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THE E. DONNALL THOMAS LECTURE
Manipulating and Visualizing T-Cell Alloresponses

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INTRODUCTION

Dr. Bruce R. Blazar was this year's honored E. Donnall Thomas lecturer at the Tandem BMT Meetings, held on February 12, 2005, in Keystone, Colorado. Dr. Blazar is a Professor in the Department of Pediatrics and Chief of the Pediatric Blood and Marrow Transplantation Program at the University of Minnesota. He has been at the forefront of the identification of immune mechanisms underlying graft-versus-host disease (GVHD) and marrow rejection. Using these observations, Dr. Blazar has developed several strategies to block alloresponses and to facilitate immune recovery after transplantation. Some of the most promising strategies are now being translated clinically. The following text is a modified transcribed version of the presentation made by Dr. Blazar.

LECTURE

In this lecture, I would like to share with you some of our studies over the past 10 years or more in the area of regulating alloresponses. First, I will illustrate the role of positive and negative T-cell costimulatory pathways in graft-versus-host disease (GVHD). I will describe imaging studies to visualize GVHD and the impact of costimulatory pathway blockade. I will then briefly discuss GVHD effects of CD4+/CD25+ regulatory T cells, and will conclude by describing studies on the use of imaging to visualize the fate of host antidonor responses resulting in engraftment and graft rejection.

There is a multistage process of GVHD pathophysiology, beginning with chemoradiotherapy induction of tissue injury. Dendritic cells and B cells are exposed to proinflammatory cytokines in uptake tissue antigens released by this injury. Antigen-presenting cells are then stimulated. These cells encounter T cells that become activated, which then clonally expand and migrate into tissues, where they cause tissue injury, and this process continues to be amplified. I will focus on this expansion and migration process of GVHD pathophysiology in the latter part of this lecture.

In keeping with the requisites for an immune response versus tolerance, GVHD as well as other productive immune responses involve engagement of major histocompatibility complex (MHC)/antigen with T-cell receptors (TCRs) along with a second set of signals that have been called costimulatory receptors. These receptors bind to ligands presented on antigen-presenting cells so that when donor T cells encounter host antigen-presenting cells, both the first signal by this engagement and a second signal by this engagement lead to a fully productive immune response. In the absence of or inability to receive costimulatory ligands, only a single signal is delivered by the engagement of MHC with TCR and results in either tolerance or apoptosis that may be able to modify adverse alloresponses in vivo. In the presence of signal 2 (the costimulatory receptor–ligand interaction) alone, there is no response. However, not only has the immune system evolved to provide positive costimulatory signals, but also there are negative regulators that provide the immune system with checks and balances. Such signals are provided by homologous determinants to positive costimulatory receptors and induce a coinhibitory receptor signal that down-modulates the immune response.

The CD28/cytotoxic T lymphocyte antigen-4 (CTLA-4)/B7 family, the first described costimulatory pathway, delivers both positive signals through CD28 and negative signals through CTLA-4. T cells that engage MHC antigens in the absence of costimulatory receptors undergo apoptosis or anergy, which is a state of alloantigen nonresponsiveness. In the presence of

the binding of CD28/B7 ligands, T cells proliferate, differentiate, and can acquire effector cell function. In the presence of CTLA-4, which binds to the same B7 ligands as CD28, there is cell cycle arrest and an abortive immune response. CD28 is expressed on resting and activated murine T cells, whereas CTLA-4 is a homolog to CD28 and is expressed primarily on activated T cells. B7 ligands bind to both the positive and negative receptor, are expressed on antigen-presenting cells, and are up-regulated by proinflammatory cytokines that can occur during conditioning, regimen injury, or GVHD responses.

To illustrate the effect of costimulatory pathway involvement in GVHD, more than 10 years ago experiments were performed using strains of mice that were fully mismatched at the MHC loci with the donor. Recipients were given lethal total body irradiation and bone marrow rescue, and then spleen cells were added as a source of GVHD-causing T cells. These and the subsequent murine studies that I will discuss in this lecture were led by my colleague, Dr. Patricia Taylor, at the University of Minnesota. Initial studies to block costimulation were performed using CTLA4-Ig fusion protein, consisting of the extracellular domain of the CTLA-4 receptor that binds with high affinity to B7 ligands, and were linked to an immunoglobulin (Ig) fusion partner to prolong systemic protein half-life in vivo. CTLA4-Ig binds to B7 ligands and therefore competes with the cell surface TCR, CD28, and CTLA4 for binding to these B7 ligands, thereby blocking CD28/CTLA4/B7 responses.

A series of experiments demonstrated that CTLA4-Ig, whether of mouse or human origin, clearly was not able to protect against GVHD in most cases, although occasionally a high level of protection was observed. Because CTLA4-Ig binds to B7 ligands and can compete with both the binding of CD28 (a positive costimulatory pathway) and CTLA4 (a negative costimulatory pathway), we investigated whether selective blockade of the positive costimulatory pathway would have a much better outcome in terms of prevention of GVHD lethality. Analogous experiments were performed with the GVHD source either wild-type donor spleen cells or spleen cells obtained from a mouse that had the CD28 gene deleted by homologous recombination.

Both wild-type cells and CD28 knockout T cells could cause GVHD lethality. A caveat to these experiments was that these CD28 knockout T cells were also deficient in regulatory or suppressor cells (to be discussed later). I should point out that we and others have shown that CD28 knockout T cells can cause very little GVHD if lower cell numbers are infused or in different strain combinations. Nonetheless, the complete absence of a positive costimulatory pathway (ie, CD28), which is critical for the initiation of T-cell alloresponses, will not prevent lethal GVHD under all conditions.

With respect to the CTLA4/B7 pathway, CTLA4-Ig had only some protective effect, which could have been greater had we been able to selectively target CD28 in the absence of blocking CTLA4/B7 interaction. Therefore, experiments were performed to determine whether blocking the CTLA4/B7 pathway by infusion of anti-CTLA4 antibody would block the inhibitory signal of T-cell alloresponses and potentially accelerate GVHD lethality. Although recipients of irrelevant antibody died of GVHD, recipients of anti-CTLA4 antibody had markedly accelerated GVHD lethality, demonstrating that CTLA4/B7 interactions serve to inhibit GVHD lethality.

In summary, blockade of the costimulatory CD28/B7 pathway inhibits, but does not eliminate GVHD. Blockade of the coinhibitory CTLA4/B7 pathway accelerates GVHD. The incomplete efficacy of blocking the CD28/B7 pathway suggested that other pathways may be contributing to the generation of a productive immune response culminating in GVHD lethality.

A second member of the CD28 superfamily pathway is the inducible costimulator (ICOS). ICOS is expressed on activated T cells, and our data indicate that it is also expressed on CD4+ and CD8+ T cells during the GVH reaction. The ligand for ICOS is expressed on antigen-presenting cells including dendritic cells, B cells, and macrophages. Like many of the ligands in these receptor families, they can be induced by proinflammatory cytokines in nonlymphoid tissue. Signaling via ICOS regulates interleukin (IL)-10, IL-4, and interferon gamma, which are effector-cell cytokines involved in expansion of activated T cells. In contrast, IL-2, which is critical to the initiation of a T-cell response, is not affected by ICOS ligation. CD28/B7 interactions can occur when resting T cells encounter host alloantigens, resulting in initiation of an immune response including clonal expansion and IL-2 production. CD28/B7 binding subsequently results in the up-regulation of ICOS on activated T cells. These cells are primed and undergo differentiation to effector cells along with expansion.

To determine the role of ICOS in GVHD lethality generation, studies were performed using donor bone marrow and either wild-type splenocytes or ICOS knockout donor splenocytes. After the infusion of 5×10^6 ICOS knockout splenocytes, recipients had a superior survival compared with wild-type control recipients. After the infusion of 25×10^6 ICOS knockout splenocytes, mice died of GVHD lethality, demonstrating that the very potent immune response is not fully dependent on this particular costimulatory pathway, although the recipients survived significantly longer than the recipients of wild-type splenocytes. Virtually identical data have been seen with an anti–ICOS-blocking antibody, indicating that the data with ICOS knockout splenocytes are due to an aberrant

function induced by using knockout cells. These findings have been found in multiple strain combinations by several laboratories.

A summary of the CD28/B7 superfamily indicates that the GVHD effects of donor T cells are the net result of positive costimulatory pathways, such as CD28/B7 and ICOS, and negative pathways, such as CTLA4/B7 or the program death 1 (PD-1) pathway (not covered in this lecture). The CD28 and ICOS pathways are not redundant, because ICOS blockade reduces GVHD when CD28 knockout T cells are infused. ICOS blockade is more effective than anti-B7 antibodies in treating GVHD, likely due to the fact that ICOS targets previously activated T cells. The degree and timing in cell and tissue expression of multiple receptor ligand pathways will likely dictate the biological effects on donor T cells capable of causing GVHD. In addition to the multiple pathways and multiple tissues, consideration must be given to the kinetics and level of expression in GVHD target tissues.

The TNF:TNF receptor (R) costimulatory pathway members are trimeric proteins expressed on the antigen-presenting cells with their counter-receptors on T cells. This lecture will review data on the CD40: CD40 ligand (L) pathway in detail and then summarize data on the remaining pathway members, the 4-1BB, OX40, and CD30 pathways. CD40L is expressed predominantly on activated CD4+ T cells. The counter-receptor, CD40, is expressed on antigen-presenting cells and on activated endothelial and epithelial cells. The binding of CD40L to CD40 regulates immunoglobulin isotype switching, indicative of T cell:B cell cooperation.

Studies were performed using an irrelevant or anti-CD40L antibody to determine whether blockade of this pathway could inhibit GVHD lethality. A similar story has evolved in which members of the TNF/TNFR superfamily can block GVHD under conditions of a more limited donor T-cell number. After infusion of 15×10^6 splenocytes, 50% of anti-CD40L antibody-treated MHC-disparate recipients survived versus 100% lethality in the control group. However, as with other costimulatory pathways, blockade of this pathway can be overwhelmed under very aggressive immune responses, as seen after the infusion of 25×10^6 splenocytes. Although biological effects were observed in terms of delaying GVHD mortality time, recipients ultimately uniformly succumbed to GVHD lethality.

The results with the TNF/TNFR pathways can be summarized as follows:

1. *Because CD4+ T cells exhibit CD40L expression during a GVHD response, GVHD systems that are mediated only by CD4+ T cells can be inhibited by CD4L: CD40 blockade. CD8+ T-cell–mediated GVHD systems are not effected, although GVHD systems in which both CD4+ and CD8+ T cells are required to cause optimal GVHD lethality can be inhibited.*
2. *Results for the CD30 pathway are similar, demonstrating the effects of CD30:CD30L blockade on CD4+ or combined CD4+ and CD8+ T-cell–mediated GVHD. Despite the expression of CD30 on CD8+ T cells analyzed during a GVHD response, little effect was seen in a CD8+ T-cell–mediated GVHD system.*
3. *For the 4-1BB pathway, although 4-1BB is expressed on alloreactive CD4+ and CD8+ cells, there is a more dominant effect on inhibiting CD4 responses. There are some effects on CD8+ T-cell–mediated GVHD and an effect on GVHD mediated by both CD4+ and CD8+ T cells.*
4. *Relevant to the OX40 pathway, alloreactive cells express OX40 on both CD4+ and CD8+ T cells, and OX40: OX40L blockade inhibits GVHD in systems in which GVHD is mediated by CD4+ T cells, CD8+ T cells, and both CD4+ and CD8+ T cells.*

With respect to the latter two pathways, of note, 4-1BB has been reported as a survival factor for CD8+ T cells responding to nominal antigens, although for GVHD lethality induction, we have noted a more dominant effect on CD4+ T-cell–mediated GVHD. In contrast, whereas OX40 has been reported to be a survival factor predominantly for CD4+ T-cell responses to nominal antigens, blockade of CD8+ T-cell–mediated GVHD provides significant biological effects on outcome. Thus, overall, CD4 T-cell–mediated GVHD can be inhibited by any of these TNF:TNFR family pathway blockades studied to date. CD8+ T-cell–mediated GVHD is most dependent on OX40 and to a lesser extent on 4-1BB signals, contrary to what would have been surmised from the literature, at least under these conditions. In addition, the CD28 and TNFR family members are nonredundant, as shown in studies using CD28 knockout T cells.

The limitations to performing in vivo antibody blockade or using knockout T cells for experiments include the presence of proinflammatory cytokine responses and tissue destructive events that occur in vivo in GVHD model systems. Thus, sufficient inhibition of T-cell priming or expansion by blockade of a single pathway in vivo may be difficult. A successful ex vivo approach could be advantageous by permitting the infusion of donor T cells that are incapable of causing alloresponses, so that when they encounter this inflammatory environment in vivo, they have already been incapacitated, and these multiple pathways then cannot be fully operative in promoting a GVHD response. Blockade of the CD40L or CD28/B7 pathways may be especially useful in CD4 T-cell tolerance, because of their critical role in initiating alloantigen responses.

Consequently, Dr. Taylor developed ex vivo tolerance approaches based on cultures containing CD4+ T cells and irradiated MHC class II-disparate

irradiated host antigen-presenting cells. These cultures were performed in the presence or absence of anti-CD40L. T cells were analyzed in vitro for immune responses, and an aliquot was infused in vivo into MHC class II-disparate recipients to determine whether these tolerized cells could cause GVHD lethality. Control cells responded vigorously in vitro to alloantigen expressed on host antigen-presenting cells. In the presence of anti-CD40L antibody, this response was markedly blunted. There was no effect of anti-CD40L antibody on day 2, because it takes 24 to 48 hours to up-regulate CD40L. In the presence of IL-2, the immune response was intact. In secondary cultures using CD4+ T cells washed free of antibody, exposure to alloantigen rechallenge in the complete absence of antibody resulted in a marked alloantigen hyporesponsiveness. After the infusion of 1×10^6 cells, all recipients survived, whereas the infusion of 3×10^4 control cultured cells caused 94% lethality. The approximate reduction in the GVHD lethality capacity of tolerized cells was estimated to be approximately 30-fold. In comparison, the administration of anti-CD40L antibody in vivo resulted in an approximate 3-fold inhibition of GVHD, which was at least 10-fold less than that achieved with ex vivo tolerized cells.

Based on these data, we investigated the possible suppressor-cell activity of these tolerized T cells, which could explain the markedly superior GVHD protective effect of tolerized cells compared with in vivo anti-CD40L antibody infusion. Additional supporting data for this hypothesis were derived from studies in which we demonstrated that CD4+ T cells deficient in CD40L had an approximate 3-fold reduction in GVHD lethality capacity. Tolerized T cells have the appearance of T cell activation by flow cytometry, as compared to naive CD4+ T cells, based on forward- and side-scatter properties. Biochemical analyses of these tolerized cells showed that some intracellular pathways were increased, indicating that tolerized cells had been activated. Collectively, these data suggested the possibility that the tolerized cells had acquired suppressor-cell features.

To determine whether tolerized cells had suppressor-cell properties, CD4+ T cells were tolerized and then analyzed in vitro to determine whether they would inhibit a naive CD4+ T-cell response to alloantigen in MLR culture. In addition, an aliquot of cells was injected in vivo to determine whether tolerized cells could down-regulate a GVHD lethality response mediated by fresh naïve CD4+ T cells. Whereas naive CD4+ T cells alone had a vigorous proliferative response to alloantigenic stimulators, the immune response in the presence of as few as 1:3 tolerized:naïve cells was markedly blunted. With as few as 1:100 tolerized:naïve cells, the immune response was reduced by 50%, indicating potent in vitro suppressor-cell activity. Whereas the in vivo infusion of 1×10^5 fresh CD4+ T cells was uniformly lethal when given to sublethally irradiated MHC class II-disparate recipients (the same strain as the stimulator cells used for tolerization), the separate injection of as few as 3×10^4 tolerized cells that are widely distributed throughout the animal results in 70% long-term survival, indicating very potent suppressor-cell activity in vivo.

These data led us to examine the role of CD4/25+ regulatory/suppressor cells. Tolerized CD4 T cells expressed high levels of CD25 antigen during our culture. Depletion of these CD4+/25+ cells completely precluded tolerance induction by either CD40L/CD40 pathway blockade or CD28/CTLA4/B7 pathway blockade. Thus CD40L antibodies favor the outgrowth of CD4+/25+ T cells with suppressor-cell activity, as we previously demonstrated in other experiments. The high degree of suppression of GVHD lethality by these tolerized cells suggested that activated CD4+/25+ cells alone were responsible. This led us to experiments to isolate CD4+/25+ cells and expand them in culture for a period of 1 to 2 weeks by providing TCR signals through CD3 engagement, which increases their potency, as well as survival factors, including IL-2 and either TGF-β or CD28 costimulatory signals. These cells can expand up to 40- to 67-fold under these conditions and have increased potency compared with naïve T cells, as seen in studies performed by Dr. Taylor and many others. To determine the extent of suppression of GVHD lethality that could be achieved, donor bone marrow and supplemental splenocytes were given to a fully MHC-disparate strain combination, as described earlier, along with the infusion of 1×10^7 ex vivo expanded CD4+/25+ T cells on days 0 and 4. In contrast to the uniform lethality of the control groups, the infusion of CD4+/25+ T cells resulted in 80% to 100% survival, a result also observed in other laboratories for other strain combinations.

These murine data prompted us to determine whether human CD4+/25+ T cells could be isolated and expanded as a possible strategy to inhibit GVHD lethality in the clinic. In contrast to the mouse, human CD4+/25+ T cells are not as readily identifiable as a discrete population in human peripheral blood. Rather, there is a continuum of CD25 antigen expression. Only cells with the highest density of CD25 expression have suppressor-cell function, and these cells are present in low frequency (typically 1% to 2% of peripheral blood CD4+ T cells). In studies initiated by Dr. Wayne Godfrey while at the University of Minnesota, a bead isolation procedure was developed to acquire the highest CD25 antigen-expressing CD4+ T cells from both adult peripheral blood and cord blood cells. In the presence of CD3/CD28 beads, similar to the procedure used in mice, we have noted that these cells can expand at least 100-fold in three weeks, potently suppressing an allo-MLR response. In about 2/3 of instances, isolated peripheral blood

CD4+/25+ T cells suppressed the immune response by ≥ 50% at ratios of 1:2 CD4+/25+:CD4+/CD25− responder T cells. In the coming year, CD4+/25+ T cells will be studied by multiple laboratories both in the United States and abroad for their potency in GVHD prevention in humans.

We sought to extend these data to determine how GVHD-suppressive approaches, such as costimulatory pathway blockade and CD4+/25+ T-cell infusion, influence GVHD T-cell effector-cell expansion and their homing and migration to GVHD target organs. A better understanding of the precise biology of these processes should allow improvement in GVHD prevention. To accomplish these studies, we used T cells from a donor mouse that expressed green fluorescent protein (GFP) using a system initially described by our collaborator, Dr. Angela Panoskaltsis-Mortari at the University of Minnesota. The use of GFP transgenic cells permit tracking of GVHD effector-cell trafficking, including analysis of GVHD target organs and the kinetics of their infiltration into these organs using animal imaging techniques beginning on day 1 after transplantation. At 1 week and 2 weeks after infusion, inguinal lymph nodes, spleen, and intestinal Peyer's patches each exhibited marked infiltration of these cells consistent with the GVHD effector-cell response. There was a diffuse infiltration throughout the small and large intestines, the liver, and the lung, which is also a target of GVHD.

This model was used to determine the effects of costimulatory pathway blockade. For the purpose of illustration, I will focus on ICOS pathway blockade in studies led by Dr. Taylor, who analyzed the effects of treating recipients of GFP transgenic T cells with irrelevant immunoglobulin or anti-ICOS antibody, followed by sequential whole-animal imaging of cohorts of mice at different times after transplantation. In the control group, intestinal loops had a marked infiltration with GFP transgenic T cells at 1 week posttransplantation that increased by 2 weeks posttransplantation. Anti-ICOS antibody markedly inhibited the infiltration in the intestine at both the 1-week and 2-week time points, although some cells clearly escaped at both time points, as was observed in the Peyer's patches. Anti-ICOS antibody delayed the infiltration into lymph nodes, although some cells escaped through this process. These probably were the cells capable of expanding in sufficient numbers under high doses (25×10^6) that were responsible for the GVHD lethality using ICOS knockout splenocytes discussed earlier.

Similarly, in the liver, infiltration was inhibited very early after transplantation, and there were some escapees that appeared later after transplantation. Thus ICOS blockade reduced or delayed GFP T-cell infiltration into lymphoid and other GVHD target organs. Similar but more profound results were seen with CD4+/25+ T-cell infusions. Therefore, for us and others using luciferase-based techniques, whole-animal imaging has proven to be a powerful tool for visualizing GVHD, providing insight into how interventions may affect the expansion, homing, and migration properties of donor effector T cells.

GVHD and host-versus-graft (HVG) responses are opposing immunological effects. Dominant GVH responses will result in GVHD, whereas dominant HVG responses will result in bone marrow rejection. Donor anti-host T cells can cause GVHD, whereas host anti-donor T cells and host natural killer (NK) cells can cause bone marrow graft rejection. The mechanism, sites, and kinetics of bone marrow graft rejection is not well understood, and such knowledge may enable the development of strategies to facilitate alloengraftment after transplantation.

To better understand the fate of these donor T cells under various conditions of engraftment and graft resistance, Dr. Taylor applied the GFP imaging system to study the fate of GFP transgeneic T-cell–depleted bone marrow cells infused into sublethally irradiated fully allogeneic recipients. Dynamic imaging of the animals revealed showed bone marrow cells in the bone marrow cavity, inguinal lymph nodes, Peyer's patches, and spleen and disseminating widely into lymphohematopoietic organs. At 1 week after transfer, GFP transgenic cells were seen in the liver, lung, intestinal loops, and colon, the same sites to which GVHD effector cells migrate and known sites of alloresponses. In an attempt to better understand the immunobiology responsible for allogeneic graft rejection, recipients were irradiated, and then host-type allogeneic T cells were infused. Under these conditions, in the absence of host T cells, recipients all survived and had a mean 74% donor cell engraftment. In the presence of adoptively transferred host T cells, these recipients now rejected their grafts and died of bone marrow aplasia, as determined by evaluation of hematocrit and other analyses.

Subsequent studies used GFP transgeneic host-type T cells to permit whole-body imaging. The coinfusion of syngeneic bone marrow stimulated very little migration of host-type T cells into the bone marrow cavity, in contrast with allogeneic bone marrow, which resulted in host-type T-cell infiltration into the bone marrow cavity on day 4. By 1 week posttransplantation, a large number of these host-type T cells infiltrated into the bone marrow cavity, suggesting the possibility that these host-type T cells may be rejecting bone marrow in situ. However, these host-type T cells do not only go to bone marrow; they also migrate to other sites where the bone marrow also homes, including the lymphoid system. For example, large numbers of host-type T cells were seen in the inguinal lymph nodes on day 7 posttransplantation in recipients receiving allogeneic bone marrow, whereas far fewer host-type T cells were seen in the inguinal lymph nodes in recipients receiving syngeneic bone

marrow. These data demonstrate that an expansion and migration of host-type T cells occurs in lymph nodes on exposure to allogeneic bone marrow. Findings for Peyer's patches and the spleen were similar, with a more intense infiltration of host-type T cells in recipients of allogeneic bone marrow than in recipients of syngeneic bone marrow, providing a vivid illustration of HVG T-cell responses.

Recipient allosensitization can be a significant barrier to bone marrow engraftment under some conditions. Multiple-transfused aplastic anemia patients are at higher risk for graft rejection due to priming of host antidonor immune responses. Alloprimed mice also can reject donor bone marrow grafts. In an effort to better understand the mechanisms that may be responsible (at least under these rodent conditions) for donor bone marrow graft rejection under allosensitization conditions, recipients were sublethally irradiated and infused with GFP transgenic donor bone marrow. Cohorts of recipients were sensitized at 1 month before transplantation to provide an alloprimimg situation. (Nonsensitized recipients given 350 cGy of irradiation would reject donor bone marrow grafts unless pan-T-cell–depleting antibodies were given.) On day 3 posttransplantation, few bone marrow cells were present in rejecting recipients. These GFP transgenic bone marrow cells expanded by day 6, but were lost by day 14 posttransplantation. In the engrafting animals that were not allosensitized but had received pan-T-cell–depleting antibodies, comparable data were observed in the first 6 days posttransplantation. In contrast, GFP transgenic bone marrow was present in higher numbers in engrafted mice on day 14 than on day 6 posttransplantation. In allosensitized mice there was a complete absence of GFP transgenic donor bone marrow in bone marrow cavities at all time points posttransplantation. At 18 hours posttransplantation (the earliest time point examined), there was a complete absence of donor bone marrow in alloprimed recipients. GFP transgenic donor bone marrow also migrated to secondary lymphoid organs and GVHD target organs. Findings in these organs mirrored those in the bone marrow cavity, such that GFP transgenic donor bone marrow cells were present in each site in engrafting mice on day 3 and in higher numbers on day 6 posttransplantation, but were lost by day 14 posttransplantation in rejecting but not engrafting mice. In contrast, no GFP transgenic donor bone marrow was seen in any site by 18 hours after transfer in rejecting mice.

To determine whether host T cells or NK cells were responsible for this primed bone marrow graft rejection, mice that had received no manipulation or had been primed with donor splenocytes 1 month before transplantation were given lethal irradiation and full allogeneic donor bone marrow rescue. Primed mice were also given irrelevant antibody or antibody that depleted CD4+ and CD8+ T cells and NK cells. Whereas nonprimed mice survived and had high levels of donor cell engraftment, primed mice given irrelevant antibody succumbed due to bone marrow aplasia. Despite T-cell and NK-cell depletion, recipients could not be rescued from bone marrow aplasia; in fact, there was no influence of pan-T-cell and NK-cell depletion on the time to mortality under these heavy irradiation conditions.

Because combined T-cell and NK-cell depletion had no observable effects in primed mice, we explored the possibility that host antidonor alloantibody was responsible for eliminating donor bone marrow cells and inducing bone marrow aplasia. Experiments were performed using nonsensitized or sensitized recipients that were irradiated and rescued with very high doses of donor bone marrow in an attempt to overcome graft rejection. Recipients were wild-type or B-cell–deficient animals due to the absence (by homologous recombination) of the μ heavy chain of immunoglobulin. Under the wild-type conditions, the unprimed mice engraft and survive, while the primed mice uniformly die. In the complete absence of B cells, recipient survival is high with complete donor chimerism despite priming. Of note, however, in experiments using a 10-fold–lower donor bone marrow cell dose infused into allosensitized B-cell–deficient mice, rejection will occur, indicating that μ-knockout mice are capable of mounting a non–antibody-mediated response after allosensitization. Furthermore, incubation of donor-type thymocytes with sera obtained from sensitized mice demonstrated binding even at high serum dilutions, whereas such binding was not observed using third-party thymocytes at these low serum concentrations.

In summary, donor bone marrow rejection is far more rapid in alloprimed mice than in unprimed mice, occurring in <1 day in the former versus >1 week in the latter. Alloantibody production can be detected, and imaging provides a vivid illustration of homing/migration patterns of donor bone marrow and host antidonor T cells, which should be useful in developing strategies to facilitate alloengraftment.

We have many opportunities to control the immune response in bone marrow transplantation by targeting the costimulatory pathway receptor/ligand-binding interactions that regulate productive immune responses, as well as taking into account the coinhibitory pathways and CD4+/25+ T cells that are attempting to down-regulate GVHD responses. Although the immune system incorporates a series of checks and balances to permit life-saving immunity and to protect against uncontrolled or unwanted T-cell activation, immune manipulation via costimulatory and coinhibitory pathways or cellular therapy based on these principles will undoubtedly improve the outcome of allogeneic transplantation of human patients in the coming years.