Editorial

Regional delivery of immunosuppression for transplantation of vascularized composite allografts: opportunities near and far

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The pathway to innovation of vascularized composite allografts (VCA), such as grafts of allogeneic limbs or face, differs in important ways from pathway to innovation of clinical organ allografts. Although innovations in organ transplantation might be tested initially in large animal models, which better represent some aspects of human biology than laboratory mice, ultimately innovations in organ transplantation must be optimized in carefully planned and well powered clinical trials before a decision is made about whether clinical application is warranted. Innovations in VCA transplantation might also undergo initial testing in large animal models, such as the model Lellouch *et al.* (1) used to explore the potential utility of FK506-impregnated disks in VCA transplantation, but ultimately decisions about application must be reached without relying on results of clinical trials like those now standard in organ transplantation. This difference between organ and VCA transplantation reflects the profound difference in experience—currently about 150,000 organ transplants are performed world-wide, while in the most prolific year 15 VCA were performed.

Optimizing immunosuppression for VCA

If recipients of VCA are scarce, the imperative for optimizing immunosuppression while minimizing risks is compelling. In contrast to organ transplants, VCA usually are not performed to rescue the recipient from a severe, potentially lethal condition but rather to correct disability and improve the quality of life. Nor are VCA considered "life-supporting" transplants, as are organ transplants. Consistent with these distinctions, the recipients of clinical VCA, such as limb or face transplants, are generally healthy and presence of a significant underlying disease might contraindicate VCA transplantation. Organ transplant recipients always have a serious underlying disease and a person with clinically stable and/or mild condition would be directed away from organ transplantation and would be among the last to be offered a donated organ. Because VCA recipients are healthy, the immunosuppressive regimens used to avert rejection should minimize the risk of life-threatening complications and toxicities. Indeed, the VCA graft and not the recipient should bear the greater burden of risk. In contrast, immunosuppressive regimens used in organ transplantation might and often do impose risks of serious complications because survival of the recipient depends on survival of the graft.

Lellouch and co-workers have long sought novel approaches to minimizing the risks of immunosuppressive therapy in VCA transplantation. As one approach, the authors have been exploring local delivery immunosuppression, reasoning that success would allow the dose and hence the risk of systemic immunosuppression to be decreased. As another approach the authors have explored the long-standing idea that induction and maintenance of allogeneic tolerance through the engrafting of allogeneic hematopoietic

cells might eliminate the need for immunosuppressive therapy thereby eliminating risk. The current report (1) describes experiments in which film-like disks impregnated with FK506, a calcineurin inhibitor also known as tacrolimus, were implanted below skin edges of allogeneic facial VCA in cynomolgus monkeys in efforts to decrease the need for systemic FK506 delivery to support VCA. The authors sought to optimize dose and formulation and to test whether local delivery of FK506 in this way could avert rejection and enable development of chimerism. Local delivery of FK506 and other drugs was previously reported to prevent rejection of VCA in rodents, but experience in randomly bred large animals and in clinical settings is quite limited.

The experiments described in this new report show that this new approach does contribute to immunosuppression and in conjunction with low doses of other agents and FK506 given systemically may help thwart rejection of VCA. Since FK506 is approved for topical use in skin diseases, such as psoriasis, evidence of efficacy of local FK506 in non-human primates might be taken to support use in clinical VCA recipients. However, several monkeys in which FK506-impregnated film was introduced exhibited toxicity. One monkey developed unacceptably high levels of the drug, which the authors ascribe to flawed formulation, and three subsequently treated with whole body irradiation and allogeneic bone marrow experienced post-transplant lymphoproliferative disease. The question then is how one should weigh evidence of biological efficacy against the evidence of toxicity in experiments performed in a small number of non-human primates?

We are more impressed by evidence of biological efficacy. The implantation of impregnated film effectively delivers FK506 since rejection is indeed prevented. Moreover, the "toxicity" Lellouch *et al.* (1) describe largely reflects effective release of the drug leading to high, potentially toxic, systemic levels. Since the number and formulation of disks and the frequency of implantation remain to be optimized, the results support the possibility that optimum use of disks as local source of FK506 could allow the doses of oral FK506 to be decreased leading to a net decrease in risks imposed by the drug. However, the small number of monkeys used cannot be taken to represent the range of responses likely to be seen in a large diverse population of treated individuals.

On the other hand, we see little evidence of toxicity of FK506 unequivocally linked to local delivery. We do not consider monkeys conditioned for hematopoietic stem cell engraftment to represent a clinically relevant test of local FK506 for VCA recipients. Such conditioning is not a standard practice in VCA transplantation. Rather, occurrence of lymphoproliferative disease could be taken to reflect effective control of development and/or functions of effector memory T cells manifest as loss of T-dependent viral latency. Hence, while we commend the conservative interpretation Lellouch *et al.* (1) apply to their results we do not believe clear evidence of toxicity was observed under conditions that represent current management of VCA.

Challenges and opportunities associated with large animal models and clinical trials in transplantation

New immunosuppressive drugs or regimens in transplantation are nearly always tested in non-human primates. However, complex models using non-human primates often under-represent efficacy and over-represent complications and toxicity that would be observed in clinical settings when use has been optimized and risks ameliorated by availability of more complete and effective monitoring (2). Regardless of the weight one might give evidence of efficacy and toxicity reported by Lellouch *et al.* (1), the findings highlight one difference between VCA from organ transplantation that has the most profound implications for how innovations can be advanced to clinical application.

Although recipients of VCA and recipients of organ transplants receive similar amounts of immunosuppression (3), rejection occurs at higher frequency in clinical VCA than in clinical organ transplants. During the first year after transplantation nearly all VCA undergo a rejection reaction, some repeatedly, while only 10–15% of kidney transplants undergo a proven rejection reaction (4,5). This difference makes it possible to test mitigation of rejection in clinically relevant conditions (e.g., with current regimens of immunosuppression) using relatively few non-human primates. Although statistical tests were not applicable for this preliminary examination local FK506, the authors claim that the considerable experience at their center indicates results of three transplants per condition in non-human primates would "provide statistical power for the detection of survival differences" (1). Regardless of one's views concerning the authors' claims, three animals do provide a power of 80% in a VCA model in which 90% of controls experienced rejection and treated animals did not. In contrast, more than 90 kidney

transplants in non-human primates would be needed to achieve the same power for testing an innovation that would mitigate rejection. Indeed, the scarcity of acute rejection of organ transplantation has made it necessary to employ sub-optimal immunosuppression in experimental models and to use endpoints other than acute rejection in clinical trials. For example, clinical trials in kidney transplantation now commonly use long term (5 or 10 years) outcomes or surrogate markers of immunity in lieu of acute rejection (6) and experiments in non-human primates might include cessation or suboptimal dosing of immunosuppression to test innovations in immunosuppression. What deserves wider recognition then is that notwithstanding technical challenges, the high frequency of rejection, makes clinical and experimental VCA (in large animals given current regimens of immunosuppression) profoundly more efficient systems for testing innovations like FK506 implants.

The high frequency of acute rejection in VCA

Why does rejection occur with such greater frequently in clinical VCA than in clinical organ transplants? Some postulate the high frequency of rejection of VCA reflects the presence of allogeneic skin, which is more immunogenic than allogeneic organ tissue. We certainly agree that skin is quite immunogenic, skin allografts in unmodified recipients nearly always evoke alloantibody responses and can reliably distinguish monozygotic from di-zygotic relationships. However, organ allografts may be more immunogenic than commonly appreciated. Clinical renal allografts always generate donor-specific B cell responses (7) and like skin allografts always fail after transplantation into unmodified recipients (8). More important however is that other differences between VCA and organ transplants may suggest avenues for fundamental and clinical progress.

Another explanation for the high frequency of rejection in VCA reflects the ability to continuously visualize the skin component of VCA. Because skin is quite sensitive to regional inflammation and changes are quickly seen or felt by recipients even minor episodes of rejection are reported and potentially treated. In contrast, rejection of organ transplants sometimes must await periodic visits to clinics and monitoring of blood or surveillance biopsies. The ready availability of skin for inspection and biopsy allows nearly continuous assessment of interventions and potentially facilitates repeated treatment with local FK506.

Local delivery of immunosuppression for cell-mediated conditions?

The report of Lellouch *et al.* (1) might prompt yet another question and that is why local delivery of FK506 would be expected to exert any biological impact. Although FK506 is thought to prevent rejection of allografts by blocking activation of naïve and memory T cells, this mechanism does not fully explain the efficacy and limitations observed in clinical transplantation or the prospects for efficacy of local delivery. Thus, while FK506 appears to hinder some functions of stimulated naïve and memory T cells tested *in vitro*, experimental and clinical experience indicates the agent suppresses naïve T cells more effectively than memory T cells (9). Thus, FK506 can prevent rejection of unsensitized recipients but by itself poorly if ever prevents rejection of sensitized recipients. Likewise, memory T cells in most transplant recipients receiving FK506 can exert at least some control of latency of ubiquitous human herpesviruses. But, since activation of all naïve T cells (and some memory T cells) occurs exclusively in lymphoid tissues efficacy of FK506 should depend on systemic levels that deliver the drug to lymphoid organs. If that is correct then the principal benefit of local delivery might reflect the relatively continuous elution of the drug from the implants, averting the fluctuation in drug levels associated with intermittent oral or parenteral administration.

But, eliminating fluctuation of drug levels cannot explain how topical FK506 controls autoimmune diseases of skin, presumably mediated by effector memory T cells, like the effector memory T cells that control viral replication and latency and resist control by calcineurin inhibitors. We would entertain an alternative explanation for the efficacy of FK506 in rejection and autoimmunity. The migration of effector T cells into target tissues and actions therein depends in part on interactions with endothelial cells that further activate the T cells and enable migration into target tissues. The interactions between T cells and endothelial cells depend in part on activation of endothelial cells. Antibodies that block these interactions are generally too toxic for clinical use, as the antibodies prevent would block the contribution of phagocytes to host defense throughout the body. Local suppression of T cell-endothelial cell interaction would seem preferable. Some years ago we showed that FK506 blocks transcription of a select set of genes in endothelial cells, including genes encoding proteins that could enable interaction with effector memory T cells

(10,11). While systemic delivery of FK506 undoubtedly suppresses activation of naïve T cells and perhaps some memory T cells, local delivery of FK506 might confer benefit by a different mechanism. If FK506 indeed does inhibit interactions between effector T cells and endothelial cells, that could explain why calcineurin inhibitors so effectively prevent rejection reactions mediated by memory T cells and how local delivery of FK506 to small areas of skin effectively thwart T cell-mediated injury at sites or lesions where the drug is applied but not elsewhere. We think the work of Lellouch *et al.* (1) could offer insights of potential import in VCA transplantation and beyond.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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