The *Discussion Forum* provides a medium for airing your views on any issues related to the pharmaceutical industry and obtaining feedback and discussion on these views from others in the field. You can discuss issues that get you hot under the collar, practical problems at the bench, recently published literature, or just something bizarre or humorous that you wish to share. Publication of letters in this section is subject to editorial discretion and company-promotional letters will be rejected immediately. Furthermore, the views provided are those of the authors and are not intended to represent the views of the companies they work for. Moreover, these views do not reflect those of Elsevier, *Drug Discovery Today* or its editorial team. Please submit all letters to Steve Carney, Features Editor, *Drug Discovery Today*, e-mail: S.Carney@elsevier.com

Targeted drug delivery systems for the intracellular delivery of macromolecular drugs ▼

The review by Torchilin and Lukyanov on peptide and protein drug delivery to and into tumors [1] represents an excellent overview regarding the current state of research in this field. The continuing quest for novel drugs, often of macromolecular nature, should go hand in hand with the development of ways to formulate them in an appropriate dosage form. Given the complexity and vulnerability of novel macromolecular drugs being developed, the issue of delivery to the site of action is crucial. Torchilin and Lukyanov successfully describe the current approaches for enhanced delivery of macromolecular drugs like peptides and proteins to tumor sites using polymeric or liposomal carriers. Nevertheless, with respect to the intracellular delivery of the drug, we feel that the authors underestimate the importance of the endocytic route and overemphasize the more controversial membrane transduction pathway.

Cell-specific targeting can be achieved by attaching targeting ligands such as antibodies, peptides or carbohydrates to the delivery system. Ideally, the presence of such targeting ligands should not influence pharmacokinetics and target localization of the drug delivery system and should result in sufficient cytosolic accumulation of the targeted drug. Torchilin and Lukyanov describe two main routes to achieve this: endosomal release and membrane transduction.

The latter of these, membrane transduction, is a natural feature of a number of proteins involving so-called protein transduction domains (PTDs). Several cationic PTDs have now been described that translocate proteins [2] and even relatively large particles (up to 200 nm) across the cell membrane [3], by an as yet unidentified mechanism. A number of reports, and especially those on the membrane translocation of large particles [3–5], appear to be miraculous and recently, serious questions have been raised about the validity of the observations [6–8]. These recent reports point out that the cellular distribution of fluorescent PTDs or PTD-protein constructs is dramatically changed after fixation of the cells compared with the distribution in living cells [6–8]. We speculate that the cationic nature of the particle-associated PTDs allows for efficient cell-membrane interaction with any cell type, and can be followed by endocytic uptake comparable to the uptake mechanism observed for cationic non-viral gene delivery systems. In addition, one might also question the *in vivo* applicability of PTD-mediated translocation of drug carriers after intravenous administration, as the cationic nature of PTDs can be expected to reduce blood residence time and to cause aspecific cell association.

Therefore, to achieve cytosolic delivery of macromolecular drugs, we would favor the use of physiological cellular uptake mechanisms combined with inclusion of elements that enable endosomal escape. This approach combines cell specificity, by using cell-specific targeting ligands, with internalization mediated by the targeted receptor and endosomal escape induced by an 'escape' moiety incorporated in the system. We recently proved the feasibility of this approach with a targeted liposomal system developed to deliver the diphtheria toxin-A subunit into the cytoplasm of ovarian carcinoma cells [9]. By using liposomes that expose tumor cell-specific antibodies on their surface, and that contain in the aqueous interior the toxin plus an escape (fusogenic) peptide derived from influenza virus, we obtained a strong antitumor effect demonstrating delivery of the toxin into the cytoplasm. This targeted fusogenic system offers promise for the cytosolic delivery of other proteins as well.

Obviously, cytosolic delivery is not the end point in many cases, as the drug target is located in a defined subcellular compartment, for example, the nucleus in the case of gene therapeutics. In our view, intracellular targeting is a crucial step in the delivery of macromolecular drugs (whether peptide, protein or DNA). The drug delivery research field is only now beginning to address the challenge of this intracellular targeting step.

References

1 Torchilin V.P. and Lukyanov A.N. (2003) Peptide and protein drug delivery to and into tumors: challenges and solutions. *Drug Discov. Today* 8, 259–266

 1359-6446/03/$ – see front matter PII: S1359-6446(03)02699-0

2 Schwarze S.R. *et al.* (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* 285, 1569–1572
3 Torchilin V.P. *et al.* (2001) TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8786–8791
4 Lewin M. et al.(2000) Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. *Nat. Biotechnol.* 18, 410–414
5 Torchilin V.P. et al. (2003) Cell transfection in vitro and in vivo with nontoxic TAT peptide-liposome-DNA complexes. *Proc. Natl. Acad. Sci .U. S. A.* 100, 1972–1977
6 Lundberg M. and Johansson M. (2002) Positively charged DNA-binding proteins cause apparent cell membrane translocation. *Biochem. Biophys. Res. Commun.* 291, 367–371
7 Leifert J.A. et al. (2002) Full-length proteins attached to the HIV tat protein transduction domain are neither transduced between cells, nor exhibit enhanced immunogenicity. *Gene Ther.* 21, 1422–1428
8 Richard J.P. *et al.* (2003) Cell-penetrating peptides. A re-evaluation of the mechanism of cellular uptake. *J Biol. Chem.* 278, 585–590
9 Mastrobattista E. *et al.*. (2002) Functional characterization of an endosome-disruptive peptide and its application in cytosolic delivery of immunoliposome-entrapped proteins. *J.Biol.Chem.* 277, 27135–27143

Gerben A. Koning and Gert Storm
Department of Pharmaceutics
Utrecht Institute for Pharmaceutical Sciences
University of Utrecht
PO Box 80082
3508 TB Utrecht
The Netherlands

Engineered T lymphocytes – powerful killers ▼

The immune system has evolved to fight against pathogens and it is against its nature to destroy the self, even when the self becomes a threat to the survival of the whole organism. Moreover, cells that undergo malignant transformation do their best to prevent themselves from being recognized and destroyed. The term immunotherapy refers to the group of strategies that increase the ability of the immune system to recognize and destroy transformed cells. In a recent review in *Drug Discovery Today*, Whelan *et al.* [1] describe several humoral- and cellular-activating immunotherapy strategies that are under clinical investigation, together with an assessment of their results. These strategies are based on the idea that tumor antigens must be presented to the immune system in such a way that the immune system no longer tolerates self antigens, allowing cytotoxic T lymphocytes (CTLs) to be induced. CTLs are able to recognize and lyse specific target cells. Tumor antigens can be presented as naked DNA, peptides, proteins or whole tumor cells. Powerful antigen-presenting cells such as dendritic cells can be loaded with tumor antigen *ex vivo* and injected back into patients to ensure optimal processing and presentation of antigenic peptide. In general, these strategies depend on the activation of CTLs *in vivo*. Some of these strategies have encouraging results but many problems still need to be overcome.

A different approach that was not covered by Whelan *et al.* [1] involves *ex vivo* genetic manipulation of CTLs to alter their specificity and activation requirements. This is achieved by introducing a receptor that recognizes a tumor antigen linked to an intracellular domain that drives activation. Recent studies using this technique have shown that systemic B-cell lymphoma and colon carcinoma were eradicated *in vivo* [2,3]. The important lesson that stems from these studies is that we can control the survival and activity of the $CD8^+$ T lymphocytes to exploit their killing potential. This strategy has the advantage of being human leukocyte antigen-independent (the target of CD8 is the tumor antigen, independent of processing and association to class I molecules), but it is limited to tumor antigens that are expressed at the plasma membrane.

In conclusion, we are learning to control delivery of tumor antigens and to prepare effective and long-lasting CTLs. Our continuing progress in understanding the mode of function of the immune system should help us to find the best way to combine different approaches to obtain clinically relevant results.

References

1 Whelan, M. *et al.* (2003) Cancer immunotherapy: an embarrassment of riches? *Drug Discovery Today* 8, 253–258
2 Brentjens, R.J. *et al.* (2003) Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nat. Med.* 9, 279–286
3 Haynes, N.M. *et al.* (2002) Rejection of syngeneic colon carcinoma by CTLs expressing single-chain antibody receptors codelivering CD28 costimulation. *J. Immunol.* 169, 5780–5786

Federica Benvenuti
Institut Curie
INSERM U520
12 Rue Lhomond
75005 Paris, France