RNA INTERFERENCE

Silence of the genes

siRNAs — short, interfering RNA duplex molecules that can mediate post-transcriptional silencing in a sequence specific manner — theoretically represent ideal drugs for the specific downregulation of unwanted gene products. However, their delivery into target cells is a key obstacle to their therapeutic application. Reporting in *Nature Biotechnology*, Song *et al.* now provide a proof-of-principle study of a systemic method to deliver siRNA into specific cell types via cell-surface receptors, with the aim of maximizing therapeutic benefit, while minimizing non-specific silencing and toxicity in bystander cells.

The authors chose HIV envelope protein (Env) as a model receptor for targeted delivery of siRNA. This was achieved with a fusion protein (F105-P) consisting of an Env-specific antibody Fab fragment (comprising the non-immunogenic antigen-recognition domains), fused to protamine, a nucleic acid-binding protein that normally nucleates DNA in sperm. Incubation with siRNAs resulted in stable fusion protein/RNA complexes, which were internalized by cells carrying the respective surface antigen and efficiently mediated the downregulation of the siRNA-targeted gene product.

In *in vitro* experiments, F105-P complexed with siRNA targeting HIV *gag* (encoding an essential viral capsid protein) significantly reduced viral replication and release of viral particles in HIV-infected primary CD-4 T cells compared with cells treated with F105-P alone. Using the same fusion protein construct, these studies were extended to *in vivo* mouse tumour models with melanoma cells that were transfected with vectors for HIV Env before their implantation in the host. Here, intratumoural or intravenous injection of F105-P complexed with a cocktail of siRNA directed against several oncogenes significantly slowed down tumour growth in Env-expressing tumours, without exerting any effect on non-transfected tumours. Furthermore, the complex seemed to be completely non-immunogenic, and was not trapped by any cells of the reticuloendothelial system that could interfere with systemic delivery. Demonstrating the flexibility of the targeting strategy, further siRNA/fusion proteins were generated that consisted of a single-chain antibody directed against ErbB2 (an antigen on many breast cancer cell lines), which also mediated targeted delivery of siRNAs.

INFLAMMATION

The nerve of macrophages

After abdominal surgery, paralysis of the bowel, or post-operative ileus, commonly leads to extended hospital stays, and is characterized by inflammation and delayed transit of contents of the gut, often accompanied with nausea, vomiting and pain. The economic burden of ileus is estimated to be several billion dollars per year in the US. In the August issue of *Nature Immunology*, De Jonge *et al.* demonstrate in a mouse model of gastrointestinal (GI) ileus that stimulation of the vagus nerve attenuates inflammation and ileus via a STAT3 pathway in macrophages.

The vagus nerve, the longest in the body, is a component of the parasympathetic nervous system and promotes normal body function, including gastric motility. Local inflammation causes afferent fibres of the vagus nerve to trigger an anti-inflammatory response through firing of the efferent vagus nerve and the release of acetylcholine (ACh). ACh binds to α7 nicotinic ACh receptors (nAChR) expressed by macrophages to suppress pro-inflammatory cytokine production. This pathway can be manipulated by stimulating the vagus nerve or by using cholinergic agonists, such as nicotine, to control undesirable inflammation.

The authors showed that nicotine exerts its anti-inflammatory effect on peritoneal macrophages via the tyrosine kinase JAK2 and the STAT3 transcription factor, *in vitro* and *in vivo*. After nicotine binding, JAK2 is recruited to the α7 subunit of nAChR, leading to JAK2 phosphorylation. This in turn leads to phosphorylation of the STAT3 transcription factor, which forms dimers and translocates to the cell nucleus, where it induces the expression of a number of pro- and anti-inflammatory proteins, as well as the suppressor of cytokine signalling (SOCS)-3. However, the authors found that blockade of SOCS3 expression did not prevent the anti-inflammatory action of nicotine, suggesting that the cholinergic deactivation of macrophages results from activation of STAT3 rather than SOCS3.

Manipulating the cholinergic anti-inflammatory pathway is a promising strategy for treating post-operative ileus; a number of vagus nerve stimulators are approved for the treatment of epilepsy and depression. The timing of treatment could be important, as earlier attempts to treat this condition using cholinergic agents had only limited success, perhaps because treatment was administered after the inflammatory process had progressed.

This study also has important implications for other inflammatory conditions that might be alleviated by activating the JAK2–STAT3 pathway. In particular, ulcerative colitis is associated with altered STAT3 expression and phosphorylation and, interestingly, the condition is ameliorated by cholinergic stimulation in the form of smoking or nicotine treatment. Unfortunately, the toxic effects of nicotine will undoubtedly prevent this cholinergic agonist from any long-term therapeutic use. Future studies are required to investigate the use of other α7nAChR agonists in a therapeutic setting. For example, galantamine hydrobromide (Reminyl; Johnson & Johnson), both a cholinesterase inhibitor and an allosteric enhancer of nicotinic receptors, is currently prescribed for the symptomatic treatment of schizophrenia and Alzheimer's disease.

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References and links

ORIGINAL RESEARCH PAPER de Jonge, W. J. *et al.* Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nature Immunol.* 17 July 2005 (doi:10.1038/ni1229)
FURTHER READING Ulloa, L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nature Rev. Drug Discov.* **4**, 673–684 (2005)