

STRATEGIES TO ENHANCE T-CELL RECONSTITUTION IN IMMUNOCOMPROMISED PATIENTS

Marcel R. M. van den Brink*, Önder Alpdogan* and Richard L. Boyd‡

Abstract | Immune deficiency, together with its associated risks such as infections, is becoming an increasingly important clinical problem owing to the ageing of the general population and the increasing number of patients with HIV/AIDS, malignancies (especially those treated with intensive chemotherapy or radiotherapy) or transplants (of either solid organs or haematopoietic stem cells). Of all immune cells, T cells are the most often affected, leading to a prolonged deficiency of T cells, which has important clinical consequences. Accordingly, strategies to improve the recovery and function of T cells, as we discuss here, should have a direct impact on reducing the morbidity and mortality of many patients and should increase the efficacy of therapeutic and prophylactic vaccinations against microbial pathogens or tumours.

Immune deficiency — a decrease in the number or function of immune cells — leads to a significant increase in the incidence and severity of infections, the occurrence and relapse of cancers, and the failure of immunotherapies, including vaccination. Apart from genetic causes (such as SEVERE COMBINED IMMUNODEFICIENCY, SCID) and autoimmune diseases, immunodeficiency is commonly associated with ageing but can also arise directly as a result of infections that target the immune system — most notably, infection with HIV — and as a consequence of common cancer treatments, such as myeloablative chemotherapy and radiation. Immunodeficiency also occurs through treatment with drugs that are frequently used to prevent rejection of foreign cell, tissue or organ transplants. In these situations, T cells are more suppressed than other immune cells and are slower to recover. T-cell deficiency is more pronounced in adults as a result of the markedly reduced function of the thymus, which undergoes atrophy early in life, particularly from the onset of puberty, lapsing to less than 10% of its maximum size by the early 20s¹.

In this review, we discuss strategies that could enhance the reconstitution of T cells after an ALLOGENEIC haematopoietic stem cell (HSC) transplant (HSCT) — which is associated with a severe deficiency in T cells, as

discussed later and in BOX 1 — but these strategies have equal potential to overcome T-cell deficiencies in general. The approaches include those that target lymphoid progenitors and promote thymopoiesis, and those that use *ex vivo* culture systems, hormones, growth factors, cytokines or co-stimulatory molecules (FIG. 1). We have focused on strategies that are currently being studied in clinical trials or have realistic potential for clinical use in the foreseeable future.

Furthermore, in patients with T-cell deficiencies, adoptive T-cell therapy has resulted in the successful treatment of Epstein–Barr-virus-induced lymphoproliferative disease and lymphoma, as well as infection with cytomegalovirus (CMV), and it is now being studied as a promising therapeutic strategy for the prophylaxis and treatment of a variety of infections and malignancies. However, the use of adoptive cell therapy to treat specific infections or tumours is beyond the scope of this article and is reviewed in REFS 2,3.

Allogeneic HSC transplantation

Allogeneic HSC transplantation is a potentially curative therapy for a variety of life-threatening diseases of lymphohaematopoietic cells and tissues, including malignancies and diseases characterized by defective

*Departments of Medicine and Immunology, Box 111-Kettering 406D, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA.

‡Department of Pathology and Immunology, Central and Eastern Clinical School, Monash University, Clayton, Victoria 3181, Australia. Correspondence to M.R.M.B. e-mail: vandenbm@mskcc.org doi:10.1038/nri1484

Box 1 | Immune reconstitution after allogeneic haematopoietic stem-cell transplantation

The post-transplant reconstitution of T cells in recipients of an allogeneic haematopoietic stem-cell transplant (HSCT) involves the clonal expansion of mature donor lymphocytes (both alloreactive and non-alloreactive) that were infused with the allograft, as well as residual host lymphocytes resistant to the conditioning regimen, and thymic-dependent or possibly thymic-independent *de novo*-generated donor lymphocytes (FIG. 2). In young hosts, the thymus can support *de novo* generation of T cells from donor haematopoietic precursor cells. However, the contribution of the thymus to post-transplant T-cell recovery depends on the following: the extent of thymic damage from conditioning of the recipient with chemotherapy and/or radiotherapy, the degree of age-associated thymic involution, the engraftment of donor-derived haematopoietic precursor cells, the occurrence of graft-versus-host disease (GVHD) and the extent of drug-induced immunosuppression from antibiotics or prophylaxis for GVHD. Studies using thymectomized mice that have received an HSCT have shown *de novo* generation of T cells (commonly of non-classical T cells, such as CD4⁺CD8⁻ or CD8 $\alpha\alpha$ ⁺ T cells) in extrathymic sites, including the mucosa-associated lymphoid tissues in the gut^{115,116}; however, their contribution to T-cell recovery in euthymic recipients of an HSCT was limited.

Studies of patients who received a T-cell-depleted HSCT have indicated that the deficient T-cell immunity in the first year after transplantation is not only due to an insufficient number of T cells but also due to an insufficient T-cell repertoire¹¹⁷. The restoration of the repertoire probably depends on the repopulation of thymus-derived naive T cells. Strategies to restore thymic function are therefore a principal aim of immune reconstitution. In addition to the importance of intact thymic precursors (BOX 2) and thymic function for post-transplant T-cell reconstitution, *de novo*-generated T cells (thymic emigrants), as well as mature T cells that are transferred in the allograft, require extrathymic support for their survival and proliferation. The factors that determine the homeostasis of peripheral T cells are beginning to be defined. For example, the process of homeostatic proliferation (BOX 3), which is particularly relevant in lymphopenic states and involves the cytokines interleukin-7 (IL-7) and IL-15, has been recognized as an important contributor to post-transplant T-cell reconstitution^{84,92,93,106}. In addition, survival signals have a crucial role in the reconstitution and homeostasis of T cells after transplantation. Adult recipients of an HSCT have a 5–10-fold increase in the number of apoptotic peripheral T cells compared with healthy controls, and this is associated with GVHD, HLA disparity between the donor and the host, time after transplant, and the expression of FAS (CD95) and B-cell lymphoma 2 (BCL-2)^{118,119}.

SEVERE COMBINED IMMUNODEFICIENCY

(SCID). Humans or mice with this rare genetic disorder lack functional T and B cells owing to a mutation in a gene that is involved in T-cell and/or B-cell development; consequently, they suffer from recurrent infections. Several forms of SCID have been described, including mutations in the common cytokine-receptor γ -chain of several interleukin receptors, Janus activated kinase 3 (JAK3) and adenosine deaminase.

ALLOGENEIC

Allogeneic tissues or cells are genetically different from the host and can elicit an immune response when transplanted, resulting in rejection or graft-versus-host disease.

GRAFT-VERSUS-HOST DISEASE

(GVHD). Tissue damage in a recipient of allogeneic transplanted tissue (usually bone marrow) that results from the activity of donor cytotoxic T cells that recognize the tissue of the recipient as foreign. GVHD varies markedly in severity, but it can be life threatening in severe cases and, in particular, affects the intestines, liver and skin.

CONGENIC

An animal strain that is genetically identical to another strain except for one or more allelic differences that do not result in an antigen that can elicit an immunological response when tissue is transferred or transplanted from one strain to another.

lymphohaematopoiesis. Myeloablative and non-myeloablative conditioning regimens, which consist of radiation, chemotherapy and/or immunosuppressive drugs to enable engraftment of the donor HSCs, specifically target T cells to prevent graft rejection by T cells of the host and GRAFT-VERSUS-HOST DISEASE (GVHD) by T cells of the donor.

In contrast to the relatively early recovery of innate immunity (mediated by myeloid and natural killer (NK) cells), all recipients (but especially adult recipients) of an allogeneic HSCT have post-transplant deficiencies in their reconstitution of B cells and T cells, and these can exceed the period of lymphocytopenia as a result of delays in the recovery of function^{4,5}. Although children commonly recover T-cell-based immunity within 6 months of an HSCT following chemotherapy, adults can require years and, even then, rarely re-establish a fully competent T-cell repertoire^{4,6}. This prolonged post-transplant lymphoid deficiency (in particular of the T-cell lineage) is associated with an increased risk of infections^{4,7}, relapse of malignancy⁸ and development of secondary malignancies⁹, and it reduces the efficacy of immunotherapeutic strategies, such as vaccination against microorganisms or tumours. The risk of opportunistic infections in the post-transplant period directly correlates with the recovery of T cells (especially CD4⁺ T cells)^{4,5,7}.

Particularly in the first weeks after transplantation, the lymphoid system of recipients of an allogeneic HSCT contains shifting populations of donor and host T cells, which include the following: *de novo*-generated donor T cells, which originate from donor haematopoietic

precursors and are produced in the thymus or possibly in extrathymic sites such as the intestinal mucosa; non-alloreactive T cells, which derive from mature donor T cells in the allograft and can undergo homeostatic proliferation in the lymphopenic host; alloreactive T cells, which are transferred in the allograft and cause GVHD; and residual host T cells, which have survived the conditioning regimen and can reject the allograft (BOX 1; FIG. 2). Therefore, any strategy that affects the T cells of transplant recipients will need to be tested for its potential risks of enhancing GVHD or graft rejection and its potential benefits of promoting thymopoiesis, homeostatic proliferation and T-cell survival.

Adoptive transfer of lymphoid progenitor cells

The recent identification of common lymphoid progenitors (CLPs) in adult bone marrow (BOX 2; FIG. 3) has allowed the development of adoptive transfer of donor lymphoid precursors to recipients of an allogeneic HSCT as a strategy to expedite and enhance *de novo* generation of donor T cells and to promote recovery of T cells. This strategy operates under the assumption that the addition of committed lymphoid precursors to the graft will result in an accelerated (but transient) recovery of the thymus before lymphoid precursors derived from the pluripotent donor HSCs begin their continuing repopulation of the thymus. Adoptive transfer of committed progenitors has been successfully applied in mouse models to enhance myeloid reconstitution through the CONGENIC transplantation of common myeloid progenitors (CMPs)

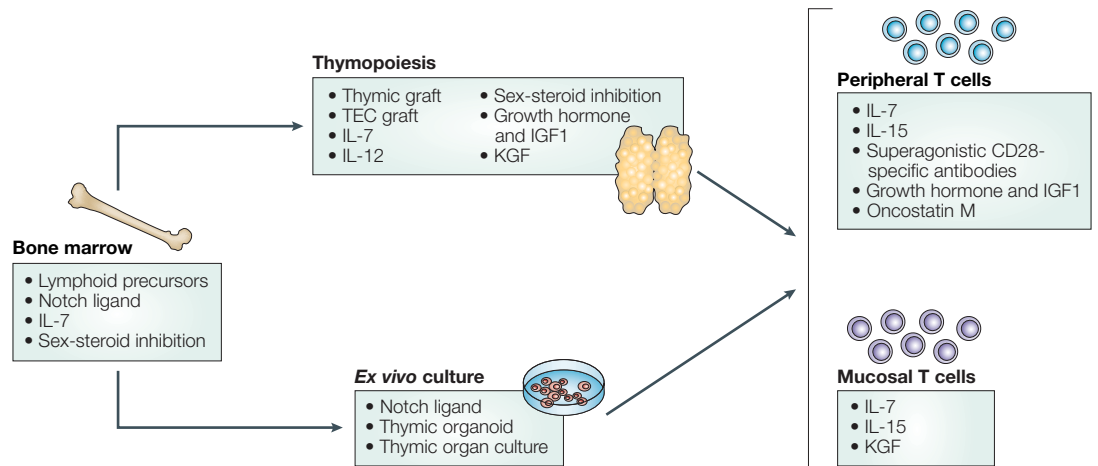


Figure 1 | **Strategies to enhance T-cell reconstitution after allogeneic bone-marrow transplantation.** After transplantation, various immunostimulatory strategies are used to improve reconstitution of T cells. The regions of the body and/or cell populations that are targeted by these strategies are depicted, and the strategies — which include the administration of various cells, tissues or factors, and the inhibition of sex steroids — are indicated. These strategies function to increase the number of lymphoid precursors in the bone marrow, stimulate thymopoiesis, and/or enhance the production of peripheral T cells and/or mucosal T cells. Some strategies promote T-cell reconstitution by affecting more than one of these areas. IGF1, insulin-like growth factor 1; IL, interleukin; KGF, keratinocyte growth factor; TEC, thymic epithelial cell.

and granulocyte/monocyte progenitors (GMPs) in recipients of an HSCT, which resulted in increased protection against infection with *Aspergillus fumigatus* or *Pseudomonas aeruginosa*¹⁰. The addition of CLPs to the allograft increased the protection of lethally irradiated recipients of an HSCT against infection with murine CMV (MCMV), which requires a complex immune response involving CD4⁺ and CD8⁺ T cells, NK cells and B cells¹¹. However, accelerated reconstitution was mainly achieved in the B-cell lineage, and only minor effects on thymic cellularity and peripheral T-cell reconstitution were observed. Moreover, thymectomized recipients of an HSCT plus CLPs also had increased protection against infection with MCMV, which might be explained by an increase in maturation of extrathymic CD8⁺ (but not CD4⁺) T cells, as well as early reconstitution of NK cells. Adoptive transfer of CLPs did not cause GVHD in allogeneic recipients, indicating that CLP-derived T cells with alloreactive potential were adequately deleted or functionally downregulated in the thymus and/or periphery.

The relatively modest effects of the transfer of CLPs on T-cell reconstitution probably result from the commitment of most CLPs to the B-cell lineage. Therefore, adoptive-transfer studies using precursors that preferentially commit to the T-cell lineage, such as the CD62L⁺ thymic precursor (BOX 2), are expected to result in stronger effects on T-cell recovery.

Enhancing thymopoiesis

Thymic grafts. The thymus is the main and most efficient (if not the only) site of T-cell lymphopoiesis (FIG. 3), and this has resulted in several studies using transplantation of thymic grafts to enhance T-cell recovery. Thymus transplants have been used in both animal models and children with SCID or DIGEOURGE SYNDROME to

establish functional lymphocyte responses or as a method for the induction of CENTRAL TOLERANCE following HSC or solid-organ transplantation.

Early human studies in which human fetal thymic tissue or cultured thymic epithelium were transplanted into recipients of an allogeneic HSCT were unsuccessful, but these might have been compromised by the immunosuppressive therapy that these patients received to prevent GVHD¹². More-recent studies, using transplantation of cultured thymic fragments (in the absence of an HSCT) to patients with DiGeorge syndrome, have been more successful^{13,14}. Thymocyte maturation and a normal thymic microenvironment were observed, and T-cell responses to mitogens and antigens were evident^{13,14}. The T-cell receptor (TCR) repertoire of these patients was initially oligoclonal and then progressed to being polyclonal, allowing for adequate immune responses to a wider variety of antigens¹⁴. MIXED LYMPHOCYTE REACTIONS showed tolerance to donor antigens, and recipients had normal antibody titres after immunization with tetanus toxoid or Pneumovax (the polyvalent pneumococcal vaccine)¹⁴.

These results in human patients were supported by similar results obtained using a mouse model of DiGeorge syndrome (NUDE MICE). Nude mice transplanted with cultured thymic fragments (both congenic and allogeneic) survived and showed relatively normal lymphopoiesis¹⁵. Antibody responses normalized, and T-cell proliferation and cytotoxicity increased from 10% of the level of wild-type mice to 100%. Third-party skin grafts were rejected, and second-party grafts (from the thymic donor) were accepted or rejected slowly in both nude mice and mice subjected to total body irradiation^{15,16}.

Thymic transplants have also been relatively successful in large animal models. Several research groups have shown the acceptance, survival and function of

DIGEOURGE SYNDROME

A syndrome characterized by cardiac malformations, facial anomalies and hypoplasia of the parathyroid gland and thymus. Most cases are the result of a deletion of the chromosomal region 22q11.2. Mice deficient in the homeobox A3 protein (HOXA3) develop a phenotype similar to patients with DiGeorge syndrome.

CENTRAL TOLERANCE

Lack of self-responsiveness that occurs as lymphoid cells develop. It is associated with the deletion of autoreactive clones. For T cells, this occurs in the thymus.

MIXED LYMPHOCYTE REACTION

A tissue-culture technique that is used for the *in vitro* testing of the proliferative response of T cells from one individual to lymphocytes from another individual.

NUDE MICE

Mice with a mutation in the forkhead box N1 gene (*Foxn1*), which results in hairlessness, defective formation of the thymus and a lack of mature T cells.

immunocompetent thymic grafts in miniature swine^{17–19}, which were rendered tolerant to renal allografts syngeneic to the grafted thymus¹⁸. In addition, transplantation of composite organs, such as thymokidneys (a kidney with vascularized autologous thymic tissue under the capsule) and thymohearts, resulted in improved survival of secondary grafts. That is, transplantation of a thymus of donor origin together with a solid-organ transplant, such as a kidney or heart, increases the chance of graft acceptance^{17,19}. These studies highlight that successful long-term acceptance of tissue grafts (in this case, a kidney or heart) requires a functioning thymus (donor derived or at least containing donor antigen-presenting cells, APCs) that can induce negative selection and/or the development of regulatory T cells.

Although thymic grafts have been successful in promoting T-cell recovery and tolerance in preclinical and clinical studies, this strategy is limited by the availability of thymic tissue.

Thymic epithelial progenitor cells. The monoclonal antibody MTS24, which was raised against membrane preparations of mouse thymic stroma²⁰, recognizes a glycoprotein that is differentially expressed throughout embryogenesis and adult life by mouse thymic epithelial cells (TECs). In the adult thymus, only isolated cells in the medulla are recognized by MTS24 (that is, MTS24⁺), but during early embryogenesis, the entire epithelium is MTS24⁺. Downregulation of expression of the glycoprotein in the thymus is coincident with the appearance of T cells. The idea that MTS24 might

recognize a population of primordial TECs was first proposed by Blackburn *et al.*²¹ after they showed that this antibody stained the thymic remnant of nude mice.

In separate studies, Gill *et al.*²² and Bennett *et al.*²³ sorted embryonic TECs (from day 15 and day 12.5 embryos, respectively) into two groups: CD45⁺MTS24⁺MHC class II⁺ and CD45⁺MTS24⁺MHC class II⁻. Following *in vitro* reaggregation of a small number (as few as 500) of these cells, cells from each group were transplanted under the kidney capsule of recipient mice. After 3 weeks²³ and 8 weeks²², capsulated, vascularized, ectopic thymi were present in those mice transplanted with MTS24⁺ TECs but not in those transplanted with MTS24⁻ TECs. In these grafts, thymocyte development — as defined by the expression of CD4 and CD8, as well as CD25 and CD44 — seemed to be normal^{22,23}. Immunohistological analysis showed that the thymi that were produced had a normal architecture and that all of the main stromal-cell components were present^{22,23}. Importantly, thymi grafted into nude mice could produce peripheral T cells. Current strategies are aimed at more precisely defining the nature of TEC progenitors in mice as a basis for identifying the equivalent population of cells in the human thymus. The rapidly expanding horizons of stem-cell research might also enable the direct derivation of TEC progenitors from primitive adult or embryonic stem cells.

Ex vivo culture systems

Several *ex vivo* culture systems have been developed to generate T cells from haematopoietic precursors: precursors are introduced into either a mouse fetal

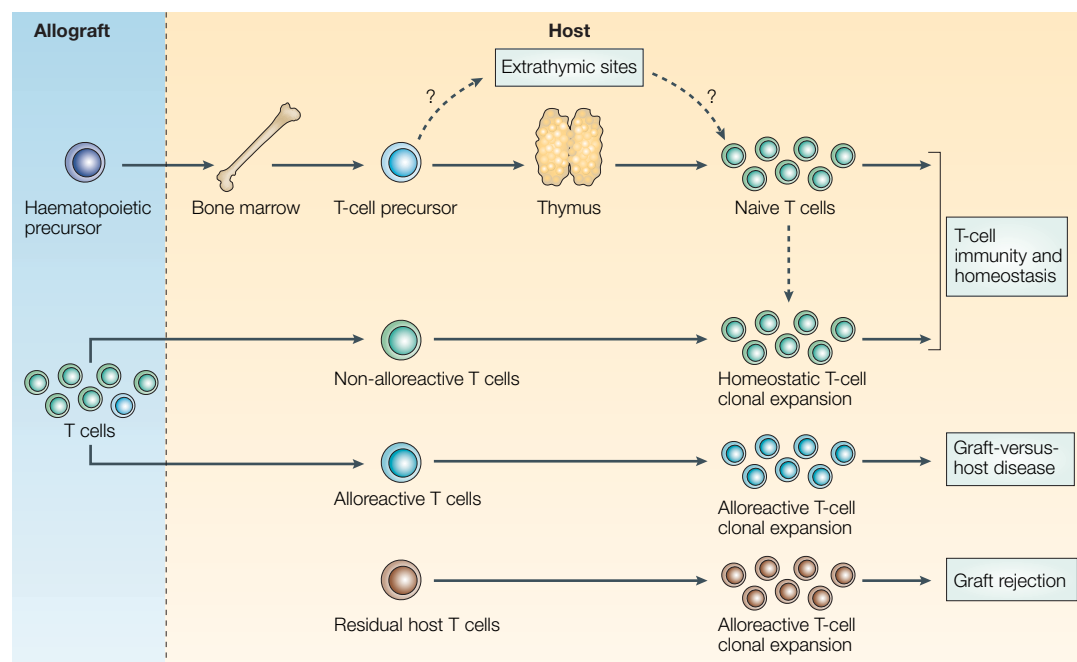


Figure 2 | T-cell reconstitution after allogeneic haematopoietic stem-cell transplantation. Five different T-cell populations might be present in recipients of an allogeneic haematopoietic stem-cell transplant (HSCT). Four of these are donor derived: thymic-dependent, newly generated T cells; non-alloreactive mature T cells that are infused with the allograft; alloreactive T cells that are also transferred with the allograft; and, possibly, a small population of donor T cells that are newly generated in extrathymic sites. The fifth population consists of residual host T cells that have survived the conditioning regimen (radiotherapy and/or chemotherapy) of the HSCT and can cause graft rejection.

Box 2 | Early T-cell lineage progenitors

Sustained thymopoiesis requires continuous seeding of the thymus with bone-marrow progenitors¹²⁰. In adult mice, lymphoid precursors enter the thymus periodically through the postcapillary venules in the corticomedullary junction^{121,122}. The search for the elusive T-cell lineage progenitor cells has resulted in several candidates (reviewed in REF. 123).

Common lymphoid progenitors

Common lymphoid progenitors (CLPs) are defined as being lineage (Lin)⁻CD44⁺cKIT^{low}SCA1^{low}THY1^{low}AA4⁺ interleukin-7 receptor α -chain (IL-7R α)⁺ fms-related tyrosine kinase 3 (FLT3)⁺, and they constitute 0.02% of adult bone-marrow cells¹²⁴. These cells were shown to have short-term (that is, early, rapid but not sustainable) reconstitution capability for T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells and dendritic cells (DCs), but they have a greater potential for lymphopoiesis of B cells than T cells. Also, they have not been isolated from the adult thymus. However, a cell population known as CLP-2 has been identified; these cells can be derived in short-term culture from CLPs (now known as CLP-1s)¹²⁵. CLP-2s are bipotent precursors of T cells and B cells, but in contrast to CLP-1s, they are cKIT⁻B220⁺ and can be detected in the thymus.

Early T-cell lineage progenitors

Early T-cell lineage progenitors (ETPs) are defined as being Lin⁻CD25⁻CD44⁺cKIT^{hi}SCA1^{hi}IL-7R α ^{low/-}, and they constitute 0.01% of all thymocytes¹²⁶. Within 3 weeks of being transferred to the thymus, they can undergo a 20,000–50,000-fold expansion. These ETPs can develop into T cells, B cells, NK cells, NKT cells and DCs, and to a lesser extent into myeloid cells.

ETPs (but not CLPs) have weak myeloid differentiation potential, whereas CLPs have a stronger B-cell differentiation potential. After intrathymic injection, ETPs produce more double-positive thymocytes for a longer period of time than do CLPs. Ikaros-deficient mice (which do not produce B cells but have normal T-cell development) have ETPs but lack CLP-1 and CLP-2 populations, which indicates that ETPs can develop independently from CLPs. These data indicate that there are two progenitor populations that exist in parallel, both with B-cell and T-cell potential.

This raises the question of whether a common progenitor of ETPs and CLPs exists in the bone marrow. CLPs arise from Lin⁻cKIT^{hi}SCA1^{hi}FLT3⁺ bone-marrow progenitors, and this process requires FLT3 ligand¹²⁷. However, the existence of a common progenitor for both ETPs and CLPs has not been shown, although two recently identified early lymphoid progenitors — recombination-activating gene 1 (RAG1)⁺Lin⁻cKIT^{hi}SCA1^{hi}IL-7R α -FLT3⁺ cells¹²⁸ and Lin⁻cKIT^{hi}SCA1^{hi}THY1⁻CD62L⁺ cells¹²⁹ — would be good candidates.

thymic organ culture²⁴ or a three-dimensional matrix (a thymic organoid)²⁵, and then co-cultured with monolayers of thymic stroma²⁶, peripheral-blood mononuclear-cell feeder layers²⁷ or combinations of growth factors and cytokines²⁸. T cells that are generated in human culture systems could be administered to patients in addition to donor HSCs to expedite post-transplant T-cell recovery. However, most of these *in vitro* T-cell generation systems are difficult to establish, have a variable outcome and yield a small number of mature T cells. There are also uncertainties regarding whether positive and negative selection can occur appropriately in these culture systems. This makes the clinical application of these systems problematic, at least in the near future, until considerable improvements are made. One recently developed promising *ex vivo* culture system involves the co-culture of haematopoietic precursor cells with a Notch ligand (with or without a bone-marrow stromal cell line), resulting in large populations of thymic precursors and mature T cells.

Notch ligand. Signalling through Notch is involved in various cell-fate decisions during the development of a multicellular organism, including survival, proliferation, lineage commitment and tissue architecture. In mammals, four members of the Notch family (the receptors Notch-1, -2, -3 and -4) and five ligands (Jagged-1 and -2, and Delta-like-1, -3 and -4) have been described. Notch-1 is essential for T-cell lineage commitment: the inhibition of Notch-1 results in a block in thymocyte differentiation at the DOUBLE-NEGATIVE 1 (DN1) TO DN2 TRANSITION and the accumulation of B cells in the thymus, whereas overexpression of constitutively active Notch-1 in haematopoietic progenitors inhibits B-cell development and promotes T-cell development up to the double-positive (DP) stage in the bone marrow. These and other studies indicate that Notch-1 is important for instructing bone-marrow-derived lymphoid precursors to select a T-cell versus B-cell fate in the thymus, as well as for promoting T-cell differentiation (reviewed in REF. 29). Consistent with this, Notch ligands are highly expressed by the thymus and less so by the bone marrow and fetal liver³⁰. Several recent studies have shown that the Notch ligand Delta-like-1 can induce T-cell development from haematopoietic precursors *in vitro*^{31,32}. Incubation of mouse bone marrow with both the extracellular domain of Delta-like-1 fused to the Fc portion of human IgG and growth factors — stem-cell factor (SCF), interleukin-6 (IL-6), IL-11 and FLT3 (fms-related tyrosine kinase 3) ligand — inhibited myeloid differentiation and led to an increase in the number of precursors with short-term lymphoid and myeloid repopulation potential³². The addition of IL-7 further enhanced early T-cell development. Overexpression of Delta-like-1 by the OP9 bone-marrow stromal cell line (known as OP9-DL1 cells) — which can support haematolymphopoiesis from embryonic stem cells, and early haematopoiesis and B-cell lymphopoiesis from HSCs — allowed these cells to support the complete differentiation of fetal liver stem cells to mature CD8⁺ $\alpha\beta$ and $\gamma\delta$ T cells³¹. At present, the OP9-DL1 culture system has two drawbacks: it does not support the positive selection of functional CD4⁺ T cells or natural killer T (NKT) cells, because OP9-DL1 cells do not express MHC class II or CD1d; and defective negative selection of self-reactive T-cell clones could occur, because OP9-DL1 cells probably have a limited capacity to present self-antigens to developing T cells³³. Additional genetic engineering of the OP9 stromal cell line to express MHC class II and CD1d molecules should further optimize this *ex vivo* culture system, which has potential for clinical use in recipients of an HSCT. Cultures could also be depleted of fully mature T cells before infusion so that only T-cell precursors are transferred, and these would then undergo positive and negative selection in the thymus of the recipient.

Hormones and growth factors

Sex-steroids. The progressive loss of cell-mediated immunity during ageing can mostly be attributed to age-related thymic atrophy³⁴, which consists of a decrease in

DOUBLE-NEGATIVE 1 (DN1) TO DN2 TRANSITION
Thymic precursors at the DN1 (CD3⁻CD4⁻CD8⁻CD25⁻CD44⁺) stage lose the ability to generate B cells, natural killer cells and dendritic cells after their transition to DN2 (CD3⁻CD4⁻CD8⁻CD25⁺CD44⁺) thymocytes.

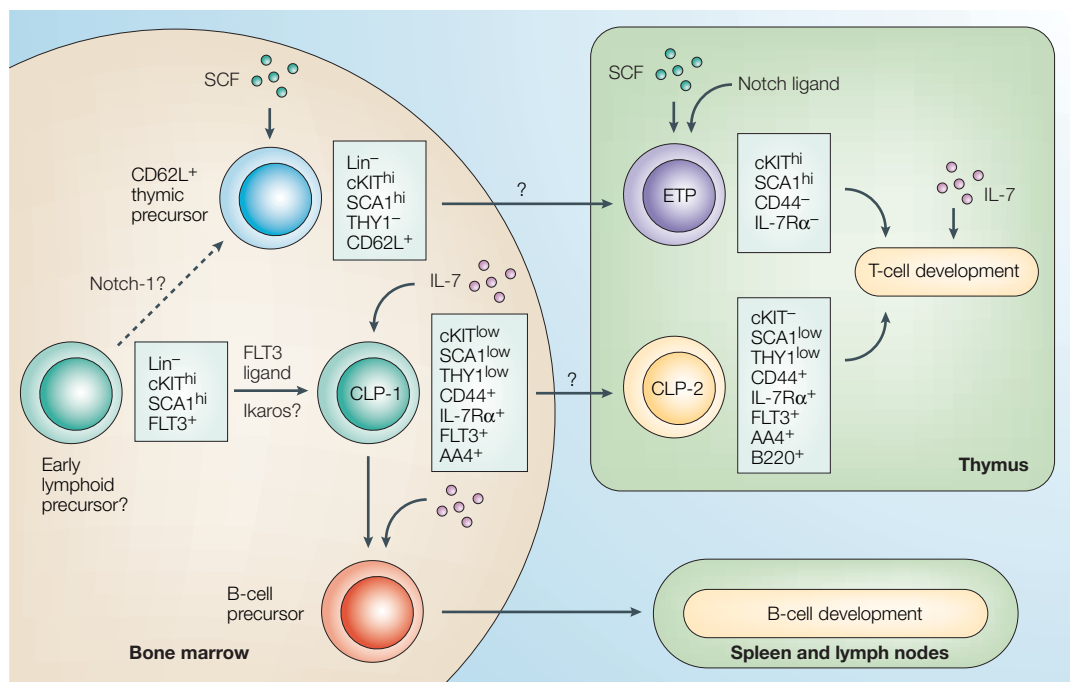


Figure 3 | T-cell precursors in mice. The relationship between early lymphoid precursors, thymic precursors and common lymphoid progenitors (CLPs) is not yet clearly understood. Here, known and potential precursors of mouse T cells are shown, together with the cytokines and other factors that are important for early T-cell development. The phenotypic features of each T-cell precursor are also indicated. Interestingly, in contrast to mice, interleukin-7 (IL-7) does not seem to be involved in B-cell development in non-human primates and humans. ETP, Early T-cell lineage progenitor; FLT3, fms-related tyrosine kinase 3; IL-7Rα, IL-7 receptor α-chain; Lin, lineage; RAG1, recombination-activating gene 1; SCF, stem-cell factor.

thymic cellularity, as well as a marked alteration of the thymic microenvironment³⁵. Thymic atrophy leads to a decrease in the number of recent thymic emigrants³⁶. The ratio of naive T cells to memory T cells is decreased in the peripheral lymphoid tissues, and the TCR repertoire is restricted for both CD4⁺ and CD8⁺ T-cell subsets, resulting in diminished peripheral T-cell responses (reviewed in REF. 37).

The main cause of thymic involution is thought to be the increased production of sex steroids (androgens, oestrogen and progesterone) after puberty, and it is well established in rodents that the ablation of sex steroids reverses age-related thymic atrophy^{35,38}. Both surgical and chemical castration (using luteinizing-hormone-releasing hormone, LHRH) of old mice and rats lead to an increase in thymic cellularity and reformation of the thymic architecture^{35,38–40}. Castration also leads to an increase in the number of peripheral T cells⁴⁰. Castrated mice show increased reactivity to sheep red blood cells and enhanced rejection of skin grafts⁴¹. These increased responses were reversed by androgen treatment⁴¹. When castration and thymectomy were carried out together, rejection of skin grafts did not occur, confirming that the effects of sex steroids are linked to thymic output⁴¹. Olsen *et al.*⁴² have shown that functional androgen receptors at the cell surface of TECs, but not thymocytes, are essential for age-related thymic involution.

From a clinical perspective, it is important that the decreased levels of sex steroids resulting from surgical castration can also be achieved chemically through the

administration of LHRH analogues, which are commonly used for the treatment of sex-steroid-exacerbated conditions, such as prostate cancer, breast cancer and endometriosis. Preliminary data from a phase II trial of patients with haematological malignancies who received an HSCT following myeloablative chemotherapy have revealed evidence of thymic rejuvenation in those treated with an LHRH agonist that is commonly used for the treatment of prostate cancer, as indicated by increased levels of naive CD4⁺ T cells (R.L.B., unpublished observations).

Growth hormone and insulin-like growth factor 1. Apart from their anabolic effects as hormones involved in the regulation of metabolism, growth hormone and insulin-like growth factor 1 (IGF1) can enhance haematopoiesis, thymopoiesis, and T-cell and B-cell function⁴³. The mechanisms responsible for the effects of growth hormone and IGF1 on T-cell development and function are poorly understood and could involve direct effects on T cells and their precursors, as well as stimulation of other cells (such as APCs or stromal cells) that can support T cells (reviewed in REF. 44). The main source of growth hormone is the anterior pituitary gland. But growth hormone is produced by many cells, and its receptor is also expressed by many cells (including haematopoietic cells)⁴⁵. Activation of the growth-hormone receptor results in downstream activation of JAK2 (Janus activated kinase 2), STAT1 (signal transducer and activator of transcription 1), STAT3 and

ACTIVATION-INDUCED CELL DEATH (AICD). The apoptotic cell death of activated lymphocytes. It ensures the rapid elimination of effector cells after their antigen-dependent clonal expansion. Defects in AICD result in lymphoproliferative diseases that are associated with autoimmune disorders.

STAT5 (REF. 45). Growth hormone mediates most of its effects on metabolism and haematopoiesis either directly or indirectly through the induction of IGF1. IGF1 can be secreted by haematopoietic cells, bone-marrow stromal cells and TECs, and IGF1 receptors are expressed by thymocytes, T cells, B cells, NK cells, monocytes and bone-marrow stromal cells⁴⁶. IGF1 receptor expression is upregulated by T cells after activation by engagement of the TCR and CD28 (REF. 47).

Under conditions of stress, Snell–Bagg dwarf mice, which have abnormal anterior-pituitary function, suffer from defects in T-cell immunity, including thymic hypoplasia and a decreased number of peripheral CD4⁺ T cells, which can be reversed by the administration of growth hormone⁴⁸. Moreover, administration of IGF1 to 9-month-old mice was shown to promote engraftment of the thymus by lymphoid precursors and to increase thymic cellularity⁴³. However, mice deficient in growth hormone or IGF1, or Snell–Bagg mice under non-stress conditions, have no defects in lymphoid development or function, which indicates that neither growth hormone nor IGF1 are required for normal lymphopoiesis and lymphoid homeostasis⁴⁹.

Both growth hormone and IGF1 can promote the survival and function of peripheral T cells, as well as increase the function of B cells, NK cells and macrophages⁴⁴. For example, growth hormone can potentiate the antigen-specific proliferative and cytokine responses of human T-cell clones⁴⁵. IGF1 and growth hormone seem to have an anti-apoptotic effect on peripheral T cells, because inhibition of the IGF1 receptor results in increased ACTIVATION-INDUCED CELL DEATH and FAS (CD95)-mediated apoptosis, and both IGF1 and growth hormone can also partially inhibit dexamethasone-induced apoptosis of CD4⁺ T cells⁵⁰. Studies using human cord-blood T cells showed that administration of IGF1 could not only decrease FAS-dependent and FAS-independent apoptosis, but also enhance T-cell proliferation. This *in vitro* proliferative effect on the T-cell response to mitogens was confirmed in 9-month-old mice treated with IGF1 (REF. 43).

Administration of growth hormone to normal adult mice increases the numbers of haematopoietic precursors in the spleen and bone marrow, and in recipients of bone-marrow transplants, it promotes multi-lineage reconstitution^{45,51}. Serum levels of IGF1 are decreased in patients who have received an allogeneic HSCT, and T-cell recovery correlates with an increase in serum IGF1 levels⁵². In mouse models, post-transplant administration of IGF1 to recipients of a syngeneic or an allogeneic HSCT resulted in an increase in thymopoiesis, peripheral T-cell numbers and proliferation, and it did not aggravate GVHD^{53,54}. Interestingly, post-transplant administration of another neuroendocrine hormone, prolactin, also resulted in increased thymic cellularity and improved T-cell (and B-cell) reconstitution⁵⁵.

Clinical studies in patients with AIDS showed that IGF1 and growth hormone were well tolerated, could increase lean body mass and could increase thymic volume in children, but they had only modest effects on T-cell function (as determined by *in vitro* production of

IL-2 in response to peptides derived from HIV)⁴⁵. So, growth hormone and IGF1 are probably not required for normal haematopoiesis, but under conditions of stress (such as during AIDS, high-dose chemotherapy or lethal conditioning for HSCT), they can promote engraftment, haematopoiesis and thymopoiesis. Further trials are required to examine the potential toxic effects, including glucose intolerance, oedema and arthralgia, and to determine the theoretical risks of autoimmunity, graft rejection, GVHD and tumour-growth enhancement.

Keratinocyte growth factor. Keratinocyte growth factor (KGF; also known as fibroblast growth factor 7, FGF7) acts through its receptor (FGF receptor 2 IIIb isoform, FGFR2IIIb) on a variety of epithelial tissues, including hepatocytes⁵⁶, gut epithelial cells⁵⁶ and skin keratinocytes⁵⁷. In the thymus, KGF is produced by both thymic stromal cells⁵⁸ and thymocytes^{58,59}, but FGFR2IIIb is only expressed by TECs^{58,59}. FGFR2IIIb-deficient mice have arrested thymic development, which leads to decreased thymic cellularity and abnormal T-cell development⁶⁰.

In models of bone-marrow transplantation, thymic reconstitution after a syngeneic or an allogeneic HSCT was considerably enhanced following treatment with KGF⁵⁸. Thymic cellularity was increased, and the developmental block that is usually observed after an HSCT, between DN and DP thymocytes, was released⁵⁸. KGF-treated HSCT-recipient mice had considerably more cells containing *Il-7* mRNA in their thymi than untreated recipients of an HSCT, and treatment with KGF had no effect on thymic reconstitution in recipients of an HSCT that were deficient in *Il-7*. Together, these results indicate a role for *Il-7* in KGF-enhanced immune reconstitution⁵⁸, although the abnormal thymic phenotype of *Il-7*-deficient mice (that is, a marked decrease in thymocyte number and abnormal thymic microenvironment) might prevent KGF from having an effect. An increase in the number of donor-derived T cells in the spleen and lymph nodes was observed after administration of KGF to recipients of a T-cell-depleted HSCT⁵⁸, and these T cells — both CD4⁺ and CD8⁺ — expressed markers of naive T cells, indicating that the increased cell number results from an increase in thymic export and not peripheral clonal expansion⁵⁸. T-cell-dependent antibody responses were also enhanced following a syngeneic or an allogeneic HSCT and pretreatment with KGF⁵⁸.

Because FGFR2IIIb is expressed by many of the organs that are damaged during GVHD, several research groups have studied the effects of treatment with KGF in the setting of acute GVHD. Administration of KGF can facilitate allo-engraftment and ameliorate the development of GVHD through a variety of mechanisms that are not related to T-cell reconstitution, including protection and repair of epithelial-cell injury in the gut mucosa, reduction in inflammatory-cytokine release and inhibition of the allogeneic T-cell response^{61–63}. Treatment with KGF also largely protected the thymic microenvironment from the alterations that are usually seen during acute GVHD⁶⁴.

EFFECTOR MEMORY CELLS

Memory T cells that home to peripheral tissues and plasma cells that home to the bone marrow and secrete antibodies. They are responsible for immediate protection.

CENTRAL MEMORY CELLS

Memory T and B cells that home to secondary lymphoid organs. These cells are heterogeneous and do not have the full range of functions that are characteristic of effector T cells or plasma cells. They are responsible for secondary or chronic responses to antigen and might be involved in long-term maintenance of effector memory cells.

KGF is currently in phase II and III clinical trials to assess its effects on both mucositis (a side-effect of chemotherapy that results in mucosal ulceration of the digestive tract) after high-dose chemotherapy and GVHD following an allogeneic HSCT⁶⁵. Hopefully, these studies will also provide data regarding the effects of KGF on T-cell recovery.

Cytokines and co-stimulation

Interleukin-2 and interleukin-12. IL-2 was the first T-cell growth factor to be used in clinical trials to enhance lymphocyte activity in patients with cancer or AIDS; however, the results were mixed, and marked toxicity was found (reviewed in REFS 66,67). Post-transplant administration of a low dose of IL-2 to recipients of an autologous or allogeneic HSCT had little effect on T cells but did increase the number of NK cells by 5–10-fold⁶⁸. Moreover, it was not associated with marked toxicity and did not exacerbate GVHD. A phase III study is in progress to determine whether the administration of IL-2 can decrease the relapse rate of patients who receive an autologous HSCT to treat haematological malignancies. Interestingly, administration of IL-2 to patients with HIV/AIDS resulted in the emergence of CD4⁺ T cells with a CD4⁺CD25⁺ regulatory T-cell phenotype⁶⁹. In addition, a recent study showed that high-level production of IL-2 increased the risk of acute GVHD in patients who received an unrelated bone-marrow transplant⁷⁰.

IL-12 is produced by thymic dendritic cells (DCs)⁷¹, and IL-12 β -deficient mice have accelerated thymic involution, which is associated with an increased number of DN1 thymocytes, degeneration of the thymic extracellular matrix and blood vessels, a decreased thymic cortex to medulla ratio and an increased number of apoptotic cells in aged mice⁷². IL-12 has a synergistic effect on both IL-7-induced and IL-2-induced proliferation of thymocytes, which indicates that a combination therapy including IL-12 could have a thymopoietic effect. However, despite the potential of IL-2 and IL-12 to enhance post-transplant T-cell recovery, the toxicities observed when these cytokines were administered to patients have diminished the enthusiasm for further clinical development.

Interleukin-7. IL-7 is produced by stromal cells (in the thymus and bone marrow), keratinocytes, intestinal epithelial cells and DCs (reviewed in REF 73). The IL-7 receptor (IL-7R) consists of a specific α -chain and the common cytokine-receptor γ -chain, which is also a component of the receptors for IL-2, IL-4, IL-9, IL-15 and IL-21 (REFS 74,75). IL-7R is expressed at a high level by lymphocyte precursors (including CLPs) (BOX 2), thymocytes (except for CD4⁺CD8⁺ (DP) thymocytes, which express only low levels of IL-7R), naive and memory T cells, and immature B cells. IL-7-deficient and IL-7R α -deficient mice have no $\gamma\delta$ T cells and have a 100-fold reduction in thymic cellularity; however, a small number of $\alpha\beta$ T cells can develop normally^{76,77}. Patients with mutations in IL-7R α or the common cytokine-receptor γ -chain develop a SCID syndrome

with a marked T-cell deficiency. IL-7 has a variety of effects on lymphocyte development and survival, and it is required at various stages in the development of T cells from lymphoid precursors to memory T cells. In the thymus, IL-7 promotes the survival (probably through the upregulation of expression of B-cell lymphoma 2, BCL-2), differentiation and proliferation of CD4⁺CD8⁻ (DN) thymocytes, as well as the survival and proliferation of CD4⁺ and CD8⁺ (single-positive) thymocytes^{78,79}. In the periphery, IL-7 has proliferative and anti-apoptotic effects on mature T cells, through upregulation of expression of the survival factors BCL-2 and lung Kruppel-like factor^{80,81}. IL-7 is not required for the initiation of an antigen-specific T-cell response, but it controls the transition of CD8⁺ T cells from EFFECTOR MEMORY CELLS TO CENTRAL MEMORY CELLS⁸². IL-7 has been identified as a key regulator of peripheral T-cell homeostasis (BOX 3) and is required for the homeostatic proliferation of CD4⁺ and CD8⁺ T cells during peripheral lymphopenia.

IL-7 secretion is relatively constant, and its regulation is still poorly understood, except for the inhibitory effect of TGF- β on IL-7 production by bone-marrow stromal cells⁸³. Serum levels of IL-7 are increased during lymphopenia, which is probably owing to a decrease in the available target cells that IL-7 can interact with and not necessarily to an increase in IL-7 production. IL-7R α is expressed by naive T cells but is downregulated after the activation of these cells and their subsequent transition to effector cells^{80,84}. However, IL-7R α is re-expressed by a small proportion of effector cells and is important for the development and survival of memory T cells⁸².

Administration of IL-7 has several stimulatory effects on T-cell development, including increased thymopoiesis in mice^{85,86} (both *in vitro* and *in vivo*) and humans⁸⁷ (in a thymic organ culture), increased numbers of peripheral CD4⁺ and CD8⁺ T cells without activation⁸⁸, enhanced antiviral or antitumour activity of cytotoxic T cells that are clonally expanded *in vitro* for adoptive T-cell therapy^{89,90}, increased homeostatic proliferation of both CD4⁺ and CD8⁺ T cells, and increased survival and proliferation of CD8⁺ memory T cells⁸⁴. These encouraging findings have resulted in considerable interest in the clinical development of IL-7 as a 'lymphoid growth factor' for clinical situations that require enhanced T-cell function, including bone-marrow transplantation, vaccination and treatment of AIDS.

Preclinical studies in mouse models of HSC transplantation have shown that administration of IL-7 after transplantation can enhance the reconstitution of T cells in recipients of a syngeneic or an allogeneic HSCT through increased thymopoiesis, increased homeostatic proliferation of transferred and *de novo*-generated mature T cells, and decreased apoptosis of peripheral T cells^{84,86,91–93}. Recipients of an HSCT that were treated with IL-7 had augmented antimicrobial and antitumour activity, but the potent effects on T cells in the post-transplant setting carry the risk of aggravating GVHD in recipients of an allogeneic HSCT. Several studies have addressed this concern and have shown that prolonged

Box 3 | **Peripheral homeostasis**

Under normal circumstances, the number of peripheral T cells is tightly regulated, and this has led to the concept of T-cell homeostasis, which is supported by several observations. First, the number of peripheral T cells in mice remains constant and depends only on strain or age. Second, the number of T cells increases to a normal level following sub-lethal irradiation or viral infection, and naive T cells proliferate after transfer to T-cell-deficient mice^{130,131}. Third, T-cell receptor (TCR)-transgenic mice maintain a normal number of T cells¹³¹. Importantly, thymic output seems to depend on overall thymic size and cellularity and is not affected by changes in the number of peripheral T cells. Therefore, alterations in the naive T-cell pool are sensed — through as-yet-undefined regulatory mechanisms that might involve interleukin-7 (IL-7) and IL-15 — and this results in the loss or proliferation of naive T cells in the periphery¹³².

Expression of MHC class I and II molecules in the periphery is one of the requirements for the survival and proliferation of naive T cells¹³³. Homeostatic proliferation of peripheral T cells is most probably driven by low-affinity interactions between TCR molecules and self-peptide–MHC complexes at the cell surface of dendritic cells, similar to the interactions during positive selection in the thymus^{134–136}.

Naive and memory T cells seem to have independent homeostatic set points and seem to occupy separate and independent homeostatic niches in the peripheral T-cell pool¹³⁴. When memory T cells are transferred to T-cell-deficient mice, they proliferate until their numbers are equal to the memory T-cell numbers in normal mice, and the transfer of a large number of memory T cells is unable to increase the size of the memory T-cell pool, even when naive T cells are absent¹³². The persistence of memory T cells in the periphery seems to be independent of exposure to peptide–MHC complexes. For example, CD8⁺ memory T cells could proliferate in MHC-class-I-deficient lymphopenic hosts, and CD4⁺ memory T cells could survive indefinitely in MHC-class-II-deficient hosts¹³⁴.

The cytokines IL-7 and IL-15 have an important role in peripheral T-cell homeostasis. IL-7 is a non-redundant cytokine for the survival and homeostasis of CD4⁺ and CD8⁺ naive and memory T cells^{137–140}, whereas IL-15 supports the homeostasis and survival of CD8⁺ naive and memory T cells^{131,139,141,142}. Recent studies have indicated that naive T cells are not quiescent cells that can persist indefinitely but, instead, require signals to survive in the periphery. These signals could involve the transcription factor nuclear factor of activated T cells 4 (NFAT4)¹⁴³, lung Kruppel-like factor¹⁴⁴ and members of the B-cell lymphoma 2 (BCL-2) family¹⁴⁵, which are all crucial for the maintenance of a naive T-cell pool.

administration of high doses of IL-7 can aggravate GVHD⁹⁴, whereas administration of IL-7 at lower doses and at shorter intervals had no effect on morbidity and mortality from GVHD. Importantly, administration of IL-7 to recipients of T-cell-depleted allografts (a highly effective strategy to prevent GVHD) did not result in the development of GVHD⁹².

Studies using non-human primates have shown that administration of IL-7 has more profound effects on peripheral T-cell proliferation than on thymopoiesis and is less effective at promoting B-cell development than in rodents⁹⁵. Importantly, marked toxicity was only observed at doses more than tenfold higher than the therapeutic dose (M. Morre, unpublished observations). Despite the lack of evidence for increased thymopoiesis in higher species, the promising effects on peripheral T cells, together with the effects observed in preclinical studies, have stimulated interest in clinical trials in patients with AIDS, tumour-associated immune deficiency and post-transplant immune deficiency, which are scheduled to begin soon.

Interleukin-15. IL-15 is a pleiotropic cytokine that is particularly important for the development, activation, trafficking and homeostasis of NK cells, NKT cells and

CD8⁺ memory T cells (reviewed in REF. 96). IL-15 binds a receptor with three chains — a unique α -chain (IL-15R α), the IL-2R β -chain and the common cytokine-receptor γ -chain. IL-15 is produced by APCs (such as macrophages and DCs) and by epithelial cells in the bone marrow, thymus, kidney, skin and intestines. Mice deficient in IL-15 or its receptor lack NK cells and have decreased numbers of CD8⁺ memory T cells^{97,98}. In mice, administration or overexpression of IL-15 results in increased proliferation, survival, cytolytic activity and cytokine secretion (including tumour-necrosis factor, interferon- γ and granulocyte colony-stimulating factor) of NK cells^{99,100} and memory CD8⁺ T cells¹⁰¹, as well as increased antimicrobial and antitumour activity^{102–104}. Administration of IL-15 to recipients of an HSCT can increase graft-versus-tumour activity after a syngeneic¹⁰⁵ or an allogeneic¹⁰⁶ HSCT and can increase reconstitution of CD8⁺ memory T cells, NK cells and NKT cells after an allogeneic HSCT — as long as the allograft was depleted of T cells to avoid GVHD¹⁰⁶. In addition, IL-15 is less toxic than IL-2 (REF. 107). These data from mouse models indicate that the administration of IL-15 could be an effective immunotherapy to enhance the number and function of CD8⁺ memory T cells, NK cells and NKT cells in various settings, including post-transplant immune reconstitution and vaccination against pathogens or tumours. *In vitro* studies have shown that IL-15 could enhance the function of HIV-specific CD8⁺ T cells¹⁰⁸, leading to the suggestion that immunotherapy with IL-15 could be effective for patients with AIDS, who have defective IL-15 production.

Superagonistic CD28-specific antibody. Co-stimulation through CD28 promotes the proliferation of T cells that have been activated through TCR engagement, by providing proliferative as well as anti-apoptotic signals¹⁰⁹. Although conventional agonistic CD28-specific antibodies have no mitogenic activity in the absence of TCR stimulation, recent studies have identified 'superagonistic' CD28-specific antibodies, which recognize a unique epitope on the CD28 molecule and can induce polyclonal T-cell proliferation in the absence of TCR engagement¹¹⁰. Administration of superagonistic CD28-specific antibodies results in polyclonal T-cell expansion, together with a marked (but transient) increase in the number of regulatory T cells and the expression of anti-inflammatory cytokines, including IL-10 (REF. 111). When tested in a syngeneic model of HSC transplantation in rats, the administration of superagonistic CD28-specific antibodies accelerated the post-transplant proliferation of a small number of mature T cells that had been transferred with the syngeneic bone-marrow graft but did not affect thymic output¹¹⁰. Interestingly, the clonal expansion of CD4⁺ T cells was greater than that of CD8⁺ T cells, and TCR-repertoire diversity and T-cell function were sustained. In contrast to polyclonal T-cell activation through stimulation of the TCR–CD3 complex — which results in an initial clonal expansion followed by clonal contraction (involving apoptosis by activation-induced cell death) — T cells induced following

administration of superagonistic CD28-specific antibodies persisted, possibly through the well-described anti-apoptotic effects of CD28 stimulation, including the upregulation of BCL-X_L¹¹². Superagonistic antibodies specific for human CD28 have been developed, and clinical trials are being planned for patients with cancer who have received myeloablative therapy.

Oncostatin M. **Oncostatin M** is a member of the IL-6 family and can stimulate haemangioblasts and fetal hepatic cells during fetal development. Athymic transgenic mice that express oncostatin M in the early T-cell lineage can transform their lymph nodes (but not their spleen, gut, liver or bone marrow) into a primary lymphoid organ similar to a normal thymus, possibly through neoangiogenesis of oncostatin-M-receptor-expressing postcapillary venules, thereby allowing the entry of T-cell precursors¹¹³. These lymph nodes can support extrathymic T-cell development and produce a diversified repertoire of functional T cells. However, although administration of oncostatin M could potentially enhance T-cell recovery, the expression of oncostatin M is increased during age-associated thymic atrophy, and administration of oncostatin M to adult mice results in thymic atrophy, possibly owing to enhanced production of corticosteroids¹¹⁴. Therefore, determining the clinical potential of oncostatin M requires further preclinical studies to analyse its thymic and extrathymic effects, as well as its toxicities.

Summary

With the remarkable progress in our understanding of lymphoid precursors, thymic development and peripheral T-cell homeostasis, as well as our improved understanding of the individual molecules involved in these processes, new targets for therapeutic intervention have become available. Several clinical trials aimed at improving thymic rejuvenation and T-cell immunity are currently in progress or are anticipated to begin soon; these include trials of thymic grafting, KGF administration and administration of superagonistic CD28-specific antibody. In particular, there is early evidence that LHRH analogues improve immune-system recovery in recipients of an HSCT after myeloablative chemotherapy for leukaemia or lymphoma. The improvements induced by sex-steroid ablation therapy, at the level of both the thymus and the bone marrow, could form a platform from which to administer other more specific therapies directed at peripheral T cells. Phase I trials of IL-7 administration to patients with cancer are currently underway and will soon be followed by studies treating recipients of an HSCT. Collectively, these novel approaches to restoring immune capacity through the translation of preclinical research could result in the development of one or more new strategies to improve the outcome for a variety of patients who incur considerable morbidity and mortality from T-cell deficiency.

- Berzins, S. P. *et al.* Thymic regeneration: teaching an old immune system new tricks. *Trends Mol. Med.* **8**, 469–476 (2002).
- O'Reilly, R. J. *et al.* Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts. *Immunol. Rev.* **157**, 195–216 (1997).
- Dudley, M. E. & Rosenberg, S. A. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nature Rev. Cancer* **3**, 666–675 (2003).
- Small, T. N. *et al.* Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. *Blood* **93**, 467–480 (1999).
- Storek, J. *et al.* Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. *Blood* **98**, 3505–3512 (2001).
- Parkman, R. & Weinberg, K. I. Immunological reconstitution following bone marrow transplantation. *Immunol. Rev.* **157**, 73–78 (1997).
- Storek, J., Gooley, T., Witherspoon, R. P., Sullivan, K. M. & Storb, R. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. *Am. J. Hematol.* **54**, 131–138 (1997).
- Maraninchi, D. *et al.* Impact of T-cell depletion on outcome of allogeneic bone-marrow transplantation for standard-risk leukaemias. *Lancet* **2**, 175–178 (1987).
- Curtis, R. E. *et al.* Solid cancers after bone marrow transplantation. *N. Engl. J. Med.* **336**, 897–904 (1997).
- BitMansour, A. *et al.* Myeloid progenitors protect against invasive aspergillosis and *Pseudomonas aeruginosa* infection following hematopoietic stem cell transplantation. *Blood* **100**, 4660–4667 (2002).
- Arber, C. *et al.* Common lymphoid progenitors rapidly engraft and protect against lethal murine cytomegalovirus infection after hematopoietic stem cell transplantation. *Blood* **102**, 421–428 (2003).
- Atkinson, K. *et al.* Thymus transplantation after allogeneic bone marrow graft to prevent chronic graft-versus-host disease in humans. *Transplantation* **33**, 168–173 (1982).
- Markert, M. L. *et al.* Transplantation of thymus tissue in complete DiGeorge syndrome. *N. Engl. J. Med.* **341**, 1180–1189 (1999).
- Markert, M. L. *et al.* Thymus transplantation in complete DiGeorge syndrome: immunologic and safety evaluations in 12 patients. *Blood* **102**, 1121–1130 (2003). **This article reports on the first series of patients with no detectable thymus function who received allogeneic cultured thymic tissue, resulting in recovery of T-cell function in 7 of 12 patients.**
- Hong, R., Schulte-Wissermann, H., Jarrett-Toth, E., Horowitz, S. D. & Manning, D. D. Transplantation of cultured thymic fragments. II. Results in nude mice. *J. Exp. Med.* **149**, 398–415 (1979).
- Waer, M., Palathumpat, V., Sobis, H. & Vandeputte, M. Induction of transplantation tolerance in mice across major histocompatibility barrier by using allogeneic thymus transplantation and total lymphoid irradiation. *J. Immunol.* **145**, 499–504 (1990).
- Yamada, K. *et al.* Thymic transplantation in miniature swine. II. Induction of tolerance by transplantation of composite thymokidneys to thymectomized recipients. *J. Immunol.* **164**, 3079–3086 (2000).
- Kamano, C. *et al.* Vascularized thymic lobe transplantation in miniature swine: thymopoiesis and tolerance induction across fully MHC-mismatched barriers. *Proc. Natl Acad. Sci. USA* **101**, 3827–3832 (2004).
- Menard, M. T. *et al.* Composite 'thymoheart' transplantation improves cardiac allograft survival. *Am. J. Transplant.* **4**, 79–86 (2004).
- Godfrey, D. I., Izon, D. J., Wilson, T. J., Tucek, C. L. & Boyd, R. L. Thymic stromal elements defined by M.Abs: ontogeny, and modulation in vivo by immunosuppression. *Adv. Exp. Med. Biol.* **237**, 269–275 (1988).
- Blackburn, C. C. *et al.* The *nu* gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. *Proc. Natl Acad. Sci. USA* **93**, 5742–5746 (1996).
- Gill, J., Malin, M., Hollander, G. A. & Boyd, R. Generation of a complete thymic microenvironment by MTS24⁺ thymic epithelial cells. *Nature Immunol.* **3**, 635–642 (2002).
- Bennett, A. R. *et al.* Identification and characterization of thymic epithelial progenitor cells. *Immunity* **16**, 803–814 (2002). **These two articles describe the identification of a TEC subpopulation based on expression of the glycoprotein MTS24. These cells can fully reconstitute the thymic epithelial microenvironment and can support normal T-cell development.**
- Ceredig, R., Jenkinson, E. J., MacDonald, H. R. & Owen, J. J. Development of cytolytic T lymphocyte precursors in organ-cultured mouse embryonic thymus rudiments. *J. Exp. Med.* **155**, 617–622 (1982).
- Poznansky, M. C. *et al.* Efficient generation of human T cells from a tissue-engineered thymic organoid. *Nature Biotechnol.* **18**, 729–734 (2000).
- Rosenzweig, M. *et al.* *In vitro* T lymphopoiesis of human and rhesus CD34⁺ progenitor cells. *Blood* **87**, 4040–4048 (1996).
- Gardner, J. P., Zhu, H., Colosi, P. C., Kurtzman, G. J. & Scadden, D. T. Robust, but transient expression of adeno-associated virus-transduced genes during human T lymphopoiesis. *Blood* **90**, 4854–4864 (1997).
- Pawelec, G., Muller, R., Rehbein, A., Hahnel, K. & Ziegler, B. L. Extrathymic T cell differentiation *in vitro* from human CD34⁺ stem cells. *J. Leukoc. Biol.* **64**, 733–739 (1998).
- Radtke, F., Wilson, A., Mancini, S. J. & MacDonald, H. R. Notch regulation of lymphocyte development and function. *Nature Immunol.* **5**, 247–253 (2004). **This recent review provides an excellent overview of the role of the Notch family and their ligands in lymphocyte development.**
- Harman, B. C., Jenkinson, E. J. & Anderson, G. Microenvironmental regulation of Notch signalling in T cell development. *Semin. Immunol.* **15**, 91–97 (2003).
- Schmitt, T. M. & Zuniga-Pflucker, J. C. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 *in vitro*. *Immunity* **17**, 749–756 (2002). **This article describes the capability of a bone-marrow stromal cell line ectopically expressing the Notch ligand Delta-like-1 to support the differentiation of haematopoietic progenitors into mature T cells *in vitro*.**
- Varnum-Finney, B., Brashem-Stein, C. & Bernstein, I. D. Combined effects of Notch signaling and cytokines induce a multiple log increase in precursors with lymphoid and myeloid reconstituting ability. *Blood* **101**, 1784–1789 (2003).
- Zuniga-Pflucker, J. C. T-cell development made simple. *Nature Rev. Immunol.* **4**, 67–72 (2004).

34. Aspinall, R. & Andrew, D. Thymic atrophy in the mouse is a soluble problem of the thymic environment. *Vaccine* **18**, 1629–1637 (2000).
35. Nabarra, B. & Andrianarison, I. Ultrastructural study of thymic microenvironment involution in aging mice. *Exp. Gerontol.* **31**, 489–506 (1996).
36. Steffens, C. M., Al-Harthi, L., Shott, S., Yogev, R. & Landay, A. Evaluation of thymopoiesis using T cell receptor excision circles (TRECs): differential correlation between adult and pediatric TRECs and naive phenotypes. *Clin. Immunol.* **97**, 95–101 (2000).
37. Linton, P. J. & Dorshkind, K. Age-related changes in lymphocyte development and function. *Nature Immunol.* **5**, 133–139 (2004).
38. Fitzpatrick, F. T., Kendall, M. D., Wheeler, M. J., Adcock, I. M. & Greenstein, B. D. Reappearance of thymus of ageing rats after orchidectomy. *J. Endocrinol.* **106**, R17–R19 (1985).
39. Greenstein, B. D., Fitzpatrick, F. T., Kendall, M. D. & Wheeler, M. J. Regeneration of the thymus in old male rats treated with a stable analogue of LHRH. *J. Endocrinol.* **112**, 345–350 (1987).
40. Windmill, K. F. & Lee, V. W. Effects of castration on the lymphocytes of the thymus, spleen and lymph nodes. *Tissue Cell* **30**, 104–111 (1998).
41. Castro, J. E. Orchidectomy and the immune response. II. Response of orchidectomized mice to antigens. *Proc. R. Soc. Lond. B* **185**, 437–451 (1974).
42. Olsen, N. J., Olson, G., Viselli, S. M., Gu, X. & Kovacs, W. J. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. *Endocrinology* **142**, 1278–1283 (2001).
43. Clark, R., Strasser, J., McCabe, S., Robbins, K. & Jardieu, P. Insulin-like growth factor-1 stimulation of lymphopoiesis. *J. Clin. Invest.* **92**, 540–548 (1993).
44. Welniak, L. A., Sun, R. & Murphy, W. J. The role of growth hormone in T-cell development and reconstitution. *J. Leukoc. Biol.* **71**, 381–387 (2002).
45. Murphy, W. J. & Longo, D. L. Growth hormone as an immunomodulating therapeutic agent. *Immunol. Today* **21**, 211–213 (2000).
46. Stuart, C. A., Meehan, R. T., Neale, L. S., Cintron, N. M. & Furlanetto, R. W. Insulin-like growth factor-1 binds selectively to human peripheral blood monocytes and B-lymphocytes. *J. Clin. Endocrinol. Metab.* **72**, 1117–1122 (1991).
47. Walsh, P. T. & O'Connor, R. The insulin-like growth factor-I receptor is regulated by CD28 and protects activated T cells from apoptosis. *Eur. J. Immunol.* **30**, 1010–1018 (2000).
48. Murphy, W. J., Durum, S. K. & Longo, D. L. Role of neuroendocrine hormones in murine T cell development. Growth hormone exerts thymopoietic effects *in vivo*. *J. Immunol.* **149**, 3851–3857 (1992).
49. Foster, M., Montecino-Rodriguez, E., Clark, R. & Dorshkind, K. Regulation of B and T cell development by anterior pituitary hormones. *Cell. Mol. Life Sci.* **54**, 1076–1082 (1998).
50. Dobashi, H., Sato, M., Tanaka, T., Tokuda, M. & Ishida, T. Growth hormone restores glucocorticoid-induced T cell suppression. *FASEB J.* **15**, 1861–1863 (2001).
51. Tian, Z. G. *et al.* Recombinant human growth hormone promotes hematopoietic reconstitution after syngeneic bone marrow transplantation in mice. *Stem Cells* **16**, 193–199 (1998).
52. Small, T. *et al.* Longitudinal analysis of serum levels of insulin-like growth factor-1 post bone marrow transplantation. *Blood* **90**, 541a (1997).
53. Jardieu, P., Clark, R., Mortensen, D. & Dorshkind, K. *In vivo* administration of insulin-like growth factor-I stimulates primary B lymphopoiesis and enhances lymphocyte recovery after bone marrow transplantation. *J. Immunol.* **152**, 4320–4327 (1994).
54. Alpdogan, O. *et al.* Insulin-like growth factor-I enhances lymphoid and myeloid reconstitution after allogeneic bone marrow transplantation. *Transplantation* **75**, 1977–1983 (2003).
55. Sun, R. *et al.* Immunologic and hematopoietic effects of recombinant human prolactin after syngeneic bone marrow transplantation in mice. *Biol. Blood Marrow Transplant* **9**, 426–434 (2003).
56. Housley, R. M. *et al.* Keratinocyte growth factor induces proliferation of hepatocytes and epithelial cells throughout the rat gastrointestinal tract. *J. Clin. Invest.* **94**, 1764–1777 (1994).
57. Pierce, G. F. *et al.* Stimulation of all epithelial elements during skin regeneration by keratinocyte growth factor. *J. Exp. Med.* **179**, 831–840 (1994).
58. Min, D. *et al.* Protection from thymic epithelial cell injury by keratinocyte growth factor: a new approach to improve thymic and peripheral T-cell reconstitution after bone marrow transplantation. *Blood* **99**, 4592–4600 (2002).
- This article describes how KGF can protect TECs against cytotoxic-therapy-induced damage, resulting in enhanced post-transplant T-cell recovery and function.**
59. Erickson, M. *et al.* Regulation of thymic epithelium by keratinocyte growth factor. *Blood* **100**, 3269–3278 (2002).
60. Revest, J. M., Suniara, R. K., Kerr, K., Owen, J. J. & Dickson, C. Development of the thymus requires signaling through the fibroblast growth factor receptor 2-IIb. *J. Immunol.* **167**, 1954–1961 (2001).
61. Panoskaltis-Mortari, A., Lacey, D. L., Vallera, D. A. & Blazar, B. R. Keratinocyte growth factor administered before conditioning ameliorates graft-versus-host disease after allogeneic bone marrow transplantation in mice. *Blood* **92**, 3960–3967 (1998).
62. Krjanovski, O. I. *et al.* Keratinocyte growth factor separates graft-versus-leukemia effects from graft-versus-host disease. *Blood* **94**, 825–831 (1999).
63. Panoskaltis-Mortari, A. *et al.* Keratinocyte growth factor facilitates allograftment and ameliorates graft-versus-host disease in mice by a mechanism independent of repair of conditioning-induced tissue injury. *Blood* **96**, 4350–4356 (2000).
64. Rossi, S. *et al.* Keratinocyte growth factor preserves normal thymopoiesis and thymic microenvironment during experimental graft-versus-host disease. *Blood* **100**, 682–691 (2002).
65. Meropoul, N. J. *et al.* Randomized phase I trial of recombinant human keratinocyte growth factor plus chemotherapy: potential role as mucosal protectant. *J. Clin. Oncol.* **21**, 1452–1458 (2003).
66. Nelson, B. H. IL-2, regulatory T Cells, and tolerance. *J. Immunol.* **172**, 3983–3988 (2004).
67. Dutcher, J. Current status of interleukin-2 therapy for metastatic renal cell carcinoma and metastatic melanoma. *Oncology (Huntington, NY)* **16**, 4–10 (2002).
68. Soiffer, R. J., Murray, C., Gonin, R. & Ritz, J. Effect of low-dose interleukin-2 on disease relapse after T-cell-depleted allogeneic bone marrow transplantation. *Blood* **84**, 964–971 (1994).
69. Sereti, I. *et al.* Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of CD4⁺/CD25⁺ T cells. *Blood* **100**, 2159–2167 (2002).
70. MacMillan, M. L. *et al.* High-producer interleukin-2 genotype increases risk for acute graft-versus-host disease after unrelated donor bone marrow transplantation. *Transplantation* **76**, 1758–1762 (2003).
71. Rizzitelli, A., Berthier, R., Collin, V., Candéas, S. M. & Marche, P. N. T lymphocytes potentiate murine dendritic cells to produce IL-12. *J. Immunol.* **169**, 4237–4245 (2002).
72. Li, L. *et al.* IL-12 inhibits thymic involution by enhancing IL-7- and IL-2-induced thymocyte proliferation. *J. Immunol.* **172**, 2909–2916 (2004).
73. Fry, T. J. & Mackall, C. L. Interleukin-7: from bench to clinic. *Blood* **99**, 3892–3904 (2002).
74. Noguchi, M. *et al.* Interleukin-2 receptor γ chain: a functional component of the interleukin-7 receptor. *Science* **262**, 1877–1880 (1993).
75. Goodwin, R. G. *et al.* Cloning of the human and murine interleukin-7 receptors: demonstration of a soluble form and homology to a new receptor superfamily. *Cell* **60**, 941–951 (1990).
76. von Freeden-Jeffry, U. *et al.* Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* **181**, 1519–1526 (1995).
77. Peschon, J. J. *et al.* Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J. Exp. Med.* **180**, 1955–1960 (1994).
78. von Freeden-Jeffry, U., Solvason, N., Howard, M. & Murray, R. The earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression. *Immunity* **7**, 147–154 (1997).
79. Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R. & Weissman, I. L. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell* **89**, 1033–1041 (1997).
80. Schluns, K. S., Kieper, W. C., Jameson, S. C. & Lefrançois, L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. *Nature Immunol.* **1**, 426–432 (2000).
- This is one of several articles to define the crucial role of IL-7 in the homeostasis of peripheral T cells.**
81. Schober, S. L. *et al.* Expression of the transcription factor lung Kruppel-like factor is regulated by cytokines and correlates with survival of memory T cells *in vitro* and *in vivo*. *J. Immunol.* **163**, 3662–3667 (1999).
82. Kaech, S. M. *et al.* Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nature Immunol.* **4**, 1191–1198 (2003).
83. Tang, J. *et al.* TGF- β down-regulates stromal IL-7 secretion and inhibits proliferation of human B cell precursors. *J. Immunol.* **159**, 117–125 (1997).
84. Alpdogan, O. *et al.* IL-7 enhances peripheral T cell reconstitution after allogeneic hematopoietic stem cell transplantation. *J. Clin. Invest.* **112**, 1095–1107 (2003).
85. Sempowski, G. D., Gooding, M. E., Liao, H. X., Le, P. T. & Haynes, B. F. T cell receptor excision circle assessment of thymopoiesis in aging mice. *Mol. Immunol.* **38**, 841–848 (2002).
86. Bolotin, E., Smogorzewska, M., Smith, S., Widmer, M. & Weinberg, K. Enhancement of thymopoiesis after bone marrow transplant by *in vivo* interleukin-7. *Blood* **88**, 1887–1894 (1996).
87. Okamoto, Y., Douek, D. C., McFarland, R. D. & Koup, R. A. Effects of exogenous interleukin-7 on human thymus function. *Blood* **99**, 2851–2858 (2002).
88. Geiselsart, L. A. *et al.* IL-7 administration alters the CD4:CD8 ratio, increases T cell numbers, and increases T cell function in the absence of activation. *J. Immunol.* **166**, 3019–3027 (2001).
89. Lynch, D. H. & Miller, R. E. Interleukin 7 promotes long-term *in vitro* growth of antitumor cytotoxic T lymphocytes with immunotherapeutic efficacy *in vivo*. *J. Exp. Med.* **179**, 31–42 (1994).
90. Wiryana, P., Bui, T., Faltynek, C. R. & Ho, R. J. Augmentation of cell-mediated immunotherapy against herpes simplex virus by interleukins: comparison of *in vivo* effects of IL-2 and IL-7 on adoptively transferred T cells. *Vaccine* **15**, 561–563 (1997).
91. Abdul-Hai, A. *et al.* Stimulation of immune reconstitution by interleukin-7 after syngeneic bone marrow transplantation in mice. *Exp. Hematol.* **24**, 1416–1422 (1996).
92. Alpdogan, O. *et al.* Administration of interleukin-7 after allogeneic bone marrow transplantation improves immune reconstitution without aggravating graft-versus-host disease. *Blood* **98**, 2256–2265 (2001).
93. Mackall, C. L. *et al.* IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation. *Blood* **97**, 1491–1497 (2001).
94. Sinha, M. L., Fry, T. J., Fowler, D. H., Miller, G. & Mackall, C. L. Interleukin 7 worsens graft-versus-host disease. *Blood* **100**, 2642–2649 (2002).
95. Storek, J. *et al.* Interleukin-7 improves CD4 T-cell reconstitution after autologous CD34 cell transplantation in monkeys. *Blood* **101**, 4209–4218 (2003).
96. Fehniger, T. A. & Caligiuri, M. A. Interleukin 15: biology and relevance to human disease. *Blood* **97**, 14–32 (2001).
97. Kennedy, M. K. *et al.* Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* **191**, 771–780 (2000).
98. Lodolce, J. P. *et al.* IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* **9**, 669–676 (1998).
99. Carson, W. E. *et al.* Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* **180**, 1395–1403 (1994).
100. Carson, W. E. *et al.* A potential role for interleukin-15 in the regulation of human natural killer cell survival. *J. Clin. Invest.* **99**, 937–943 (1997).
101. Zhang, X., Sun, S., Hwang, I., Tough, D. F. & Sprent, J. Potent and selective stimulation of memory-phenotype CD8⁺ T cells *in vivo* by IL-15. *Immunity* **8**, 591–599 (1998).
102. Maeurer, M. J. *et al.* Interleukin-7 or interleukin-15 enhances survival of *Mycobacterium tuberculosis*-infected mice. *Infect. Immun.* **68**, 2962–2970 (2000).
103. Yajima, T. *et al.* Memory phenotype CD8⁺ T cells in IL-15 transgenic mice are involved in early protection against a primary infection with *Listeria monocytogenes*. *Eur. J. Immunol.* **31**, 757–766 (2001).
104. Klebanoff, C. A. *et al.* IL-15 enhances the *in vivo* antitumor activity of tumor-reactive CD8⁺ T cells. *Proc. Natl Acad. Sci. USA* **101**, 1969–1974 (2004).
105. Katsanis, E. *et al.* IL-15 administration following syngeneic bone marrow transplantation prolongs survival of lymphoma bearing mice. *Transplantation* **62**, 872–875 (1996).
106. Alpdogan, O. *et al.* Interleukin-15 enhances immune reconstitution after allogeneic bone marrow transplantation. *Blood* July 27 2004 (doi:10.1182/blood-2003-09-3344).
107. Munger, W. *et al.* Studies evaluating the antitumor activity and toxicity of interleukin-15, a new T cell growth factor: comparison with interleukin-2. *Cell. Immunol.* **165**, 289–293 (1995).
108. Castelli, J., Thomas, E. K., Gilliet, M., Liu, Y. J. & Levy, J. A. Mature dendritic cells can enhance CD8⁺ cell noncytotoxic anti-HIV responses: the role of IL-15. *Blood* **103**, 2699–2704 (2004).
109. Acuto, O. & Michel, F. CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nature Rev. Immunol.* **3**, 939–951 (2003).

110. Effein, K., Rodríguez-Palmero, M., Kerkau, T. & Hunig, T. Rapid recovery from T lymphopenia by CD28 superagonist therapy. *Blood* **102**, 1764–1770 (2003).
This article shows that a novel class of superagonistic CD28-specific antibodies can induce polyclonal T-cell proliferation without TCR engagement.
111. Lin, C. H. & Hunig, T. Efficient expansion of regulatory T cells *in vitro* and *in vivo* with a CD28 superagonist. *Eur. J. Immunol.* **33**, 626–638 (2003).
112. Kerstan, A. & Hunig, T. Distinct TCR- and CD28-derived signals regulate CD95L, Bcl-x_l, and the survival of primary T cells. *J. Immunol.* **172**, 1341–1345 (2004).
113. Clegg, C. H., Ruffles, J. T., Wallace, P. M. & Haugen, H. S. Regulation of an extrathymic T-cell development pathway by oncostatin M. *Nature* **384**, 261–263 (1996).
114. Sempowski, G. D. *et al.* Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J. Immunol.* **164**, 2180–2187 (2000).
115. Mackall, C. L. & Gress, R. E. Pathways of T-cell regeneration in mice and humans: implications for bone marrow transplantation and immunotherapy. *Immunol. Rev.* **157**, 61–72 (1997).
116. Dulude, G. *et al.* Thymic and extrathymic differentiation and expansion of T lymphocytes following bone marrow transplantation in irradiated recipients. *Exp. Hematol.* **25**, 992–1004 (1997).
117. Roux, E. *et al.* Analysis of T-cell repopulation after allogeneic bone marrow transplantation: significant differences between recipients of T-cell depleted and unmanipulated grafts. *Blood* **87**, 3984–3992 (1996).
118. Hebib, N. C. *et al.* Peripheral blood T cells generated after allogeneic bone marrow transplantation: lower levels of Bcl-2 protein and enhanced sensitivity to spontaneous and CD95-mediated apoptosis *in vitro*. Abrogation of the apoptotic phenotype coincides with the recovery of normal naive/primed T-cell profiles. *Blood* **94**, 1803–1813 (1999).
119. Lin, M. T. *et al.* Increased apoptosis of peripheral blood T cells following allogeneic hematopoietic cell transplantation. *Blood* **95**, 3832–3839 (2000).
120. Scollay, R., Smith, J. & Stauffer, V. Dynamics of early T cells: prothymocyte migration and proliferation in the adult mouse thymus. *Immunol. Rev.* **91**, 129–157 (1986).
121. Foss, D. L., Donskoy, E. & Goldschneider, I. The importation of hematogenous precursors by the thymus is a gated phenomenon in normal adult mice. *J. Exp. Med.* **193**, 365–374 (2001).
122. Petrie, H. T. Role of thymic organ structure and stromal composition in steady-state postnatal T-cell production. *Immunol. Rev.* **189**, 8–20 (2002).
123. Bhandoola, A., Sambandam, A., Allman, D., Meraz, A. & Schwarz, B. Early T lineage progenitors: new insights, but old questions remain. *J. Immunol.* **171**, 5653–5658 (2003).
124. Kondo, M., Weissman, I. L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* **91**, 661–672 (1997).
This article describes the identification of a CLP in adult bone marrow that can give rise to T cells, B cells and NK cells.
125. Martin, C. H. *et al.* Efficient thymic immigration of B220⁺ lymphoid-restricted bone marrow cells with T precursor potential. *Nature Immunol.* **4**, 866–873 (2003).
126. Allman, D. *et al.* Thymopoiesis independent of common lymphoid progenitors. *Nature Immunol.* **4**, 168–174 (2003).
This article shows that early T-cell lineage progenitors are present in the thymus, and therefore the production of T-cell lineage progeny could be sustained by a CLP-independent pathway.
127. Sitnicka, E. *et al.* Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. *Immunity* **17**, 463–472 (2002).
128. Igarashi, H., Gregory, S. C., Yokota, T., Sakaguchi, N. & Kincaid, P. W. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* **17**, 117–130 (2002).
129. Perry, S. *et al.* L-selectin defines a bone marrow analog to the thymic early T-lineage progenitor. *Blood* **103**, 2990–2996 (2004).
130. Rocha, B., Dautigny, N. & Pereira, P. Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios *in vivo*. *Eur. J. Immunol.* **19**, 905–911 (1989).
131. Ku, C. C., Murakami, M., Sakamoto, A., Kappler, J. & Marrack, P. Control of homeostasis of CD8⁺ memory T cells by opposing cytokines. *Science* **288**, 675–678 (2000).
132. Tanchot, C. & Rocha, B. The organization of mature T-cell pools. *Immunol. Today* **19**, 575–579 (1998).
133. Freitas, A. A. & Rocha, B. Peripheral T cell survival. *Curr. Opin. Immunol.* **11**, 152–156 (1999).
134. Goldrath, A. W. & Bevan, M. J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402**, 255–262 (1999).
135. Viret, C., Wong, F. S. & Janeway, C. A. Jr. Designing and maintaining the mature TCR repertoire: the continuum of self-peptide:self-MHC complex recognition. *Immunity* **10**, 559–568 (1999).
136. Dulude, G., Roy, D. C. & Perreault, C. The effect of graft-versus-host disease on T cell production and homeostasis. *J. Exp. Med.* **189**, 1329–1342 (1999).
137. Tan, J. T. *et al.* IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl Acad. Sci. USA* **98**, 8732–8737 (2001).
138. Schluns, K. S., Kieper, W. C., Jameson, S. C. & Lefrançois, L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. *Nature Immunol.* **1**, 426–432 (2000).
139. Goldrath, A. W. *et al.* Cytokine requirements for acute and basal homeostatic proliferation of naive and memory CD8⁺ T cells. *J. Exp. Med.* **195**, 1515–1522 (2002).
140. Seddon, B., Tomlinson, P. & Zamoyska, R. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nature Immunol.* **4**, 680–686 (2003).
141. Alves, N. L., Hooibrink, B., Arosa, F. A. & van Lier, R. A. IL-15 induces antigen-independent expansion and differentiation of human naive CD8⁺ T cells *in vitro*. *Blood* **102**, 2541–2546 (2003).
142. Tan, J. T. *et al.* Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8⁺ cells but are not required for memory phenotype CD4⁺ cells. *J. Exp. Med.* **195**, 1523–1532 (2002).
143. Oukka, M. *et al.* The transcription factor NFAT4 is involved in the generation and survival of T cells. *Immunity* **9**, 295–304 (1998).
144. Kuo, C. T., Veselits, M. L. & Leiden, J. M. LKLF: a transcriptional regulator of single-positive T cell quiescence and survival. *Science* **277**, 1986–1990 (1997).
145. Veis, D. J., Sorenson, C. M., Shutter, J. R. & Korsmeyer, S. J. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* **75**, 229–240 (1993).

Acknowledgements

This work was supported by grants to M.R.M.B. from the National Institutes of Health, United States. M.R.M.B. is also the recipient of a Damon Runyon Scholar Award from the Damon Runyon Cancer Research Foundation, United Kingdom. This work was also supported by grants to R.L.B. from the National Health and Medical Research Council, Australia. The authors thank G. Goldberg for her many valuable contributions to the manuscript.

Competing interests statement

The authors declare **competing financial interests**: see Web version for details.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 CD28 | growth hormone | IGF1 | IL-7 | IL-12 | IL-15 | KGF | oncostatin M | Notch-1

FURTHER INFORMATION

Marcel van den Brink's laboratory:

<http://www.mskcc.org/mskcc/html/10937.cfm>

Richard Boyd's laboratory:

<http://www-personal.monash.edu.au/~malin/Boyd/>

Access to this interactive links box is free online.