The Kinetics of Immune Reconstitution After Human Marrow Transplantation

By Lawrence G. Lum

Human marrow transplantation for the treatment of malignant and nonmalignant disorders is becoming an established modality of therapy. As in any aggressive therapeutic modality, the benefits must be balanced with the risks of the therapy. The aggressive chemoradiotherapy used to prepare patients for marrow transplantation creates a transient immunodeficiency disorder postgrafting until the transferred donor marrow reestablishes a competent immune system. Immune reconstitution posttransplant follows a general pattern developing from immature to mature immune functions. Immune reactivity during the first month postgrafting is extremely low. Cytotoxic and phagocytic functions recover by day 100, while more specialized and cooperative functions of T and B cells remain impaired up to one year or more postgrafting. After the first year postgrafting, the various components of the immune systems of most healthy marrow recipients begin to work synchronously, whereas the immune systems of recipients with chronic graft-v-host disease (GVHD) remain crippled. Recent evidence shows that transfer of specific immunity from marrow donors to marrow recipients plays a role in reestablishing immunocompetence. Transferred antigen-specific immunity may explain why more recipients do not die from overwhelming infections.

Bone marrow transplantation is an accepted mode of therapy for immunodeficiency disorders, aplastic anemia, or hematologic malignancies. The chemoradiotherapy used to prepare marrow graft recipients ablates existing normal and abnormal immunohematopoietic marrow elements, provides immunosuppression to permit engraftment, and creates space in the marrow microenvironment for the donor marrow to develop. Although the immunodeficiency is created under carefully controlled circumstances, the marrow graft recipients are extremely susceptible to life-threatening infections with a variety of bacterial, viral, and fungal pathogens.

The first 100 days postgrafting is the interval of maximum risk; however, marrow graft recipients with chronic graft-v-host disease (GVHD) continue to be at risk for bacterial, viral, and, less frequently, fungal infections.

Marrow transplantation offers a model of iatrogenic immunodeficiency that is unique in that immunity generally recovers if the transplant recipient does not succumb to transplant related complications or recurrent disease. This model permits dissection of the various elements of the human immune system as they reappear under the influence of various treatment modalities postgrafting. An understanding of the kinetics of immune reconstitution in the marrow transplant model will enhance our understanding of immunodeficiency disorders. Parallels can be drawn between the transplant model and the defects seen in immunodeficiency disorders and the regressing immune systems of patients with acquired immunodeficiency syndrome (AIDS).

The various in vivo and in vitro immune function tests of the marrow recipients will be discussed in terms of the first 100 days (short-term recipients) and from 100 days to years (long-term recipients) postgrafting. The areas that will be reviewed include: (1) general considerations of immunity, (2) issues related to acute GVHD in short-term recipients, (3) issues related to chronic GVHD in long-term recipients, (4) mucosal defense system, (5) recovery of granulocyte and accessory cell functions, (6) recovery of cytotoxic functions of lymphocytes, (7) T cell-mediated immunity early postgrafting, (8) T cell-mediated immunity in long-term recipients, (9) B cell-mediated immunity early postgrafting, (10) B cell-mediated immunity in long-term recipients, (11) use of immunomodulators to accelerate immune reconstitution, (12) reimmunizations for marrow recipients, (13) recapitulation of ontogeny, and (14) future directions.

General Considerations of Immunity

Studies at a number of transplant centers have shown that the repopulation of the immune and hematopoietic systems is dependent on appropriate proliferation, maturation, and differentiation of donor cells. The signals that direct immune

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reconstitution are largely unknown. However, the key factor in immune reconstruction is time after grafting. Success-
ful immune reconstruction is dependent on the orchestrated emergence of many different components of the immune
system. All marrow graft recipients have a profound impair-
ment of most immune functions during the first four to five
months postgrafting, regardless of the type of graft (auto-
logous, syngeneic, or allogeneic), the type of underlying dis-
ease, the conditioning regimen, the type of postgrafting
immunosuppression, the preparative regimen, or the pres-
ence of acute GVHD. Immune reconstitution in most healthy long-term survivors is successfully completed by one
to two years postgrafting, whereas the same process in
recipients with chronic GVHD is delayed, leaving them
susceptible to infections.

The marrow recipient progresses from an immunologic
state characterized by primitive cytotoxic systems such as
natural killer activity, and the predominance of cells with
suppressive functions to a more mature immunologic state
characterized by finely tuned immune responses. Initially
there is reestablishment of phagocytic functions followed by
repopulation of the early lymphoid elements responsible first
for primitive and then for more specific cytotoxic functions.
Subsequently T cells responsible for regulatory functions
emerge. The early suppressive T cell signals are followed by
the development of mature helper T cell signals. Evidence
suggests that the transition from a predominance of suppres-
sion to a predominance of helper activity in the recipient can
be disrupted by chronic GVHD or its treatment. Transfer of
existing donor immunity may play a role in achieving anti-
gen-specific immune reconstitution in both the T and B cell
compartments of the recipient immune system.

The role of the host thymus in this reconstitution process
remains elusive in human marrow transplantation. Age of
recipient at transplant, irradiation, and chemotherapy could
all affect the functional capacity of the thymic microenviron-
ment to properly facilitate the maturation of donor T cells.
Furthermore, other nont hymic components of the immune
system that may play roles in controlling the recapitulation
of immune ontogeny could also have been damaged during
the preparative regimen. Though ill defined, both thymic and
nonthymic factors influence immune reconstitution.

Although host immunity is severely compromised by the
preparative regimens, it is not absent. Graft rejections or lack
of sustained engraftment in patients with severe aplastic
anemia or patients who receive T cell-depleted marrow are
probably mediated by persistent host immunity. Pretrans-
plant transfusion sensitization increases graft rejections in
patients with aplastic anemia, whereas untransfused aplas-
tic anemia patients have a lower incidence of graft rejection
than their transfused counterparts.

Graft failures in recipients who receive T cell-depleted
bone marrows to prevent acute GVHD suggest that removal
of all T cells may be a two-edged sword. Vigorous removal of
T cells has decreased the incidence of acute GVHD but has
increased the incidence of graft failure and recurrent leukem-
ia. Graft failure occurs presumably due to residual irradia-
tion-resistant host cells that destroy the marrow graft. (The
role of cytotoxic T cells in GVHD and host defense is
reviewed on later pages.) The general consensus is that
certain T cell populations in the marrow inoculum may be
needed to facilitate sustained engraftment in the face of host
resistance and to maintain immune surveillance against the
expansion of residual leukemic clones.

Low levels of persistent host immunity may play a protec-
tive role against bacterial, fungal, and viral pathogens in the
first three months after marrow grafting. Persistent host-
type isohemagglutinins can be detected up to three to four
months postgrafting in recipients who receive ABO incom-
patible marrows. These findings suggest that surviving chemoradioreistant host lymphocytes or persistent antigen
may have a role in the education of naive donor cells.

ISSUES RELATED TO ACUTE GVHD
IN SHORT-TERM RECIPIENTS

When the emerging immune system in the recipient does
not recognize antigens on host tissues as self, it may mount
an appropriate immunologic reaction against foreign anti-
gens (acute GVHD). This ability to attack host tissues
demonstrates “misdirected” immunocompetence of the
graft. This misdirected immunocompetence may be due to
improper thymic reeducation, altered thymic microenviron-
ment, or host-recipient histoincompatibility. Unfortunately,
this attack often has very serious effects on the marrow
recipient. The principal target organs of acute GVHD in
animals and man are the skin, liver, and gastrointestinal (GI)
tract. Cytotoxic cells are thought to play a major role in
the immunologic attack. The clinical manifestations of acute
GVHD range from mild skin rashes, impairment of liver
function, and GI disturbances such as nausea, vomiting, and
diarrhea to severe disease with full thickness skin eruptions
with bullae formation, liver failure, bloody diarrhea from
denuded intestinal epithelium, and depressed immune func-
tions leading to life-threatening sepsis. Roughly half the
marrow recipients transplanted with allogeneic bone marrow
from HLA-identical siblings develop acute GVHD. Approxi-
ately 10% to 15% of all marrow recipients die of acute
GVHD or its associated complications. Acute GVHD, per se,
results in immunosuppression.

Acute GVHD may be enhanced by the presence of entero-
bacteria in the gut. The enterobacteria invading the gut
mucosa may share antigenic determinants with host epithe-
lium resulting in enhanced cytotoxic immune responses
directed at the mucosal lining. Observations that support
this hypothesis are (1) recipients with acute GVHD have
more infections than those without GVHD, and (2) the
protective environments of laminar air flow rooms reduce the
number of infections and delay the onset of acute GVHD in
recipients transplanted for aplastic anemia.

Since acute GVHD is a major impediment to successful
marrow transplantation, immunosuppressive agents have
been given pre- or postgrafting to prevent the development of
acute GVHD. These agents may further immunosuppress
the recipient’s immune system and prolong the susceptibility
to infections. Methotrexate, cyclophosphamide, and cyclo-
sporine (Cs) have been commonly used to prevent GVHD in
man. The advantage of Cs over methotrexate is faster
engraftment, less mucositis, decreased platelet support, and a shorter hospital stay. However, immune function tests in those who received Cs prophylaxis for GVHD were not different from those who received methotrexate. The recent combination of methotrexate and Cs to prevent GVHD has been more successful than methotrexate alone in reducing the incidence of acute GVHD and in improving long-term survival.

Adapting the well-characterized murine transplant models using T cell depletion for preventing GVHD in man has been cumbersome at best. Since cytotoxic T cells are thought to mediate the damage to tissues in acute GVHD, methods to remove T cells from marrow inocula have been used to prevent GVHD. These methods include treatment with antithymocyte globulin, monoclonal antibodies (MoAbs) directed at T cells, lectin agglutinin sedimentation, or sheep erythrocyte rosetting. Although T cell depletion procedures have decreased the incidence and severity of acute GVHD, the general consensus is that over-zealous T cell depletion has led to graft failure in recipients transplanted for acute leukemias. Furthermore, vigorous T cell depletion may be removing T cells that mediate a graft-vs-leukemia effect. Most transplant groups agree that the incidence of leukemic relapse has increased with T cell depletion of the marrow graft. These procedures may also lead to increased abnormalities of immune reconstitution.

Recent studies show that depleting CD4 cells from the marrow may prevent acute GVHD and still permit sustained engraftment. Although most models implicate cytotoxic CD8 cells in the pathogenesis of GVHD, there is no evidence that rules out the participation of CD4 cells or both CD4 and CD8 cells in the pathogenesis of acute GVHD. Follow-up studies on recipients who receive T cell-depleted marrow grafts will help sort out such issues.

No data directly support the hypothesis that specific cytotoxic cells or specific antibodies mediate the immune reaction seen in clinical GVHD. Incriminating immunologic evidence for the existence of specific alloreactive T cells or specific antibodies directed at tissues bearing non-HLA antigens is absent. Mixed lymphocyte culture assays using donor-derived cells from marrow recipients with acute GVHD failed to detect proliferative responses to cryopreserved host lymphocytes. One recent study showed that T cell clones isolated from skin biopsies involved in acute GVHD could proliferate in response to host lymphocytes; however, direct cytotoxicity mediated by such cells was not shown. New approaches for understanding the immunobiology of GVHD are needed. Simple questions remain unanswered, such as “What are the effector and effecter cells mediating GVHD in human transplant recipients?” and “What are the target cells in the various organs systems affected by GVHD?”

A promising predictive test for acute GVHD was recently developed. Donor lymphocytes were sensitized against recipient lymphocytes in vitro and cocultured with recipient skin. The coculture system showed histologic changes similar to acute GVHD and was able to predict acute GVHD. Such a test may be useful in selecting donors for transplantation and selecting the type of GVHD prophylaxis.

**MUCOSAL DEFENSE SYSTEM**

Although difficult to quantify, chemoradiotherapy damage to the mucosa in the marrow recipient is a major insult to the host immune system. The mucosal defense systems are then further damaged by acute GVHD. Alterations in the integrity of mucosal linings and the impaired local IgA
secretion by B cells in the linings of the intestinal, respiratory, and urinary tracts may provide easy access for invading pathogens. It is clear from the types of infections that occur and the timing of such infections that mucosal immunity ("the first line of defense") is severely impaired in the first 100 days postgrafting and continues to be impaired in recipients who have chronic GVHD. Skin biopsies from long-term recipients with chronic GVHD show histologic evidence of sialadenitis, stomatitis, or both with sialodochitis. Salivary gland involvement was associated with decreased or absent levels of IgA, decreased salivary flow rates, and increased concentrations of sodium, albumin, and IgG. If one adds these findings to the low serum IgG and IgM levels in the first several months after grafting and serum IgA levels that remain low for more than one year postgrafting in the majority of marrow graft recipients, it is not surprising that fatal infections occur despite protection afforded by antibiotics, laminar air flow rooms, granulocyte transfusions, and immune globulin transfusions.

PHAGOCYTIC AND ACCESSORY CELL FUNCTIONS

Studies on neutrophils show that their numbers normalize early, but they have impaired chemotaxis up to four months postgrafting. Neutrophil iodination capacity, a measure of the ability of neutrophils to phagocytize and activate oxygen-dependent mechanisms of intracellular killing, were not different from normal controls during the early period. These findings partially explain why fungal and bacterial infections are common postgrafting when neutrophils are absent and neutrophil chemotaxis is impaired.

Peripheral blood monocytes from marrow recipients can be shown to be of donor origin by cytogenetic markers as early as 41 days postgrafting. Tissue-fixed macrophages in the lung and liver are of donor type by day 80 postgrafting. Monocytes have been reported to exhibit normal chemotaxis, adherence, phagocytosis, and cytotoxicity early postgrafting.

Studies on the antigen-presenting accessory cell functions of peripheral blood monocytes from marrow recipients show that monocytes can successfully present killed Escherichia coli bacteria in proliferation assays. Enriched monocyte populations from the majority of short-term and long-term marrow recipients were capable of providing accessory cell function in T cell-dependent pokeweed mitogen-stimulated immunoglobulin production. Suppressor activity in pokeweed mitogen-stimulated immunoglobulin production mediated by monocytes was detected in only a few marrow recipients with chronic GVHD. Interleukin 1 (IL 1) production by monocytes from marrow recipients is normal by five to six weeks postgrafting. By six weeks posttransplant, monocyte functions are generally intact.

CYTOTOXIC FUNCTIONS OF LYMPHOCYTES

Impaired T cell-mediated or natural killer (NK) cell-mediated cytotoxicity directed at viruses may account for nearly one half of the allogeneic recipients developing infections with the herpes group of viruses postgrafting. Cytomegalovirus (CMV) accounts for roughly 60% of the interstitial pneumonias that occur during the first three months postgrafting. Attempts to prevent or treat CMV pneumonias with various agents or CMV immune globulin have met with variable success.

The role of NK cells directed at viruses and cytotoxic T cells directed at viruses during the immediate postgrafting period remains controversial. Most studies show that NK activity against K562 or Chang targets, lectin-dependent cellular cytotoxicity, and antibody dependent cellular cytotoxicity recover rapidly to normal levels in the majority of marrow recipients by 30 to 50 days postgrafting, but up to 20% of the marrow recipients continue to have deficient cytotoxic functions a year or more postgrafting. One study showed that HLA-restricted CMV-specific cytotoxic T lymphocyte responses to CMV and natural killer cell responses to CMV develop postgrafting and that low or nonexistent cytotoxic responses occur at the onset of CMV infections in recipients who develop fatal infections. Another study showed that NK activity fell from normal levels when recipients developed severe viral infections, and there was a correlation between the incidence of acute GVHD and high NK activity. Other studies show no significant correlations between cytotoxic activity and clinical findings such as infections, GVHD, and recurrent leukemia.

Impaired cell-mediated lympholysis (CML) occurred in cells from recipients with acute and chronic GVHD, whereas most short-term and long-term recipients without GVHD had normal CML responses. Interleukin 2 (IL 2) production was reduced in short-term recipients with acute GVHD. The addition of IL 2 to the mixed lymphocyte cultures during the sensitization phase could restore CML reactivity in lymphocytes from recipients with acute GVHD but not in lymphocytes from recipients with chronic GVHD. One simple explanation for the heterogeneous and conflicting reports on cytotoxic cell function would be the differences in the techniques used to study recipients and the differences in the study populations. A unifying and biologically relevant explanation is that the current studies may reflect a spectrum of maturational stages of cytotoxic cells.

T CELL-MEDIATED IMMUNITY EARLY POSTGRAFTING

Donor-derived T and B cells repopulate the peripheral blood in allogeneic marrow recipients within the first three months. However, repopulation of T cell subsets as defined by surface phenotypic markers using monoclonal antibodies or Fc-receptor rosetting occurs at different rates for both subsets and individual recipients. The consensus of most studies using phenotypic markers for the helper-inducer phenotypes (CD4) and the suppressor/cytotoxic phenotype (CD8) postgrafting is that the absolute number of CD4 cells is lower than normal in the first three to six months postgrafting, and the absolute number of CD8 cells is higher than normal in the first six months postgrafting resulting in lower than normal helper-inducer/suppressor-cytotoxic cell ratios. Similarly, the proportions of Fe-IgG receptor-bearing T cells (Tg, suppressor phenotype) were increased, and the proportions of Fe-IgM receptor-bearing T cells (Th, helper phenotype) were decreased early after transplant.
leading to decreased T<sub>G</sub>/T<sub>M</sub> ratios observed in recipients up to three years posttransplant. Immature T cells or thymocytes (OKT10) were found circulating in small numbers up to six to 12 months postgrafting. The expression of HLA-DR (Ia-like) antigens was increased after three months postgrafting. Several groups have shown associations between viral infections and decreased CD4/CD8 ratios; but other groups have not shown such an association. In autologous marrow graft recipients it has been suggested that low CD4/CD8 ratios may be predictive of CMV infections. Some groups showed a correlation between low CD4/CD8 ratios and acute GVHD. However, other studies indicate that most short-term autologous marrow recipients have low CD4/CD8 ratios. Long-term recipients with chronic GVHD persist in having low proportions of CD4 cells and high proportions of CD8 cells.

The expression of IL<sub>2</sub> receptors, identified by the MoAb anti-TAC, on T cells from marrow recipients less than six months postgrafting is controversial. One group did not find IL<sub>2</sub> receptor positive T cells, whereas a more recent report showed >25% of the cells in the peripheral blood of marrow recipients undergoing graft rejection or acute GVHD were IL<sub>2</sub> receptor positive. In our studies peripheral blood lymphocytes from five of 12 short-term recipients with or without GVHD between 45 and 83 days postgrafting expressed IL<sub>2</sub> receptors ranging from 1.0% to 12.3% (Lum, unpublished). The expression of IL<sub>2</sub> receptors may represent in vivo activation of T cells by any number of clinical events or treatments. Investigators have found that OKT10 and Ia were expressed on the T cells from short-term recipients. Inverted CD4/CD8 ratios and the expression of OKT10 and Ia antigens may simply reflect immature and/or regenerating T cell populations early after marrow grafting with CD8 cells arising first. The effects of chronic GVHD on this sequence of events are discussed later.

In vivo cellular immunity as measured by delayed-type hypersensitivity is impaired in the first 100 days postgrafting. In vitro proliferative responses after stimulation with mitogens, specific antigens, and alloantigens measured by <sup>3</sup>H-thymidine uptake are low during the first 100 days postgrafting.

A number of investigators have used a variety of in vitro systems for testing the ability of T and B cells from short-term marrow recipients to produce immunoglobulin after nonspecific activation with agents such as pokeweed mitogen or Protein A from Staphylococcus aureus. T cells from two thirds of the recipients did not provide T cell helper activity and had increased suppressor T cell activity. More recent studies designed to identify T cell subsets responsible for abnormalities in T cell function show that CD4 cells failed to prevent and frequently exhibited suppression, and CD8 cells did not routinely exert suppressor activity. Overall, T cells failed to help their B cell counterparts during the early period postgrafting to produce immunoglobulin. Such functional data indicate that T cells are not ready to perform in a concerted fashion with B cells early after grafting. In addition, T cells responsible for suppressor activity directed at Epstein-Barr virus (EBV)-driven B cell clones may not function, thereby permitting the development of B cell lymphomas early after transplantation in those receiving T cell-depleted bone marrow. This notion is supported by observations in our laboratory showing that EBV-driven immunoglobulin secretion by donor B cells is often not suppressed by recipient T cells, whereas normal T cells do suppress the EBV-driven immunoglobulin secretion response of B cells.

T CELL-MEDIATED IMMUNITY IN LONG-TERM RECIPIENTS

After one year postgrafting, marrow recipients without chronic GVHD normalize their CD4/CD8 ratios, but those with chronic GVHD have persistently elevated proportions of CD8 cells with decreased proportions of CD4 cells in their peripheral blood. Low numbers of circulating CD4 cells in recipients with chronic GVHD may be a result of selective in vivo sequestration and/or depletion by ongoing GVHD reactions occurring in the skin, liver, or gut. This inverted ratio is associated with a number of in vitro findings described in the following sections.

Greater than 90% of marrow recipients make at least one positive response to a battery of five recall skin test antigens by four years postgrafting. Proliferative responses to mitogens, alloantigens, and specific antigens return to normal or near normal values after six months to one year postgrafting in recipients without chronic GVHD. Early studies performed in the pokeweed mitogen system using T cells from long-term marrow recipients with and without chronic GVHD showed that roughly one half of those with chronic GVHD had T cells that failed to provide helper activity and exhibited excessive suppressor activity. Fractionation of T cells from recipients with chronic GVHD into Fc-IgG receptor positive T cells (T<sub>G</sub>) and Fc-IgG receptor negative T cells (T<sub>G</sub>) showed heterogeneous helper and suppressor activity in both T cell subsets. Such T cell defects were less frequent in healthy long-term survivors. One study showed increased spontaneous Ig secretion by B cells from recipients with chronic GVHD.

Later studies showed that excessive suppressor T cell activity, for the most part, was due to increased proportions of CD8 cells. CD4 cells from one third of the patients with chronic GVHD did not provide helper activity, whereas CD4 cells from healthy long-term survivors consistently provided helper activity in the pokeweed mitogen system. Studies using immunoglobulin production assays show heterogeneous functional responses in the CD4 and CD8 T cell subsets from recipients with and without chronic GVHD.

In a recent study, tetanus toxoid, herpes simplex type 1 virus, and pokeweed mitogen were used to develop functional profiles of CD4 and CD8 subsets from long-term marrow graft recipients. The study showed that immune reconstitution occurs in a variable fashion associated with extremely heterogeneous T and B cell subset functions. Recipients with chronic GVHD had delayed reacquisition of normal function within each T cell subset. The spectrum of functional responses found in the T cell subsets may reflect developmental stages within each phenotypic lineage postgrafting. However, these nonspecifically stimulated functional assays of immunoglobulin production did not correlate
with in vivo specific antibody titers to tetanus toxoid or herpes simplex virus on a per patient basis.\textsuperscript{105}

**B CELL-MEDIATED IMMUNITY EARLY POSTGRAFTING**

Levels of IgG and IgM in the serum return to normal after three to six months postgrafting, but levels of IgA may remain low for years.\textsuperscript{7-10,12} IgE levels have been reported by several groups to be elevated in association with acute GVHD.\textsuperscript{106-108} Levels of the total hemolytic complement, the third component of complement, and the fourth component of complement are normal in the first three months postgrafting.\textsuperscript{12,28} Allogeneic and syngeneic marrow recipients have severely depressed antibody responses to immunizations with the neoantigens keyhole-limpet hemocyanin, pneumococcal polysaccharide type 3 antigen, and bacteriophage 0X174 in the first six months postgrafting.\textsuperscript{12,23,35} An early study used specific antibody titers to keyhole-limpet hemocyanin and yellow fever virus as immunologic probes in a short-term marrow recipient to prove lymphoid engraftment after transplantation from an identical twin donor who was immunized to keyhole-limpet hemocyanin and yellow fever virus pretransplant.\textsuperscript{109} The recipient developed antibody titers to keyhole-limpet hemocyanin. Recent data from this and other laboratories show that IgG antibody titers to recall antigens such as tetanus toxoid, diphtheria toxoid, and measles virus can be detected within the first 100 days postgrafting with or without booster immunizations to the test antigens.\textsuperscript{110-112} Two hundred and twenty-one of 235 (94\%) short-term patients had titers to tetanus toxoid, 176 of 232 (76\%) short-term recipients had titers to diphtheria toxoid, and seven of eight (87\%) short-term recipients had titers to measles virus\textsuperscript{112} (Lum, unpublished). Specific antibody titers to recall antigens in short-term marrow recipients were in the normal range without booster immunizations, whereas responses to primary and secondary immunizations with neoantigens postgrafting were severely depressed during the early period postgrafting.

The source of the specific antibodies to recall antigens during the first 100 days remains unclear. The likely source of antibody is B cells of donor origin. However, persistent host antibodies, persistent host B cells producing antibody, passively acquired antibody via blood product support, and the stimulation of already precommitted memory B cells transferred from the donor may all contribute to antibody titers to recall antigens. Antigen-specific helper activity may not be required for in vivo antibody synthesis by precommitted B cells. This hypothesis is supported by one report that shows that titers to tetanus toxoid and hepatitis B are detectable in short-term recipients who received T cell-depleted marrow by immunizing donors alone or both the donors and the recipients pretransplant.\textsuperscript{113}

The probability of detecting host-type isohemagglutinin in ABO-incompatible marrow graft recipients is less than 0.20 by day 80 postgrafting and is zero by day 120 postgrafting.\textsuperscript{114} Therefore, the levels of specific antibody to recall antigens detected at 100 days postgrafting suggest a major contribution produced by transferred donor B cells. Immunoglobulin allotypes of donor origin have been detected as early as 113 days postgrafting.\textsuperscript{114} Clearly, the synthesis of antibodies of donor allotype must begin before this time to be detected without interference from host-type allotypes.

B cell phenotyping data in the first two months postgrafting show that B cells from allogeneic marrow recipients have normal or near normal numbers of surface immunoglobulin positive (sIg\textsuperscript{+}) B cells as measured by polyclonal antisera directed at human immunoglobulin.\textsuperscript{9-10,12} However, recent phenotyping data shows the appearance of B cells bearing the CD5\textsuperscript{+} T cell antigen 30 days postgrafting.\textsuperscript{115} The CD5\textsuperscript{+} B cells were present in healthy recipients. High proportions of CD5\textsuperscript{+} B cells are found in fetal spleen (30\% to 50\%).\textsuperscript{91} These B cells may represent an early stage of differentiation of normal B cells or a separate lineage.\textsuperscript{116} Data from this laboratory show that populations of sIgG, sIgM, sIgA, or sIgD\textsuperscript{+} B cells in peripheral blood lymphocytes may be absent in certain recipients (Lum, unpublished). The most likely explanation for these findings is that B cells lacking a specific surface marker may be associated with specific maturational defects. This notion is supported by a study that showed B cells from marrow recipients had higher numbers of primitive \(\mu\)-determinants per B cell than normal B cells\textsuperscript{117}; these findings are similar to those found in murine splenic \(B_2\) cells that display increased \(\mu\)-determinants early in ontogeny.

B cell-enriched populations from marrow recipients in the first three months postgrafting fail to produce in vitro antibody after stimulation with specific or nonspecific activators in the presence of normal T cells.\textsuperscript{93-95,97,118} B cells from a few marrow recipients in the early period produced nonspecific immunoglobulin after EBV (T-independent) stimulation.\textsuperscript{97} Stimulation with EBV to polyclonally activate production of IgG antitetanus toxoid antibodies (anti-TT) was not successful during the early period.\textsuperscript{119} Furthermore, B cells from short-term recipients failed to produce anti-TT after stimulation with tetanus toxoid in the presence of donor helper T cells.\textsuperscript{118}

In assays for B cell activation, proliferation, and differentiation, B cells from short-term marrow recipients cannot be activated by staphylococcal aureus Cowan I bacteria (SAC) to subsequently proliferate or differentiate in the presence of normal T cell factors containing B cell growth factor (BCGF), B cell differentiation factor (BCDF), and IL 2.\textsuperscript{120} With the exception of preprogrammed spontaneous antibody secretion, the B cells from marrow recipients seem incapable of responding to exogenous signals.

**B CELL-MEDIATED IMMUNITY IN LONG-TERM RECIPIENTS**

Primary and secondary responses to neoantigens (keyhole-limpet hemocyanin and bacteriophage 0X174) recover to normal levels by one year in healthy recipients but remain impaired in those with chronic GVHD. Those with chronic GVHD not only have defective primary responses to bacteriophage 0X174, but they fail to switch from IgM production to IgG production in secondary responses.\textsuperscript{13}

Recent studies using recall antigens have been informative. In healthy long-term recipients, 85 of 125 (68\%) studied had IgG titers to tetanus toxoid; 62 of 104 (60\%) had IgG titers to diphtheria toxoid, and 12 of 18 (66\%) had IgG titers to measles virus. In recipients with chronic GVHD, 79 of 166
of 36 (29%) had IgG titers to diphtheria toxoid, and 6 of 15 (40%) had IgG titers to measles virus. None of the recipients (ranging from one year to nine years postgrafting) were boosted to the test antigens postgrafting. Furthermore, a number of long-term recipients who received T cell-depleted marrow grafts also had detectable antibody titers to tetanus toxoid and diphtheria toxoid. Such data show that there was transfer of antigen-specific B cell memory from the marrow donors to the marrow recipients, and the transferred immunity persisted for years posttransplant. One report showed transfer of anti-acetylcholin-receptor immunity from a marrow donor with myasthenia gravis to the marrow recipient who developed myasthenia gravis postgrafting. Recent studies show that bone marrow mononuclear cells could produce specific antitetanus toxoid antibody after in vitro stimulation with pokeweed mitogen or tetanus toxoid with or without tetanus toxoid booster immunizations.

B cells from three fourths of the recipients with chronic GVHD have impaired B cell function, whereas only one third of the healthy recipients have B cell defects in vitro immunoglobulin production systems stimulated with pokeweed mitogen. Furthermore, B cells from the same marrow recipient have different responses to different activators of immunoglobulin production when studied at the same time in systems activated by pokeweed mitogen, EBV, tetanus toxoid, and herpes simplex type 1 virus. These suggest that there are functionally distinct groups for each activator of polyclonal immunoglobulin production that mature at different times postgrafting. Although the above studies document B cell failure, none specify the types of defects responsible for the lack of immunoglobulin secretion. These results provided the rationale in our laboratory for developing assays for assessing antigen-specific immunity and identifying the types of B cell defects that occur in marrow recipients.

Our latest studies focused on antigen-stimulated specific antibody synthesis in lymphocytes obtained from long-term recipients. Anti-TT synthesis was induced using a tetanus toxoid-specific system. An early study showed that tetanus toxoid-specific T cell helper activity can be detected in cocultures of recipient T cells and donor B cells. However, recipient B cells cocultured with donor T cells failed to produce anti-TT. The results of this study suggest that antigen-specific T cells can be transferred in marrow transplantation. In another study designed to rule out the possibility of low antigen-specific B cell precursor frequency, we used high numbers of T and B lymphocytes per culture and found that only a minority of marrow recipients had B cells capable of producing in vitro anti-TT when increased numbers of lymphocytes were placed in culture. These studies demonstrated that antigen-specific precursor frequency is low in long-term marrow recipients. Roughly one third of long-term marrow recipients had B cells capable of producing anti-TT after EBV stimulation. These data suggested that the majority of B cell defects were primarily due to deficient function rather than a low antigen-specific B cell precursor frequency.

In assays that assess nonspecific B cell activation, proliferation, and differentiation, B cells from long-term marrow recipients exhibited two patterns in their B cell functions. B cells from some recipients could be activated with SAC but had decreased proliferative and differentiative responses to T cell supernatants containing B cell growth and differentiation factors. B cells from other recipients could proliferate but could not synthesize immunoglobulin after SAC activation and stimulation with T cell supernatants. Healthy long-term recipients had fewer, less severe B cell defects in these B cell function assays. Such B cell defects were not observed in the normal control subjects. There was a high degree of correlation between low IgG levels in marrow recipients and the failure of B cells from marrow recipients to synthesize IgG after costimulation with SAC and T cell supernatants. These studies suggest that the normal maturation of B cells is interrupted by specific blockades at one or more stages after marrow grafting.

ATTEMPTS TO ACCELERATE RECONSTITUTION

Attempts to accelerate the immune reconstitution with the use of thymic tissue transplantation or thymosin fraction V have not been successful nor have they influenced the development of chronic GVHD thought to be due to low or absent thymic tissue function in older (>30 years) marrow graft recipients. However, the results of such studies indicate that future administration of thymic hormones or related molecules may be done safely. Other hormones that might be considered for use in immune reconstitution are thymosin alpha-1, the pentapeptide thymopoietin, and serum thymic factor. Varicella zoster-specific transfer factor did not reconstitute varicella zoster-specific immunity or prevent recurrent varicella zoster infections when given between day 50 and day 64 postgrafting.

The development and availability of recombinant T and B cell growth factors will permit clinicians to administer specific molecules to determine if certain T and B cell immune defects could be constituted in marrow recipients and other patients with immune deficiency disorders.

REIMMUNIZATIONS FOR MARROW RECIPIENTS?

Most transplant centers have not reimmunized patients postgrafting. However, questions that are frequently asked include: (1) Do transplant recipients need to be routinely reimmunized to the childhood series of immunizations? (2) Should they be immunized to specific pathogens such as Streptococcus pneumoniae or Hemophilus influenzae? (3) Should live viral vaccines be used in the reimmunization of marrow recipients?

Recent studies that show transfer of antigen-specific immunity from the marrow donors to marrow recipients suggest that a significant number of marrow recipients will develop specific antibody titers to recall antigens without reimmunization to the specific antigens. Obviously, those who develop antibody titers need not be reimmunized to the specific antigens. However, those who do not develop antibody titers need to be considered for reimmunizations with diptheria toxoid, tetanus toxoid, and inactivated polio vac-
cines and followed for the development of specific antibody titers. Herd immunity and transferred specific immunity may help explain why more marrow recipients do not succumb to measles, polio, tetanus, diphtheria, and pertussis infections. Vaccinations with live viral vaccines are not recommended. The inactivated Salk polio vaccine would be useful for those at risk during outbreaks of polio. The value of specific viral or bacterial vaccines (herpes vaccine, Pneumovax) in marrow recipients remains debatable. In some immunodeficiency states the efficacy of such immunizations is poor.

Furthermore, it is unclear how many immunizations with a specific vaccine would be necessary to obtain specific immune responses in the transplant population. Those undergoing reimmunization schedules would likely require two or three immunizations before they would develop circulating antibody titers. Recipients being treated for active chronic GVHD should have their immunizations deferred until their disease is resolved, since it is likely that they would not respond to immunizations. Titers to the specific antigen should be followed to determine if an immune response was obtained.

**RECAPITULATION OF ONTOGENY**

Immune reconstitution after human marrow grafting has offered a unique model for studying stages of normal immune ontogeny, transfer of immunity, and immune defects that may parallel those seen in immunodeficiency disorders. The kinetics of immune reconstitution follow a general pattern. First to appear are cytotoxic and phagocytic cells. More mature systems characterized by highly specialized cellular functions such as proliferation, helper activity, differentiation, lymphokine production, and antibody biosynthesis appear later. The latter functions require complex and finely tuned cell-cell interactions mediated by cell contact and soluble factors. As the elements of the immune system regain their roles, healthy long-term recipients develop immunocompetence sooner than those with chronic GVHD. During the recapitulation of ontogeny over a time period ranging up to four years postgrafting, a spectrum of functional and nonfunctional stages within various T and B cell phenotypes are expressed. The development of immunocompetence includes antigen-specific immunity transferred from the marrow donor to the marrow recipient as shown by (1) the detection of specific antibody titers to recall antigens and (2) the ability of T and B cells from long-term recipients to produce specific recall antibodies in vitro. The overall pattern of immune reconstitution is delayed in recipients with chronic GVHD. The delay may be due to failure of lymphocyte subsets to progress through certain developmental stages. On the other hand, the syndrome of chronic GVHD with autoantibody formation and other autoimmune phenomena may be a result of immune dysregulation due to unregulated overactivity of certain lymphocyte subsets. (Perhaps with appropriate thymic education this would not occur.)

For the sake of discussion, the immune reconstruction process was artificially divided into early and late phases and into the various cell types. However, one must emphasize that synchronization of various components of the lymphoid system is essential for complete reconstitution. Without appropriate synchronization of the various elements of the immune system the result would be immune dysfunction. Of the multiple factors studied for their effects on immune reconstitution, time continues to be a key factor affecting the reestablishment of immunocompetence. It remains unclear whether certain immune deficits lead to the development of GVHD or immune deficits are a result of GVHD or its treatment. Furthermore, the effects of T cell depletion of bone marrow or HLA-mismatched transplantation on immune reconstitution are unknown and remain areas of active investigation.

**FUTURE DIRECTIONS**

The questions that emerge from this review suggest future laboratory and clinical investigations revolving around the following issues: (1) augmenting specific immunity to pathogens before and/or after transplant by administering vaccines or vaccine substitutes, (2) the clinical use of growth factors to restore specific T and B cell functions, (3) the use of adoptively transferred donor-derived cytotoxic T cell lines directed at specific viral or bacterial pathogens to treat recipients with specific infections, and (4) the insertion of specific genes into the bone marrow inoculum using retroviral vectors to reconstitute specific immune defects. These maneuvers are approaches that may accelerate immune reconstitution and shorten the immunodeficiency experienced by marrow recipients.

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