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LENTIVIRAL-MEDIATED INTERLEUKIN-10 GENE THERAPY FOR LUNG TRANSPLANTATION

To the Editor:

We read with great interest the article published by Oishi and colleagues,¹ which reported that lentiviral-mediated interleukin-10 (IL-10) gene therapy decreased allograft lung rejection. We would like to congratulate Oishi and colleagues¹ for this elegant study. In addition, we admire the work that has been done by that laboratory (led by Dr Keshavjee) regarding gene delivery in lung transplantation, and personally we² share the overall enthusiasm of Oishi and colleagues.¹ We would like to add several discussion points that we believe will increase the interest in this emerging topic.

Because of well-known limitations of adenoviral gene therapy, Oishi and colleagues¹ used lentiviral vectors, which demonstrated capacity to mediate stable, long-term transgene expression in vivo. Most recently, lentiviral vectors have become a promising tool for safely manipulating the mammalian genome. Despite some well-documented safety concerns with lentivirus, it is important to note that the total number of clinical trials worldwide with lentivirus has increased to 7.3% in 2017, as opposed to 2.9% in 2012. The most pressing safety concern regarding lentiviral vectors are that they are derived from HIV type 1 and have the potential to cause insertional mutagenesis at the site of lentivirus integration. Deleterious serious side effects of an induced host response could include oncogenic, infectious, and other transformative changes in the treated cells. Another major disadvantage is that lentiviruses can target multiple genes and pathways simultaneously in both target and off-target cells, which can cause further complications with time.³

One concern that we would like to address to expand the discussion is the idea that leveraging one cytokine can address a complex problem, such as allograft rejection. Oishi and

colleagues¹ demonstrated that IL-10 gene therapy has a positive effect in a mouse transplantation model. It is well known that IL-10 is a pleiotropic cytokine that not only functions as an anti-inflammatory cytokine but also has been shown to have a role in immune stimulation and to regulate key lymphoid and myeloid cells. In a previous study, Oishi and colleagues¹ showed that most of the transduced lung cells after intratracheal administration of lentivirus-mediated marker gene are alveolar macrophages. IL-10 may cause downregulation of protective cytokines such as interferon- γ , however, with concurrent inhibition of alveolar macrophages function and their microbicidal capacity with impaired bacterial clearance of respiratory tree.⁴ For example, when mice are treated with recombinant IL-10, disease caused by *Streptococcus pneumoniae* is much more severe.⁵ Moreover, it is well known that sequence homology of human and mouse IL-10 are not the same, and Oishi and colleagues¹ demonstrated that exogenous delivery of human IL-10 increased messenger RNA expression of mouse IL-10 by a factor of greater than several times. So which IL-10, exogenous or endogenous, can actually prevent allograft rejection?

We agree with the key assertion of Oishi and colleagues¹ that ex vivo gene delivery to the lung graft before transplantation is very attractive because of its reducing to a certain extent the collateral overexpression to other organs. Human IL-10 level in the graft at day 1 was 6.7 pg/mg protein after only ex vivo administration, which is not a low concentration compared with day 28 after multiple in vivo injection. We believe that this study will be improved if Oishi and colleagues¹ added an experimental group of animals treated only by ex vivo gene delivery. Answering the question of what level of IL-10 concentration is necessary to produce a positive effect on rejection is of most importance weighted against the risks in vivo.

In summary, Oishi and colleagues¹ have documented that lentiviral-mediated IL-10 delivery decreased lung allograft rejection in mice. Future small and large animal studies are needed to confirm the efficacy of this protocol.

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Funded by the National Institutes of Health, grant 7R01 HL083078.

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<https://doi.org/10.1016/j.jtcvs.2018.07.052>

LENTIVIRAL INTERLEUKIN-10 GENE THERAPY: SAFETY AND QUESTIONS

Reply to the Editor:

Gene therapy and gene editing (functional modification of genes) represent a future direction for treatment of human diseases. Viral vector-based gene delivery or gene transfer is crucial for this purpose. Thoracic and cardiovascular surgery provides great opportunities for translating experimental discoveries toward clinical applications. Awareness of the challenges and limitations of gene delivery is essential for clinician investigators in this field.

We are very glad that our recent article¹ has drawn interest and stimulated discussion on this subject. Katz and colleagues raise several questions related to our work for further dialogue. Clarification of these concerns may improve understanding of the scientific work related to gene therapy and help us to further develop our research focused on prevention and treatment of chronic lung allograft dysfunction, a major complication after lung transplantation.

In our report, we chose to use recombinant lentivirus (LV) as a delivery vehicle specifically for its ability to infect a variety of cells and generate persistent transgene expression in vivo. Katz and colleagues raise concerns about the safety of using LV. The first approved clinical trial using recombinant LV was in 2002. According to Gene Therapy Clinical Trials Worldwide (<http://www.abedia.com/wiley/>), there are at least 196 independent LV-based clinical trials that have opened worldwide. Fortunately, the “deleterious serious side effects of an induced host response” that concern Katz and colleagues have not been seen in clinical trials. Furthermore, although recombinant LV vectors were derived from human immunodeficiency virus (HIV), patients with HIV display negligible increases in blood cancers that are not due to immune dysfunction. In contrast to wild-type HIV, recombinant

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LVs integrate once and do not replicate in treated cells because these recombinant virions do not establish a productive infection themselves nor do they produce HIV proteins from their integrated proviral state. Lastly, whereas LV integration is relatively random, the effective multiplicity of infection we are achieving in productively infected cells is likely not high enough to cause a large number of integrative effects that could disrupt many pathways simultaneously. The safety of viral-based gene delivery is important. More evidence from experimental research and clinical trials should be collected and closely monitored.

Katz and colleagues further challenge the use of interleukin (IL) 10 as the choice of therapeutic gene. We agree that IL-10 has well known and important immunomodulatory effects on monocytes, macrophages, and dendritic cells, and it can inhibit T cell activation.²⁻⁴ Conversely, IL-10 can drive B cell immunoglobulin class switching and thus promote humoral immune responses.⁵ It is these immunomodulatory functions and anti-inflammatory properties that make us interested in IL-10 as a candidate to prevent the development of chronic lung allograft dysfunction, a process with complicated immunologic and inflammatory mechanisms. Whether long-lasting IL-10 gene expression in the lung allograft is beneficial was the subject of our study. Nevertheless, further mechanistic and safety studies are warranted before clinical use.

Our observation that LV-driven human IL-10 increased mouse IL-10 expression in the grafts certainly supports the concept that regulation of the alloimmune response may result from such a therapy. Whether the attenuated chronic rejection we observed is attributable primarily to the exogenously administered human IL-10 or to the endogenously upregulated mouse IL-10 is unclear from our study and merits further investigation. At the same time, we concede that tonic IL-10 expression may impair protective immunity, and we were unable to test this possibility in our specific pathogen-free mice. Effects of IL-10 gene therapy on secondary infection susceptibility in a lung transplant setting are indeed important and interesting and merit further study. Treatment of the allograft with IL-10 ex vivo before transplantation without a subsequent in vivo component is also an attractive approach that we are currently examining.

Overall, the safety of viral vectors should be considered both in experimental studies and through clinical trials.