Strategies for inhibition of tumor necrosis factor *in vivo*

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A new technique to deliver *in vivo*, very efficiently, an inhibitor of tumor necrosis factor α (TNF-α) has been described by J. Kolls, K. Peppel, M. Silva and B. Beutler[1]. TNF-α is a central mediator in sepsis and several other immunopathological conditions. Under experimental conditions, *in vivo* inhibition of TNF-α is efficient at preventing a wide array of tissue lesions, and so blocking the activity of this cytokine has therapeutic potential[2–6]. Ongoing clinical trials also indicate that anti-TNF-α monoclonal antibody treatment has promising applications, for example, in the treatment of Crohn's disease (S. van Deventer and colleagues[7]) and of rheumatoid arthritis (M. Feldman and colleagues[8]). However, there are drawbacks to passive immunization (see Ref. 1 for a discussion), as there are with other methods of blocking TNF-α activity, for example, by deletion of the genes encoding TNF-α or the TNF-α receptor in experimental animals (represented schematically in Fig. 1). Thus, the method developed by Kolls *et al.* for the *in vivo* delivery of a chimeric TNF-α inhibitor using an adenovirus vector is promising, especially for use in other fields of experimental biology.

The adenoviral construct used for the *in vivo* transduction led to the expression of the inhibitor in endothelial cells and hepatocytes. In fact, while adenoviruses can infect many types of cells *in vitro*, *in vivo*, they infect almost exclusively hepatocytes and endothelial cells. This is because the endothelium is susceptible to infection, but forms an effective barrier against the virus, preventing it from infecting most other cells except hepatocytes, which can be infected because of the fenestration of the endothelium there (B. Beutler, pers. commun.). The inhibitor was expressed in high titers over a long time and the level and duration of expression were correlated: the higher the titer of inhibitor adenovirus that is given, the longer expression persists. As a result of this sustained inhibition of TNF-α, these transduced animals have an impaired resistance to bacterial infection. Thus, *in vivo* transduction by this method obviously allows extremely high levels of expression of any gene of interest.

One of the most impressive and important aspects of this method is the uncommonly high level of production of the TNF-α inhibitor (as much as 1 mg ml^{-1} is found in circulating blood). However, the expression stops abruptly and the mechanisms by which this occurs remain incompletely understood. The lifespan of the transduced cells may be limited, but experiments to tackle this question have not been carried out. Advantages and disadvantages of this technique of *in vivo* delivery of inhibitors over others are summarized in Table 1. The major advantage of *in vivo* expression of inhibitors over the application of monoclonal antibodies is the guaranteed administration of the proteins for a longer period of time. Compared with the technology of generating transgenic animals, the *in vivo* transduction or transfection methods involve impressively simple manipulations, and there is no triggering of compensatory mechanisms and/or developmental changes. These latter two 'side effects' seem to have been largely overlooked in recent papers. Another major advantage is the possibility to transfect with a combination of inhibitors, which, in the case of gene knockout, would require double- or triple-knockout mice to be generated.

Other approaches to expression of inhibitor molecules *in vivo* have already demonstrated the value of this technique for possible gene therapy. Stable transfection of synovial cells *ex vivo* with the gene encoding the interleukin-1-receptor antagonist protected rabbits from arthritis[9,10]. In this case, the inhibitor was expressed only transiently in the synovium. A further positive aspect, especially of organ-specific expression, is that optimal protection against pathology can be achieved in this way. Localized delivery of a given inhibitor by gene transfer may be more biologically effective than delivery of this inhibitor by injection[9,10]. Successful *in vivo* transfection with liposome–DNA complexes has also been reported in a model involving inhalation of liposomes containing the gene encoding the cystic fibrosis transmembrane conductance regulator for the treatment of cystic fibrosis[11].

Using viral vectors for *in vivo* transduction always carries the potential risk of infection. Kolls *et al.* claim that the TNF-α-receptor–immunoglobulin construct does not become integrated into the cell genome, but rather remains episomal. The adenoviral genome used was adapted to be replication defective, and this may be sufficient to ensure, as the authors claim, that the transferred gene remains episomal. While this seems to be safe enough for generating conditional knockouts that are used for basic research, for the purpose of gene therapy, it is absolutely required to prove formally that the transgene stays episomal and does not become integrated. This could be addressed by Southern blotting, but even this approach may not

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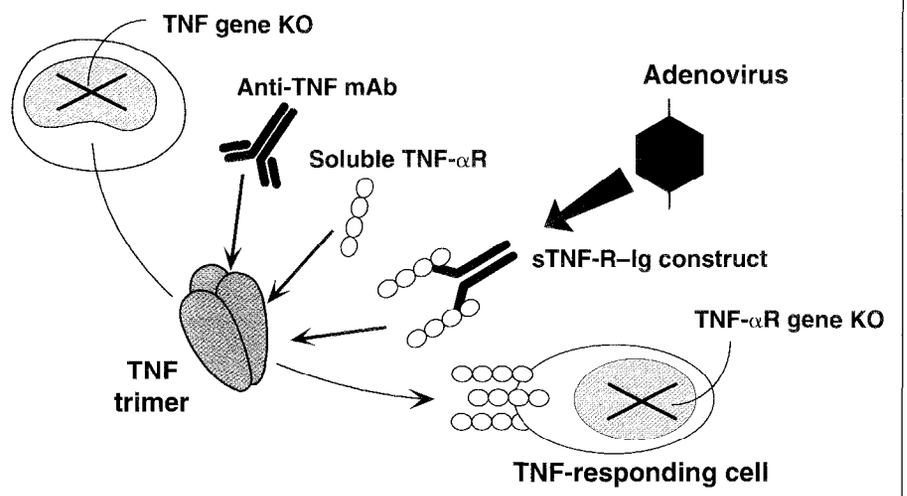

Fig. 1. Tumor necrosis factor α (TNF-α) functions can be inhibited in various ways: by addition of polyclonal or monoclonal neutralizing antibodies, soluble TNF-α receptors (TNF-αR; schematically represented by their four cysteine-rich domains) or soluble receptor–immunoglobulin constructs (sTNF-R–Ig). Alternatively, the genes encoding either TNF-α or TNF-αR can be knocked out (KO). These methods have been used by several groups to decipher mechanisms of TNF-α action and to suggest potential therapeutic approaches (see text). mAb, monoclonal antibody.

be sensitive enough to provide a definitive answer.

The reduced resistance to bacterial infection that is the physiological consequence of inhibition of TNF-α is not surprising, but rather proves the efficacy of the method. However, possible side effects of the adenoviral transduction need to be kept in mind in interpreting the data. Despite the absence of viral replication, there seem to be signs of viral infection, such as liver inflammation, in the transduced animals. Furthermore, the production of interferon is likely to be upregulated and might modulate the effects of TNF-α responses (B. Beutler, pers. commun.). With the method used by Kolls *et al.*, TNF-α can be blocked efficiently without leading to the abnormalities of development (that is, failure to thrive and lymphoid-tissue aplasia) that occur in mice injected at birth with anti-TNF-α antibody[12]. The adenoviral vector has also been injected into newborn mice via the external jugular vein (B. Beutler, pers. commun.): the growth rate of these mice was similar to that of littermates that received a control virus construct containing the gene for β-galactosidase; in particular, their thymuses and lymph nodes were of normal weight. Interestingly, however, a complete lymph-node aplasia has also been reported recently in TNF-α, TNF-β knockout mice[13]. Taken together, the data from all three experimental systems suggest that some of the effects seen using polyclonal anti-TNF antibodies are due to inhibition of TNF-β (or lymphotoxin, LT). The polyclonal antibody used in Ref. 12 also neutralizes the activity of TNF-β (N.H. Ruddle, pers. commun.). In fact, the whole discussion on blocking TNF-α may be useful for blocking functions of TNF-β, the role of which may well soon be understood better and recognized as being of equal importance as that of TNF-α. Indeed, while TNF-α does not bind to the

Table 1. Advantages and disadvantages of some methods for inhibition of tumor necrosis factor

	Alteration of gene expression by:			
	Mutation (gene knockout)	**Viral transduction**	**Liposome–DNA transfection**	**Inhibitor administration**
Advantages	Stable Any health status Complete	Very high expression Any genetic background Any age Any health status Any gene (even if required for embryonic development)	Technically simple Any genetic background Any age Any health status Any gene (even if required for embryonic development)	Any genetic background Any age Any protein
Disadvantages	Compensatory mechanisms likely to be triggered Demanding technology	Symptoms of viral infection Triggering of immune response Demanding technology Nonpermanent expression Incomplete (locally restricted) Potential risk of insertion into the genome	Lower efficiency (Neuro)toxicity Nonpermanent expression Incomplete (locally restricted) Potential risk of insertion into the genome	Difficult to access certain sites Requires large amounts of purified proteins

LT receptor, TNF-β (or LT) can bind to both p55 and p75 TNF-α receptors.

It will be attractive to evaluate the response of these TNF-inhibitor-expressing mice in the context of several pathological conditions. The technique described by Kolls *et al.* will certainly become a very useful tool to analyse cell–cell interactions on a molecular level in *in vivo* models. The significance of the method of Kolls *et al.* may not be as a suggested method for gene therapy, but rather as an elegant technique to replace the use of knockout mice, in that it might create a 'conditional knockout' for any gene of interest.

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Plant-virus movement: *de novo* process or redeployed machinery?

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Three papers have appeared recently that provide a basis for the view that cell-to-cell trafficking of macromolecules through plant plasmodesmata is not unique to virus infection, but that plant viruses exploit an existing mechanism of cellular communication in plants to spread the infection through the tissues[1–3]. This view has been expanded recently to include the idea that plasmodesmata mediate a 'supracellular' control of plant processes[4].

It has long been recognized that plant viruses encode proteins that mediate viral movement through plasmodesmata, and that these proteins can modify plasmodesmal structure and function[4,5]. Pioneering work in this field used the tobacco mosaic virus (TMV) movement protein (MP) P30 and the technique of microinjection to assess the size exclusion limit (SEL) of plasmodesmata in the presence and absence of the MP. By microinjecting fluorescently labelled dextrans of different sizes into cells in transgenic tobacco plants expressing P30 and monitoring their diffusion into adjacent cells, the normal SEL of <1 kDa in non-transformed tissue was shown to increase to >10 kDa in the presence of P30 (Ref. 6). This increase corresponds to a change in the functional diameter of the microchannels within plasmodesmata from 1.2–1.8 nm to 2.4–3.1 nm, which is still too small to allow the passage of the intact virus or of its genomic RNA. However, P30 can bind to and denature single-stranded (ss) RNA to produce long, thin (<2 nm) ribonucleoprotein complexes[7], which might be able to pass through the altered microchannels.

Some other viruses have been shown to have similar properties: their MP can bind to nucleic acids and their coat protein is not necessary for cell-to-cell movement. However, this is not always the case: cell biological observations of virus particles within structurally modified plasmodesmata suggest that a further class of viruses, including the comoviruses[8] and the caulimoviruses[9], move as intact virions.

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MPs traffic through plasmodesmata

TMV and two functionally related viruses, red clover necrotic mosaic virus (RCNMV) and bean dwarf mosaic virus (BDMV), are the subjects of the three recent papers mentioned above[1–3]. Whereas TMV and RCNMV have ssRNA genomes and single MPs (TMV P30; RCNMV 35 kDa protein), BDMV (a geminivirus) has a genome of ssDNA and two proteins (BL1 and BR1) that are necessary for a spreading infection in plants. These papers all report a novel and elegant approach to assess the effect of MPs on plasmodesmal function. The proteins were expressed in *Escherichia coli*, and the purified recombinant proteins were directly comicroinjected with fluorescent dextrans into mesophyll cells of host plants and the SEL assessed. This approach was extended in two cases[1,3] by studying the fate of fluorescently labelled MP and by directly measuring the ability of the MP to transport nucleic acids by co-injecting MP and fluorescently labelled RNA or DNA. P30, RCNMV 35 kDa protein and BDMV BL1 protein all increase the SEL of plasmodesmata