Consequences of Immunodepletion:

Restoration of T-cell homeostasis after T-cell depletion

T cell immune reconstitution following lymphodepletion

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Review

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Abstract

T cell reconstitution following lymphopenia from chemotherapy or stem cell transplant is often slow and incompetent, contributing to the development of infectious diseases, relapse, and graft-versus-host disease. This is due to the fact that *de novo* T cell production is impaired following cytoreductive regimens. T cells can be generated from two pathways: (1) thymus derived through active thymopoiesis and (2) peripherally expanded clones through homeostatic proliferation. During recovery from lymphopenia, the thymic pathway is commonly compromised in adults and T cells rely upon peripheral expansion to restore T cell numbers. This homeostatic proliferation exploits the high cytokine levels following lymphopenia to rapidly generate T cells in the periphery. Moreover, this early peripheral expansion of T cells can also be driven by exogenous antigen. This results in loss of T cell repertoire diversity and may predispose to auto- or allo-immunity. Alternatively, the high homeostatic proliferation following lymphopenia may facilitate expansion of anti-tumor immunity. Murine and human studies have provided insight into the cytokine and cellular regulators of these two pathways of T cell generation and the disparate portraits of T cell immunity created through robust thymopoiesis or peripheral expansion following lymphopenia. This insight has permitted the manipulation of the immune system to maximize anti-tumor immunity through lymphopenia and led to an appreciation of mechanisms that underlie graft versus host disease.

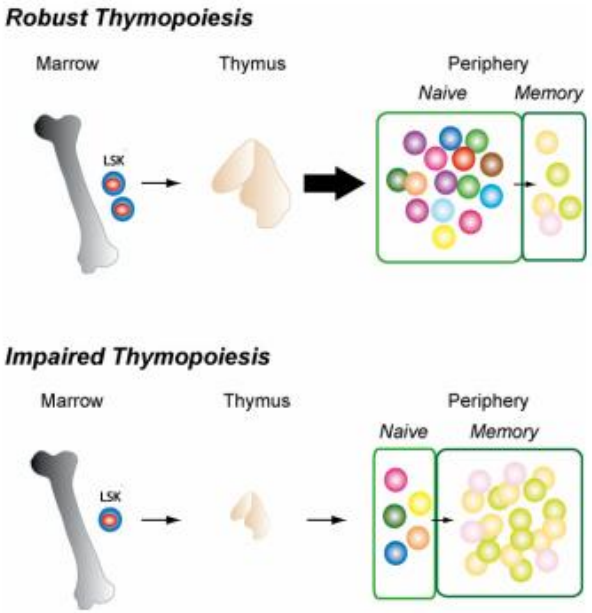

Fig. 1. Diagrammatic representation of the consequences of robust vs. impaired thymopoiesis. In young individuals without GVHD, thymic renewal rapidly ensues following transplant induced lymphopenia, with an increase in thymic size and a subsequent increase in thymic emigrants. The naïve pool is thus enlarged and enriched for newly derived thymic T cells and displays a diverse T cell repertoire, shown diagrammatically as multi-colored cells. In contrast, when thymopoiesis is impaired by age or GVHD, the thymus remains small and releases few new naïve T cells to the peripheral pool during immune reconstitution. The memory pool is expanded and there is less diversity within both the naïve and memory pool.

Factors affecting reconstitution of the T cell compartment in allogeneic haematopoietic cell transplant recipients

Bone Marrow Transplantation (2003) 32, 1001–1014

PR Fallen[1,4], L McGreavey[1,5], JA Madrigal[1,2], M Potter[2], M Ethell[2], HG Prentice[2], A Guimarães[3] and PJ Travers[1,2,]

Summary:

The factors affecting T cell reconstitution post haematopoietic cell transplantation (HCT) are not well characterised. We carried out a longitudinal analysis of T cell reconstitution in 32 HCT recipients during the first 12 months post transplant. We analysed reconstitution of naïve, memory and effector T cells, their diversity and monitored thymic output using TCR rearrangement excision circles (TRECs). Thymic-independent pathways were responsible for the rapid reconstitution of memory and effector T cells less than 6 months post HCT. Thymic-dependent pathways were activated between 6 and 12 months in the majority of patients with naïve T cell numbers increasing in parallel with TREC levels. Increasing patient age, chronic GVHD and T cell depletion (with or without pretransplant Campath-1H) predicted low TREC levels and slow naïve T cell recovery. Furthermore, increasing patient age also predicted high memory and effector T cell numbers. The effects of post HCT immunosuppression, total body irradiation, donor leucocyte infusions, T cell dose and post HCT infections on T cell recovery were also analysed. However, no effects of these single variables across a variety of different age, GVHD and T cell depletion groups were apparent. This study suggests that future analysis of the factors affecting T cell reconstitution and studies aimed at reactivating the thymus through therapeutic intervention should be analysed in age-, GVHD- and TCD-matched patient groups.

n.b. the oldest patient in this trial was 53

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Restoration of T-cell homeostasis after T-cell depletion

Crystal L. Mackall, Frances T. Hakim and Ronald E. Gress*†*

T-cell homeostasis appears to be maintained throughout much of normal adult life independent of de-novo production from hematopoietic stem cells via thymopoiesis. Instead, peripheral mechanisms are generally sufficient to maintain normal T-cell number, function and adequate TCR repertoire diversity in healthy hosts. Studies of T-cell regeneration in animals, however, have shown that full restoration of T-cell homeostasis after profound T-cell depletion is primarily dependent upon thymopoiesis. In this setting, thymic-deficient hosts have prolonged reductions in total T-cell number, restricted TCR repertoire diversity, and limited immunocompetence. In humans, age-related reductions in thymic regenerative capacity as early as young adulthood result in incomplete restoration of T-cell homeostasis after T-cell depletion.

In summary, regeneration of $CD4^+$ T cells after lymphocyte depletion in adults occurs primarily via peripheral expansion of mature $CD4^+$ T cells, giving rise to quantitative deficiencies, an increased susceptibility to programmed cell death and limited T-cell receptor repertoire diversity. For $CD8^+$ cells, while quantitative restoration of total $CD8^+$ T-cell number is commonly observed, important alterations in subset composition remain for a prolonged period.

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Restoration of T-cell homeostasis after T-cell depletion

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Adults

In contrast to the relatively prompt and complete restoration of T-cell homeostasis observed in children, adults typically show prolonged abnormalities in T-cell number and function after T-cell depletion. These abnormalities are characterized by persistent depletion of peripheral blood $CD4^+$ T cells, relative and sometimes absolute expansion of atypical $CD8^+$ populations, an increased susceptibility of T cells to activation-induced cell death and contraction of the T-cell repertoire. Recent evidence suggests that such abnormalities can be accounted for by limitations in thymic regenerative pathways, increased peripheral expansion in response to T-cell depletion and thymic-independent generation and/or expansion of atypical $CD8^+$ subsets.

After BMT, adults typically show peripheral blood $CD4^+$ T-cell depletion for greater than one year, with a predominance of memory-type $CD4^+$ T cells and a relative paucity of naive-type $CD4^+$ T cells.[34,39] In adults treated with chemotherapy at doses which induce moderate $CD4^+$ depletion, rises in peripheral blood $CD4^+$ cells are observed in the first six months post-therapy, concurrent with other lymphocyte populations. However, this is associated primarily with an increase in the number of 'memory' $CD4^+$ cells ($CD45RA^-CD45RO^+$) rather than an increase in cells expressing the 'naive' phenotype, as is observed in children. This period of initial expansion is followed by a decline in $CD4^+CD45RO^+$ cell number to levels comparable to the chemotherapy-induced nadir. Post-chemotherapy T cells in this setting are generally poor responders to in-vitro assays of lymphocyte function, in part related to a propensity for these populations to undergo activation-induced programmed cell death. Indeed, a positive correlation exists between the number of activated cells in the peripheral blood in this setting (as measured by expression of HLA-DR) and apoptotic frequency of $CD4^+$ T cells after mitogenic stimulation (F. Hakim, manuscript submitted). These results suggest that, in humans, as in mice, the process of peripheral expansion is inherently unable to restore T-cell number *in vivo* to normal levels after significant T-cell depletion. Furthermore, populations derived via this process are functionally limited, in part related to an increased susceptibility to activation-induced cell death. Indeed, restoration of $CD4^+$ T-cell homeostasis, as measured

Because thymic-independent peripheral expansion is driven by antigen-specific activation of mature T-cell populations, it is predicted that hosts which have regenerated T cells in this manner will have a contracted TCR repertoire, limited by the diversity of the starting inocula and the diversity of the antigens present within the host at the time of expansion. Indeed, several recent reports have provided examples of severely contracted TCR repertoires in adults after T-cell regeneration.[40,41] In one report showing the TCR repertoire.[45,46] Taken together, the data strongly suggest that thymic-dependent $CD4^+$ T-cell regenerative pathways in adults are insufficient for restoration of lymphocyte homeostasis after profound T-cell depletion.

Distinctions Between CD8$^+$ and CD4$^+$ T-Cell Regenerative Pathways Result in Prolonged T-Cell Subset Imbalance After Intensive Chemotherapy

By Crystal L. Mackall, Thomas A. Fleisher, Margaret R. Brown, Mary P. Andrich, Clara C. Chen, Irwin M. Feuerstein, Ian T. Magrath, Leonard H. Wexler, Dimiter S. Dimitrov, and Ronald E. Gress

Rapid recovery of CD4$^+$ T cells after intensive chemotherapy is limited by an age-dependent decline in thymopoiesis. Here we sought to determine whether similar limitations exist for CD8$^+$ T-cell regeneration. After intensive chemotherapy, CD8$^+$ T cells had a faster effective doubling time than CD4$^+$ T cells (median, 12.6 *v* 28.2 days, $P < .05$). Accordingly, at 3 months posttherapy, mean CD8$^+$ T-cell number had returned to baseline, whereas mean CD4$^+$ T-cell number was only 35% of pretherapy values ($P < .05$). These differences were primarily due to very rapid expansion of CD8$^+$CD57$^+$ and CD8$^+$CD28$^-$ subsets. At 3 months posttherapy, there was no relationship between age and CD8$^+$ T-cell number ($R = -.02$), whereas CD4$^+$ T-cell number was inversely related to age ($R = -.66$) and there were no discernible differences in CD8$^+$ recovery among patients with or without thymic enlargement, whereas CD4$^+$ recovery was enhanced in patients with thymic enlargement after chemotherapy ($P < .01$). Therefore thymic-independent pathways of T-cell regeneration appear to rapidly regenerate substantial numbers of CD8$^+$, but not CD4$^+$ T cells, resulting in prolonged T-cell subset imbalance after T-cell depletion. These inherent distinctions between CD4$^+$ *v* CD8$^+$ T-cell regeneration may have significant implications for immunotherapeutic strategies undertaken to eradicate minimal residual neoplastic disease after cytoreductive chemotherapy.

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Table 3. Lymphocyte Subsets After Completion of Chemotherapy in Patients With and Without Thymic Rebound

Subset	Time Posttherapy	Thymic Rebound* +	Thymic Rebound* −	*P* Value
CD8$^+$CD3$^+$	3 mos	594 ± 244	476 ± 197	.78
CD8$^+$CD3$^+$	6 mos	695 ± 197	331 ± 184	.20
CD8$^+$CD45RA$^+$	3 mos	93 ± 37	46 ± 12	.32
CD8$^+$CD45RA$^+$	6 mos	217 ± 52	116 ± 43	.25
CD4$^+$CD3$^+$	3 mos	343 ± 119	182 ± 25	.47
CD4$^+$CD3$^+$	6 mos	507 ± 77	203 ± 39†	.006
CD4$^+$CD45RA$^+$	3 mos	106 ± 21	13 ± 4†	.004
CD4$^+$CD45RA$^+$	6 mos	320 ± 123	23 ± 9†	.001

* Thymic rebound was determined based on either a twofold increase in thymic volume during the first year after chemotherapy compared with thymic volume pretherapy as measured by CT scanning or thymic uptake by gallium^{67} scanning as delineated in Materials and Methods.

† *P* value using Mann-Whitney U test.

In summary, we have shown that biologic distinctions between CD8$^+$ and CD4$^+$ T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. CD8$^+$ T cells show rapid regeneration without relationships to age or thymic enlargement, while rapid CD4$^+$ T-cell regeneration is only observed in younger patients with thymic enlargement after completion of chemotherapy. These observations are best explained by the rapid recovery of CD8$^+$CD28$^-$ and CD8$^+$CD57$^+$ subsets via thymic-independent pathways, which are not available for regeneration of CD4$^+$ T cells.

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IMMUNOLOGY REVIEW ARTICLE

$CD8^+$ $CD28^-$ and $CD8^+$ $CD57^+$ T cells and their role in health and disease

Summary

Chronic antigenic stimulation leads to gradual accumulation of late-differentiated, antigen-specific, oligoclonal T cells, particularly within the $CD8^+$ T-cell compartment. They are characterized by critically shortened telomeres, loss of CD28 and/or gain of CD57 expression and are defined as either $CD8^+CD28^-$ or $CD8^+CD57^+$ T lymphocytes. There is growing evidence that the $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cell population plays a significant role in various diseases or conditions, associated with chronic immune activation such as cancer, chronic intracellular infections, chronic alcoholism, some chronic pulmonary diseases, autoimmune diseases, allogeneic transplantation, as well as has a great influence on age-related changes in the immune system status. $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cell population is heterogeneous and composed of various functionally competing (cytotoxic and immunosuppressive) subsets thus the overall effect of $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cell-mediated immunity depends on the predominance of a particular subset. Many articles claim that $CD8^+CD28^-$ ($CD8^+CD57^+$) T cells have lost their proliferative capacity during process of replicative senescence triggered by repeated antigenic stimulation. However recent data indicate that $CD8^+CD28^-$ ($CD8^+CD57^+$) T cells can transiently up-regulate telomerase activity and proliferate under certain stimulation conditions. Similarly, conflicting data is provided regarding $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cell sensitivity to apoptosis, finally leading to the conclusion that this T-cell population is also heterogeneous in terms of its apoptotic potential. This review provides a comprehensive approach to the $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cell population: we describe in detail its origins, molecular and functional characteristics, subsets, role in various diseases or conditions, associated with persistent antigenic stimulation.

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Quantitative analysis of T cell receptor diversity in clinical samples of human peripheral blood ☆, ☆☆

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A B S T R A C T

The analysis of T cell receptor diversity provides a clinically relevant and sensitive marker of repertoire loss, gain, or skewing. Spectratyping is a broadly utilized technique to measure global TCR diversity by the analysis of the lengths of CDR3 fragments in each Vβ family. However the common use of large numbers of T cells to obtain a global view of TCR Vβ CDR3 diversity has restricted spectratyping analyses when limited T-cell numbers are available in clinical setting, such as following transplant regimens.

We here demonstrate that one hundred thousand T cells are sufficient to obtain a robust, highly reproducible measure of the global TCR Vβ repertoire diversity among twenty Vβ families in human peripheral blood. We also show that use of lower cell number results not in a dwindling of observed diversity but rather in non-reproducible patterns in replicate spectratypes. Finally, we report here a simple to use but sensitive method to quantify repertoire divergence in patient samples by comparison to a standard repertoire profile we generated from fifteen normal donors. We provide examples using this method to statistically evaluate the changes in the global TCR Vβ repertoire diversity that may take place during T subset immune reconstitution after hematopoietic stem cell transplantation or after immune modulating therapies.

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Lymphocyte Depletion and Immunosuppression With Repeated Leukapheresis by Continuous Flow Centrifugation

By Daniel G. Wright, Jacob Karsh, Anthony S. Fauci, John H. Klippel, John L. Decker, Joseph F. O'Donnell, and Albert B. Deisseroth

Leukapheresis by continuous flow centrifugation (CFC) was studied in normal volunteers and patients with rheumatoid arthritis to determine whether this procedure, when carried out repeatedly, can cause lympocytopenia and immunosuppression similar to that produced by thoracic duct drainage. The removal of lymphocytes from blood by CFC was maximal in our hands at centrifuge speeds of 900–1300 rpm and could be related directly to blood flow rates. In addition, it was found that the proportional removal of different lymphocyte subpopulations is affected by the position within the plasma-blood cell interface at which lymphocytes are removed during CFC. Repeated leukapheresis, with techniques designed to favor the removal of T lymphocytes, was carried out in 8 patients with rheumatoid arthritis at a rate of 2–3 CFC/wk for periods of 5–7 wk (total CFC were 13–18/patient). Up to 18.6 $\times$ 10^{10} lymphocytes were removed from the patients (mean 13.0 $\times$ 10^{10} lymphocytes) at an average rate of 3.5 $\times$ 10^{9} lymphocytes/day, resulting in significant lymphocytopenia in each patient (mean decrease of blood lymphocyte counts, 72%). As has been observed with thoracic duct drainage, in similar patients, decreases in lymphocyte counts occurred most rapidly during the first 10 days of repeated leukapheresis. Lymphocytopenia reflected predominately a loss of circulating T lymphocytes, and in vitro lymphocyte responses to T-cell mitogens were reduced. Lymphocytopenia (lymphocyte counts <50% preleukapheresis values) persisted for up to 12 mo following repeated leukapheresis. A consistent fall in circulating IgM was also observed with lymphocyte depletion. An analysis of lymphocyte counts in normal volunteers who were repeat leukapheresis donors indicated that a minimum rate of $<10^{9}$ lymphocytes removed/day by CFC is necessary for there to be measurable declines in blood lymphocyte counts. These studies demonstrate that repeated leukapheresis by CFC can produce lymphocyte depletion and immunologic changes analogous to those observed with thoracic duct drainage.