REVIEW: IMMUNOLOGY

Deficient Cellular Immunity—Finding and Fixing the Defects

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The critical role of cellular immunity in resistance to infectious diseases is glaringly revealed by life-threatening infections if T cell function is disrupted by an inherited or acquired immunodeficiency. Although treatment has historically focused on infectious complications, understanding of the cellular and molecular basis of immunodeficiency and technologies useful for enhancing cellular immunity have both been rapidly evolving. A new era of molecular and cellular therapy is emerging as approaches to correct abnormal genes, the loss of T cell subpopulations, and aberrant T cell homeostasis make the transition from bench to bedside.

The impact of primary (inherited) and secondary (acquired) immunodeficiencies on the health care system has been enormous. Inherited immunodeficiencies have an estimated incidence of 1 per 10,000 births. Acquired immunodeficiencies are now much more frequent for two reasons: the common use of lymphoablative cytotoxic therapy to treat malignancies, and the AIDS epidemic. Our understanding of immunodeficient states has become increasingly clear with characterization of the biochemical and cellular events essential to T cell development, function, and survival, and with elucidation of the operative principles in the maintenance of T cell homeostasis. Advances in cellular and molecular engineering make it possible to begin specifically targeting these recently defined underlying defects and restoring immune competence.

Inherited T Cell Immunodeficiency States

The primary immunodeficiency diseases are a heterogeneous set of disorders that have been extensively reviewed (*1*). This discussion focuses only on those that involve deficient T cell function and provide models for treatment with modern cellular and molecular approaches.

Severe combined immunodeficiency (SCID) syndromes. These X-linked and autosomal recessive disorders result from defects affecting maturation of both T and B cells, and require intervention beginning in the first months of life to ameliorate life-threatening infections. X-linked SCID, accounting for 50 to 60% of the cases, is most commonly caused by mutations in the common γ chain (γ_c), an essential component of the interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15 cytokine receptors. Signaling via γ_c requires activation of the associated tyrosine kinase JAK3, and an autosomal recessive form of SCID with the identical phenotype is associated with deficiency of JAK3 (*2*). Patients lack mature T and natural killer (NK) cells and often have increased but dysfunctional B cells. Studies in knockout mice suggest that the absence of T cells results from the lack of IL-7–dependent T cell proliferation during thymic maturation.

The development of functional T and B cells requires recombination of the genes encoding antigen receptors, and autosomal recessive SCID, associated with an absence of mature T and B cells, can result from mutations of either of the recombinase activating genes, *RAG-1* or *RAG-2* (*3*). Other abnormalities in the recombination machinery, such as enzymes involved in double-strand break repair, also cause sporadic cases of human SCID (*4*).

Deficiencies in enzymes responsible for purine salvage cause about 40% of autosomal recessive SCID. The most common syndrome, adenosine deaminase (ADA) deficiency, results in nearly complete absence of T cells and a variable decrease in B cells (*5*). Although all cells in the body lack ADA enzyme activity, lymphocytes appear to be uniquely sensitive to toxicity resulting from the accumulation of the metabolite, deoxyadenosine triphosphate.

Interference with the ability of T cells to interact with antigen-presenting cells (APCs) and B cells can also produce severe immunodeficiencies. Major histocompatibility complex class II deficiency, resulting from a block in class II gene transcription, precludes effective $CD4^+$ T cell development and T cell–B cell collaboration. A somewhat less severe defect, denoted clinically as hyper-IgM (immunoglobulin M) syndrome, results from the failure of $CD4^+$ T cells to express the ligand required to activate CD40 on APC and B cells, and is manifested by frequent bacterial infections due to failure of T cell–dependent Ig class-switching by B cells, as well as by opportunistic infections due to defective T cell stimulation by inadequately activated APC (*6*).

T cell–specific immunodeficiencies. T cell activation is initiated through the T cell receptor (TCR), and deficiencies in critical components of this multimeric signaling complex, such as the CD3γ chain, the CD3ε chain, or the tyrosine kinase ZAP70 that associates with the ζ chain, can lead to selective T cell immunodeficiency (*7*). Alternatively, after a competent TCR signal, failure of the responding T cells to expand can result from defects in IL-2 production or IL-2 receptor function (*8*).

More limited immunodeficiencies, associated with susceptibility only to particular pathogens, have helped in deciphering the unique contributions of individual components of the T cell response. X-linked lymphoproliferative syndrome (XLP) is associated with progressive Epstein-Barr virus (EBV) infection leading to acutely fatal mononucleosis or subsequent EBV-associated B cell lymphoma. The normally very potent cellular immune response to EBV-infected B cells is blunted in XLP patients because of the absence of SAP (SLAM-associated protein), an adaptor protein that associates with members of a family of accessory signaling molecules expressed on T and NK cells, preventing binding of a phosphatase that dampens the activation signal (*9*). A childhood syndrome, associated with severe disseminated infections with nontuberculous mycobacteria and occasionally other intracellular bacteria, results from defective interferon-γ receptor (IFN-γR) signaling from receptor mutations or from defective induction of IFN-γ from mutations in the IL-12 gene or receptor, suggesting a nonredundant requirement for IFN-γ in the handling of these infections by T cells and macrophages (*10*).

Thymic deficiency. The thymus gland, the site where T cells mature from bone marrow–derived progenitors, is required for T cell development. Deletions in the short arm of chromosome 22 are associated with faulty embryogenesis of pharyngeal pouch and cephalic neural crest tissue, resulting in DiGeorge syndrome. The variable extent of de-

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letions results in a spectrum of defects affecting multiple organs, including thymic hypoplasia (*11*). A small subset of these individuals have complete DiGeorge syndrome; they lack all thymic tissue, have only low numbers of dysfunctional peripheral T cells, and exhibit susceptibility to life-threatening infections.

Approaches to Treating Primary T Cell Immunodeficiencies

Hematopoietic stem cell transplantation (SCT). Providing normal bone marrow–derived precursors that can mature into T and B cells in the host has been the mainstay of therapy for children with severe immunodeficiencies, but the outcome depends on the nature of the donor, the underlying disorder, and the timing of transplantation. Patients who receive unfractionated or T cell–depleted bone marrow cells from a human leukocyte antigen (HLA)–identical sibling donor have survival rates exceeding 90%. However, transplantation of T cell–depleted marrow cells from a haploidentical parental donor or from an unrelated donor has been less successful (*12*). This in large part reflects either the development of graft-versus-host disease (GVHD), mediated by residual mature donor T cells contaminating the stem cell graft that recognize the patient as foreign, or rejection of the foreign graft by the host, resulting in failure to reconstitute immune function. Unfortunately, these problems are intertwined, because complete depletion of donor T cells prevents GVHD but increases the risk of graft rejection. Efforts to decrease graft rejection by ablating residual host immune function with cytotoxic chemotherapy and radiotherapy have had little impact on survival, because of toxicity, infections, and interference with immunologic recovery. Therefore, more targeted immunosuppressive strategies, such as administration of monoclonal antibodies (mAbs) to the lymphocyte adhesion molecules LFA-1 and CD2, are being explored (*13*). The development and testing of mAbs that selectively delete NK cells is awaited, because the rejection rate is highest in the large fraction of SCID patients who have functional NK cells (*12*).

Transplantation of haploidentical parental donor cells during the neonatal period (<3.5 months) reduces the risk of graft rejection because of immature recipient immune function, and improves survival (*12*). Indeed, in settings in which SCID can be diagnosed prenatally, in utero transplantation of parental stem cell preparations has been attempted, with encouraging preliminary results (*14*). This method has the potential advantage of facilitating tolerance between donor and host, but because of practical and technical obstacles, it is not clearly superior to neonatal transplantation.

Analysis of immune function has revealed that SCT reconstitutes T cell number and function very rapidly in children with SCID. However, B cell engraftment is more variable and many individuals continue to require immunoglobulin infusions, suggesting a need for better therapy.

Genetic modification of T cells. In ADA deficiency, therapy with purified ADA conjugated to polyethylene glycol (PEG) is noncurative but provides sufficient enzyme activity to rescue some peripheral T cells. The genetic defect in these T cells can potentially be permanently corrected by insertion of the ADA gene into in vitro activated cells via a retroviral vector. Clinical trials in which polyclonal autologous T cells have been modified and reinfused into patients have encouragingly demonstrated long-term persistence of functional ADA^+ T cells. However, obstacles remain; the percentage of ADA^+ T cells achieved is still insufficient to stop PEG-ADA therapy (*15*), and the repertoire of corrected T cells is progressively limited because developing T cells remain ADA-deficient.

Applications of gene therapy to other settings in which peripheral T cells can be isolated, such as deficiencies in TCR components, face additional obstacles. Self-reactive T cells are usually deleted in the thymus during development as a result of signals delivered by TCR engagement. However, T cells with deficient TCR signaling components may escape this negative selection, and establishment of full TCR function by gene insertion after thymic maturation could potentially result in autoimmunity.

Genetic modification of hematopoietic stem cells. In murine models, gene transfer into stem cells has corrected SCID (*16*). Although human stem cells have been less willing recipients of genes, administration of bone marrow progenitor cells transduced with an ADA-containing retroviral vector into ADA-deficient patients did result in accumulation of small numbers of T cells derived from the stem cells (*15*). Umbilical cord stem cells, which cycle more than marrow-derived stem cells, may be better targets for the current gene transfer vectors, which require cell division for integration. Although ADA-deficient newborns treated with transduced cord blood stem cells have also demonstrated gradual accumulation of ADA^+ T cells, it still has not been possible to eliminate concurrent therapy with PEG-ADA (*17*). Thus, broader use of stem cell therapy awaits further evolution of vectors, such as the len-

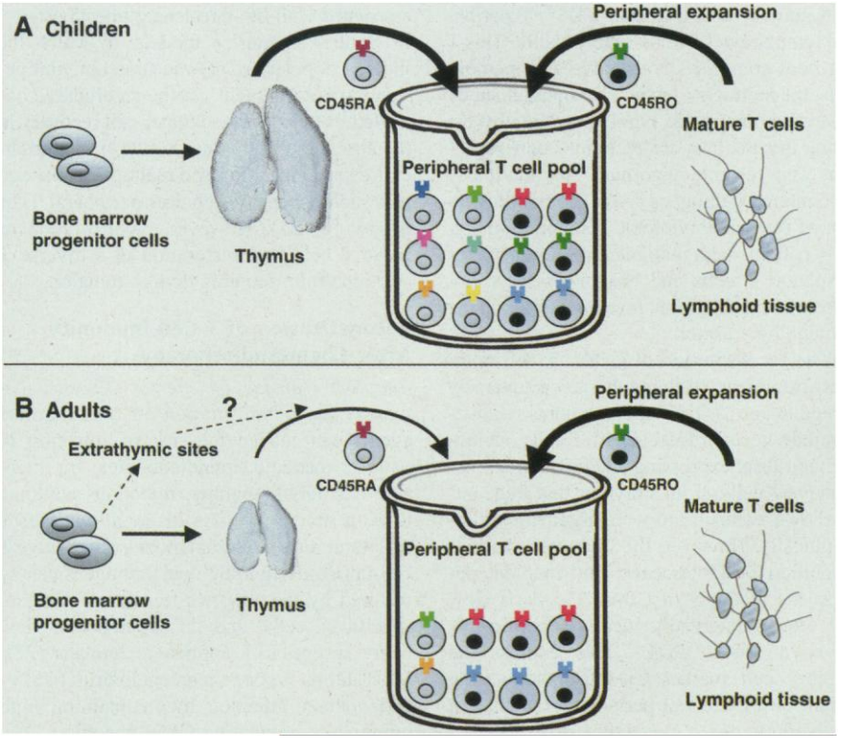

Fig. 1. T cell recovery from chemotherapy. Most lymphocytes are destroyed by cytotoxic chemotherapy. Recovery in children (**A**) results from expansion of mature memory T cells (CD45RO) in the periphery and production of naïve T cells (CD45RA) in the thymus, leading to restoration of normal T cell numbers and a diverse repertoire. As a result of thymic involution, in adults (**B**) the production of naïve T cells is reduced, resulting in a prolonged immunodeficient state characterized by reduced T cell numbers and a restricted repertoire.

tivirus-based vectors, that can insert genes into quiescent stem cells (*18*). However, additional issues still need to be resolved before many SCID syndromes can be successfully treated, such as how to attain the expression of genes like *RAG-1* and *RAG-2* at only the appropriate developmental stages, or how to physiologically regulate the expression of genes like those that encode IL-2 or SAP.

Thymic transplantation. Improvements in techniques for culturing thymic tissue fragments removed from immunocompetent individuals undergoing cardiac surgery have made it possible to consider tissue transplantation to restore deficient thymic function. Indeed, in the subset of patients with DiGeorge syndrome who completely lack a functional thymus, T cell maturation in grafted allogeneic thymic tissue has been observed, with resultant development of a normal repertoire of peripheral T cells (*19*). However, general application of allogeneic thymic transplantation to correct less complete T cell deficiencies due to inadequate thymic function will require the development of methods to prevent rejection by existing host T cells.

Acquired Immunodeficiency After Cytotoxic Chemoradiotherapy

In the normal immunocompetent adult, the total number of $CD4^+$ and $CD8^+$ T peripheral lymphocytes is remarkably stable. This T cell pool comprises both naïve and memory cells, the numbers of which are maintained by separate homeostatic regulators, thereby assuring that the host can recognize new pathogens and respond vigorously to previously encountered pathogens (*20*). The administration of intensive cytotoxic chemoradiotherapy to patients with malignant disease depletes peripheral T cells and challenges these homeostatic mechanisms, revealing kinks in the immunologic armor.

The reconstitution of $CD4^+$ T cell numbers after intensive chemotherapy is inversely related to age (*21*). Younger patients relatively rapidly recover total $CD4^+$ T cells, including the subset expressing CD45RA (a marker of naïve T cells) from a thymus that frequently shows compensatory enlargement radiographically. However, the thymus undergoes involution in adolescence, and in older patients the recovery of $CD4^+$ T cells is slow and often numerically incomplete (Fig. 1). Moreover, most $CD4^+$ cells express the memory cell marker CD45RO, suggesting expansion of residual peripheral T cells that survived cytotoxic chemotherapy but inadequate thymic production (*21*).

Surprisingly, recovery of $CD8^+$ T cell numbers after intensive chemotherapy is more rapid than $CD4^+$ T cell recovery and unrelated to age (*22*). However, the early recovering $CD8^+$ T cells are mostly $CD45RO^+$ and $CD28^-57^+$, representing terminally differentiated cells with limited remaining replication capacity that are also found in increased numbers in elderly patients. By contrast, naïve $CD8^+$ T cell recovery requires a thymic contribution and parallels the recovery of naïve $CD4^+$ T cells (*22*).

Before a SCT for treatment of malignancy, patients receive doses of chemotherapy, or radiotherapy, or both sufficient to completely ablate all endogenous hematopoietic cells, making T cell recovery entirely dependent on the infused cells. Children, even if transplanted with stem cell preparations depleted of mature T cells, rapidly recover naïve $CD45RA^+$ T cells from differentiation of donor progenitor cells in the thymus (*23*). In adult allogeneic SCT recipients, recovery of $CD45RA^+$ cells is delayed, with reconstitution dominated by $CD45RO^+$ T cells of limited TCR diversity; this suggests peripheral expansion of mature T cells infused with the stem cell inoculum (*24*). Depletion of the small number of mature T cells contaminating the stem cell preparation given to adults results in more pronounced immunoincompetence, with further delay in recovery of T cell number and repertoire diversity (*25*). Although the eventual appearance of $CD45RA^+$ cells in adults suggests diminished but persistent thymic function, the prolonged perturbation in T cell number and repertoire is associated with life-threatening infections.

Studies in murine models, in which the infused population, thymic function, and peripheral environment can be rigorously controlled, have confirmed that T cell recovery in the absence of a thymus results from peripheral expansion of infused mature T cells, and that such expansion requires peripheral TCR triggering (*26*). However, reconstitution of naïve T cells and restoration of a diverse T cell repertoire requires thymic function.

Reconstitution of T Cell Immunity After Chemoradiotherapy

Adoptive transfer of effector T cells. Although the administration of antimicrobial agents can reduce the risk of infection in patients rendered immunodeficient by intensive chemoradiotherapy, infections continue to be a severe problem in the absence of T cell immunity. In animal models, protective T cell immunity to individual pathogens can be restored by the adoptive transfer of antigen-specific T cells, and this approach is now being successfully applied to humans (*27*). Most adults harbor cytomegalovirus (CMV) and contain infection by maintaining high numbers of circulating CMV-specific $CD8^+$ cytotoxic T cells. However, the loss of this cytotoxic T lymphocyte (CTL) response after allogeneic SCT permits life-threatening virus replication (*28*). It is now possible to isolate virus-specific $CD8^+$ CTL clones from the blood of the immunocompetent allogeneic stem cell donor, expand these to large numbers in vitro, and transfer the cells to the recipient early after transplant. The infusion of 10^9 cells per square meter of body surface area of cloned CTLs specific for CMV is nontoxic and restores the host CTL response to CMV (*29*). However, maintenance of $CD8^+$ T cell immunity to chronic viral infections requires a concurrent $CD4^+$ T helper (T_H) response, and, although transferred CTLs remain detectable for more than 3 months, a strong CTL response persists only in patients who recover $CD4^+$ CMV-specific T_H cells (*29*). Preliminary studies suggest that adoptive transfer of both $CD8^+$ and $CD4^+$ CMV-specific T cell clones can safely and fully restore persistent T cell immunity to deficient SCT recipients (*30*).

Life-threatening EBV-induced B cell lymphoproliferative disease (EBV-LPD) often develops in recipients of T cell–depleted allogeneic SCT during the period when CTLs to EBV are deficient. Although infusion of unselected polyclonal donor T cells as treatment can lead to sustained regression of EBV-LPD due to in vivo expansion of donor EBV-reactive CTLs, alloreactive T cells present in the infused population mediate GVHD in most patients (*31*). Prophylactic infusions of EBV-reactive T cell lines that have been generated in vitro from donor peripheral blood lymphocytes (PBLs), and contain virus-reactive $CD4^+$ as well as predominantly $CD8^+$ T cells, prevent the development of EBV-LPD and reduce the risk of GVHD (*32*). These transferred T cells with an inserted marker gene can be detected by polymerase chain reaction beyond 18 months. These studies in CMV and EBV disease have established the transfer of virus-specific T cells as an effective strategy for restoring selected T cell responses in immunodeficient patients; this may become standard therapy as the T cell culture technology is simplified.

Reconstituting the diverse T cell repertoire by T cell transfer to achieve full immunocompetence represents a more formidable challenge. Concurrent in vitro stimulation of PBLs with mAbs to CD3 (mimicking TCR signaling) and to CD28 (for costimulation) can induce rapid, exuberant expansion of T cells. In the setting of autologous SCT, the effects of infusion of more than 10^{10} of such polyclonally expanded T cells are currently being evaluated, and preliminary results suggest that the recovery of normal T cell numbers is hastened (*33*). However, many issues remain, including the diversity of the T cell repertoire achieved, the long-term effects on immune reconstitution, and particularly the safety of this procedure, because activating T cells in this manner may bypass normal regulatory mechanisms and expand potentially autoreactive T cells.

Augmenting T cell production. Nonspecif-

ic expansion of donor T cells ex vivo is not suitable for T cell reconstitution after allogeneic SCT because of the risk of GVHD from host-reactive T cells. Thus, interventions to increase host production of naïve $CD4^+$ and $CD8^+$ T cells need to be pursued. Studies in mice have identified potential extrathymic sites of T cell development and have shown that administration of oncostatin M, a member of the IL-6 cytokine family, can promote in athymic mice the development and accumulation of mature functional $CD4^+$ and $CD8^+$ T cells in lymph nodes (*34*). However, convincing evidence for the development of human T cells with a diverse repertoire in the absence of thymic function is still lacking.

Increasing thymic T cell production remains the most physiologic approach to restoring T cell immunity, but is hindered by the decline in thymic mass and function observed with advancing age. However, recent studies using sensitive molecular techniques to identify excision products from rearranged TCR genes in newly matured T cells suggest the thymus of adults beyond age 50 retains a reduced but detectable capacity to generate new T cells (*35*). This is providing renewed impetus to pursue the administration of cytokines or hormones to augment residual thymic function. There are many candidates, such as epidermal growth factors (which can stimulate proliferation of thymic epithelium) or factors that normally stimulate thymopoiesis but have declined with age (such as growth hormone and its mediator, insulin-like growth factor–I). Two promising approaches are suggested by recent murine studies: (i) administration of IL-7, which is normally produced by thymic epithelium and can increase thymic cellularity and output in mice undergoing SCT (*36*), and (ii) ablating production of sex steroids, which can lead to rapid regeneration of thymic tissue and function in aged mice with an atrophic gland. The effects of such manipulations in humans await clinical investigation.

T Cell Immunodeficiency After Infection with HIV

HIV infection is now the most common etiology of acquired T cell immunodeficiency. The virus binds and enters T cells expressing the CD4 molecule, but subsequently integrates and replicates most efficiently in activated T cells. This process predictably leads to preferential depletion of memory $CD45RO^+CD4^+$ T cells, which are more likely than naïve T cells to be engaged by antigen (*37*). However, the perturbations of T cell immunity induced by HIV are considerably more complex than a direct cytopathic effect on memory $CD4^+$ T cells (Fig. 2). HIV-infected adults also have declines in naïve $CD45RA^+CD4^+$ and, surprisingly, in $CD45RA^+CD8^+$ T cells, because of a composite of events including infection of immature developing $CD4^+8^+$ thymocytes, damage to thymic architecture and function, and aberrant sequestration of T cell subsets (*38*). In some children infected intrapartum, HIV infection of thymic epithelium leads to complete disruption of T cell development, similar to individuals with DiGeorge syndrome (*39*).

Uninfected T cells are also lost as a result of apoptosis mediated by several mechanisms, including a partial activation signal delivered when the HIV protein gp120 cross-links CD4, Fas (a death receptor)–mediated death of $CD4^+$ and $CD8^+$ T cells after interaction with $CD4^+$ cells and macrophages induced by HIV to express Fas ligand, and toxic effects of the HIV tat protein (*40*). Apoptosis may be responsible for the $CD8^+$ T cell lymphopenia observed late in disease, a time when HIV isolates that use the CXCR-4 coreceptor usually predominate. Engagement of CXCR-4 by these isolates induces expression of tumor necrosis factor–α receptor II (TNFRII) on $CD8^+$ T cells and membrane-bound TNF-α on macrophages, with subsequent cellular interaction leading to apoptosis of the $CD8^+$ cells (*41*).

Potential compensatory mechanisms to replenish the lost T cells include increasing thymic production of naïve cells and peripheral expansion of residual memory cells. However, thymic function is impaired, and memory $CD4^+$ T cells are preferentially eliminated by the virus. Thus, a progressive

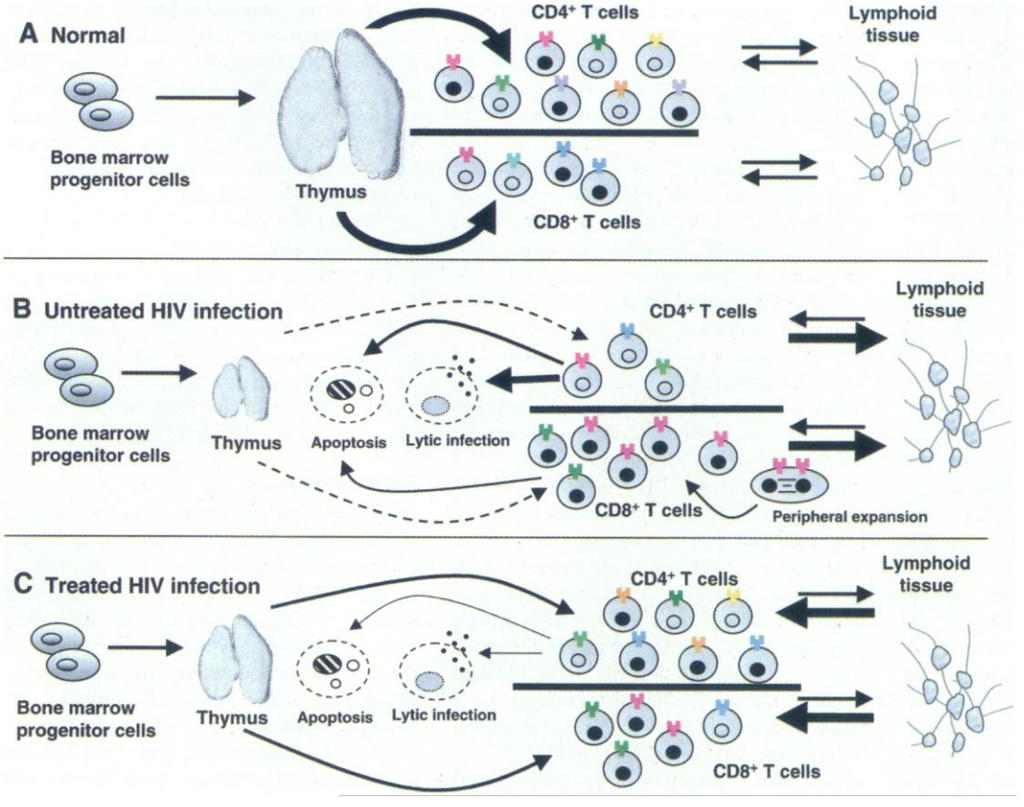

Fig. 2. Peripheral T cell pool in HIV disease. HIV infection decreases the peripheral pools of both $CD4^+$ and $CD8^+$ T cells because of diminished thymic production of new T cells, marginalization of cells to lymphoid tissues, and apoptosis of uninfected cells, in addition to selected $CD4^+$ cell loss due to lytic infection. The $CD8^+$ population initially overcompensates by expanding peripheral memory cells. These changes result in a shrunken T cell population with a limited T cell repertoire and reversal of the CD4/CD8 ratio. After antiretroviral treatment, T cell numbers increase because of improved thymic output, decreased cell loss, and redistribution from lymphoid organs. However, the repertoire remains contracted and can only be corrected by continued output of new cells by the thymus.

immunodeficiency ensues, characterized by severe depletion of $CD4^+$ T cells, an initial peripheral expansion followed by gradual loss of memory $CD8^+$ T cells, and shrinkage of the $CD4^+$ and $CD8^+$ T cell repertoires.

Controlling HIV Replication and Reconstituting Immunity

Antiretroviral drug therapy. Restoring T cell immunity requires control of HIV replication to halt destruction of the immune system. New combination antiviral drug regimens with protease and reverse transcriptase inhibitors can markedly reduce viral load in most patients, often leading to a rapid increase in memory $CD4^+$ and $CD8^+$ T cells due to redistribution from lymphoid organs, peripheral expansion, and reduction in cell death (*42*). However, repair of the contracted T cell repertoire is delayed for many months, and ultimately requires thymus-dependent production of new T cells (*43*). Unfortunately, current regimens often do not completely inhibit viral replication and do not eliminate the reservoir of latent virus in resting $CD4^+$ T lymphocytes. Thus, many patients eventually fail therapy because of the emergence of drug-resistant viruses, or resurgence of virus replication due to cessation of therapy because of drug toxicity or to poor compliance with the complex regimen (*44*).

Adoptive transfer of HIV-specific T cells. Considerable evidence suggests that $CD8^+$ T cells are the primary effector cells in host defense to HIV, limiting HIV replication after primary infection and delaying progression to disease, but ultimately are unable to contain the virus (*45*). Thus, adoptive transfer of autologous virus-specific CTLs isolated from the blood and expanded in vitro is being evaluated as a means to quantitatively boost HIV immunity (*46*). Modification of $CD8^+$ Gag-specific CTL clones with a retroviral vector containing a marker gene has made it possible to monitor in vivo activities. The infusion of more than 10^9 CTLs/m^2 achieved frequencies of 1 to 4% of the $CD8^+$ T cells in the peripheral blood, and also increased HIV-specific cytolytic activity (*47*). Over the next several days, the transferred CTLs localized to lymph nodes and accumulated at sites adjacent to $CD4^+$ cells actively replicating HIV. Concurrently, CTL-mediated antiviral activity was evidenced by a decline in the number of circulating HIV-infected cells. However, the HIV-specific CTLs persisted very briefly in vivo, resulting in only a transient antiviral effect. This brief survival likely reflected an inadequate endogenous HIV-specific $CD4^+$ T_H response required to sustain the $CD8^+$ response (*48*). Improving therapeutic efficacy will require not only sustaining CTL immunity by providing or augmenting helper function, but also achieving a much stronger CTL response, as suggested by quantitative studies of the frequency in peripheral blood of virus-specific CTLs required to mediate clearance of viruses (*49*). Trials addressing these issues are currently being performed.

Genetic modification of T cells and hematopoietic stem cells. Attempting to restore deficient $CD4^+$ T_H responses in the context of continued HIV infection is enigmatic. Predictably, infusions of normal $CD4^+$ cells into patients from an uninfected identical twin donor do not lead to sustained reconstitution (*50*). Genetic modification of susceptible cells can impart resistance to HIV by interfering with critical steps in the virus life cycle, and many strategies are being evaluated. RNA-based inhibitors include trans-activation response (TAR) element and Rev response element (RRE) decoys that sequester Tat or Rev or ribozymes engineered to cleave targeted sites in the HIV genome (*51*). Protein-based inhibitors include a dominant negative mutant Rev protein (RevM10) and engineered intracellular antibodies or chemokines that bind and sequester specific HIV proteins or cellular coreceptors, respectively (*52*).

In an early clinical trial, autologous polyclonal $CD4^+$ T cells modified with RevM10 infused into HIV-infected patients survived longer than unprotected cells in vivo (*53*). Transferred CMV-specific $CD4^+$ T_H clones modified to express both TAR and RRE decoys similarly survived longer than control clones in HIV-infected hosts (*30*). This latter approach is now being extended to determine whether an effective host immune response to HIV can be established by the transfer of expanded numbers of $CD4^+$ HIV-specific T cell clones rendered genetically resistant to HIV.

Genetic modification of hematopoietic progenitor cells could potentially lead to protection of all hematopoietic cells at risk of infection, including lymphocytes, monocytes, and dendritic cells. The major obstacles are quantitative—inefficient transduction of quiescent hematopoietic stem cells and limited in vivo selection of protected stem cells that are not targets of HIV. Thus, reconstitution of infected individuals with a new resistant immune system remains a conceptually attractive but practically elusive goal.

Cytokine therapy. HIV infection is associated with abnormal production of many cytokines that may be amenable to replacement therapy. Deficient IL-12 production by APCs may contribute to the inadequate production of IFN-γ by T cells, which in turn may lead to decreased resistance to the virus. However, administration of IFN-γ has had minimal activity in clinical trials, and IL-12 administration did not decrease viral burden in a primate model for HIV (*54*).

The administration of IL-2, a potent T cell growth factor that is deficient in HIV-infected patients, has shown more in vivo activity. Therapy with high doses of IL-2 led to increased numbers of $CD4^+$ T cells, but transient increases in plasma HIV concentrations were noted and toxicity limited the duration of therapy (*55*). Lower doses of subcutaneous IL-2 cause less toxicity and do not increase plasma HIV, but result in smaller increases in $CD4^+$ T cells (*56*). Although persistent therapy with IL-2 may substantially increase $CD4^+$ T cell numbers in some patients, this approach expands existing T cells and is not likely to correct the defects in the T cell repertoire. An alternative use of IL-2 now being pursued is to activate latently infected cells in patients receiving antiretroviral therapy, because inducing HIV gene expression might render this reservoir susceptible to elimination.

Restoration of thymic function. HIV can infect thymic epithelial and dendritic cells. This not only results in disruption of thymic architecture, abnormal cytokine production, and a decline in output of naïve T cells, but can potentially permanently impair thymic function (*57*). However, preclinical studies in SCID-hu mice grafted with a human HIV-infected thymus suggests that antiretroviral therapy can restore thymic T cell production (*58*). Indeed, effective control of HIV replication with drug therapy, particularly in infected young adults, appeared to result in a rapid increase in thymic output of new T cells. However, thymic production remained subnormal in comparison to age-matched controls after 8 months of therapy in seven of nine HIV-infected individuals, and did not increase at all in patients over the age of 60 (*35*). These results indicate that thymopoiesis is surprisingly resilient in HIV-infected individuals, but still suggest that approaches to boost thymic output would be beneficial, especially in older patients.

Thymic transplantation has been proposed as a means to restore thymopoiesis and hasten T cell recovery in HIV-infected patients. However, as previously discussed, procuring and preventing rejection of transplanted allogeneic thymic tissue remain serious obstacles. Thus, alternative approaches that reverse thymic atrophy or improve output from residual thymic tissue will need to be explored.

Future Prospects

Restoring T cell immunity by directly targeting the responsible defect represents a relatively new therapeutic endeavor, and has required the development of both means to precisely define the nature of the deficiency and reagents to specifically enhance T cell development and function. Major challenges remain. For many primary deficiencies, developing methods to improve engraftment of purified haploidentical stem cells should yield an effective therapy. Gene therapy that

will selectively provide a normal gene product is a promising alternative, but will require the evolution of improved vectors to efficiently deliver regulated genes to quiescent stem cells. For acquired deficiencies, adoptive transfer of expanded numbers of specific T cells should prove increasingly useful for providing protection from specific pathogens, but broader correction of the underlying immunodeficiency will require methods to increase the production of new T cells. Designing strategies to promote formation of competent mature T cells from progenitors either in tissue culture or by augmenting in vivo thymic function should be an area of fruitful effort in the next several years.

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