Gillis S, Ruscetti FW. Functional and molecular characteristics of T-cell growth factor. Mol Immunol 1980;17:579–589. [PubMed: 6971398]
59. Craiu A, Barouch DH, Zheng XX, Kuroda MJ, Schmitz JE, Lifton MA, Steenbeke TD, Nickerson CE, Beaudry K, Frost JD, Reimann KA, Strom TB, Letvin NL. An IL-2/Ig fusion protein influences CD4+ T lymphocytes in naive and simian immunodeficiency virus-infected Rhesus monkeys. AIDS Res Hum Retroviruses 2001;17:873–886. [PubMed: 11461674]
60. Farel CE, Chaitt DG, Hahn BK, Tavel JA, Kovacs JA, Polis MA, Masur H, Follmann DA, Lane HC, Davey RT Jr. Induction and maintenance therapy with intermittent interleukin-2 in HIV-1 infection. Blood 2004;103:3282–3286. [PubMed: 14726376]
61. Kovacs JA, Lempicki RA, Sidorov IA, Adelsberger JW, Sereti I, Sachau W, Kelly G, Metcalf JA, Davey RT Jr, Falloon J, Polis MA, Tavel J, Stevens R, Lambert L, Hosack DA, Bosche M, Issaq HJ, Fox SD, Leitman S, Baseler MW, Masur H, Di Mascio M, Dimitrov DS, Lane HC. Induction of prolonged survival of CD4+ T lymphocytes by intermittent IL-2 therapy in HIV-infected patients. J Clin Invest 2005;115:2139–2148. [PubMed: 16025158]
62. Sereti I, Imamichi H, Natarajan V, Imamichi T, Ramchandani MS, Badralmaa Y, Berg SC, Metcalf JA, Hahn BK, Shen JM, Powers A, Davey RT, Kovacs JA, Shevach EM, Lane HC. In vivo expansion of CD4CD45RO-CD25 T cells expressing foxP3 in IL-2-treated HIV-infected patients. J Clin Invest 2005;115:1839–1847. [PubMed: 15937547]
63. Morrissey PJ, Goodwin RG, Nordan RP, Anderson D, Grabstein KH, Cosman D, Sims J, Lupton S, Acres B, Reed SG. Recombinant interleukin 7, pre-B cell growth factor, has costimulatory activity on purified mature T cells. J Exp Med 1989;169:707–716. [PubMed: 2522498]
64. Conlon PJ, Morrissey PJ, Nordan RP, Grabstein KH, Prickett KS, Reed SG, Goodwin R, Cosman D, Namen AE. Murine thymocytes proliferate in direct response to interleukin-7. Blood 1989;74:1368–1373. [PubMed: 2788467]
65. Suda T, Murray R, Guidos C, Zlotnik A. Growth-promoting activity of IL-1(alpha), IL-6, and tumor necrosis factor-alpha in combination with IL-2, IL-4, or IL-7 on murine thymocytes. Journal of Immunology 1990;144:3039–3045.
66. Geiselhart LA, Humphries CA, Gregorio TA, Mou S, Subleski J, Komschlies KL. IL-7 Administration Alters the CD4:CD8 Ration, Increases T Cell Numbers, and Increases T Cell Function in the Absence of Activation. J Immuno 2001;166:3019–3027.
67. Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. J Exp Med 2003;198:1797–1806. [PubMed: 14662907]
68. Nugeyre MT, Monceaux V, Beq S, Cumont MC, Ho Tsong Fang R, Chene L, Morre M, Barre-Sinoussi F, Hurtrel B, Israel N. IL-7 stimulates T cell renewal without increasing viral replication in simian immunodeficiency virus-infected macaques. J Immunol 2003;171:4447–4453. [PubMed: 14530372]
69. Storek J, Gillespy T 3rd, Lu H, Joseph A, Dawson MA, Gough M, Morris J, Hackman RC, Horn PA, Sale GE, Andrews RG, Maloney DG, Kiem HP. Interleukin-7 improves CD4 T-cell reconstitution after autologous CD34 cell transplantation in monkeys. Blood 2003;101:4209–4218. [PubMed: 12543864]
70. Ma A, Boone DL, Lodice J. The pleiotropic functions of interleukin 15: not so interleukin 2-like after all. Journal of Experimental Medicine 2000;191:753–755. [PubMed: 10704456]
71. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity 1998;8:591–599. [PubMed: 9620680]
72. Villinger F, Miller R, Mori K, Mayne AE, Bostik P, Sundstrom JB, Sugimoto C, Ansari AA. IL-15 is superior to IL-2 in the generation of long-lived antigen specific memory CD4 and CD8 T cells in rhesus macaques. Vaccine 2004;22:3510–3521. [PubMed: 15308379]
73. Mueller YM, Petrovas C, Bojczuk PM, Dimitriou ID, Beer B, Silvera P, Villinger F, Cairns JS, Gracely EJ, Lewis MG, Katsikis PD. Interleukin-15 increases effector memory CD8+ t cells and NK Cells

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

in simian immunodeficiency virus-infected macaques. J Virol 2005;79:4877–4885. [PubMed: 15795273]

74. Geginat J, Sallusto F, Lanzavecchia A. Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4(+) T cells. J Exp Med 2001;194:1711–1719. [PubMed: 11748273]
75. Picker LJ, Reed-Inderbitzin EF, Hagen SI, Edgar JB, Hansen SG, Legasse A, Planer S, Piatak M, Lifson JD, Maino VC, Axthelm MK, Villinger F. IL-15 induces CD4 effector memory T cell production and tissue emigration in nonhuman primates. J Clin Invest 2006;116:1514–1524. [PubMed: 16691294]
76. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 2006;311:1924–1927. [PubMed: 16484453]
77. Rubin JS, Osada H, Finch PW, Taylor WG, Rudikoff S, Aaronson SA. Purification and characterization of a newly identified growth factor specific for epithelial cells. Proc Natl Acad Sci U S A 1989;86:802–806. [PubMed: 2915979]
78. Anderson G, Harman BC, Hare KJ, Jenkinson EJ. Microenvironmental regulation of T cell development in the thymus. Seminars in Immunol 2000;12:457–464.
79. Panoskaltsis-Mortari A, Lacey DL, Vallera DA, Blazar BR. Keratinocyte growth factor administered before conditioning ameliorates graft-versus-host disease after allogeneic bone marrow transplantation in mice. Blood 1998;92:3960–3967. [PubMed: 9808590]
80. Min D, Taylor PA, Panoskaltsis-Mortari A, Chung B, Danilenko DM, Farrell C, Lacey DL, Blazar BR, Weinberg KI. Protection from thymic epithelial cell injury by keratinocyte growth factor: a new approach to improve thymic and peripheral T-cell reconstitution after bone marrow transplantation. Blood 2002;99:4592–4600. [PubMed: 12036893]
81. Rossi S, Blazar BR, Farrell CL, Danilenko DM, Lacey DL, Weinberg KI, Krenger W, Hollander GA. Keratinocyte growth factor preserves normal thymopoiesis and thymic microenvironment during experimental graft-versus-host disease. Blood 2002;100:682–691. [PubMed: 12091365]
82. Alpdogan O, Hubbard VM, Smith OM, Patel N, Lu S, Goldberg GL, Gray DH, Feinman J, Kochman AA, Eng JM, Suh D, Muriglan SJ, Boyd RL, van den Brink MR. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. Blood 2006;107:2453–2460. [PubMed: 16304055]
83. Seggewiss R, Lore K, Guenaga FJ, Pittaluga S, Mattapallil J, Chow CK, Koup RA, Camphausen K, Nason MC, Meier-Schellersheim M, Donahue RE, Blazar BR, Dunbar CE, Douek DC. Keratinocyte growth factor augments immune reconstitution after autologous hematopoietic progenitor cell transplantation in rhesus macaques. Blood. 2007
84. Miller RA. Aging and immune function: cellular and biochemical analyses (Nathan Shock Memorial Lecture, 1992). 1994;29:21–35.
85. Eren R, Globerson A, Abel L, Zharhary D. Quantitative analysis of bond marrow thymic progenitors in young and aged mice. Cell Immunol 1990;127:238–246. [PubMed: 2328529]
86. Ku CC, Kotzin B, Kappler J, Marrack P. CD8+ T-cell clones in old mice. Immunological Reviews 1997;160:139–144. [PubMed: 9476672]
87. Freitas AA, Rocha BB. Lymphocyte lifespans: homeostasis, selection and competition. Immunol Today 1993;14:25–29. [PubMed: 8442858]
88. Gardiner I. The effect of aging on the susceptibility to infection. 1980:2801.
89. Gavazzi G, Krause K-H. Ageing and Infection. The Lancet 2002;2:659–666.
90. Castle S. Clinical Relevance of Age-Related Immune Dysfunction. 2000;31:578–585.
91. Mouton C, Bazaldua O, Pierce B, Espino D. Common Infections in Older Adults. American Family Physician 2001;63:257–268. [PubMed: 11201692]
92. Haynes L, Eaton SM, Buns EM, Randall TD, Swain SL. Newly Generated CD4 T cells in aged animals do not exhibit age-related defects in response to antigen. J Exp Med. 2005in press
93. Haynes L, Eaton SM, Burns EM, Rincon M, Swain SL. Inflammatory cytokines overcome age-related defects in CD4 T cell responses in vivo. J Immunol 2004;172:5194–5199. [PubMed: 15100256]
94. Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev 2005;126:913–922. [PubMed: 15885745]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

95. Walford RL. Studies in immunogerontology. J Am Geriatr Soc 1982;30:617–625. [PubMed: 7119327]

96. Chen J, Astle CM, Harrison DE. Delayed Immune Aging in Diet-Restricted B6CBAT6 F1 Mice Is Associated With Preservation of Naive T Cells. 1998;53A:B330–B337.

97. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, Blazar BR, Millar JL, Malin MA, Chidgey AP, Boyd RL. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol 2005;175:2741–2753. [PubMed: 16081852]