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Urinary nanosensors of early transplant rejection

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Abstract

Nanoparticle sensors of the activity of the protease granzyme B detect early T-cell-mediated rejection of transplanted skin grafts in mice via the release of a proteolytically cleaved fluorescence reporter that filters into urine.

Organ replacement in end-stage organ failure started with the successful transplantation of a kidney between two monozygotic twins¹. In this case, the twins' genetic similarity prevented the immunological rejection of the transplanted organ. However, most transplanted organs are from deceased or living donors, and the genetic disparities between the donor organ and the recipient host can trigger immune responses that often lead to the rejection of the allograft. Life-long pharmacological immunosuppression is thus necessary to maintain allograft survival. Yet chronic immunosuppression carries the risk of adverse effects and complications, such as opportunistic infections, malignancies, diabetes mellitus and hypertension. And even with adequate immunosuppression regimens, acute rejection can occur within days or weeks of the transplantation as a result of pre-sensitization to immunogenic donor antigens (and can occur much later in the case of inadequate immunosuppression²). Acute rejection may be caused by humoral or cellular processes; the latter is mediated by T cells that recognize donor histocompatibility antigens in the transplanted organ, leading to complications such as interstitial inflammation, tubulitis, endoarteritis and, in the case of renal transplants, glomerulitis³.

The need to detect transplant rejection early is critical to ensure graft survival and to initiate contingency measures. The diagnostic gold-standard for detecting transplant rejection involves the collection of a core biopsy — an invasive procedure that has limited predictive power and carries a morbidity risk. Allograft rejection can also be diagnosed non-invasively, for instance via urine analysis by detecting relevant mRNA transcripts or proteins biomarkers^{4,5}. By building on observations of cytotoxic CD8⁺ T cells infiltrating the allograft during acute rejection, Andrew Adams, Gabriel Kwong and colleagues now report in *Nature Biomedical Engineering* nanoparticles that sense granzyme B — a serine protease released by cytotoxic CD8⁺ T cells — and thus serve as a biomarker of T-cell-mediated rejection in a mouse model of skin-allograft rejection⁶. The nanoparticles, which accumulate preferentially in the allograft tissue, can detect granzyme-B activity early via reporter

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substrates that, after being specifically cleaved by granzyme B, are filtered by the kidney into the urine, where they can be detected non-invasively (Fig 1b).

Kwong and co-authors first identified a peptide, cleaved by granzyme B but not by coagulation proteases, that does not promote the activation of the complement cascade. The nanosensors consist of a formulation of iron oxide (approved by the United States Food and Drug Administration for clinical use) coated with polyethylene glycol to reduce its uptake by the reticuloendothelial system and conjugated to a near-infrared-fluorophore–quencher pair linked by the peptide. On granzyme-B-mediated cleavage of the peptide, the quenched fluorophore is released, resulting in the restoration of fluorescence (Fig. 1b). The authors first tested the ability of the nanosensors to detect, in a dose-dependent manner, the release of granzyme B following T-cell killing of cellular target cells in vitro. Data from a mouse model of skin-graft transplantation then revealed that, owing to increased permeability of local blood vessels as well as immune-cell infiltration after transplantation, the systemically administered nanosensors preferentially accumulated in the rejecting allograft, resulting in the enhanced detection of granzyme-B activity. The authors also observed minimal accumulation of the nanosensors in the contralateral isograft or in the bladder, although more of them accumulated in organs with fenestrated endothelium (the liver, spleen and lymph nodes) than in non-fenestrated organs (such as the brain, heart, kidney and lung). Importantly, the fluorescence in the urine of the animals bearing the allografts was approximately two-fold higher than that in the urine of the mice that received skin isografts.

Kwong and co-authors also show via histopathology that CD8⁺ T cells and granzyme B accumulated in the implanted skin allografts and in the draining lymph nodes of the allograft recipients after graft transplantation, that the nanosensors accumulated in the allogeneic skin in larger numbers than in skin isografts as early as day 3 after transplantation, and that a higher fluorescence signal than that before transplant was detected in the animals' urine as early as 5 days after transplantation in the recipients of allografts. Moreover, when the nanosensors were administered to skin-allograft recipients treated with a subtherapeutic daily dose of the immunosuppressant tacrolimus (which delayed allograft rejection by about 4 days), fluorescence in urine after transplantation (with respect to the pre-transplantation urinary signals) was significantly elevated on days 7 and 12 for the immunosuppressed recipients. Expectedly, these signals were significantly weaker than those from the untreated allograft-rejecting recipients. This reduction in signal could be the result of a slower rejection rate, owing to reduced cytotoxic-T-cell response within the allograft, or of immunosuppression-related inhibition of granzyme-B release by the cytotoxic T cells infiltrating the allograft. This could be clarified by testing whether a fully efficacious immunosuppression dose delivered at the same time as the nanosensors significantly blunts the release of granzyme B, and thus the sensitivity of the assay (calcineurin inhibitors such as Tacrolimus can inhibit granzyme-B granule exocytosis by CD8⁺ T-cell clones on T-cell-receptor activation⁷).

From a clinical standpoint, the nanosensors would in principle require repeated administration if they were to be useful for monitoring T-cell-mediated graft rejection over a patient's life. And because they comprise multiple potentially immunogenic antigens — the granzyme-B peptide, the quencher and the fluorochrome — it is reassuring that even when

inoculated intraperitoneally with complete Freund's adjuvant, the nanosensors did not elicit a detectable total immunoglobulin-G (IgG) response in the mice. Nevertheless, a number of caveats apply: first, a more sensitive quantification of specific IgG responses to the nanosensor components would be needed to ascertain their immunogenicity; second, the IgG response measured was limited only to primary immunization, and repeated delivery might elicit stronger recall antigen-specific IgG responses; and third, the IgG response was measured up to day 14 post-immunization, leaving open the possibility that an increase in titers of IgG specific to nanosensor components occurs at later time points. If either an antibody or even a T-cell-effector response is elicited by any of the nanosensor components, its utility for monitoring T-cell mediated rejection would be significantly curtailed. Moreover, in clinical use the fluorescence signals in the urine, which in Kwong and co-authors' study were normalized to those before graft transplantation, would need a baseline that is appropriate for patients who succumb to rejection years after graft transplantation. Finally, it remains to be determined if granzyme B is invariably upregulated in clinical T-cell-mediated rejection, and whether the nanoparticles could discriminate between rejection and antiviral immunity, which may also elicit protective CD8⁺ cytotoxic T cell responses^{9,10} yet require a different therapeutic response.

Kwong and colleagues' innovative approach to developing a non-invasive assay for detecting acute transplant rejection will need thorough testing of performance and immunogenicity in large animals. As the gold standard for the diagnosis of rejection requires an invasive core biopsy, such a non-invasive approach would enable a more frequent monitoring to diagnose T-cell-mediated rejection early, thereby allowing for prompt intervention and the successful preservation of long-term graft function. The ability to ensure the survival of a single allograft for the lifetime of the recipient is a worthy goal, and would go a long way towards alleviating the demand for more donor organs for the many patients facing end-stage organ failure.

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Fig. 1 |

Nanosensors for detecting acute T-cell-mediated transplant rejection. **a**, Release of fluorescence reporters by nanosensors exposed to the serine protease granzyme B, which is produced primarily by CD8⁺ T cells infiltrating an allograft during T-cell mediated rejection. The nanosensor consists of an iron oxide core conjugated to a fluorophore–quencher reporter pair via a peptide that is proteolytically cleavable by granzyme B. On cleavage, the fluorophore signal is restored. **b**, Granzyme B is detected by intravenously injected nanosensors that accumulate preferentially in the skin allograft. Following proteolytic cleavage of the Granzyme-B-specific reporter, the cleaved reporters undergo kidney filtration into the urine, enabling the non-invasive early detection of the cytotoxic-T-cell activity that is associated with graft rejection. Figure reproduced from ref. 6, Springer Nature.

[AE: The panels are from Fig. 2a and 1 in the Kwong Article.]