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Composite Tissue Allotransplantation: Current Challenges

K.V. Ravindra, S. Wu, M. McKinney, H. Xu, and S.T. Ildstad

ABSTRACT

Composite tissue allotransplantation (CTA) in the clinic is taking firm root. Success at hand, face, knee, trachea, and laryngeal transplantation has led to widespread interest and increasing application. Despite this, skepticism is common, particularly in the realm of reconstructive surgeons. The risks of immunosuppression remain a barrier to the advancement of the field, as these are perceived by many to be prohibitive. Significant progress in the field require the development of newer immunosuppressive agents with less toxicity and methods to achieve donor specific tolerance. This review focuses on the current state of CTA—both in the clinic and the laboratory. A thorough understanding of the immunology of CTA will allow the widespread application of this promising field.

COMPOSITE TISSUE allotransplantation (CTA) transplantation is the dream of plastic and reconstructive surgeons. Major trauma to the face and extremities often leaves such massive defects in soft tissue and or bone that are nearly impossible to correct with native tissue. Even if a cosmetic repair is achieved, often the functional result is far from satisfactory. There are often no good options available and the only chance of restoring function, appearance, and dignity to these patients is composite tissue transplantation. An estimated 7 million Americans every year would benefit from composite tissue reconstruction owing to oncologic surgery, traumatic injuries, and congenital anomalies.[1] This potential patient population far outnumbers those on the solid organ transplantation waiting list.[1] The concept of limb transplantation dates back as far as the 4th century AD. The legend of twin saints Cosmos and Damian described the restoration of an extremity by miraculous transplantation from a deceased donor (Fig 1).[2] An interesting account is recorded from the 16th century where Gaspare Tagliacozzi, who is often regarded as the father of plastic surgery, reconstructed the nose of man using a flap of forearm tissue donated by a slave.[3]

The advances in vascular surgery at the beginning of the 20th century served as the stimulus for early transplantation experiments. The first whole joint transplantations in animals and in humans were performed by Judet[4] and Lexer[5] in 1908. However, in both cases the allografts were nonvascular and immunosuppression was not used. In 1936, Schwind[6] described the successful heterotopic transplantation of rat hind limbs by parabiosis in which 1 rat was surgically sutured to another at the site of partial hind limb amputation. However, the animals used in this study were of the same strain, thereby excluding the possibility of an immune reaction to the transplanted tissue. These studies showed the technical feasibility of limb transplantation.

The first reported clinical human hand transplant was performed in Ecuador in 1964.[7] A team led by Robert Gilbert performed this unprecedented surgery on a young man who had lost both hands in an explosion. Despite the use of systemic steroids and azathioprine, severe rejection developed 2 weeks after the operation and amputation was performed.

The development of more efficacious and mechanistically driven immunotherapy in the 1980s moved the possibility of

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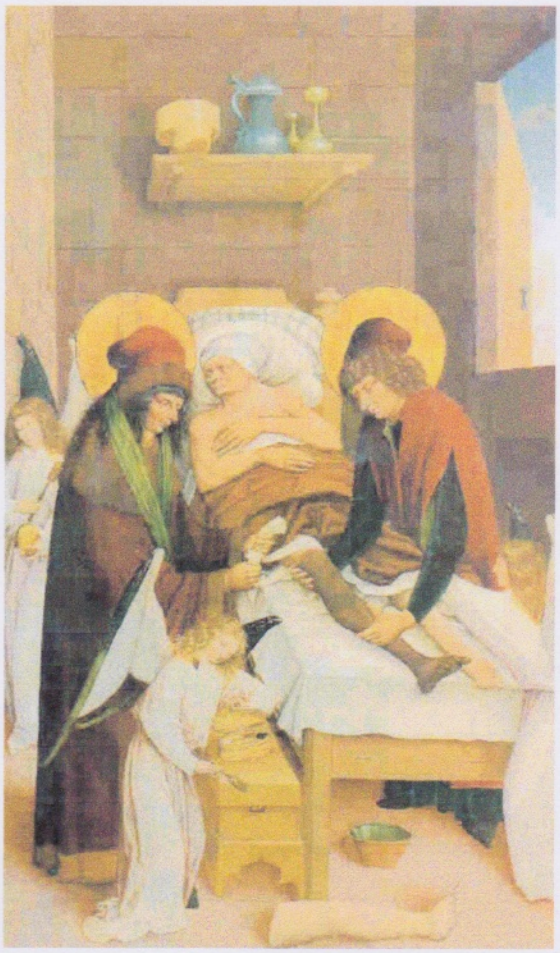

Figure 1. History of CTA. The legends surrounding Saints Cosmos and Damien included the following, which was the subject of many paintings: the grafting of a leg from a recently deceased Ethiopian to replace a patient's ulcerated leg.

successful CTA closer to reality. Calcineurin inhibitors, first cyclosporine A and later FK506, contributed significantly to the increased success in liver, heart, and lung transplantation outcomes.[8–12] By combining agents with nonoverlapping mechanism of action and side effects, effective prevention of rejection was achieved with reduced toxicity compared with single drug therapy.[13] Currently the incidence of acute rejection is significantly lower in organ transplantation. However, the conundrum of chronic graft dysfunction has not yet been solved.

The truly modern era of CTA emerged in 1998, when an international team performed a successful hand transplant in Lyon, France.[14] Unfortunately, the patient was noncompliant and the rejected hand was ultimately amputated. On the heels of this first, short-term success, Jones et al[15] performed the first long-term successful hand transplant in the world, showing that the same triple drug immunosuppressive regimen used in maintenance of renal allografts was all that was needed to control rejection of transplanted hands. As of March 2009, the international registry on hand and composite tissue transplantation (http://www.handregistry.com) has recorded the performance of hand transplantation in 32 patients (20 unilateral and 12 bilateral). Other forms of CTA were reported in 42 patients including larynx ($n = 16$), abdominal wall ($n = 9$), face ($n = 6$), knee ($n = 6$), femoral diaphysis ($n = 3$), uterus ($n = 1$), and lower limb ($n = 1$). Seven of the transplanted hands are >8 years posttransplant and only a few graft failures have been reported, nearly all owing to noncompliance.[16] The longest surviving patient with a hand is currently >10 years posttransplant.[17]

RISKS ASSOCIATED WITH THE IMMUNOSUPPRESSIVE DRUGS IN CTA

Despite such favorable early outcomes in this relatively small number of patients, controversy remains as to whether the risks associated with the immunosuppressive drugs required to prevent rejection outweigh their benefits in CTA.[1] The immunosuppressive drugs act nonspecifically to suppress the immune system, resulting in an associated risk of tumors, opportunistic infections, and organ toxicity.[18] The hand transplant registry reports opportunistic infections in 63.6% and metabolic complications in 50% of the patients. The most serious complications have been steroid-related aseptic necrosis of both hips in 1 patient[19] and renal compromise in the first face transplant recipient.[20] Although other complications such as malignancies, cardiovascular related disease, and nephrotoxicity have not yet been reported, the incidence among these is predicted to parallel that in solid organ transplant recipients.[21]

Chronic rejection was predicted to be another major challenge for CTA.[14] It is believed to have a multifactorial etiology, with ischemic damage at the time of transplantation and chronic immune reactivity to the allograft the major contributing factors.[22] Although acute rejection rates have been reported to be 85% in the first 12 months in hand allografts[23]; these have been easily reversed with escalation of immunosuppression. To date, chronic rejection has not been adequately characterized in clinical CTA.[24–26] The first hand transplant recipient who required amputation of the graft following a prolonged period of noncompliance with medication, demonstrated cutaneous chronic rejection in the explanted graft. The rejected allograft demonstrated a histologic picture that resembled chronic lichenoid graft-versus-host disease (GVHD).[27,28] The first report of graft loss due to chronic rejection after the recipient was tapered to monotherapy occurred at the American Transplant Congress 2009 Annual Meeting.[29]

The main challenges facing CTA are to minimize immunosuppression while simultaneously avoiding acute and chronic rejection. This goal has caused research in the field of transplantation immunology to shift its major focus towards donor-specific tolerance. Tolerance can be defined as a state of hyporesponsiveness toward the donor, in the

absence of immunosuppression, while maintaining adequate immune responses to third party antigens.[30] Tolerance is the most sought after goal in the field of transplantation.

ESTABLISHING TOLERANCE BY CHIMERISM

One of the earliest and most well-established approaches to conferring tolerance is through hematopoietic stem cell chimerism.[31] In chimerism, tissues from 2 genetically distinct organisms reside successfully in a single organism. Chimerism can be divided into 2 primary types: macro- and microchimerism. Macrochimerism is usually established through isolated bone marrow (BM) transplantation to a conditioned recipient. Conditioning ablates the recipient's BM to make space for allogeneic BM and immunosuppresses the recipient, thereby preventing rejection of the transplanted BM. After donor pluripotent hematopoietic stem cells engraft in the recipient BM and produce all its lineages, a new hybrid immune system results with reciprocal bidirectional donor–host tolerance. Newly developing T lymphocytes that recognize the donor or host antigens are clonally deleted in the thymus. It has been shown experimentally that as little as 1% donor chimerism is sufficient to induce a robust state of donor-specific tolerance.[32,33]

Microchimerism occurs from migration of passenger leukocytes from a allograft into an unconditioned recipient.[34] These leukocytes from the transplanted allograft are proposed to lead to clonal exhaustion and/or donor-specific tolerance after interaction with recipient leukocytes.[35] In microchimerism, donor pluripotent hematopoietic stem cells do not engraft in recipient marrow; instead hematopoietic-derived cells (primarily dendritic cells) are produced in the donor organ and migrate systemically. As a result, not all stem-cell–derived lineages are generated and very low levels of donor cells are detectable in the recipient's peripheral blood. It is debated whether microchimerism is responsible for tolerance or is a side effect of tolerance.

In the spring of 1992, 1 experiment involving the search for donor leukocytes in the blood and tissues of 30 long-surviving human liver or kidney allograft recipients, with sensitive cytostaining and polymerase chain reaction techniques, showed that microchimerism was present in ≥1 peripheral recipient locations in all 30 patients.[36] The long-term persistence of multilineage microchimerism implied, and ultimately proved,[37] that hematolymphopoietic precursor and stem cells are part of the passenger leukocyte population of organ grafts. Although rejection of organ allografts in the presence of microchimerism and long-term allograft survival in the absence of microchimerism have also been reported,[38,39] some make a strong argument that microchimerism is essential for the maintenance of clonal exhaustion–deletion resulting from the initial flood of passenger leukocytes during the first several weeks after transplantation.[40]

ACHIEVING CHIMERISM THROUGH VASCULARIZED BM TRANSPLANTATION

Because hematopoietic tissue accompanies the bone in hand allografts, immunologists anticipated that this procedure may induce chimerism even if the recipient did not receive conditioning before transplantation. In such cases, hand allografts are considered vascularized BM transplantation (VBMT), and the donor BM cells are contained within their own stromal microenvironment, functioning immediately upon transfer to provide a ongoing supply of donor hematolymphopoietic cells.[41] Studies using the rat limb transplantation model have confirmed that systemic immune reconstitution is more rapid with a VBMT compared with isolated cellular BM transplantation using comparable cell numbers.[42] Hewitt et al[43] transplanted vascularized limb allografts from Lewis × Brown-Norway F1 to Lewis and reported long-term survival of 8 recipients treated with cyclosporine. In 2 of 8 animals, the immunosuppression was successfully discontinued, resulting in no histologic evidence of rejection, and indefinite graft acceptance. One important limitation to these findings is that the 2 strains of rat used have relatively weak immune responsiveness; therefore, graft acceptance is more likely to occur in this setting than in stronger donor–recipient combinations. These findings have yet to be extended to more robust transplant models.

Although the BM component within the hand allograft could also potentially increase the risk of GVHD, many experimental studies addressing this issue failed to confirm this prediction.[44,45] Hewitt et al,[46] using the rat hind-limb transplantation model, observed that 37.5% of the recipients developed lethal GVHD, whereas the remainder of the animals recovered from a self-limiting course of GVHD and developed long-term tolerance. Some studies used irradiation of the hand limb before transplantation for GVHD prevention.[47,48] Removal of the popliteal lymph nodes without graft irradiation eliminated GVHD,[49] suggesting that VBMT was not the cause of GVHD, but rather the mature lymphocytes in the graft. Significantly, GVHD has not occurred in any of the hand transplant recipients so far.

These results have not yet been replicated in a large animal model. Bourget et al[50] have shown that peripheral chimerism was present only in the immediate postoperative period and that it was not necessary for maintenance of tolerance in a swine model of induction of tolerance to musculoskeletal allografts across minor antigen disparities. In the clinical setting, Granger et al[51] performed kinetic studies on peripheral blood of two subjects after hand transplantation and evaluated donor-specific reactivity in vitro and chimerism. Donor-specific hyporesponsiveness did not develop in mixed lymphocyte reaction and donor macrochimerism was not detectable. Peripheral microchimerism was observed in the early posttransplant period and was undetectable thereafter. A human hand allograft contains only small amounts of functionally active donor mar-

row, and therefore will not significantly affect a human recipient.

MACROCHIMERISM INDUCED BY CELLULAR BM TRANSPLANTATION

Two types of bone marrow chimeras exist: fully allogeneic and mixed allogeneic. In full chimerism, the donor marrow replaces the recipient hematopoietic system. This typically results when the recipient is myeloablated before allogeneic BM transplantation. Myeloablative conditioning is a well-established therapy for leukemia and other immunohematopoietic disorders.[52] However, it is becoming increasingly clear that it is not the high-dose chemotherapy/radiation from the conditioning that promotes remission in allogeneic BM transplantation for malignancies, but rather the immunotherapeutic potential of donor lymphocytes,[53,54] leading to a paradigm shift in conditioning for BM transplantation. It is currently believed that conditioning mechanistically functions to immunosuppress the host and prevent donor BM graft rejection rather than to physically prepare vacant niches. This has led to the successful development of nonmyeloablative transplants with significant reduction of morbidity and mortality related to the conditioning regimen.[55,56] These findings have even greater potential advantage for BM transplantation in nonmalignant disease, such as organ transplants, autoimmune disorders, and CTA, where treatment-related toxicities must be minimal.

In mixed chimerism, a dual immune system comprising the donor and recipient hematopoietic cells is successfully established (Fig 2).[3,32] Mixed chimerism was first intentionally established in conditioned adult recipients transplanted with a mixture of T-cell–depleted syngeneic plus T-cell–depleted allogeneic marrow.[32] Mixed chimerism is associated with donor specific transplantation tolerance in vivo and in vitro[32,57] and has been shown to effectively induce donor-specific tolerance to a variety of allografts such as skin,[32] heart,[58,59] lung,[60] pancreatic islets,[61] trachea,[62] esophagus in rodents, large animals,[63] and primates,[64] eliminating the need for immunosuppressant drugs. In humans, BM transplantation–induced mixed chimerism has been shown to confer acceptance of donor-specific skin[65] and kidney allografts[66,67] in the absence of immunosuppression. Another advantage is that mixed chimerism prevents chronic rejection,[4,62,68] which is the leading cause of late graft loss.

Mixed chimerism has 3 advantages over fully allogeneic chimerism: (1) it is linked with a lower incidence and severity of GVHD; (2) it preserves immunocompetence for primary immune responses[33,69]; and (3) it can be induced by means other than myeloablative conditioning. Therefore, mixed chimerism represents the optimal approach for the induction of sustained transplantation tolerance, as well as for treatment of a number of benign hematopoietic disorders.

DIFFICULTIES IN THE APPLICATION OF CHIMERISM PROTOCOLS IN CLINICAL CTA

Despite the potential of mixed chimerism for inducing tolerance to CTA, several important considerations must be addressed before it can achieve widespread clinical application. The most important challenges are (1) avoidance of GVHD; (2) toxicity of the conditioning regimens; and (3) the logistics of simultaneous CTA and BM transplantation in the setting of deceased donor transplantation. It is worth mentioning that most experimental protocols for inducing tolerance have included a 28-day delay between donor BM transplantation and CTA.

APPROACHES TO AVOID GVHD

Despite the availability of potent novel immunosuppressive agents, GVHD continues to be a significant clinical problem, particularly for patients receiving unmodified BM grafts from unrelated donors. The severity of GVHD is directly correlated with the degree of mismatch between donor and recipient.[70,71] Despite matching all 6 major HLA antigens, there is still a 40% incidence of GVHD and a 20%

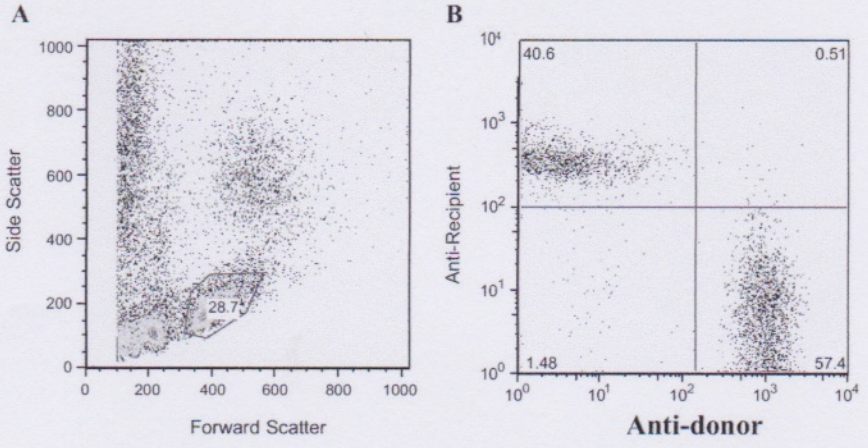

Figure 2. Detection of donor- and host-derived cells of lymphoid lineage in mixed allogeneic chimeras using 2-color flow cytometry. Recipient WF rats received anti-TCR-$\alpha\beta$ mAb on day −3 and followed by 300 cGy TBI and were transplanted with 100 × 10^6 T-cell–depleted ACI BM cells on day 0. Chimeric typing was performed 1 month after reconstitution. **(A)** Lymphocytes were gated based on forward and side scatter. **(B)** In mixed allogeneic chimeras, cell lines of lymphoid of host and donor origin were present.

mortality rate for those with GVHD. If 5 of 6 HLA antigens are matched, there is a 60% incidence of GVHD with a mortality of 50%. If ≤4 major antigens are matched, there is a 100% incidence of GVHD with the mortality rate increasing to 80%.[72] Primary effector cells for GVHD are donor cytotoxic $CD8^+$ T cells and natural killer (NK) cells.[73] The incidence and severity of GVHD has been effectively reduced in animal models and in humans by depleting T cells from the donor BM.[74] Although GVHD was successfully avoided, a significant increase in failure of engraftment was observed.[74] This observation of T-cell–depleted BM graft failure gave rise to 2 hypotheses: either T cells are essential for engraftment so that engraftment cannot be achieved without GVHD or cells that enhance engraftment are different from T cells but removed by the T-cell depletion procedure.

Rare event cell sorting was utilized to phenotypically and functionally determine precisely which cell facilitates engraftment of purified allogeneic BM stem cells in a major histocompatibility complex (MHC)-specific fashion while avoiding GVHD. Ildstad et al[75] were the first to characterize graft facilitating cells (FC) as $CD8^+/TCR^-$.[76] The FC population forms only 0.4% of the total BM and constitutes <1.6% of the total lymphoid gate (Fig 3). An ablated recipient will survive when 1,000 syngeneic purified stem cells are transplanted. However, the same recipient will not survive after infusion of 10,000 allogeneic purified stem cells owing to failure of engraftment. The addition of 30,000 $CD8^+/TCR^-$ FC from the same donor enables purified allogeneic stem cells to engraft.[77]

Another distinct T-cell population that is critical to peripheral regulation of GVHD are T regulatory cells (T_{reg}).[78] The best studied T_{reg} subsets are $CD4^+$ T cells arising during T-cell development[79] in the thymus. These cells constitutively express CD25, the alpha chain of the interleukin (IL)-2 receptor T_{reg}, and comprise 5%–10% of peripheral $CD4^+$ T cells in healthy mice and humans.[80,81] Very recently, it has become clear that human peripheral blood $CD4^+/CD25^+$ T cells are heterogeneous and contain both $CD4^+/CD25^{high}/CD127^-$ cells (2%–3% of $CD4^+$ T cells), which represent bona fide T_{reg} with potent suppressive T_{reg} activity, as well as many more $CD4^+/CD25^{medium}$ $CD127^+$ nonregulatory effector T cells.[82] The suppressive mechanism of $CD4^+/CD25^{high}$ T_{reg} is unclear at present but is believed to be mainly cell-contact dependent in vitro, although suppressive cytokines like IL-10 and transforming growth factor (TGF)-β have been reported to play a role, particularly in vivo.[83] The $CD4^+/CD25^{high}$ T_{reg} are best identified by their expression of the transcriptional regulatory factor FoxP3, which seems to serve as a master control gene for T_{reg} development and function.[84] Distinct from these natural T_{reg} that tend to be specific for self-antigens are the induced T_{reg} that are induced during inflammation in peripheral tissues or generated in the laboratory. These cells typically have a specificity for distinct cell types, tumors, or exogenous antigens, making them ideal responders to pathogenic microbes[85] and as a cell-based method to induce tolerance. With a murine experimental autoimmune thyroiditis (EAT) model, Morris et al[86] demonstrated the existence of $CD4^+/CD25^+/FoxP3^+$ T_{reg} influencing thyroiditis development in naive susceptible mice. These $FoxP3^+$ T_{reg} were required for induction of antigen-specific tolerance. Their data reinforce the important role of T_{reg} in mediating self-tolerance.

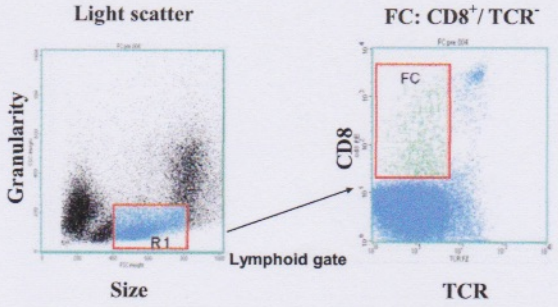

Figure 3. Sorting for FC. FC are isolated from the lymphoid gate for $CD8^+/TCR^-$. Each dot represents a cell. The $CD8^+/TCR^-$ cell population is enclosed in the red box.

One study of patients with acute GVHD reported an inverse correlation between FoxP3 expression and the grade of GVHD.[87] Expression of FoxP3 mRNA was almost undetectable in patients with grade III–IV GVHD and significantly reduced (by almost 2-fold) in patients with grade I–II GVHD, compared with patients who did not develop clinical GVHD. Sequential analysis of peripheral blood lymphocytes from patients with acute GVHD evolving into chronic GVHD revealed that FoxP3 expression was consistently reduced where the disease was active, but returned to normal after resolution of GVHD. Taken together, these data suggest that these T_{reg} are recent thymic emigrants and play a crucial role in downregulating GVHD.

There is accumulating evidence demonstrating that FoxP3 is transiently expressed in activated T cells where it can be detected within 24 hours and peaks at 72 hours.[88] The observation that abundant FoxP3 messenger RNA was detected in the recently activated $CD4^+/CD25^+$ cells lacking regulatory function[89] suggests that FoxP3 expression alone is not sufficient to indicate regulatory activity of $CD4^+/CD25^+$ cells. Besides, conflicting data have been reported with regard to the role of T_{reg} in the development of chronic GVHD in humans. The first study was performed by Clark et al[90] in which they defined T_{reg} as $CD4^+/CD25^{high}$ and used flow cytometry to measure the size of the T_{reg} pool in peripheral blood of 40 patients who survived >100 days after allogeneic hematopoietic cell transplantation (alloHCT). The authors reported that patients with chronic GVHD had significantly increased T_{reg}, expressed both as a percentage of $CD4^+$ T cells or as absolute counts, compared with patients without chronic GVHD. Sanchez et al[91] conducted a similar study on 35 consecutive patients who underwent alloHCT. They found

a small, but not significant, increase in the absolute number of $CD4^+/CD25^{high}$ T_{reg} in patients with chronic GVHD compared with patients without the disease. The authors also examined the ratio of activated nonregulatory $CD134^+$ ($OX40^+$) T cells over $CD4^+/CD25^{high}$ T_{reg} and found that the $CD134^+/CD25^{high}$ ratio was remarkably higher in patients with active chronic GVHD compared with patients without chronic GVHD or with resolved chronic GVHD. Therefore, some investigators have proposed the use of the ratio of T_{reg} relative to T effector cells (T_{eff}) under their immunosuppressive control as a measure of T_{reg} activity in vivo.[92] In fact, the results obtained so far appear to support the view that the balance of T_{reg}/T_{eff} tips toward T_{eff} in patients with GVHD compared with patients without the disease.

Recent publications in the hand transplant literature have shown that $Foxp3^+$ T_{reg} infiltrate the skin of hand allografts.[93] This may explain the observation that rejection has not been a major limitation for what is believed to be a highly antigenic tissue source.[94] These T_{reg} comprise the cutaneous immune system.

A ROLE FOR T_{reg} IN CUTANEOUS IMMUNITY

Another possible explanation for the apparent discrepancies about T_{reg} function in these studies is that they simply reflect the varying strategies employed to measure the T_{reg} population. Additional biomarkers of human T_{reg} cells may help to resolve these seemingly contradicting results. Hirahara et al[95] recently reported that nearly all peripheral blood $CD4^+/CD25^{high}$ $FoxP3^+$ T_{reg} expressed high levels of the chemokine receptor CCR4. Moreover, 80% of T_{reg} expressed cutaneous lymphocyte antigen (CLA) and 73% expressed CCR6. These molecules were functional, as CLA^+ T_{reg} showed CD62E ligand activity and demonstrable chemotactic responses to the CCR4 ligands CCL22 and CCL17 and to the CCR6 ligand CCL20. The phenotype and chemotactic response of these T_{reg} were significantly different from those of $CD4^+/CD25^{medium}$ nonregulatory T cells. Liu et al[96] found that IL-7 receptor (CD127) is downregulated on T_{reg} $CD4^+$ T cells in peripheral blood. The majority of these cells are $FoxP3^+$ and a combination of $CD4^+$, $CD25^+$, and $CD127^-$ resulted in selection of a highly purified population of T_{reg} cells. Thus, utilization of new cell surface markers such as CCR4 and CD127 might be beneficial for the selection and expansion of T_{reg} cells for diagnostic and therapeutic applications.

CONDITIONING OF THE RECIPIENT

The recipient's hematopoietic system must be immunologically modified or conditioned to allow engraftment of allogeneic stem cells. The fact that chimeras with 1% donor chimerism are just as tolerant as those with 100% donor chimerism led to a search for approaches to minimally condition rather than ablate the recipient to intentionally establish mixed donor:host chimerism. To avoid the adverse effects of ablative conditioning, alternative strategies such as polyclonal and monoclonal antibodies (anti-CD4/CD8, anti-NK, anti–TCR-$\alpha\beta$/TCR-$\gamma\delta$), and immunosuppressive drugs (tacrolimus, cyclophosphamide) have been successfully used either alone or in combination with low doses of irradiation.[97,98] The underlying discovery in these studies was the recognition that the dominant role for conditioning was immunologic rather than to prepare vacant niches. The first demonstration that ablation was not essential to engraftment was reported in a mouse model in the early 1970s. Chimerism could be established using antilymphocyte serum alone if the donor and recipient were closely matched genetically.[99] The administration of alkylating drugs like cyclophosphamide after donor marrow transplantation also significantly reduces the minimum total body irradiation (TBI) dose for conditioning. Conditioning of mice with 200 cGy TBI followed by a single dose of cyclophosphamide on day +2 relative to the marrow infusion established mixed chimerism in MHC-disparate recipients.[100] If the recipients were conditioned and not transplanted, endogenous hematopoiesis resumed, demonstrating the nonmyeloablative nature of this regimen.[101] This 200 cGy based approach was validated in dogs and is now widely used in the clinic as an outpatient procedure.[56] In a rat model, Ozer et al[102] found that administration of cyclosporine and ALS for 21 days induced donor-specific tolerance in the recipients of the rat hind limb composite tissue allografts.

The minimum dose of TBI for establishing chimerism has also been reduced by targeting costimulatory molecules. Costimulatory molecules comprise signal 2 in T-cell activation (Fig 4). A number of costimulatory molecules are required to mediate T-cell activation. For T-cell activation to occur, ≥2 signals are required: signal 1 is characterized by T-cell receptor recognition of a MHC:antigen complex; signal 2 involves the binding of B7 or CD40 on an antigen-presenting cell to CD28 or CD154 on the surface of T cells, a process known as co-stimulation. If T-cell receptors recognize antigen in the context of MHC (signal 1) without receiving costimulatory signals, anergy is induced. Wekerle et al[103] found that treatment of naive mice with a high dose of fully MHC-mismatched allogeneic BM, followed by 1 injection each of monoclonal antibody against CD154 and cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4-Ig), resulted in multilineage hematopoietic macrochimerism (approximately 15% donor) that persisted for up to 34 weeks. Foster et al[104] demonstrated that CD28 blockade (CTLA4-Ig, 2 mg/kg per day, alternate days), in combination with tacrolimus (1 mg/kg per day, daily) from day 0 through day +10, a single dosage antilymphocyte serum (10 mg at day +10), and 300 cGy TBI before BMT resulted in multilineage mixed chimerism (of 17% four weeks post-BMT) and effectively inhibited the development of acute and chronic rejection of vascularized hindlimb allografts in rats.

Nonmyeloablative conditioning has been successfully translated to large animals. BM infusion into dogs conditioned with 200 cGy TBI and treated with mycophenolate

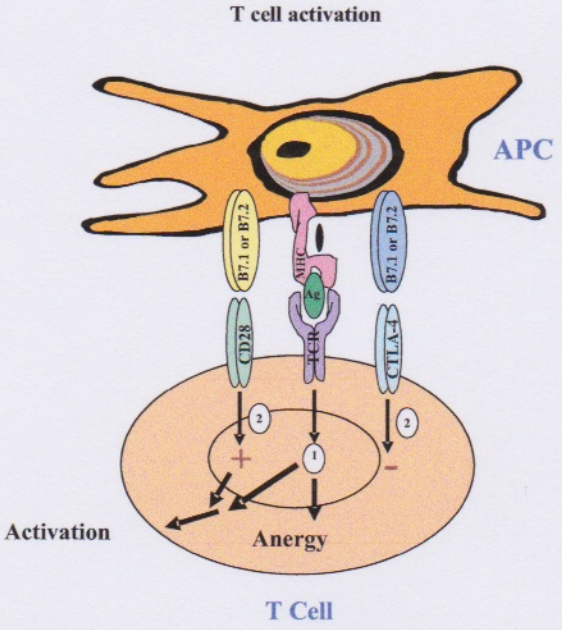

Figure 4. T cells require 2 signals from APC to be activated. The first signal is provided by binding of the TCR to an antigen presented by MHC on APC. The second signal involves the interaction of costimulatory molecules between T cells and APC. Activation of T cells requires the engagement of both signals 1 and 2. Delivery of signal 1 without signal 2 induces anergy and immune deviation toward tolerance.

mofetil and cyclosporine posttransplant resulted in stable mixed chimerism in 50% of recipients up to 97 weeks. The addition of fludarabine to this conditioning approach achieved engraftment in 100% of recipients.[105] Addition of CTLA4-Ig to the conditioning approach allowed durable chimerism to be established with only 100 cGy TBI. More recently, the TBI was replaced by the administration of anti-CD45 monoclonal antibody conjugated to Bismuth 213, an α-emitter that is short lived (half-life of 45 minutes).[105] CD45 is expressed on all hematopoietic stem cell-derived lineages and thus allows a very specific target for conditioning.

The successful establishment of mixed chimerism in humans was a significant advance toward the clinical use of this approach for the induction of tolerance in CTA recipients. Conditioning of recipients with 200 cGy TBI in combination with fludarabine and cyclophosphamide administered both pre- and post-BM infusion from haploidentical donors resulted in acceptable rates of GVHD and actuarial overall survival at 2 years of 36%.[103] Additionally, this nonmyeloablative conditioning approach is now widely used in treatment of several benign conditions. Consequently, the morbidity and mortality owing to BMT have declined markedly. Spitzer et al[106] took this approach 1 step further by performing a combined renal and BM transplantation in patients with end-stage renal disease secondary to multiple myeloma. After a nonmyeloablative conditioning regimen (cyclophosphamide, thymic irradiation, and peritransplant antithymocyte globulin), a combined BM and kidney transplant was performed. Although 4 of the subjects lost donor chimerism between 71 and 123 days posttransplant, all 6 patients accepted their kidney grafts long term. Moreover, 3 of the 6 were successfully tapered off immunosuppression for 1.3 to >7 years. Three patients are in sustained complete remission of multiple myeloma, despite loss of chimerism in 2. More recently, a dual approach with simultaneous renal and BM transplantation has been utilized. This strategy was used in 1 HLA matched and 5 haplomatched renal allograft recipients without underlying malignancy.[67,107] Despite antibody-mediated rejection episodes in 3 of the 5, and loss of chimerism within 2 weeks in all, 4 of 5 were subsequently tapered off all immunosuppression. Although the overall goal to establish demonstrable durable chimerism was not achieved, this represents a novel new approach to minimize immunosuppression. This early success has enormous implications for application in composite tissue allograft recipients.

SUMMARY

The field of CTA is expected to grow exponentially in the next decade. The long-term success of hand transplantation and the early success of complex facial allotransplantation has generated tremendous excitement and stirred great debate both in the clinic and the laboratory. However, future growth in the field will hinge on the development of novel methods aimed at reduction of immunosuppression and more individualized management, as well as the clinical application of basic research in tolerance. The transplanted tissue in CTA lends itself to easy observation and safe biopsy, thus providing a unique opportunity to study immunology in real time. The lessons learned could have vast ramifications for the entire field of transplantation.

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