Dendritic Cells: Efficient Transfection and its Applications

Antigen presentation is essential in vaccine development, cancer treatment and other immunotherapies, as well as autoimmune disease therapies of many kinds. Various antigen presenting cells (APCs), such as macrophages, B cells, fibroblasts and dendritic cells (DCs), have been used in research studies and clinical applications. Of all the natural APCs, bone-marrow-derived DCs remain the most desirable APCs because they can prime naïve T cells and possess the highest potency for inducing differentiation of T cells into antigen-specific effector cells. However, until recently, the success of applications utilizing DCs has been largely compromised by inefficient transfection. Recent developments in cancer immunotherapy have centered on tumor-associated antigens delivered to, and presented by DCs. One such study by Heiser *et al.* demonstrated successful delivery of prostate-specific antigen (PSA) mRNA into human DCs (1). The PSA delivered by this method had no toxicity or adverse effects, as is often encountered with viral-mediated delivery of antigen-encoding DNA. Furthermore, a PSA-specific T-cell response was detected, and transient clearance of tumor cells was seen in patients.

In addition to cancer therapy, in which antigen-presenting DCs are used to potentiate T-cell responses, manipulation of DCs to diminish the T-cell response is also important in therapy for autoimmune diseases and organ transplantation. In a recent study by *Hill et al.* (2), human DCs were transfected with synthetic siRNA designed to silence the transcripts of the cytokine IL-12 *p*35 using a cationic lipid, GenePORTER® (Gene Therapy Systems). Through the mechanism of RNA interference (RNAi), IL-12 production was significantly reduced and resulted in suppressed DC allostimulatory effect in a mixed leukocyte reaction (MLR). Such 'tolerogenic' DCs are potentially important therapeutic and research tools for the future.

Central to all such applications is the robust and efficient transfection of DCs. Dendritic cells are notoriously difficult to transfect, and therefore the use of DCs for applications has been limited. Multiple factors determine the success of transfection, and recently, novel innovations have brought multiple breakthroughs for basic and applied scientists. Some of these breakthroughs include the preparation and maturation of DCs, choice of material to be delivered, (e.g., DNA, RNA, mRNA, dsRNA), and transfection reagents. Of these, the most exciting recent finding is that *in vitro* transcribed RNA appears to be easier to transfect into DCs than DNA (3). The obvious advantage of RNA delivery is that it does not involve viral vectors that could potentially cause adverse immunogenic effects in the host. Another significant advantage is the relatively short half-life of RNA, a trait that minimizes the potential adverse effects associated with prolonged expression by DNA, especially for tumor antigens. Finally, liposomal reagents have proven to be more effective than electroporation in RNA delivery to DCs (3). While mRNA transfection of DCs by liposomal

reagents can reach an impressive transfection efficiency of 20%, transfection of small interference RNA (siRNA) is remarkably higher. In fact, Hill, *et al.* (2) recently showed that the cationic lipid, GenePORTER®, could transfect DCs at an efficiency as high as 88%. Importantly, as an alternative to DNA or RNA delivery, protein antigens may be directly delivered to DCs. Doolan and Graber showed that the novel lipid reagent BioPORTER® (Gene Therapy Systems) successfully delivered a human malaria antigen protein, *circumsporozoite protein* (PfCSP), to DCs, which effectively presented the antigen, as demonstrated by ELIspot assays (4).

In summary, efficient transfection of dendritic cells provides a powerful tool for vaccine development, cancer immunotherapy and autoimmune disease therapy. Although DCs remain one of the most difficult cells to transfect, promising new methods combining cationic lipids and RNA have recently been successful. To expand the scope of applications with DCs, especially for vaccine development, novel methods of transfecting DNA into DCs have been, and will need to continuously be, significantly improved.

References

1. Heiser, A, *et al.* (2002) Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL response against metastatic prostate tumors. *J. Clin. Invest.* **109**: 409-417.
2. Hill, JA, *et al.* (2003) Immune modulation by silencing IL-12 production in dendritic cells using small interfering RNA. *J. Immun.* **171**: 691-696.
3. Strobel, I, *et al.* (2000) Human dendritic cells transfected with either RNA or DNA encoding influenza matrix protein M1 differ in their ability to stimulate cytotoxic T lymphocytes. *Gene Ther.* **7**: .2028-2035.
4. Doolan, DL and Graber NL, (2002) A simple method for delivery and antigen presentation of a malaria protein in human dendritic cells. *Delivery (GTS Newsletter)* **2** (2): 6-7.