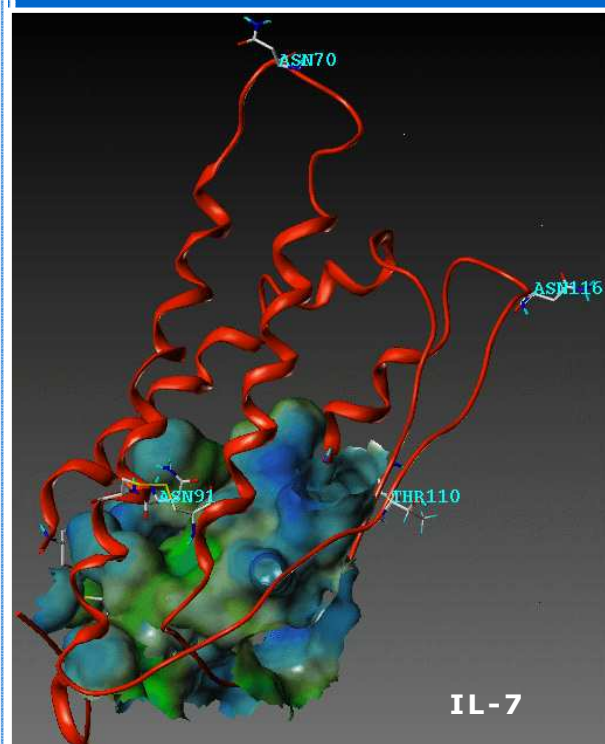




IL-7: Selected Bibliography

A PubMed search in Q1 2009 using the keyword "IL-7" returned more than 3250 citations. Below you will find a selection of these scientific papers, many from peer-reviewed journals.



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1. IL-7 and T cell Ontogeny and Homeostasis

1.1 Reviews

- **Blood.** 2002 Jun 1; 99(11):3892-904.

Interleukin-7: from bench to clinic. Fry TJ, Mackall CL.

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Interleukin-7 (IL-7) was initially isolated more than 10 years ago.¹⁻⁴ Nevertheless, the complete set of physiologic roles for this cytokine, especially those involving lymphocyte homeostasis, have only recently been elucidated. After the initial descriptions of effects on B-cell precursors, recognition that IL-7 also has marked activity on immature⁵⁻⁷ and mature⁸ T cells soon followed. Information from gene-deleted mice showed IL-7 is a non redundant cytokine for murine T and B lymphopoiesis.^{9,10} Mutations in the γ chain of the IL-7 receptor in patients with severe combined immunodeficiency (SCID) confirmed that IL-7 is indispensable for T-cell development in humans. However, the presence of B cells in these individuals suggests important differences between the role of IL-7 in murine and human lymphocyte development.¹¹ IL-7 also has potent effects on mature T cells. Recent work has shown that IL-7 is a critical modulator of low-affinity peptide-induced proliferation, which is a central feature of the homeostatic regulation of T-cell populations.^{12,13} Furthermore, circulating levels of IL-7 increase in response to T-cell depletion, suggesting a role in T-cell regeneration.¹⁴⁻¹⁶ Importantly, the primary sources of IL-7 are non-marrow-derived stromal and epithelial cells. Thus, IL-7 is a pleiotropic cytokine with central roles in modulating T- and B-cell development and T-cell homeostasis. The potency and breadth of effects suggest that IL-7 administration or neutralization of IL-7 may allow the modulation of immune function in patients with lymphocyte depletion, vaccine administration, or autoimmunity.

- **Trends Immunol.** 2001 Oct; 22(10):564-71.

Interleukin-7: master regulator of peripheral T-cell homeostasis?
Fry TJ, Mackall CL.

Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Building 10, Room 13N240, 10 Center Drive, MSC 1928, Bethesda, MD 20892-1928, USA.

Recent evidence has implicated interleukin-7 (IL-7) as a master regulator of T-cell homeostasis, based upon its essential role in the homeostatic expansion of naive T-cell populations in response to low-affinity antigens (Ags) and its capacity to enhance dramatically the expansion of peripheral T-cell populations in response to high-affinity Ags. Furthermore, T-cell-depleted humans have a unique inverse relationship between the peripheral CD4(+) T-cell count and the level of circulating IL-7. Together, these data suggest that increased amounts of IL-7 become available following T-cell depletion, thus, enhancing the high- and low-affinity Ag-driven expansion of the population of residual, mature T cells and boosting thymic regenerative capacity, as a means to restore T-cell homeostasis.

1.2 Cytheris Associated Publications

1.2.1 T cell Ontogeny

- **Rejuvenation Res.** 2007 Mar;10(1):5-17.

Old rhesus macaques treated with interleukin-7 show increased TREC levels and respond well to influenza vaccination. Aspinall R, Pido-Lopez J, Imami N, Henson SM, Ngom PT, Morre M, Niphuis H, Remarque E, Rosenwirth B, Heeney JL.

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Old age is accompanied by an increased incidence of infection and poorer responses to vaccination. In this proof of principle study, old female rhesus macaques (aged 18.5 to 23.9 years) were treated with recombinant simian interleukin-7 (IL-7) or saline, according to a two-phase regime. Treatment was not associated with bone loss as judged by plasma carboxy terminal telopeptide of type I collagen (ICTP) levels, nor with neutropenia. IL-7-treated animals showed an increase in the number of blood CD4(+) CD3(+) and CD8(+) CD3(+) T cells after both phases of treatment and a transient increase in the number of naïve (CD62L(+) CD45RA(+)) T cells for both CD4(+) and CD8(+) subsets after only the first treatment. Increases in TREC levels per T cell followed both phases of treatment, but were more prolonged after the second phase. Following vaccination with inactivated influenza strain A/PR/8/34, hemagglutination inhibition assays showed that half of the IL-7-treated animals showed a greater than eight-fold increase in antibody titer following the first challenge with the vaccine. In addition IL-7-treated animals showed higher numbers of central memory CD8(+) T cells compared to pretreatment levels with numbers greater than in the saline-treated group. Animals with the highest hemagglutination inhibition titers and the best proliferation against flu antigen were among those with the highest TREC per T cell levels after the second phase of treatment. Treatment of the elderly with IL-7 may provide an effective therapy to improve the immune system.

- **Blood.** 2007 Feb 1; 109(3):1034-42. Epub 2006 Oct 5.

IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Swainson L, Kinet S, Mongellaz C, Sourisseau M, Henriques T, Taylor N.

Institut de Génétique Moléculaire de Montpellier, France.

The IL-7 cytokine promotes the survival of a diverse T-cell pool, thereby ensuring an efficient immune response. Moreover, IL-7 induces the proliferation of recent thymic emigrants (RTEs) in neonates. Here, we demonstrate that the survival and proliferative effects of IL-7 on human RTEs can be distinguished on the basis of dose as well as duration of IL-7 administration. A dose of 0.1 ng/mL IL-7 is sufficient to promote viability, whereas cell-cycle entry is observed only at doses higher than 1 ng/mL. Moreover, a short 1-hour exposure to high-dose IL-7 (10 ng/mL) induces long-term survival but continuous IL-7 exposure is necessary for optimal cell-cycle entry and proliferation. We find that distinct signaling intermediates are activated under conditions of IL-7-induced survival and proliferation; STAT5 tyrosine phosphorylation does not correlate with proliferation, whereas up-regulation of the glucose transporter Glut-1 as well as increased glucose uptake are markers of IL-7-induced cell cycle entry. Glut-1 is directly regulated by PI3K and, indeed, inhibiting PI3K activity abrogates IL-7-induced proliferation. Our finding that the survival and proliferation of RTEs are differentially modulated by the dose and kinetics of exogenous IL-7 has important implications for the clinical use of this cytokine.

1.1.1.1 T cell Homeostasis

- **J Virol.** 2004 Sep;78(18):9740-9.

Recombinant interleukin-7 induces proliferation of naive macaque CD4+ and CD8+ T cells in vivo. Moniuszko M, Fry T, Tsai WP, Morre M, Assouline B, Cortez P, Lewis MG, Cairns S, Mackall C, Franchini G.

Animal Models and Retroviral Vaccines Section, National Cancer Institute, Bldg. 41, Rm. D804, Bethesda, MD 20892-5065, USA.

Interleukin-7 (IL-7) regulates T-cell homeostasis, and its availability is augmented in lymphopenic hosts. Naive CD8+ T cells transferred to lymphopenic mice acquire a memory-like phenotype, raising the possibility that IL-7 is the biological mediator of this effect. Here, we provide direct evidence that IL-7 induces the acquisition of memory-cell markers not only in CD8+ T cells but also in CD4+ T-cell subsets in immune-competent Indian rhesus macaques. The increase of these memory-like populations was dependent on the dose of the cytokine, and these cells were found in the blood as well as secondary lymphoid organs. Memory-like CD4+ and CD8+ T cells acquired the ability to secrete tumor necrosis factor alpha and, to a lesser extent, gamma interferon following stimulation with a cognate antigen. The phenotypic change

observed in naive T cells was promptly reversed after discontinuation of IL-7. Importantly, IL-7 induced cycling of both CD4+ and CD8+ central memory and effector memory T cells, demonstrating its contribution to the maintenance of the entire T-cell pool. Thus, IL-7 may be of benefit in the treatment of iatrogenic or virus-induced T-cell depletion.

- **J Clin Invest.** 2005 May;115 (5):1177-87. Epub 2005 Apr 7.

Adjuvant IL-7 or IL-15 overcomes immunodominance and improves survival of the CD8+ memory cell pool. Melchionda F, Fry TJ, Milliron MJ, McKirdy MA, Tagaya Y, Mackall CL.

Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA.

Current models of T cell memory implicate a critical role for IL-7 in the effector-to-memory transition, raising the possibility that IL-7 therapy might enhance vaccine responses. IL-7 has not been studied, to our knowledge, before now for adjuvant activity. We administered recombinant human IL-7 (rhIL-7) to mice during immunization against the male antigen HY and compared these results with those obtained from mice immunized with rhIL-2 and rhIL-15. Administration of rhIL-7 or rhIL-15, but not rhIL-2, increased effector cells directed against these dominant antigens and dramatically enhanced CD8(+) effectors to subdominant antigens. The mechanisms by which the cytokines augmented effector pool generation were multifactorial and included rhIL-7-mediated costimulation and rhIL-15-mediated augmentation of the proliferative burst. The contraction phase of the antigen-specific response was exaggerated in cytokine-treated mice; however, CD8(+) memory pools in rhIL-7- or rhIL-15-treated groups demonstrated superior long-term survival resulting in quantitative advantages that remained long after the cytokines were discontinued, as demonstrated by improved survival after challenge with an HY-expressing tumor undertaken several weeks after cytokine cessation. These results confirm the adjuvant activity of rhIL-15 and demonstrate that rhIL-7 also serves as a potent vaccine adjuvant that broadens immunity by augmenting responses to subdominant antigens and improving the survival of the CD8(+) T cell memory pool.

- **Proc Natl Acad Sci U S A.** 2007 Jul 10;104(28):11730-5. Epub 2007 Jul 3.

Expression of IL-7 receptor alpha is necessary but not sufficient for the formation of memory CD8 T cells during viral infection. Hand TW, Morre M, Kaech SM.

Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA.

During many acute viral and bacterial infections, IL-7 receptor alpha-chain (IL-7Ralpha) is expressed on a subset of effector CD8 T cells that preferentially develop into long-lived memory CD8 T cells. These cells functionally require IL-7Ralpha, but it is unclear

whether IL-7Ralpha acts mainly to induce their differentiation into memory cells or to sustain their long-term survival. To examine this question, IL-7Ralpha was constitutively overexpressed on all antigen-specific effector CD8 T cells during viral infection. Constitutive IL-7Ralpha expression had minimal effects on the numbers or function of effector and memory CD8 T cells formed. This indicated that IL-7Ralpha expression is not sufficient to drive memory cell development. In particular, the forced IL-7Ralpha expression did not rescue the killer cell lectin-like receptor G1 (KLRG1)(hi) short-lived effector CD8 T cells from death, showing that the majority of effector CD8 T cells die in an IL-7Ralpha-independent manner. Moreover, we found that, regardless of the ectopic expression of IL-7Ralpha, the KLRG1(hi), but not the KLRG1(lo) effector CD8 T cells, were unable to proliferate well to IL-7, which may be due to increased amounts of p27(kip) in KLRG1(hi) cells. Because IL-7 can destabilize p27(kip), this result suggested that KLRG1(hi) and KLRG1(lo) effector CD8 T cells naturally differ in their ability to transmit IL-7 signals. Altogether, these results reveal that IL-7Ralpha expression is permissive, but not instructive, to the creation of memory CD8 T cells.

- **J Clin Invest.** 2008 Mar;118(3):1027-39.

Effects of IL-7 on memory CD8 T cell homeostasis are influenced by the timing of therapy in mice. Nanjappa SG, Walent JH, Morre M, Suresh M.

Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA.

IL-7 is integral to the generation and maintenance of CD8(+) T cell memory, and insufficient IL-7 is believed to limit survival and the persistence of memory CD8(+) T cells. Here, we show that during the mouse T cell response to lymphocytic choriomeningitis virus, IL-7 enhanced the number of memory CD8(+) T cells when its administration was restricted to the contraction phase of the response. Likewise, IL-7 administration during the contraction phase of the mouse T cell response to vaccinia virus or a DNA vaccine potentiated antigen-specific CD8(+) memory T cell proliferation and function. Qualitatively, CD8(+) T cells from IL-7-treated mice exhibited superior recall responses and improved viral control. IL-7 treatment during the memory phase stimulated a marked increase in the number of memory CD8(+) T cells, but the effects were transient. IL-7 therapy during contraction of the secondary CD8(+) T cell response also expanded the pool of memory CD8(+) T cells. Collectively, our studies show differential effects of IL-7 on memory CD8(+) T cell homeostasis and underscore the importance of the timing of IL-7 therapy to effectively improve CD8(+) T cell memory and protective immunity. These findings may have implications in the clinical use of IL-7 as an immunotherapeutic agent to bolster vaccine-induced CD8(+) T cell memory.

- **J Immunol.** 2006 Jun 1;176(11):6702-8.

IL-7R alpha gene expression is inversely correlated with cell cycle progression in IL-7-stimulated T lymphocytes. Swainson L, Verhoeyen E, Cosset FL, Taylor N.

Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5535, Montpellier, France.

IL-7 plays a major role in T lymphocyte homeostasis and has been proposed as an immune adjuvant for lymphopenic patients. This prospect is based, at least in part, on the short-term expansion of peripheral T cells in rIL7-treated mice and primates. Nevertheless, in vivo, following initial increases in T cell proliferation and numbers, lymphocytes return to a quiescent state. As the bases for this cell cycle exit have not yet been elucidated, it is important to assess the long-term biological effects of IL-7 on quiescent human T lymphocyte subsets. In this study, we find that IL-7-stimulated CD4+ naive lymphocytes enter into cell cycle with significantly delayed kinetics as compared with the memory population. Importantly though, these lymphocytes exit from the cell cycle despite the continuous replenishment of rIL-7. This response is distinct in memory and naive CD4+ lymphocytes with memory cells starting to exit from cycle by day 10 vs day 18 for naive cells. Return to quiescence is associated with a cessation in IL-7R signaling as demonstrated by an abrogation of STAT-5 phosphorylation, despite an up-regulation of surface IL-7R α . Indeed, an initial 10-fold decrease in IL-7R α mRNA levels is followed by increased IL-7R α expression in naive as well as memory T cells, with kinetics paralleling cell cycle exit. Altogether, our data demonstrate that IL-7 promotes the extended survival of both naive and memory CD4+ T cells, whereas cycling of these two subsets is distinct and transient. Thus, IL-7 therapy should be designed to allow optimal responsiveness of naive and memory T cell subsets.

➤ **Blood**. 2009 April 7; doi: 10.1182/blood-2008-11-191288

Injection of Glycosylated Recombinant Simian IL-7 Provokes Rapid and Massive T-cell Homing in Rhesus Macaques. Stephanie Beq ¹, Sandra Rozlan ^{2*}, David Gautier ^{1*}, Raphaëlle Parker ¹, Véronique Mersseman ¹, Clémentine Schilte ¹ Brigitte Assouline ², Iann Rancé ², Pascal Lavedan ³, Michel Morre ² and Rémi Cheynier ¹

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Interleukin-7 (IL-7), the principal cytokine implicated in thymopoiesis and peripheral T-cell homeostasis, is presently under evaluation in human diseases characterized by persistent lymphopenia. Unexpectedly, prior to the eventual IL-7-driven T-cell expansion, all treated patients showed a profound T-cell depletion 24 hours following injection. The current study utilizes the Rhesus macaque model to investigate the mechanisms involved in this IL-7-induced T-cell depletion. We identify a new critical function of IL-7 that induces massive and rapid T-cell migration from the blood into various organs, including lymph nodes, parts of the intestine and the skin. This homing process was initiated following the induction of chemokine receptor

expression by circulating T-cells and the production of corresponding chemokines in target organs. Finally, we demonstrate that the IL-7-induced cell cycling is initiated within these organs before T-cells migrate back into the blood stream, indicating that T-cell homing is required for *in vivo* IL-7 function.

- **Blood.** 2009. April 20; doi: 10.1182/blood-2008-10-186601

IL-7 administration drives T cell cycle entry and expansion in HIV-1 infection. Irini Sereti, Richard M. Dunham, John Spritzler, Evgenia Aga, Michael A. Proschan, Kathy Medvik, Catherine A. Battaglia, Alan L. Landay, Savita Pahwa, Margaret A. Fischl, David M. Asmuth, Allan R. Tenorio, John D. Altman, Lawrence Fox, Susan Moir, Angela Malaspina, Michel Morre, Renaud Buffet, Guido Silvestri and Michael M. Lederman.

Departement de Virologie, Institut Pasteur, Paris, France.

Interleukin-7 (IL-7), the principal cytokine implicated in thymopoiesis and peripheral T-cell homeostasis, is presently under evaluation in human diseases characterized by persistent lymphopenia. Unexpectedly, prior to the eventual IL-7-driven T-cell expansion, all treated patients showed a profound T-cell depletion 24 hours following injection. The current study utilizes the Rhesus macaque model to investigate the mechanisms involved in this IL-7- induced T-cell depletion. We identify a new critical function of IL-7 that induces massive and rapid T-cell migration from the blood into various organs, including lymph nodes, parts of the intestine and the skin. This homing process was initiated following the induction of chemokine receptor expression by circulating T-cells and the production of corresponding chemokines in target organs. Finally, we demonstrate that the IL-7-induced cell cycling is initiated within these organs before T-cells migrate back into the blood stream, indicating that T-cell homing is required for *in vivo* IL-7 function.

1.3 Other Main Publications

- **J Exp Med.** 1994 Nov 1;180(5):1955-60.

Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB, Meyer JD, Davison BL.

Immunex Research and Development Corporation, Seattle, Washington 98101.

Interleukin 7 (IL-7) stimulates the proliferation of B cell progenitors, thymocytes, and mature T cells through an interaction with a high affinity receptor (IL-7R) belonging to the hematopoietin receptor superfamily. We have further addressed the role of IL-7 and

its receptor during B and T cell development by generating mice genetically deficient in IL-7R. Mutant mice display a profound reduction in thymic and peripheral lymphoid cellularity. Analyses of lymphoid progenitor populations in IL-7R-deficient mice define precisely those developmental stages affected by the mutation and reveal a critical role for IL-7R during early lymphoid development. Significantly, these studies indicate that the phase of thymocyte expansion occurring before the onset of T cell receptor gene rearrangement is critically dependent upon, and mediated by the high affinity receptor for IL-7.

- **J Exp Med.** 1995 Apr 1;181(4):1519-26.

Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. von Freeden-Jeffrey U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R.

Department of Immunology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, California 94304, USA.

Interleukin (IL)-7 is a potent stimulus for immature T and B cells and, to a lesser extent, mature T cells. We have inactivated the IL-7 gene in the mouse germline by using gene-targeting techniques to further understand the biology of IL-7. Mutant mice were highly lymphopenic in the peripheral blood and lymphoid organs. Bone marrow B lymphopoiesis was blocked at the transition from pro-B to pre-B cells. Thymic cellularity was reduced 20-fold, but retained normal distribution of CD4 and CD8. Splenic T cellularity was reduced 10-fold. Splenic B cells, also reduced in number, showed an abnormal population of immature B cells in adult animals. The remaining splenic populations of lymphocytes showed normal responsiveness to mitogenic stimuli. These data show that proper T and B cell development is dependent on IL-7. The IL-7-deficient mice are the first example of single cytokine-deficient mice that exhibit severe lymphoid abnormalities.

- **Blood.** 2001 May 15;97(10):2983-90.

A potential role for interleukin-7 in T-cell homeostasis. Fry TJ, Connick E, Falloon J, Lederman MM, Liewehr DJ, Spritzler J, Steinberg SM, Wood LV, Yarchoan R, Zuckerman J, Landay A, Mackall CL.

Pediatric Oncology Branch, Biostatistics and Data Management Section, and HIV and AIDS Malignancy Branch of the National Cancer Institute, the National Institutes of Health, Bethesda, MD, USA. tf60y@nih.gov

Interleukin (IL)-7 is known to up-regulate thymopoietic pathways of T-cell regeneration. Recent work also has shown it to potently enhance thymic-independent peripheral expansion and to restore immunocompetence in athymic T-cell-depleted hosts. We hypothesized that endogenous IL-7 could contribute to the restoration of T-cell homeostasis following T-cell depletion. To analyze this, we evaluated circulating IL-7 levels and lymphocyte subsets in multiple clinical cohorts with T-cell depletion of

varying etiologies. In pediatric (n = 41) and adult (n = 51) human immunodeficiency virus-infected CD4-depleted patients, there were strong inverse correlations between IL-7 levels and CD4 counts (r = -0.77, P <.0001, and r = -0.68, P <.0001). Declines in IL-7 were temporally correlated with recovery of CD4 counts. Similar patterns were observed in CD4-depleted patients receiving cancer chemotherapy (r = -0.65, P =.009). Therefore, in 2 disparate clinical scenarios involving CD4 depletion, IL-7 levels dynamically respond to changes in CD4 T-cell number, making this cytokine uniquely suited as a candidate regulator of T-cell homeostasis. Furthermore, in patients with idiopathic CD4 lymphopenia, a much weaker relationship between IL-7 levels and peripheral blood CD4 counts was observed, suggesting that an impaired IL-7 response to CD4 depletion may contribute to the impaired lymphocyte homeostasis observed in this population. In light of the known effects of IL-7 on T-cell regeneration, we postulate that increased availability of IL-7 could play a critical role in restoring T-cell homeostasis following T-cell depletion.

- **Proc Natl Acad Sci U S A.** 1993 Oct 1;90(19):9125-9.

Expression and function of the interleukin 7 receptor in murine lymphocytes. Sudo T, Nishikawa S, Ohno N, Akiyama N, Tamakoshi M, Yoshida H, Nishikawa S.

Basic Research Laboratories, Toray Industries, Inc., Kamakura, Japan.

A monoclonal antibody, A7R34, that recognizes the high-affinity interleukin 7 receptor (IL-7Ra) and blocks the binding between IL-7 and IL-7Ra has been produced. Cell surface staining with A7R34 demonstrated that IL-7Ra is expressed in both B- and T-cell lineages. In the bone marrow, immature B-lineage cells that do not express surface IgM were IL-7Ra+. In the thymus, IL-7Ra was detected in CD4-8- T cells and also in CD4 or CD8 single-positive cells but not in CD4+8+ double-positive cells. In the peripheral lymphoid tissues, both CD4 and CD8 single-positive cells were the major cell types that express IL-7Ra. Addition of A7R34 to a long-term B-precursor-cell culture inhibited proliferation of the B-lineage cells, indicating that IL-7 is an absolute requirement for in vitro B-cell genesis. Consistent with this in vitro result, continuous injection of A7R34 into an adult mouse resulted in a decrease of B-precursor cells and also of thymocytes, whereas a considerable fraction of mature B and T cells in the peripheral tissues persisted over 2 weeks of the experiment. When A7R34 injection is started from day 14 of gestation, it is possible to produce mice that lack B cells. These results indicate that IL-7 is an essential molecule for generation of both B and T cells in murine bone marrow and thymus, respectively. Moreover, IL-7Ra would be the sole receptor system regulating these processes.

- **J Exp Med.** 1993 Jul 1;178(1):257-64.

Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. Grabstein KH, Waldschmidt TJ, Finkelman FD, Hess BW, Alpert AR, Boiani NE, Namen AE, Morrissey PJ.

Immunex Research and Development Corporation, Seattle, Washington 98101.

The effects of interleukin 7 (IL-7) on the growth and differentiation of murine B cell progenitors has been well characterized using in vitro culture methods. We have investigated the role of IL-7 in vivo using a monoclonal antibody that neutralizes IL-7. We find that treatment of mice with this antibody completely inhibits the development of B cell progenitors from the pro-B cell stage forward. We also provide evidence that all peripheral B cells, including those of the B-1 and conventional lineages, are derived from IL-7-dependent precursors. The results are consistent with the rapid turnover of B cell progenitors in the marrow, but a slow turnover of mature B cells in the periphery. In addition to effects on B cell development, anti-IL-7 treatment substantially reduced thymus cellularity, affecting all major thymic subpopulations.

2. IL-7 and HIV/SIV

2.1 Reviews

- **Trends Microbiol.** 2008 Dec;16(12):567-73. Epub 2008 Oct 27.

Loss of CD127 expression links immune activation and CD4(+) T cell loss in HIV infection. Kiazzyk SA, Fowke KR.

Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada.

Although chronic immune activation correlates with CD4(+) T cell loss in HIV infection, an understanding of the factors mediating T cell depletion remains incomplete. We propose that reduced expression of CD127 (IL-7 receptor alpha chain, IL-7Ralpha), induced by immune activation, contributes to CD4(+) T cell loss in HIV infection. In particular, loss of CD127 on central memory CD4(+) T cells (T(CM)) severely restrains the regenerative capacity of the memory component of the immune system, resulting in a limited ability to control T cell homeostasis. Studies from both pathogenic and controlled HIV infection indicate that the containment of immune activation and preservation of CD127 expression are critical to the stability of CD4(+) T cells in infection. A better understanding of the factors regulating CD127 expression in HIV disease, particularly on T(CM) cells, might unveil new approaches exploiting the IL-7/IL-7R receptor pathway to restore T cell homeostasis and promote immune reconstitution in HIV infection.

- **Eur Cytokine Netw.** 2004 Oct-Dec; 15(4):279-89.

Interleukin-7 (IL-7): immune function, involvement in the pathogenesis of HIV infection and therapeutic potential. Beq S, Delfraissy JF, Theze J.

Unité d'Immunogénétique Cellulaire, Département de Médecine Moléculaire, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France.

Interleukin 7 (IL-7), which is constitutively produced particularly by stromal cells from the bone marrow and thymus, plays a crucial role in T cell homeostasis. This cytokine is implicated in thymopoiesis since it sustains thymocyte proliferation and survival. It regulates peripheral naive T cell survival by modulating the expression of the anti-apoptotic molecule Bcl-2, and sustains peripheral T cell expansion in response to antigenic stimulation. Infection by the human immunodeficiency virus (HIV) leads to severe T lymphopenia and general immune dysfunction. Increased IL-7 plasma levels are generally observed in HIV-infected patients. The existence of an inverse correlation between IL-7 plasma levels and the CD4+ T cell count suggests that a direct feedback mechanism is working to restore peripheral T cell counts in lymphopenic patients. Here, IL-7 plasma levels are a good predictive marker of CD4+ T cell restoration during therapy. Combinations of antiretroviral treatments considerably slow disease progression. They drastically decrease the viral load and, in most patients, significantly increase peripheral CD4+ T cell counts. However, despite their usual ability to reduce viral replication, such treatments often fail to reverse lymphopenia and do not restore specific antiviral immune responses. IL-7, based therapy, combined with efficient antiretroviral treatment, may be beneficial to HIV-infected patients by promoting thymic output, sustaining naive T cell counts and increasing memory T cell activation.

➤ **Curr HIV Res.** 2009 Jan;7(1):83-90.

IL-2, IL-7 and IL-15 as immuno-modulators during SIV/HIV vaccination and treatment. Leone A, Picker LJ, Sodora DL.

Seattle Biomedical Research Institute, Seattle, WA, USA.

While highly active antiretroviral therapy (HAART) regimens have proven to be effective in controlling active HIV replication, complete recovery of CD4+ T cells does not always occur, even among patients with high level virologic control. Recent advances in understanding the biology of T cell production and homeostasis have created the potential to augment anti-viral therapies with immunotherapies designed to facilitate recovery of the HIV-damaged immune system, in particular, the recovery of CD4+ T cell populations. The common gamma-chain cytokines IL-2, IL-7 and IL-15 are primary regulators of T cell homeostasis and thus have been considered prime candidate immunotherapeutics, both for increasing T cell levels/function and for augmenting vaccine-elicited viral-specific T cell responses. Recent studies have established that these cytokines have distinct functional roles in immune homeostasis, which focus on specific T cell populations. The ability of these cytokines to provide immunotherapeutic benefit to HIV+ patients will depend on their ability to stably increase or functionally enhance the desired T cell target population without adverse virologic or clinical consequences.

2.2 Cytheris Associated Publications

1.1.1.2 HIV

- **J Immunother.** 2006 May-Jun;29(3):313-9

IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. Rosenberg SA, Sportès C, Ahmadzadeh M, Fry TJ, Ngo LT, Schwarz SL, Stetler-Stevenson M, Morton KE, Mavroukakis SA, Morre M, Buffet R, Mackall CL, Gress RE.

Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-1201, USA. sar@nih.gov

Lymphopenia is a serious consequence of HIV infection and the administration of cancer chemotherapeutic agents. Although growth factors can be administered to patients to increase circulating neutrophils, there is no effective method to stimulate CD8+ lymphocyte production in humans, in vivo. This report is the first to describe the administration of recombinant interleukin-7 to humans and demonstrates the ability of this cytokine to mediate selective increases in CD4+ and CD8+ lymphocytes along with a decrease in the percentage of CD4+ T-regulatory cells. These studies suggest an important role for interleukin-7 in the treatment of patients with lymphopenia.

- **J Clin Invest.** 2009 Mar 16. pii: 38052. doi: 10.1172/JCI38052. [Epub ahead of print]

Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelièvre JD, Boué F, Molina JM, Rouzioux C, Avettand-Fénoël V, Croughs T, Beq S, Thiébaud R, Chêne G, Morre M, Delfraissy JF.

HIV infection results in CD4+ T cell deficiency, but efficient combination antiretroviral therapy (c-ART) restores T cells and decreases morbidity and mortality. However, immune restoration by c-ART remains variable, and prolonged T cell deficiency remains in a substantial proportion of patients. In a prospective open-label phase I/IIa trial, we evaluated the safety and efficacy of administration of the T cell regulator IL-7. The trial included 13 c-ART-treated HIV-infected patients whose CD4+ cell counts were between 100 and 400 cells/ μ l and plasma HIV RNA levels were less than 50 copies/ml. Patients received a total of 8 subcutaneous injections of 2 different doses of recombinant human IL-7 (rhIL-7; 3 or 10 μ g/kg) 3 times per week over a 16-day period. rhIL-7 was well tolerated and induced a sustained increase of naive and central memory CD4+ and CD8+ T cells. In the highest dose group, 4 patients experienced transient increases in viral replication. However, functional assays showed that the expanded T cells responded to HIV antigen by producing IFN-gamma and/or IL-2. In conclusion, in lymphopenic HIV-infected patients, rhIL-7 therapy induced substantial functional and quantitative changes in T cells for 48 weeks. Therefore, patients may benefit from intermittent therapy with IL-7 in combination with c-ART.

- **Clin Exp Immunol.** 2006 Mar;143(3):398-403.

CD127 expression and regulation are altered in the memory CD8 T cells of HIV-infected patients--reversal by highly active anti-retroviral therapy (HAART). Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Delfraissy JF, Thèze J.

Unité Immunogénétique Cellulaire, Département de Médecine Moléculaire, Institut Pasteur, 25-28 rue du Dr. Roux, 75724 Paris Cedex 15, France.

HIV infection activates abnormally the immune system and the chronic phase is accompanied by marked alterations in the CD8 compartment. The expression of CD127 (IL-7R alpha chain) by memory CD8 T lymphocytes in HIV-infected patients is analysed and reported. The memory CD8 T cell subset was characterized by expression of CD45RA and CD27 markers, and CD127 cell surface expression was measured ex vivo by four-colour flow cytometry. HIV infection was associated with a fall in the proportion of CD127(+) cells among memory CD8 lymphocytes that resulted in a higher CD127(-) CD45RA(-)CD27(+) CD8 T cell count in HIV-infected patients. Diminished CD127 cell surface expression [mean fluorescence intensity (MFI)] by positive cells was also observed in this subset. The data suggest that these defects were reversed by highly active anti-retroviral therapy (HAART). The regulation of CD127 expression was also studied in vitro. Down-regulation of CD127 by interleukin (IL)-7 was observed in memory CD8 lymphocytes from healthy donors and HAART patients. Expression of CD127 by memory CD8 lymphocytes cultured in the absence of IL-7 confirmed that IL-7R regulation is altered in viraemic patients. Under the same experimental conditions, memory CD8 lymphocytes from HAART patients were shown to express CD127 at levels comparable to cells from healthy individuals. Altered CD127 cell surface expression and defective CD127 regulation in the memory CD8 T lymphocytes of HIV-infected patients are potential mechanisms by which these cells may be impeded in their physiological response to endogenous IL-7 stimulatory signals. Our data suggest that these defects are reversed during the immune reconstitution that follows HAART.

- **J Acquir Immune Defic Syndr.** 2006 Jul;42(3):277-85.

Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients--effects of antiretroviral therapy. Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Jacod S, Delfraissy JF, Thèze J.

Unité Immunogénétique Cellulaire, Département de Médecine Moléculaire, Institut Pasteur, Paris, France.

Despite an increase in plasma IL-7 levels, the CD4 T-cell pool decrease progressively in HIV-infected patients. Here we report on our tests to check the hypothesis that defects in the IL-7 receptor system might be involved in this phenomenon. The cell surface expression of CD127 was measured ex vivo in CD4 and CD8 T lymphocytes drawn from 3 groups of HIV patients. IL-7 function was also followed in vitro by measuring IL-7-

driven T-cell proliferation, the induction of the CD25 activation marker, and overexpression of the antiapoptotic molecule Bcl-2. Untreated viremic patients showed a slight but significant decrease in CD127 expression on the surface of their CD4 lymphocytes. By contrast, CD127 expression was substantially altered on the surface of CD8 T lymphocytes taken from untreated viremic patients. IL-7-induced overexpression of the antiapoptotic molecule Bcl-2 was dramatically altered in viremic patients, whereas IL-7-dependent CD25 induction and T-cell proliferation were reduced. Highly active antiretroviral therapy partially corrected these defects in patients with an undetectable viral load and CD4 counts of more than 400 cells/microL. The effects of HAART were less pronounced in patients with undetectable VL but low CD4 counts (<250 cells/microL). The IL-7 receptor is dysfunctional in the CD4 and CD8 lymphocytes of HIV-infected patients. This may be due to abnormal activation of the immune system in HIV-infected patients and may contribute to the reduced CD4 count and the altered function of the CD8 compartment.

➤ **J Immunol.** 2007 Apr 15;178(8):5340-50.

Potential role for IL-7 in Fas-mediated T cell apoptosis during HIV infection. Fluor C, De Milito A, Fry TJ, Vivar N, Eidsmo L, Atlas A, Federici C, Matarrese P, Logozzi M, Rajnavölgyi E, Mackall CL, Fais S, Chiodi F, Rethi B.

Department of Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden.

IL-7 promotes survival of resting T lymphocytes and induces T cell proliferation in lymphopenic conditions. As elevated IL-7 levels occur in HIV-infected individuals in addition to high Fas expression on T cells and increased sensitivity to Fas-induced apoptosis, we analyzed whether IL-7 has a regulatory role in Fas-mediated T cell apoptosis. We show that IL-7 up-regulates Fas expression on naive and memory T cells through a mechanism that involves translocation of Fas molecules from intracellular compartments to the cell membrane. IL-7 induced the association of Fas with the cytoskeletal component ezrin and a polarized Fas expression on the cell surface. The potential role of IL-7 in Fas up-regulation in vivo was verified in IL-7-treated macaques and in HIV-infected or chemotherapy treated patients by the correlation between serum IL-7 levels and Fas expression on T cells. IL-7 treatment primed T cells for Fas-induced apoptosis in vitro and serum IL-7 levels correlated with the sensitivity of T cells to Fas-induced apoptosis in HIV-infected individuals. Our data suggest an important role for IL-7 in Fas-mediated regulation of T cell homeostasis. Elevated IL-7 levels associated with lymphopenic conditions, including HIV-infection, might participate in the increased sensitivity of T cells for activation-induced apoptosis.

1.1.1.3 SIV

- **J Immunol.** 2006 Jan 15;176(2):914-22.

IL-7 induces immunological improvement in SIV-infected rhesus macaques under antiviral therapy. Beq S, Nugeyre MT, Ho Tsong Fang R, Gautier D, Legrand R, Schmitt N, Estaquier J, Barré-Sinoussi F, Hurtrel B, Cheynier R, Israël N.

Unité de Régulation des Infections Rétrovirales, Institut Pasteur, Paris, France.

Despite efficient antiretroviral therapy (ART), CD4+ T cell counts often remain low in HIV-1-infected patients. This has led to IL-7, a crucial cytokine involved in both thymopoiesis and peripheral T cell homeostasis, being suggested as an additional therapeutic strategy. We investigated whether recombinant simian IL-7-treatment enhanced the T cell renewal initiated by ART in rhesus macaques chronically infected with SIVmac251. Six macaques in the early chronic phase of SIV infection received antiretroviral treatment. Four macaques also received a 3-wk course of IL-7 injections. Viral load was unaffected by IL-7 treatment. IL-7 treatment increased the number of circulating CD4+ and CD8+ memory T cells expressing activation (HLA-DR+, CD25+) and proliferation (Ki-67+) markers. It also increased naive (CD45RAbrightCD62L+) T cell counts by peripheral proliferation and enhanced de novo thymic production. The studied parameters returned to pretreatment values by day 29 after the initiation of treatment, concomitantly to the appearance of anti-IL-7 neutralizing Abs, supporting the need for a nonimmunogenic molecule for human treatment. Thus, IL-7, which increases T cell memory and de novo renewal of naive T cells may have additional benefits in HIV-infected patients receiving ART.

- **J Immunol.** 2003 Oct 15;171(8):4447-53.

IL-7 stimulates T cell renewal without increasing viral replication in simian immunodeficiency virus-infected macaques. Nugeyre MT, Monceaux V, Beq S, Cumont MC, Ho Tsong Fang R, Chêne L, Morre M, Barré-Sinoussi F, Hurtrel B, Israël N.

Unité de Biologie des Rétrovirus Institut Pasteur, Paris, France.

The main failure of antiretroviral therapy is the lack of restoration of HIV-specific CD4(+) T cells. IL-7, which has been shown to be a crucial cytokine for thymopoiesis, has been envisaged as an additive therapeutic strategy. However, in vitro studies suggest that IL-7 might sustain HIV replication in thymocytes and T lymphocytes. Therefore, in the present study, we evaluated the effect of IL-7 on both T cell renewal and viral load in SIVmac-infected young macaques in the absence of antiretroviral therapy. This evaluation was conducted during the asymptomatic phase in view of a potential treatment of HIV patients. We show that IL-7 induces both a central renewal and a peripheral expansion of T lymphocytes associated with cell activation. No

alarming modulation of the other hemopoietic cells was observed. No increase in the viral load was shown in blood or lymph nodes. These data strengthen the rationale for the use of IL-7 as an efficient immunotherapy in AIDS.

- **J Med Primatol.** 2007 Aug;36(4-5):228-37.

Rapid modifications of peripheral T-cell subsets that express CD127 in macaques treated with recombinant IL-7. Dereuddre-Bosquet N, Vaslin B, Delache B, Brochard P, Clayette P, Aubenque C, Morre M, Assouline B, Le Grand R.

SPI-BIO, CEA, Fontenay-aux-Roses, Cedex, France.

BACKGROUND: Interleukin-7 (IL-7) is a key regulator of thymopoiesis and T-cell homeostasis, which increases blood T-cell number by enhancing thymic output of naive cells and peripheral proliferation. **METHODS:** We explored the effects of unglycosylated recombinant simian IL-7 (rsIL-7) administration on peripheral T-cell subpopulations in healthy macaques. **RESULTS:** RsIL-7 was well tolerated. Mean half-life ranged between 9.3 and 13.9 hours. Blood CD3(+)CD4(+) and CD3(+)CD8(+) lymphocyte counts decreased rapidly after each rsIL-7 administration, the duration of these effects being dependent on the frequency of administration. At treatment completion, the increased of CD3(+) lymphocytes was marked at 100 microg/kg every 2 days. CD3(+) lymphocytes that harbour the alpha chain of IL-7 receptor (CD127) and CD3(+)CD8(+) lymphocytes that expressed the proliferation marker Ki-67 exhibited a similar initial profile. The expression of the anti-apoptotic marker Bcl-2 increased in CD3(+) lymphocytes during the treatment and post-treatment period in a dose/frequency dependent manner. **CONCLUSION:** RsIL-7 was well tolerated in macaques and induces rapid modifications of T-cells that express CD127.

- **J Immunol.** 2007 Mar 15;178(6):3492-504.

Interleukin-15 but not interleukin-7 abrogates vaccine-induced decrease in virus level in simian immunodeficiency virus mac251-infected macaques. Hryniewicz A, Price DA, Moniuszko M, Boasso A, Edghill-Spano Y, West SM, Venzon D, Vaccari M, Tsai WP, Trynieszewska E, Nacsa J, Villinger F, Ansari AA, Trindade CJ, Morre M, Brooks D, Arlen P, Brown HJ, Kitchen CM, Zack JA, Douek DC, Shearer GM, Lewis MG, Koup RA, Franchini G.

Animal Models and Retroviral Vaccines Section, National Cancer Institute, Building 41, Bethesda, MD 20892, USA.

The loss of CD4(+) T cells and the impairment of CD8(+) T cell function in HIV infection suggest that pharmacological treatment with IL-7 and IL-15, cytokines that increase the homeostatic proliferation of T cells and improve effector function, may be beneficial. However, these cytokines could also have a detrimental effect in HIV-1-infected individuals, because both cytokines increase HIV replication in vitro. We assessed the

impact of IL-7 and IL-15 treatment on viral replication and the immunogenicity of live poxvirus vaccines in SIV(mac251)-infected macaques (*Macaca mulatta*). Neither cytokine augmented the frequency of vaccine-expanded CD4(+) or CD8(+) memory T cells, clonal recruitment to the SIV-specific CD8(+) T cell pool, or CD8(+) T cell function. Vaccination alone transiently decreased the viral set point following antiretroviral therapy suspension. IL-15 induced massive proliferation of CD4(+) effector T cells and abrogated the ability of vaccination to decrease set point viremia. In contrast, IL-7 neither augmented nor decreased the vaccine effect and was associated with a decrease in TGF-beta expression. These results underscore the importance of testing immunomodulatory approaches in vivo to assess potential risks and benefits for HIV-1-infected individuals.

- **Blood.** 2003 Mar 15;101(6):2294-9. Epub 2002 Oct 31.

IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Fry TJ, Moniuszko M, Creekmore S, Donohue SJ, Douek DC, Giardina S, Hecht TT, Hill BJ, Komschlies K, Tomaszewski J, Franchini G, Mackall CL.

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Interleukin-7 (IL-7) is important for thymopoiesis in mice and humans because IL-7 receptor alpha (IL-7Ralpha) mutations result in a severe combined immunodeficiency phenotype with severe thymic hypoplasia. Recent evidence has indicated that IL-7 also plays an important role as a regulator of T-cell homeostasis. Here we report the immunologic effects of recombinant human IL-7 (rhIL-7) therapy in normal and simian immunodeficiency virus (SIV)-infected nonhuman primates. *Cynomolgus* monkeys receiving 10 days of rhIL-7 showed substantial, reversible increases in T-cell numbers involving a dramatic expansion of both naive and nonnaive phenotype CD4(+) and CD8(+) subsets. Although IL-7 is known to have thymopoietic effects in mice, we observed marked declines in the frequency and absolute number of T-cell receptor excision circle-positive (TREC(+)) cells in the peripheral blood and dramatic increases in the percentage of cycling T cells in the peripheral blood as measured by Ki-67 expression (baseline less than 5% to approximately 50% after 6 days of therapy) and ex vivo bromodeoxyuridine (BrdU) incorporation. Similarly, moderately CD4- depleted SIV-infected macaques treated with rhIL-7 also had significant increases in peripheral blood CD4(+) and CD8(+) T cells following rhIL-7 therapy. Thus, rhIL-7 induces dramatic alterations in peripheral T-cell homeostasis in both T-cell-replete and T-cell-depleted nonhuman primates. These results further implicate IL-7 as a promising immunorestorative agent but illustrate that a major component of its immunorestorative capacity reflects effects on mature cells. These results also raise the possibility that IL-7 therapy could be used to temporarily modulate T-cell cycling in vivo in the context of immunotherapies such as vaccination.

2.3 Other main publications

- **Nat Med.** 2001 Jan;7(1):73-9.

Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, Herndier BG, Andersson J, McCune JM.

Gladstone Institute of Virology and Immunology, San Francisco General Hospital, and Department of Medicine, University of California at San Francisco, San Francisco, California 94110-9100, USA.

We hypothesized that HIV-1-mediated T-cell loss might induce the production of factors that are capable of stimulating lymphocyte development and expansion. Here we perform cross-sectional (n = 168) and longitudinal (n = 11) analyses showing that increased circulating levels of interleukin (IL)-7 are strongly associated with CD4+ T lymphopenia in HIV-1 disease. Using immunohistochemistry with quantitative image analysis, we demonstrate that IL-7 is produced by dendritic-like cells within peripheral lymphoid tissues and that IL-7 production by these cells is greatly increased in lymphocyte-depleted tissues. We propose that IL-7 production increases as part of a homeostatic response to T-cell depletion.

- **J Virol.** 2001 Nov;75(21):10319-25.

Interleukin-7 in plasma correlates with CD4 T-cell depletion and may be associated with emergence of syncytium-inducing variants in human immunodeficiency virus type 1-positive individuals. Llano A, Barretina J, Gutiérrez A, Blanco J, Cabrera C, Clotet B, Esté JA.

Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain.

Human immunodeficiency virus type 1 (HIV-1) primary infection is characterized by the use of CCR5 as a coreceptor for viral entry, which is associated with the non-syncytium-inducing (NSI) phenotype in lymphoid cells. Syncytium-inducing (SI) variants of HIV-1 appear in advanced stages of HIV-1 infection and are characterized by the use of CXCR4 as a coreceptor. The emergence of SI variants is accompanied by a rapid decrease in the number of T cells. However, it is unclear why SI variants emerge and what factors trigger the evolution of HIV from R5 to X4 variants. Interleukin-7 (IL-7), a cytokine produced by stromal cells of the thymus and bone marrow and by keratin, is known to play a key role in T-cell development. We evaluated IL-7 levels in plasma of healthy donors and HIV-positive patients and found significantly higher levels in HIV-positive patients. There was a negative correlation between circulating IL-7 levels and CD4(+) T-cell count in HIV-positive patients ($r = -0.621$; $P < 0.001$), suggesting that IL-7 may be involved in HIV-induced T-cell depletion and disease progression. IL-7 levels were higher in individuals who harbored SI variants and who had progressed to having CD4

cell counts of lower than 200 cells/microl than in individuals with NSI variants at a similar stage of disease. IL-7 induced T-cell proliferation and up-regulated CXCR4 expression in peripheral blood mononuclear cells in vitro. Taken together, our results suggest a role for IL-7 in the maintenance of T-cell regeneration and depletion by HIV in infected individuals and a possible relationship between IL-7 levels and the emergence of SI variants.

- **Blood.** 2004 Feb 1;103(3):973-9. Epub 2003 Oct 2.

Elevated interleukin-7 levels not sufficient to maintain T-cell homeostasis during simian immunodeficiency virus-induced disease progression. Muthukumar A, Wozniakowski A, Gauduin MC, Paiardini M, McClure HM, Johnson RP, Silvestri G, Sodora DL.

University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390, USA.

Elevated levels of interleukin 7 (IL-7) have been correlated with various T-cell depletion conditions, including HIV infection, and suggested as an indicator of HIV disease progression (AIDS and death). Here, the assessment of pathogenic simian immunodeficiency virus (SIVmac239) infection in rhesus macaques demonstrated a clear association between a significant elevation in IL-7 levels and disease progression. In 5 macaques that progressed to simian AIDS and death, elevated IL-7 levels were unable to restore T-cell homeostasis. In contrast, increased IL-7 levels were followed by relatively high and stable T-cell numbers in the SIV-infected macaques with a slow-progressing phenotype. Further, studies in sooty mangabeys that do not progress to simian AIDS and that maintain stable T-cell numbers despite high levels of viral replication support the importance of IL-7 and T-cell homeostasis in disease progression. These data suggest that during pathogenic SIV infection with high viral replication, elevated IL-7 levels are unable to recover T-cell homeostasis, thereby leading to disease progression. The utility of IL-7 as a potential immunotherapeutic agent to improve HIV/SIV-related T-cell depletion may therefore depend on controlling the pathogenic effects of viral replication prior to the administration of IL-7.

3. IL-7 and Hepathic Chronic Viral Infections (HCV and HBV)

3.1 Other Main Publications

- **Hepatology.** 2006 Nov;44(5):1098-109.

Loss of IL-7 receptor alpha-chain (CD127) expression in acute HCV infection associated with viral persistence. Golden-Mason L, Burton JR Jr, Castelblanco N, Klarquist J, Benlloch S, Wang C, Rosen HR.

Division of Gastroenterology & Hepatology, Hepatitis C Center, and Integrated Program in Immunology, University of Colorado Health Sciences Center & National Jewish Hospital, Denver, CO, USA.

Interleukin-7 (IL-7) is required for the establishment and maintenance of memory CD4(+) and CD8(+) T lymphocytes, and cells lacking IL-7Ralpha (CD127) demonstrate impaired IL-2 secretion and have a short life-span. Chronic HCV is characterized by T cells that are functionally impaired and exhibit an immature phenotype. To investigate the potential role of IL-7/IL-7Ralpha in the outcome of HCV infection, we used multiparameter flow cytometry to characterize patients with acute infection (n = 24), long-term chronic infection (12) and normal subjects (13). HCV infection per se resulted in downregulation of CD127 on total CD4(+) and CD8(+) T lymphocytes as compared to normal controls. Total expression was lowest in those patients who subsequently developed persistence and intermediate in those patients with acute-resolving infection. This reduction affected both naïve and effector/memory T cells. CD127 correlated phenotypically with upregulation of chemokine receptors CCR7 and CXCR4, expression of the anti-apoptotic molecule B cell leukemia/lymphoma 2 (Bcl-2), and enhanced IL-2 production. In six HLA A2-positive patients, we longitudinally tracked tetramer responses to HCV and CMV epitopes; at baseline, reflecting the expression of CD127 on whole T cell populations, viral-specific CTLs in patients who became chronic demonstrated lower CD127. In conclusion, CD127 is a useful marker of functional CD4(+) and CD8(+) T cells and its expression correlates with virologic outcome of acute HCV. These data provide a mechanistic basis for the observation that CTLs generated in early infection rapidly decline as chronicity is established; CD127 expression should be considered in the design of novel immunotherapeutic approaches.

➤ **Immunity.** 2009 Mar;30(3):447-57. Epub 2009 Mar 12.

Hepatic interleukin-7 expression regulates T cell responses. Sawa Y, Arima Y, Ogura H, Kitabayashi C, Jiang JJ, Fukushima T, Kamimura D, Hirano T, Murakami M.

Laboratory of Developmental Immunology, Graduate School of Frontier Biosciences, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan.

Systemic cytokine activity in response to Toll-like receptor (TLR) signaling induces the expression of various proteins in the liver after infections. Here we show that Interleukin-7 (IL-7), the production of which was thought to occur at a constant rate in vivo, was a hepatically expressed protein that directly controlled T cell responses. Depletion of IL-7 expression in the liver abrogated several TLR-mediated T cell events, including enhanced CD4+ T cell and CD8+ T cell survival, augmented CD8+ T cell cytotoxic activity, and the development of experimental autoimmune encephalitis, a Th17 cell-mediated autoimmune disease. Thus, T cell responses are regulated by hepatocyte-derived IL-7, which is expressed in response to TLR signaling in vivo. We suggested that TLR-induced IL-7 expression in the liver, which is an acute-phase response, may be a good diagnostic and therapeutic target for efficient vaccine

developments and for conditions characterized by TLR-mediated T cell dysregulation, including autoimmune diseases.

J Virol. 2007 Mar;81 (6):2545-53. Epub 2006 Dec 20.

Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. Radziewicz H, Ibegbu CC, Fernandez ML, Workowski KA, Obideen K, Wehbi M, Hanson HL, Steinberg JP, Masopust D, Wherry EJ, Altman JD, Rouse BT, Freeman GJ, Ahmed R, Grakoui A.

Emory University School of Medicine, 954 Gatewood Road N.E., Atlanta, GA 30329, USA.

The majority of people infected with hepatitis C virus (HCV) fail to generate or maintain a T-cell response effective for viral clearance. Evidence from murine chronic viral infections shows that expression of the coinhibitory molecule PD-1 predicts CD8+ antiviral T-cell exhaustion and may contribute to inadequate pathogen control. To investigate whether human CD8+ T cells express PD-1 and demonstrate a dysfunctional phenotype during chronic HCV infection, peripheral and intrahepatic HCV-specific CD8+ T cells were examined. We found that in chronic HCV infection, peripheral HCV-specific T cells express high levels of PD-1 and that blockade of the PD-1/PD-L1 interaction led to an enhanced proliferative capacity. Importantly, intrahepatic HCV-specific T cells, in contrast to those in the periphery, express not only high levels of PD-1 but also decreased interleukin-7 receptor alpha (CD127), an exhausted phenotype that was HCV antigen specific and compartmentalized to the liver, the site of viral replication.

➤ **J Virol.** 2006 Apr;80(7):3532-40.

Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific CD8+ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, Thimme R.

Department of Medicine II, University Hospital Freiburg, Hugstetter Strasse 55, D-79106 Freiburg, Germany.

Virus-specific CD8+ T cells play a central role in the outcome of several viral infections, including hepatitis B virus (HBV) infection. A key feature of virus-specific CD8+ T cells is the development of memory. The mechanisms resulting in the establishment of T-cell memory are still only poorly understood. It has been suggested that T-cell memory may depend on the survival of virus-specific CD8+ T cells in the contraction phase. Indeed, a population of effector cells that express high levels of the interleukin-7 receptor alpha chain (CD127) as the precursors of memory CD8+ T cells has recently been identified in mice. However, very little information is currently available about the kinetics of CD127 expression in an acute resolving viral infection in humans and its association with

disease pathogenesis, viral load, and functional and phenotypical T-cell characteristics. To address these important issues, we analyzed the HBV-specific CD8+ T-cell response longitudinally in a cohort of six patients with acute HBV infection who spontaneously cleared the virus. We observed the emergence of CD127 expression on antigen-specific CD8+ memory T cells during the course of infection. Importantly, the up-regulation of CD127 correlated phenotypically with a loss of CD38 and PD-1 expression and acquisition of CCR7 expression: functionally with an enhanced proliferative capacity and clinically with the decline in serum alanine aminotransferase levels and viral clearance. These results suggest that the expression of CD127 is a marker for the development of functionally and phenotypically defined antigen-specific CD8+ memory T cells in cleared human viral infections.

3.2 IL-7 and Oncology

3.3 Reviews

- **Nat Med.** 2003 Mar;9(3):269-77.

Immunotherapy: past, present and future. Waldmann TA.

Metabolism Branch, Center for Cancer Research National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.

Harnessing the immune system to treat chronic infectious diseases or cancer is a major goal of immunotherapy. Among others, impediments to this aim include host failure to identify tumor antigens, tolerance to self and negative immunoregulatory mechanisms. But with recent progress, active and passive immunotherapy are proving themselves as effective therapeutic strategies.

- **Semin Hematol.** 2009 Jan;46(1):89-99.

Immunotherapy in acute leukemia. Leung W.

Division of Bone Marrow Transplantation and Cellular Therapy, Department of Oncology, St. Jude Children's Research Hospital, and Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN 38105, USA. wing.leung@stjude.org

Recent advances in immunotherapy of cancer may represent a successful example in translational research, in which progress in knowledge and technology in immunology has led to new strategies of immunotherapy, and even past failures in many clinical trials have led to a better understanding of basic cancer immunobiology. This article reviews the latest concepts in antitumor immunology and its application in the treatment of cancer, with particular focus on acute leukemia.

- **Cancer Immunol Immunother.** 2009 Feb 24. [Epub ahead of print]

Cancer, aging and immunotherapy: lessons learned from animal models. Lustgarten J.

Department of Immunology, Mayo Clinic Arizona, Mayo Clinic College of Medicine, 13400 East Shea Boulevard, Scottsdale, AZ, 85259, USA

Aging of the immune system is associated with a dramatic reduction in responsiveness as well as functional dysregulation. This deterioration of immune function with advancing age is associated with an increased incidence of cancer. Although there is a plethora of reports evaluating the effect of immunotherapy in stimulating antitumor immune responses, the majority of these studies do not pay attention to the effect aging has on the immune system. Studies from our group and others indicate that immunotherapies could be effective in the young, are not necessarily effective in the old. To optimally stimulate an antitumor immune response in the old, it is necessary to (1) identify and understand the intrinsic defects of the old immune system and (2) use relevant models that closely reflect those of cancer patients, where self-tolerance and aging are present simultaneously. The present review summarizes some defects found in the old immune system affecting the activation of antitumor immune responses, the strategies used to activate stronger antitumor immune response in the old and the use of a tolerant animal tumor model to target a self-tumor antigen for the optimization of immunotherapeutic interventions in the old.

3.4 Cytheris Associated Publications

- **Nat Med.** 2009 26 April | doi:10.1038/nm.1953.

Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. Pellegrini M, Calzascia T, Elford AR, Shahinian A, Lin AE, Dissanayake D, Dhanji S, Nguyen LT, Gronski MA, Morre M, Assouline B, Lahl K, Sparwasser T, Ohashi PS, Mak TW.

Identifying key factors that enhance immune responses is crucial for manipulating immunity to tumors. We show that after a vaccine-induced immune response, adjuvant interleukin-7 (IL-7) improves antitumor responses and survival in an animal model. The improved immune response is associated with increased IL-6 production and augmented T helper type 17 cell differentiation. Furthermore, IL-7 modulates the expression of two ubiquitin ligases: Casitas B-lineage lymphoma b (Cbl-b), a negative regulator of T cell activation, is repressed, and SMAD-specific E3 ubiquitin protein ligase-2 (Smurf2) is enhanced, which antagonizes transforming growth factor-beta signaling. Notably, we show that although short term IL-7 therapy potently enhances vaccine-mediated immunity, in the absence of vaccination it is inefficient in promoting antitumor immune responses, despite inducing homeostatic proliferation of T cells. The ability of adjuvant IL-7 to antagonize inhibitory networks at the cellular and molecular level has major implications for immunotherapy in the treatment of tumors.

- **J Exp Med.** 2008 Jul 7;205(7):1701-14. Epub 2008 Jun 23.

Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. Sportès C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, Fleisher TA, Krumlauf MC, Babb RR, Chow CK, Fry TJ, Engels J, Buffet R, Morre M, Amato RJ, Venzon DJ, Korngold R, Pecora A, Gress RE, Mackall CL.

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Interleukin-7 (IL-7) is a homeostatic cytokine for resting T cells with increasing serum and tissue levels during T cell depletion. In preclinical studies, IL-7 therapy exerts marked stimulating effects on T cell immune reconstitution in mice and primates. First-in-human clinical studies of recombinant human IL-7 (rhIL-7) provided the opportunity to investigate the effects of IL-7 therapy on lymphocytes in vivo. rhIL-7 induced in vivo T cell cycling, bcl-2 up-regulation, and a sustained increase in peripheral blood CD4(+) and CD8(+) T cells. This T cell expansion caused a significant broadening of circulating T cell receptor (TCR) repertoire diversity independent of the subjects' age as naive T cells, including recent thymic emigrants (RTEs), expanded preferentially, whereas the proportions of regulatory T (T reg) cells and senescent CD8(+) effectors diminished. The resulting composition of the circulating T cell pool more closely resembled that seen earlier in life. This profile, distinctive among cytokines under clinical development, suggests that rhIL-7 therapy could enhance and broaden immune responses, particularly in individuals with limited naive T cells and diminished TCR repertoire diversity, as occurs after physiological (age), pathological (human immunodeficiency virus), or iatrogenic (chemotherapy) lymphocyte depletion.

- **Proc Natl Acad Sci U S A.** 2008 Feb 26;105(8):2999-3004. Epub 2008 Feb 14.

CD4 T cells, lymphopenia, and IL-7 in a multistep pathway to autoimmunity. Calzascia T, Pellegrini M, Lin A, Garza KM, Elford AR, Shahinian A, Ohashi PS, Mak TW.

The Campbell Family Institute for Breast Cancer Research, University Health Network, Toronto, ON, Canada.

There are many inhibitory mechanisms that function at the cellular and molecular levels to maintain tolerance. Despite this, self-reactive clones escape regulatory mechanisms and cause autoimmunity in certain circumstances. We hypothesized that the same mechanisms that permit T cells to expand during homeostatic proliferation may inadvertently promote autoimmunity under certain conditions. One major homeostatic cytokine is IL-7, and studies have linked it or its receptor to the development of multiple sclerosis and other autoimmune diseases. We show in a model of beta-islet cell self-reactivity that the transfer of activated autoreactive CD4 T cells can prime and expand endogenous autoreactive CD8 T cells in a CD28- and CD40-dependent manner through

the licensing of dendritic cells. Despite this, mice do not develop diabetes. However, the provision of exogenous IL-7 or the physiological production of IL-7 associated with lymphopenia was able to profoundly promote the expansion of self-reactive clones even in the presence of regulatory T cells. Autoimmune diabetes rapidly ensued with CD4 help and the subsequent activation of CD8 T cells, which contributed to disease progression. With the advent of many biologicals targeting TNFalpha, IL-6, and IL-1 and their effective use in the treatment of autoimmune diseases, we propose that IL-7 and its receptor may be promising targets for biological agents in the treatment of autoimmunity.

- **Clin Immunol.** 2007 May;123 (2):155-65. Epub 2007 Feb 22.

Recombinant IL-7 enhances the potency of GM-CSF-secreting tumor cell immunotherapy. Li B, VanRoey MJ, Jooss K.

Cell Genesys Inc., 500 Forbes Boulevard, South San Francisco, CA 94080, USA.

IL-7 is known for its role in lymphopoiesis and T-cell homeostasis. In addition, its capacity to augment the immune response to weak or low affinity antigens makes it an ideal candidate to evaluate in combination with a GM-CSF-secreting tumor cell immunotherapy, which has been shown to elicit broad humoral and cellular immune responses. The studies reported here show that IL-7, when combined with a GM-CSF-secreting tumor cell immunotherapy, significantly prolonged the survival of tumor-bearing mice. The enhanced anti-tumor protection correlated with an increased number of activated dendritic cells (DC) and T cells in lymphoid tissues, such as the draining lymph nodes (DLN) and spleen. Moreover, an increased number of activated effector T cells were found in the tumor microenvironment, correlating with a more potent systemic tumor-specific T-cell response than each monotherapy alone. Taken together, these studies demonstrate that IL-7 augments the anti-tumor response of a GM-CSF-secreting tumor cell immunotherapy in preclinical models.

3.4.1 Other Main Publications

- **J Exp Med.** 1991 Dec 1;174(6):1291-8.

Interleukin 7 induces CD4+ T cell-dependent tumor rejection. Hock H, Dorsch M, Diamantstein T, Blankenstein T.

Institut für Immunologie, Universitätsklinikum Steglitz, Freie Universität Berlin, Federal Republic of Germany.

The potential of interleukin 7 (IL-7) to induce an antitumor response in vivo was analyzed. Therefore, the IL-7 gene was expressed in the plasmacytoma cell line J558L. Although the growth of IL-7-producing cells was not retarded in vitro, the IL-7-producing cells were completely rejected upon injection into mice. Tumor rejection was observed only in syngeneic but not in nude mice. The tumor-suppressive effect could be

abolished by the parallel injection of an anti-IL-7 monoclonal antibody. Immunohistochemical analysis revealed IL-7-dependent infiltration of the tumor tissue by CD4+ and CD8+ T lymphocytes, and also type 3 complement receptor-positive (CR3+) cells, predominantly macrophages. Depletion of T cell subsets in tumor-bearing mice showed the absolute dependence of the antitumor response on CD4+ cells, whereas tumor rejection was unaffected by depletion of CD8+ cells. In addition to CD4+ cells, CR3+ cells were also needed for tumor rejection. The antitumor effect of IL-7 was confirmed by expression of the IL-7 gene in a second tumor cell line of different cellular origin. Together, our results demonstrate that a high local IL-7 concentration at the tumor site obtained by tumor cell-targeted gene transfer leads to tumor rejection involving a cellular mechanism that seems to be different from the ones observed in analogous experiments with other cytokines.

➤ **J Exp Med.** 1994 Jan 1;179(1):31-42.

Interleukin 7 promotes long-term in vitro growth of antitumor cytotoxic T lymphocytes with immunotherapeutic efficacy in vivo.
Lynch DH, Miller RE.

Department of Immunobiology, Immunex Research and Development Corp., Seattle, Washington 98101.

A major obstacle to the effective use of adoptive immunotherapeutic treatment of cancer is the difficulty of obtaining tumor-reactive lymphocytes in either sufficient numbers or with appropriate in vivo function to make such an approach feasible. Previous studies have shown that antitumor cytotoxic T lymphocytes (CTL) with in vivo efficacy can be generated in vitro from lymphoid cells obtained from lymph nodes that drain the anatomical site of a tumor. Results presented here demonstrate that inclusion of interleukin 7 (IL-7) into the medium in which such CTL are cultured can support their growth in vitro for prolonged periods of time in the absence of repeated stimulation with either tumor stimulator cells or tumor antigen. More importantly, antitumor CTL propagated in medium containing IL-7 have retained both their antigenic specificity and their ability to reject tumors in vivo subsequent to intravenous injection. Parallel cultures of antitumor CTL similarly cultured in medium containing only IL-2 could only be maintained for 5-6 wk, after which the number and proportion of viable cells that were recoverable from such cultures progressively decreased. Phenotypic analysis of CTL maintained after extended culture (i.e., 22 mo) in medium containing IL-7 demonstrated them to be CD3+4-8+ T cells. These cells were also found to express lymphocyte function associated 1, intercellular adhesion molecule 1, and Mel-14 cell interaction molecules. The data also demonstrate that these CTL do not require the presence of antigen-presenting cell populations to mount a proliferative response to tumor stimulator cells. Cells in these cultures were also demonstrated to produce IL-2 after stimulation with irradiated tumor cells, thereby indicating that these CTL have become independent of the requirement for CD4+ helper cells to survive and function either in vitro or in vivo. Collectively, the findings that IL-7 can beneficially augment the generation, and propagate the long-term growth, of antitumor CTL from lymph nodes

draining a tumor site may have profound implications for promoting the immunotherapeutic treatment of cancer in humans.

- **Cancer Immunol Immunother.** 2009 Mar;58 (3):373-81. Epub 2008 Jul 19.

A prostate cancer vaccine comprising whole cells secreting IL-7, effective against subcutaneous challenge, requires local GM-CSF for intra-prostatic efficacy. Schrotten-Loef C, de Ridder CM, Reneman S, Crezee M, Dalgleish A, Todryk SM, Bangma CH, Kraaij R.

Erasmus MC, Department of Urology, Josephine Nefkens Institute, Rotterdam, The Netherlands.

A panel of cytokine-secreting RM-9 prostate cancer cells were tested as whole cell vaccines to determine their capacity to evoke an anti-prostate cancer immune response. In our model, vaccines secreting mGM-CSF or mL-7 resulted in the highest increase in circulating T lymphocytes after vaccination, prolonged survival and, in a proportion of animals, tumor-free survival. Anti-tumor effects were more evident after a subcutaneous RM-9 challenge than after an intraprostatic challenge. However, when the RM-9/mGM-CSF cell line was used as intraprostatic tumor challenge, protection after RM-9/mL-7 vaccination was restored.

4. IL-7 and Bone Marrow Transplantation

4.1 Reviews

- **Leuk Lymphoma.** 2006 Jul;47(7):1222-8.

IL-7 in allogeneic transplant: clinical promise and potential pitfalls. Snyder KM, Mackall CL, Fry TJ.

Immunology Section, Pediatric Oncology Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892, USA.

Much progress has been made in the field of allogeneic stem cell transplantation. However, one major barrier is the delay in immune recovery that can persist for months post-transplant and results in increased susceptibility to infection and relapse of malignancy. Strategies to improve immune recovery must be balanced with the potential for those therapies to exacerbate graft vs host disease. Interleukin 7 is a member of the gammac cytokine family that is required for T-cell development and maintenance of naïve T-cell populations. In addition, IL-7 plays a major role in the expansion of mature T-cells that occurs during lymphopenia and therapeutic IL-7 can enhance both quantitative and functional immune recovery following T-cell depletion. Thus, this agent holds much promise as an immunorestorative agent and as an adjuvant to vaccines or adoptive immunotherapy. Clinic trials with IL-7 are underway. Murine

studies with IL-7 in the allogeneic transplant have demonstrated that the potent immune effects of this agent can also be achieved in this setting. However, these studies have indicated that the potential for IL-7 to worsen GVHD exists and that this effect may abrogate the immune benefits. Thus, careful consideration of how best to incorporate IL-7 into allogeneic trials will be needed if the full potential of this agent is to be realized.

4.2 Cytheris Associated Publications

1.1.1.4 Monkey model

- **Blood.** 2003 May 15;101(10):4209-18. Epub 2003 Jan 23.

Interleukin-7 improves CD4 T-cell reconstitution after autologous CD34 cell transplantation in monkeys. 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.

Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

In mice, interleukin-7 (IL-7) hastens T-cell reconstitution and might cause autoimmune diseases, lymphoma, and osteoporosis. We assessed the effect of IL-7 on T-cell reconstitution and toxicity in baboons that underwent total body irradiation followed by autologous transplantation of marrow CD34 cells. Three baboons received placebo and 3 baboons received recombinant human IL-7 (rhIL-7, 75 microg/kg twice a day subcutaneously) between 6 and 10 weeks after transplantation. The mean increase in blood absolute CD4 T-cell counts was 0.9-fold in the placebo-treated animals versus 9.0-fold in those treated with IL-7 ($P = .02$). The increase observed in the IL-7-treated animals appeared attributable to peripheral expansion rather than de novo generation. The IL-7-treated animals had greater mean increases in the volumes of the spleen (2.0-fold with placebo versus 4.5-fold with IL-7, $P = .02$) and lymph nodes (1.8-fold with placebo versus 4.1-fold with IL-7, $P = .10$) but not the thymus (3.4-fold with placebo versus 1.1-fold with IL-7, $P = .18$). Side effects of IL-7 included thrombocytopenia and possibly neutropenia and hemolytic anemia. One IL-7-treated animal failed to thrive due to a disease resembling graft-versus-host disease. No animals developed lymphoma. Bone density was not decreased. In conclusion, IL-7 raises CD4 T-cell counts in irradiated primates. It remains to be determined whether this is associated with clinical benefit.

4.2.1 Murine Model

- **J Immunol.** 2004 Mar 1;172(5):3328-36.

Host conditioning is a primary determinant in modulating the effect of IL-7 on murine graft-versus-host disease. Gendelman M, Hecht T, Logan B, Vodanovic-Jankovic S, Komorowski R, Drobyski WR.

Bone Marrow Transplant Program, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI 53226, USA.

Interleukin-7 has been shown to enhance T cell reconstitution after allogeneic bone marrow transplantation, in part, by expansion of mature donor T cells, but whether IL-7 also exacerbates graft-vs-host disease (GVHD) remains unresolved. To address this issue, we examined the effect of IL-7 on GVHD induction using a well-defined murine GVHD model (B6-->B6AF1/J). Administration of IL-7 to nonirradiated B6AF1/J recipients of B6 T cells resulted in expansion of splenic donor CD4(+) and CD8(+) T cells and increased GVHD mortality. In contrast, administration of IL-7 on the same schedule failed to increase GVHD mortality in either sublethally or lethally irradiated animals that received graded doses of T cells designed to induce varying degrees of GVHD severity. Moreover, IL-7 failed to increase the number of alloreactive T cells when examined in a murine model (B6-->BALB.B) that allowed for direct quantitation of graft-vs-host-reactive T cells. The combination of irradiation and transplantation of alloreactive donor T cells resulted in significantly increased levels of endogenous splenic IL-7 mRNA when compared with nonirradiated transplanted animals, providing a potential explanation for why exogenous IL-7 did not increase GVHD severity in these mice. We conclude that host conditioning modulates the ability of exogenous IL-7 to exacerbate GVHD and that this occurs through induction of endogenous IL-7 production.

- **I Clin Invest.** 2003 Oct;112(7):1095-107.

IL-7 enhances peripheral T cell reconstitution after allogeneic hematopoietic stem cell transplantation. Alpdogan O, Muriglian SJ, Eng JM, Willis LM, Greenberg AS, Kappel BJ, van den Brink MR.

Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

We used clinically relevant murine allogeneic bone marrow transplantation (BMT) models to study the mechanisms by which IL-7 administration can improve posttransplant peripheral T cell reconstitution. After transplant we could distinguish two populations of mature donor T cells: (a) alloreactive T cells with decreased expression of CD127 (IL-7 receptor alpha chain) and (b) nonalloreactive T cells, which express CD127 and undergo homeostatic proliferation. IL-7 administration increased the homeostatic proliferation of nonalloreactive T cells, but had no effect on alloreactive T cells and the development of graft-versus-host disease. Allogeneic transplant of purified hematopoietic stem cells and adoptive transfer of thymocytes into lethally irradiated hosts suggested that recent thymic emigrants can undergo homeostatic proliferation and acquire a memory-like phenotype. We found by BrdU pulse-chase, cell cycle, and annexin V analyses that IL-7 administration has significant proliferative and antiapoptotic effects on posttransplant peripheral T cells. We conclude that homeostatic expansion is important for T cell reconstitution after allogeneic BMT and involves both transferred mature T cells and recent thymic emigrants. Apart from its thymopoietic effects, IL-7 promotes peripheral T cell reconstitution through its selective proliferative

and antiapoptotic effects on nonalloreactive and de novo-generated T cells, but has no effect on alloreactive T cells.

- **Blood.** 2001 Oct 1;98(7):2256-65.

Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. Alpdogan O, Schmaltz C, Muriglian SJ, Kappel BJ, Perales MA, Rotolo JA, Halm JA, Rich BE, van den Brink MR.

Department of Medicine and Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

Prolonged immunodeficiency after allogeneic bone marrow transplantation (BMT) causes significant morbidity and mortality from infection. This study examined in murine models the effects of interleukin-7 (IL-7) given to young and middle-aged (9-month-old) recipients of major histocompatibility complex (MHC)-matched or -mismatched allogeneic BMT. Although administration of IL-7 from day 0 to 14 after syngeneic BMT promoted lymphoid reconstitution, this regimen was ineffective after allogeneic BMT. However, IL-7 administration from day 14 (or 21) to 27 after allogeneic BMT accelerated restoration of the major lymphoid cell populations even in middle-aged recipients. This regimen significantly expanded donor-derived thymocytes and peripheral T cells, B-lineage cells in bone marrow and spleen, splenic natural killer (NK) cells, NK T cells, and monocytes and macrophages. Interestingly, although recipients treated with IL-7 had significant increases in CD4(+) and CD8(+) memory T-cell populations, increases in naive T cells were less profound. Most notable, however, were the observations that IL-7 treatment did not exacerbate graft-versus-host disease (GVHD) in recipients of an MHC-matched BMT, and would ameliorate GVHD in recipients of a MHC-mismatched BMT. Nonetheless, graft-versus-leukemia (GVL) activity (measured against 32Dp210 leukemia) remained intact. Although activated and memory CD4(+) and CD8(+) T cells normally express high levels of IL-7 receptor (IL-7R, CD127), activated and memory alloreactive donor-derived T cells from recipients of allogeneic BMT expressed little IL-7R. This might explain the failure of IL-7 administration to exacerbate GVHD. In conclusion, posttransplant IL-7 administration to recipients of an allogeneic BMT enhances lymphoid reconstitution without aggravating GVHD while preserving GVL.

- **Haematologica.** 2007 Aug;92(8):1099-106.

IL-7-mediated protection against minor-antigen-mismatched allograft rejection is associated with enhanced recovery of regulatory T cells. Broers AE, Bruinsma M, Posthumus-van Sluijs SJ, Wils EJ, Spits H, Löwenberg B, Braakman E, Cornelissen JJ.

Department of Hematology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands.

BACKGROUND AND OBJECTIVES: Interleukin-7 (IL-7) has been studied for its possible immunorestorative capacities following stem cell transplantation and has been shown to enhance post-transplant immune recovery predominantly by peripheral T-cell expansion. A major concern of IL-7 is its possible aggravating effect on graft-versus-host and host-versus-graft reactivity. **DESIGN AND METHODS:** To study the effect of IL-7 on host-versus-graft reactivity, we applied IL-7 in an experimental transplantation model using RAG-1-/- mice supplied with B6 CD45.1 congenic T cells as recipients of T-cell depleted allogeneic bone marrow grafts. **RESULTS:** Rejection of minor antigen-mismatched bone marrow was significantly reduced in IL-7 treated recipients compared with PBS treated control mice. Rejection was observed in 2 out of 18 IL-7 treated mice compared with 9 out of 17 PBS treated mice (11% vs. 53%; $p=0.012$). IL-7 administration resulted in enhanced recovery of peripheral blood CD4+CD25+ regulatory T cells (Treg) with a concomitant increase in peripheral blood Foxp3 mRNA expression. IL-7Ra (CD127) was expressed by the vast majority of CD4+Foxp3+ T cells. The incidence of graft rejection following fully MHC mismatched bone marrow transplantation was not reduced nor enhanced by IL-7 administration. **INTERPRETATION AND CONCLUSIONS:** Post-transplant IL-7 administration protects against minor antigen-mismatched bone marrow rejection, which may be due to enhanced Treg recovery.

➤ **J Immunol.** 2007 Jun 1;178(11):7473-84.

Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. Goldberg GL, Alpdogan O, Muriglan SJ, Hammett MV, Milton MK, Eng JM, Hubbard VM, Kochman A, Willis LM, Greenberg AS, Tjoe KH, Sutherland JS, Chidgey A, van den Brink MR, Boyd RL.

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Delayed immune reconstitution in adult recipients of allogeneic hemopoietic stem cell transplantations (HSCT) is related to age-induced thymic atrophy. Overcoming this paucity of T cell function is a major goal of clinical research but in the context of allogeneic transplants, any strategy must not exacerbate graft-vs-host disease (GVHD) yet ideally retain graft-vs-tumor (GVT) effects. We have shown sex steroid ablation reverses thymic atrophy and enhances T cell recovery in aged animals and in congenic bone marrow (BM) transplant but the latter does not have the complications of allogeneic T cell reactivity. We have examined whether sex steroid ablation promoted hemopoietic and T cell recovery following allogeneic HSCT and whether this benefit was negated by enhanced GVHD. BM and thymic cell numbers were significantly increased at 14 and 28 days after HSCT in castrated mice compared with sham-castrated controls. In the thymus, the numbers of donor-derived thymocytes and dendritic cells were significantly increased after HSCT and castration; donor-derived BM precursors and developing B cells were also significantly increased. Importantly, despite restoring T cell function, sex steroid inhibition did not exacerbate the development of GVHD or ameliorate GVT activity. Finally, IL-7 treatment in combination with castration had an

additive effect on thymic cellularity following HSCT. These results indicate that sex steroid ablation can profoundly enhance thymic and hemopoietic recovery following allogeneic HSCT without increasing GVHD and maintaining GVT.

- **Br J Haematol.** 2004 Sep;126(6):844-51.

IL-7 effect on immunological reconstitution after HSCT depends on MHC incompatibility. André-Schmutz I, Bonhomme D, Yates F, Malassis M, Selz F, Fischer A, Cavazzana-Calvo M.

INSERM U 429, Hôpital Necker Enfants Malades, Paris, France.

Considerable progress has been recently accomplished in the management of patients who have undergone haplo-incompatible haematopoietic stem cell transplantation (HSCT) in terms of intake and prevention of graft-versus-host disease. Nevertheless haplo-incompatible HSCT is a procedure limited to a small number of patients because of the long-lasting immunodeficiency that is responsible for more than 50% of deaths within the first 3 months. Interleukin (IL)-7, which plays a unique and key role in T-cell development both in the mouse and in the human, is particularly attractive for attempting to speed up T-cell reconstitution. However, controversial results have been obtained after bone marrow graft in murine and primate models. To elucidate the impact of IL-7 treatment, we have performed HSCT in irradiated murine recombination activating gene (RAG) immunodeficient recipients, using donors that exhibited increased major histocompatibility complex (MHC) incompatibility. Although irradiation performed prior to HSCT lead to a profound defect in the thymic stromal cells responsible for IL-7 production, IL-7 treatment had no significant effect on immune reconstitution in the MHC compatible and partially compatible settings. Interestingly, in the MHC fully incompatible setting in which only one-third of the recipients demonstrated active thymopoiesis, probably because of the rejection of donor cells by host natural killer cells, IL-7 treatment had a beneficial effect on T-cell development.

4.3 Other Main Publications

- **Blood.** 1996 Sep 1;88(5):1887-94.

Enhancement of thymopoiesis after bone marrow transplant by in vivo interleukin-7. Bolotin E, Smogorzewska M, Smith S, Widmer M, Weinberg K.

Department of Pediatrics, Childrens Hospital Los Angeles, University of Southern California School of Medicine, Los Angeles, USA.

Bone marrow transplantation (BMT) is followed by a period of profound immune deficiency, during which new T lymphocytes are generated from either stem cells or immature thymic progenitors. Interleukin-7 (IL-7) induces proliferation and differentiation of immature thymocytes. We examined whether the in vivo

administration of IL-7 to mice receiving BMT would alter thymic reconstitution. Lethally irradiated C57BL/6 mice received syngeneic BMT, followed by either IL-7 or placebo from days 5 to 18 post-BMT. At day 28, BMT recipients that had not received IL-7 had profound thymic hypoplasia (< 5% of normal), with relative increases in the numbers of immature thymocytes, decreased numbers of mature peripheral (splenic) T lymphocytes, and severely impaired T- and B-cell function. In contrast, transplanted mice treated with IL-7 had normalization of thymic cellularity, with normal proportions of thymic subsets and T-cell receptor beta variable gene (TCRV beta) usage, normal numbers of peripheral CD4+ T lymphocytes, and improved antigen-specific T- and B-cell function. In the BMT-IL-7 mice, there was an eightfold increase in the number of immature CD3-CD4-CD8- thymocytes in G2-M of the cell cycle, indicating that restoration of thymic cellularity was due to enhanced proliferation of immature thymic progenitors. Similar effects following IL-7 administration were also observed when donor bone marrow was depleted of mature T lymphocytes, indicating that IL-7 administration affected immature hematopoietic progenitors. IL-7 promotes thymic reconstitution after BMT, and may be useful in preventing post-BMT immune deficiency.

➤ **J Immunol.** 2001 Jan 1;166(1):170-81.

IL-7 enhances the responsiveness of human T cells that develop in the bone marrow of athymic mice. Tsark EC, Dao MA, Wang X, Weinberg K, Nolta JA.

Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital of Los Angeles, Los Angeles, CA 90027, USA.

The beige/nude/xid/human (bnx/hu) model of human hematopoiesis provides a unique opportunity to study extrathymic human T lymphocyte development in an in vivo system. Purified human hematopoietic stem cells develop into mature T lymphocytes and immature progenitors in the bone marrow of athymic bnx mice. The human T cells are all TCR alpha beta(+) and display a restricted TCRV beta repertoire. In the current studies, we examined the effects of systemic human IL-7 (huIL-7) administration on the phenotype and the activation status of the bnx/hu T cells. In the majority of the mice that did not have huIL-7 administration, a higher frequency of human CD3(+)/CD8(+) than CD3(+)/CD4(+) T cells developed in the bone marrow. This phenomenon is also frequently observed in human bone marrow transplant recipients. Extremely low levels of IL-2 were expressed by human CD3(+) cells isolated from these mice, in response to PMA plus ionomycin and to CD3 and CD28 cross-linking. IL-4 was not expressed by cells exposed to either stimulus, demonstrating a profound inability of the bnx/hu T cells to produce this cytokine. Systemic production of huIL-7 from engineered stromal cells transplanted into the mice increased the human CD4 to CD8 ratios, and increased the ratio of memory to naive CD4(+) and CD8(+) T cells. The human CD3(+) cells recovered from mice that had systemic huIL-7 and equivalent numbers of CD3(+)/CD4(+) and CD3(+)/CD8(+) cells in the marrow were still unable to produce IL-4 in response to any condition tested, but were capable of normal levels of IL-2 production following stimulation.

- **Blood.** 2001 Sep 1;98(5):1601-6.

Radiosensitivity of thymic interleukin-7 production and thymopoiesis after bone marrow transplantation. Chung B, Barbara-Burnham L, Barsky L, Weinberg K.

Department of Pediatrics, Division of Research Immunology and Bone Marrow Transplantation, Children's Hospital Los Angeles, University of Southern California School of Medicine, 90027, USA.

Interleukin-7 (IL-7) is the major thymopoietic cytokine. Injections of IL-7 after murine bone marrow transplantation (BMT) correct defects in thymic differentiation, including thymic hypocellularity, abnormal differentiation of CD3⁻ CD4⁻ CD8⁻ (triple-negative [TN]) thymocytes into CD4⁺ CD8⁺ (double-positive [DP]) cells, and antigen-specific mature T-lymphocyte proliferation. To determine whether IL-7 production is decreased in BMT recipients, BMT was performed with congenic murine donor-recipient strains and escalating doses of pre-BMT conditioning. Increasing doses of radiation resulted in decreased thymic cellularity and maturation from the TN to the DP stage. Quantitative reverse transcription-polymerase chain reaction analyses demonstrated that intrathymic production of IL-7 was significantly decreased in irradiated mice than in nonirradiated controls. Decline in IL-7 transcript levels was correlated with the dose of radiation administered. Analyses of the numbers of CD45⁻ major histocompatibility complex class II⁺ thymic stromal cells suggested that the mechanism for the decreased IL-7 production was loss of IL-7-producing thymic stromal cells. Experiments indicated that pre-BMT conditioning with radiation led to decreased stromal production of IL-7 and consequent blocks in the maturation of thymocytes. They provided a mechanism for both the abnormal thymopoiesis observed after BMT and the previously observed beneficial effects of IL-7 administration in murine models. Impaired production of IL-7 by thymic stroma may be a general model for the clinically observed adverse effects of cytotoxic therapy on thymopoiesis.

- **Blood.** 2003 Aug 15;102(4):1534-40. Epub 2003 Apr 24.

Interleukin-7 improves T-cell recovery after experimental T-cell-depleted bone marrow transplantation in T-cell-deficient mice by strong expansion of recent thymic emigrants. Broers AE, Posthumus-van Sluijs SJ, Spits H, van der Holt B, Löwenberg B, Braakman E, Cornelissen JJ.

Department of Hematology, Erasmus MC/Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

Interleukin-7 (IL-7) has been shown to enhance thymic output of newly developed T cells following bone marrow transplantation (BMT) in mice. In addition, IL-7 may affect peripheral expansion of T cells. In order to study the relative contribution of thymopoiesis versus peripheral T-cell expansion in the setting of compromised thymopoiesis, we have applied IL-7 in an experimental stem cell transplantation model

using T cell-deficient RAG-1(-/-) mice. C57BL/6 RAG-1(-/-) mice received transplants of syngeneic T-cell-depleted (TCD) bone marrow (Ly5.1) with or without supplemented T cells (Ly5.2). IL-7 was administered until day 63 after BMT. Peripheral blood T- and B-cell recovery was quantified by flow cytometry and thymopoiesis was studied by quantification of T-cell receptor rearrangement excision circles (TRECs). In mice receiving a T-cell-replete BMT, IL-7 selectively expanded mature CD45.2+ T cells without affecting the recovery of new bone marrow-derived CD45.1+ T cells. In contrast, IL-7 significantly enhanced the recovery of bone marrow-derived T cells after TCD BMT. Quantification of TRECs in mice receiving a TCD BMT revealed that enhanced T-cell recovery following IL-7 treatment resulted from a strong expansion of newly developed naive T cells. These results suggest that peripheral expansion of recent thymic emigrants or mature T cells may be a preferential mechanism by which IL-7 enhances T-cell recovery after BMT.

➤ **Blood.** 2001 Mar 1;97 (5):1491-7.

IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation. Mackall CL, Fry TJ, Bare C, Morgan P, Galbraith A, Gress RE.

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Thymic-dependent differentiation of bone marrow (BM)-derived progenitors and thymic-independent antigen-driven peripheral expansion of mature T cells represent the 2 primary pathways for T-cell regeneration. These pathways are interregulated such that peripheral T-cell expansion is increased in thymectomized versus thymus-bearing hosts after bone marrow transplantation (BMT). This study shows that this interregulation is due to competition between progeny of these 2 pathways because depletion of thymic progeny leads to increased peripheral expansion in thymus-bearing hosts. To test the hypothesis that competition for growth factors modulates the magnitude of antigen-driven peripheral expansion during immune reconstitution in vivo, a variety of T-cell active cytokines were administered after BMT. Of the cytokines (interleukins) tested (IL-3, IL-12, IL-6, IL-2, and IL-7), IL-2 modestly increased peripheral expansion in the face of increasing numbers of thymic emigrants, whereas IL-7 potently accomplished this. This report also demonstrates that the beneficial effect of IL-7 on immune reconstitution is related to both increases in thymopoiesis as well as a direct increase in the magnitude of antigen-driven peripheral expansion. Therefore, the administration of exogenous IL-7, and to a lesser extent IL-2, abrogates the down-regulation in antigen-driven peripheral expansion that occurs in thymus-bearing hosts after BMT. These results suggest that one mechanism by which T-cell-depleted hosts may support antigen-driven T-cell expansion in vivo is via an increased availability of T-cell-active cytokines to support clonal expansion.

5. IL-7 and Vaccines

5.1 Reviews

- **Lett Drug Des Discov.** 2006;3(8):586-592.

Utilizing IL-12, IL-15 and IL-7 as Mucosal Vaccine Adjuvants.
Stevceva L, Moniuszko M, Ferrari MG.

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In this paper we review and discuss three of the most exciting and promising cytokines for therapeutic intervention and immunomodulation of immune responses including those on mucosal surfaces. The main properties of IL-12, IL-15 and IL-7 are described and the studies utilizing these cytokines as immunomodulators and vaccine adjuvants discussed.

- **Curr HIV Res.** 2009 Jan;7(1):83-90.

IL-2, IL-7 and IL-15 as immuno-modulators during SIV/HIV vaccination and treatment. Leone A, Picker LJ, Sodora DL.

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While highly active antiretroviral therapy (HAART) regimens have proven to be effective in controlling active HIV replication, complete recovery of CD4+ T cells does not always occur, even among patients with high level virologic control. Recent advances in understanding the biology of T cell production and homeostasis have created the potential to augment anti-viral therapies with immunotherapies designed to facilitate recovery of the HIV-damaged immune system, in particular, the recovery of CD4+ T cell populations. The common gamma-chain cytokines IL-2, IL-7 and IL-15 are primary regulators of T cell homeostasis and thus have been considered prime candidate immunotherapeutics, both for increasing T cell levels/function and for augmenting vaccine-elicited viral-specific T cell responses. Recent studies have established that these cytokines have distinct functional roles in immune homeostasis, which focus on specific T cell populations. The ability of these cytokines to provide immunotherapeutic benefit to HIV+ patients will depend on their ability to stably increase or functionally enhance the desired T cell target population without adverse virologic or clinical consequences.

- **Immunol Cell Biol.** 2008 Jul;86(5):385-6. Epub 2008 May 13

Enhancing T cell memory: IL-7 as an adjuvant to boost memory T-cell generation. Purton JF, Martin CE, Surh CD.

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Cytokines play a central role in regulating the homeostasis of naïve and memory T cells and their responses to pathogens. Surprisingly, not much is known about the ability of exogenously administered cytokines to affect the formation of memory T cells. In a recent study, Nanjappa et al.¹ have shown that infusion of interleukin (IL)-7 during the contraction phase of a T-cell response can augment the accumulation of functional viral-specific memory T cells. As the formation of a large pool of memory T cells is the main goal of vaccination, these findings suggest that cytokines could be applied therapeutically to enhance vaccine efficacy.

5.2 Cytheris Associated Publications

- **J Clin Invest.** 2008 Mar;118(3):1027-39.

Effects of IL-7 on memory CD8 T cell homeostasis are influenced by the timing of therapy in mice. Nanjappa SG, Walent JH, Morre M, Suresh M.

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IL-7 is integral to the generation and maintenance of CD8(+) T cell memory, and insufficient IL-7 is believed to limit survival and the persistence of memory CD8(+) T cells. Here, we show that during the mouse T cell response to lymphocytic choriomeningitis virus, IL-7 enhanced the number of memory CD8(+) T cells when its administration was restricted to the contraction phase of the response. Likewise, IL-7 administration during the contraction phase of the mouse T cell response to vaccinia virus or a DNA vaccine potentiated antigen-specific CD8(+) memory T cell proliferation and function. Qualitatively, CD8(+) T cells from IL-7-treated mice exhibited superior recall responses and improved viral control. IL-7 treatment during the memory phase stimulated a marked increase in the number of memory CD8(+) T cells, but the effects were transient. IL-7 therapy during contraction of the secondary CD8(+) T cell response also expanded the pool of memory CD8(+) T cells. Collectively, our studies show differential effects of IL-7 on memory CD8(+) T cell homeostasis and underscore the importance of the timing of IL-7 therapy to effectively improve CD8(+) T cell memory and protective immunity. These findings may have implications in the clinical use of IL-7 as an immunotherapeutic agent to bolster vaccine-induced CD8(+) T cell memory.

5.3 Other Main Publications

- **Clin Immunol.** 2005 Jan;114(1):30-41.

Interleukin-7 improves reconstitution of antiviral CD4 T cells. Lu H, Zhao Z, Kalina T, Gillespy T 3rd, Liggitt D, Andrews RG, Maloney DG, Kiem HP, Storek J.

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We evaluated whether long-term (2 months) administration of interleukin-7 (IL7) hastens immune recovery in baboons rendered severely lymphopenic by total body irradiation and antithymocyte globulin (ATG). Four baboons were treated with recombinant baboon IL7 and three baboons with placebo. Median CD4 T cell count at the end of IL7/placebo treatment was higher in the IL7-treated animals (2262 vs. 618/microl, $P = 0.03$). This appeared to be a result of peripheral expansion rather than de novo generation. Median cytomegalovirus (CMV)-specific IFN γ -producing CD4 T cell count at the end of IL7/placebo treatment was higher in the IL7-treated animals (122 vs. 1/microl, $P = 0.03$). All animals were pretransplant cytomegalovirus-seropositive. One animal died at the end of IL7 treatment; necropsy showed extensive T cell infiltration of kidneys and lungs. In conclusion, IL7 stimulates the expansion of CD4 T cells, including functional antiviral cells. Clinical risk-benefit ratio needs to be evaluated.

- **Proc Natl Acad Sci U S A.** 2004 Jun 22;101(25):9357-62. Epub 2004 Jun 14.

IL-7 regulates basal homeostatic proliferation of antiviral CD4+T cell memory. Lenz DC, Kurz SK, Lemmens E, Schoenberger SP, Sprent J, Oldstone MB, Homann D.

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Heightened protection from infectious disease as conferred by vaccination or pathogen exposure relies on the effective generation and preservation of specific immunological memory. T cells are irreducibly required for the control of most viral infections, and maintenance of CD8(+)T cell memory is regulated by at least two cytokines, IL-7 and IL-15, which support survival (IL-7, IL-15) and basal homeostatic proliferation (IL-15) of specific CD8(+) memory T cells (T(M)). In contrast, the factors governing the homeostasis of pathogen-specific CD4(+)T(M) remain at present unknown. Here, we used a physiologic in vivo model system for viral infection to delineate homeostatic features and mechanisms of antiviral CD4(+)T(M) preservation in direct juxtaposition to CD8(+)T cell memory. Basal homeostatic proliferation is comparable between specific CD4(+) and CD8(+)T(M) and independent of immunodominant determinants and functional avidities but regulated in a tissue-specific fashion. IL-7, identified as the dominant cytokine, and IL-15, an accessory cytokine, regulate basal homeostatic proliferation and survival of antiviral CD4(+)T(M). Interestingly, a role for these cytokines in regulation of CD4(+)T cell memory is not readily discernible in the generic "memory-phenotype" population, apparently a consequence of its heterogeneous

composition. We also describe a prominent, nonredundant role for IL-7 in supporting basal homeostatic proliferation of CD8(+)T(M). We propose that homeostatic control of antiviral CD4(+) and CD8(+) T cell memory is fundamentally similar and characterized by quantitative, rather than qualitative, differences.

- **Int J Cancer.** 1992 Sep 9;52(2):261-5.

Influence of recombinant human interleukin (IL)-7 on disease progression in mice infected with Friend virus complex. Lu L, Zhou Z, Wu B, Xiao M, Shen RN, Williams DE, Kim YJ, Kwon BS, Ruscetti S, Broxmeyer HE.

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Recombinant human (rhu) IL-7 was evaluated for its influence on disease progression in mice infected with the polycythemia-inducing strain of the Friend virus complex (FVC). DBA/2 mice were injected i.v. with FVC, and then treated s.c. with rhuIL-7. IL-7 significantly prolonged survival time and decreased spleen focus-forming virus (SFFV) levels, expression of SFFV mRNA and SFFV protein production in FVC-infected mice. IL-7 did not appear to directly inactivate SFFV. Although both splenic weight and cellularity in FVC-infected mice treated with IL-7 were higher than those of normal mice, they were respectively 58% and 66% lower than those of the untreated FVC-infected mice. NK-cell activity was substantially lower in FVC-infected mice than in normal mice, while IL-7 restored NK-cell activity to normal levels. IL-6 and IFN-gamma levels were markedly reduced in FVC-infected mice compared to normal mice, but treatment of FVC-infected mice with IL-7 restored these cytokine levels. While the actual mechanisms of these effects are not yet known, the results suggest the potential therapeutic efficacy of IL-7 for certain hematopoietic and viral disorders, possibly mediated through an action on accessory cells and cytokine production.

6. IL-7 and Tuberculosis

6.1 Reviews

- **Immunol Rev.** 2008 Dec;226:191-204.

The role of cytokines in the initiation, expansion, and control of cellular immunity to tuberculosis. Cooper AM, Khader SA.

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Tuberculosis (TB) results from an interaction between a potent immune response and a chronically persistent pathogen. The ability of Mycobacterium tuberculosis (Mtb) to induce a strong immune response while being able to resist the ability of the host to

clear bacteria provides an excellent tool with which to investigate the role of specific cytokine pathways on the induction, expansion, and control of the effector T-cell response. In this review, the role of interleukin-12p40 (IL-12p40), IL-12p70, IL-23, and IL-27 in the immune response to Mtb are described. We show that IL-12(p40)(2) acts to mediate the activation of dendritic cells to become responsive to homeostatic chemokines. We also show that IL-12p70 is required for the optimal interferon-gamma (IFN-gamma) T-cell response, which is required for control of Mtb growth. IL-23 can induce IFN-gamma responses in the lung if IL-12 is not present, but its major role is in supporting the IL-17 response within the lung. Neither IL-23 nor IL-17 is required for early control of Mtb in the lung. IL-23 and IL-17, however, can be instrumental in vaccine-induced protection. Finally, IL-27 limits protective immunity in the lung, but it is also required for long-term survival. These cytokines are therefore key players in the immune response to TB.

- **Curr Opin Immunol.** 2006 Aug;18(4):438-48. Epub 2006 Jun 13.

Progress in tuberculosis vaccine development. Baumann S, Nasser Eddine A, Kaufmann SH.

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The first tuberculosis vaccine candidates have reached clinical testing. Novel subunit vaccine candidates aimed at boosting previous BCG-prime vaccination and novel viable attenuated vaccine candidates aimed at substituting BCG have both completed the preclinical stage. Despite these achievements, rational vaccine design against tuberculosis has not come to an end. Novel findings in basic immunology and microbiology will advance further improvements in vaccine development. These include the potential role of crosspriming to induce more potent T-cell responses, the role of memory T cells and regulatory T cells in sustaining or curtailing optimal immune responses, respectively, as well as the involvement of cytokines in T-cell migration to nonimmunologic tissue sites and in the generation of memory. Knowledge about basic mechanisms underlying optimum protection will not only have a direct impact on future vaccine design against tuberculosis but also help in the formulation of a set of biomarkers with predictive value for vaccine efficacy assessment.

6.2 Cytheris Associated Publications

- **Infect Immun.** 2000 May;68(5):2962-70.

Interleukin-7 or interleukin-15 enhances survival of Mycobacterium tuberculosis-infected mice. Maeurer MJ, Trinder P, Hommel G, Walter W, Freitag K, Atkins D, Störkel S.

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Both antigen-presenting cells and immune effector cells are required to effectively eradicate or contain Mycobacterium tuberculosis-infected cells. A variety of cytokines

are involved to ensure productive "cross talk" between macrophages and T lymphocytes. For instance, infection of macrophages with mycobacteria leads to effective interleukin-7 (IL-7) and IL-15 secretion, and both cytokines are able to maintain strong cellular immune responses of alpha/beta and gamma/delta T cells. Here we show that either cytokine is able to enhance survival of *M. tuberculosis*-infected BALB/c mice significantly compared to application of IL-2, IL-4, or phosphate-buffered saline (as a control). Enhanced survival could be achieved only when IL-7 or IL-15 was delivered as a treatment (i.e., 3 weeks postinfection), not when it was administered at the time of infection. Increased survival of *M. tuberculosis*-infected animals was observed following passive transfer of spleen cells harvested from *M. tuberculosis*-infected, IL-7- or IL-15-treated animals, but not after transfer of spleen cells obtained from mice which received either cytokine alone. Histological examination revealed that IL-7 and IL-15 failed to significantly impact on the number and composition of granulomas formed or the bacterial load. Our data indicated that administration of IL-7 or IL-15 to *M. tuberculosis*-treated animals resulted in a qualitatively different cellular immune response in spleen cells as reflected by increased tumor necrosis factor alpha and decreased gamma interferon secretion in response to *M. tuberculosis*-infected antigen-presenting cells.

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