Gene Therapy in Surgery

Part II: Application to Septic Shock and to Organ Transplantation

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Key-words: Gene therapy – cancer – septic shock – organ transplantation.

Summary: Background: With the increasing body of knowledge in molecular biology, gene therapy respectively gene therapy becomes more and more a valid therapeutic option.

Methods: This is a critical review of gene therapy protocols for treatment of different types of cancer. Furthermore, the pathophysiological mechanism, therapeutically strategies as well as experimental approaches toward gene transfer in septic shock and organ transplantation are critically elucidated.

Results: Gene transfer as a therapeutic option was first successfully applied in children with severe combined immunodeficiency (SCID) in 1990. The majority of gene marking or gene therapy protocols approved for human clinical trials to date are related to the treatment of cancer. Besides viral vectors for brain tumors, non-viral vectors, liposomes particularly, with almost no side effects are increasingly used.

Conclusions: Different approaches of gene transfer in cancer patients are under investigation. Experimental data of septic shock treatment and rejection therapy of the allograft in organ recipients with gene transfer are encouraging for future applications in clinical trials.

Schlüsselwörter: Gentherapie – Karzinome – septischer Schock – Organtransplantation.

Zusammenfassung: Grundlagen: Die Fortschritte in der molekularbiologische Zusammen mit der Erforschung des „Humanen Genom“-Projektes lassen den Gentransfer bzw. die Gentherapie zunehmend als therapeutische Option bei verschiedenen Erkrankungen Eingang gewinnen.

Methodik: Die folgende Arbeit stellt einen kritischen Überblick über die klinischen Protokolle der Gentherapiestudien bei Karzinomen dar. Weiters werden der pathophysiologische Mechanismus, die verschiedenen Therapieansätze sowie die erfolgreichen, experimentelle Gentransfer im septischen Schock wie auch bei der Organabstoßung nach Transplantation dargestellt.

Ergebnisse: Der erste erfolgreiche Gentransfer wurde bereits 1990 bei Kindern mit „schwerem kombiniertem Immundefekt“ (SCID) durchgeführt. Die meisten Gentransferprotokolle behandeln verschiedene Karzinome. Neben viralen Vektoren, die sich besonders gut für nichtproliferierende Gewebe, z. B. zentrales Nervensystem, eignen, werden zunehmend nichtvirale Vektoren, insbesondere Liposomen verwendet.

Einsatz des Gentransfers beim septischen Schock und bei der Abstoßungstherapie nach Organtransplantation erwarten.

Background

The reported mortality to septic shock varies between 25 and 50%. Although controversy exists over the frequency and morbidity associated with sepsis and septic shock, there is agreement that the incidence of septic shock is increasing and mortality rates are remaining relatively constant despite marked improvements in anti microbial therapies and pulmonary and cardiovascular support. Even with constant improvements in supportive care, increases in immunocompromized diseases like AIDS, as well as the aging of the population, have resulted in an increased proportion to sepsis and septic shock.

In 1986/87, Beutler, Tracey, Cerami and Lowry demonstrated that overproduction of the proinflammatory cytokine, TNF-α, was antecedent to shock and death (36-39). Initial studies demonstrated that the panoply of host responses seen in lethal endotoxemia or gram negative bacteremia could be reproduced in healthy animals simply by administering recombinant TNF-α. In subsequent studies in mice and Papio (baboon), the authors demonstrated that an exaggerated endogenous TNF-α response inhabitable with polyclonal and monoclonal antibodies, contributed to the mortality associated with endotoxemia and gram negative bacteremia (40). Since 1987 when the studies were first reported there have been at least 15 studies confirming the central role that TNF-α plays in acute gram negative bacteremia and endotoxemia (for review see Bone [41]).

Similarly, in 1988, Waage et al. (43) and Schreiber et al. (44) each reported that TNF-α toxicity could be potentiated by co-administration of either IL-1 or lipopolysaccharide. As early as 1989, Fong et al. (45) reported that blocking an endogenous TNF-α response in gram negative septic shock with monoclonal antibodies led to an attenuated IL-1 and IL-6 response. Olsén et al. (46) and subsequently Diat and colleagues (47), Norton et al. (48) and Fisher et al. (49) reported that blocking an endogenous IL-1 response with IL-1 receptor antagonist (IL-1ra) also improved survival and reduced tissue damage associated with lethal gram negative bacteremia.

Since then a considerable body of knowledge has developed to explain the mechanism and pathways by which the proinflammatory cytokines initiate and propagate shock, tissue damage and the sepsis syndrome (50). Investigators have implicated additional pro-inflammatory cytokines in the pathogenesis of overwhelming gram negative infections or endotoxemia, including interferon-γ, IL-6, IL/IF/IFD and IL-12 (51, 52, 53). Although the role that each of these specific cytokines play in the pathogenesis of septic shock is still being resolved, there is general agreement that endogenous production of TNF-α and IL-1 are central to in- fluencing and sustaining the inflammatory cytokine cascade. These 2 mediators in particular TNF-α appear very early in the inflammatory response, and their synthesis and release begins within minutes of macrophage activation (50, 54). The early re-
lease of TNF-α initiates a subsequent cascade of other cytokines and mediators. When TNF-α or IL-1 are inhibited with either antibodies or receptor antagonists, the major components of the inflammatory response are suppressed (39, 55).

It is now generally accepted that the catastrophic host responses to overwhelming bacterial infections represent an aberrant relationship between proinflammatory cytokines, TNF-α and IL-1, and their naturally occurring inhibitors (56). In lethal bacteremia and endotoxemia the concentrations of TNF-α and IL-1β in the plasma have far greater than can be suppressed by the protein-binding levels of shed TNF receptors (TNFR p55 & p75) or IL-1ra. Similarly, in ongoing inflammatory processes, such as those which occur in hospitalized patients with systemic inflammatory response syndrome (SIRS) or sepsis syndrome, the mechanisms which ultimately down-regulate proinflammatory cytokine release are ineffective. This is due in part to the continued external stimuli which ongoing infectious or inflammatory processes invoke. In such cases repeated or persistent proinflammatory cytokine synthesis (TNF-α, IL-1β) contributes to the hemodynamic instability, coagulopathy, and multiorgan dysfunction that occurs. In both septic shock and SIRS, the beneficial aspects of proinflammatory cytokine production (including stimulation of non-specific host immunity, increased antigen specific T-cell proliferation, macrophage and NK-cell bactericidal capacity) are offset by the adverse consequences of continued exposure to elevated TNF-α and IL-1 concentrations.

Clinical trials

Successful anticytokine approaches to the treatment of septic shock or sepsis syndrome associated with bacteremia or endotoxemia have failed at either suppressing the proinflammatory cytokine (TNF-α or IL-1β) response, such as with IL-10 or TGF-β, or blocking TNF-α and IL-1 activity with antibodies or by increasing pharmacologically the levels of cytokine inhibitors with recombinant IL-1ra and soluble TNF receptors. The preclinical rodent and subsequent primate studies which demonstrated efficacy with either antibodies (anti-TNF-α mAb) or cytokine inhibitors (IL-1ra or soluble TNF receptors TNFR) in lethal endotoxemia and gram negative bacteremia prompted the initiation of clinical trials in patients with sepsis syndrome and shock. At the present time, there have been at least 6 clinical trials initiated with either anti-IL-1 or anti-TNF-α therapies. Reports of preliminary results have suggested a variable clinical response (57, 58). The initial promising Phase II report of improved outcome in patients with septic syndrome treated with IL-1ra could only be confirmed in the Phase III trials with a subgroup of critically ill patients with predicted mortalities of greater than 24% by APACHE III scores (59). In fact, clinical trials with IL-1ra have been discontinued, and IL-1ra is no longer under clinical investigation. Beneficial results from the anti TNF-α monoclonal antibody studies have also been conditiona. For example, Fisher reported an improvement in outcome only in those patients with detectable plasma TNF-α (58).

In light of the observation that these clinical studies can only confirm the utility of anticytokine therapies for the treatment of shock and sepsis syndrome in very selected patient populations, interest has focused primarily on identifying prospectively patient that may benefit from such therapies. In fact, retrospective analysis of Phase II and III clinical trials with TNF-α antibodies and IL-1 inhibitors revealed that only some patients sufficiently benefited from anticytokine therapies, whereas there was a trend towards increased mortality in other patient populations (57, 58, 59). In particular, anti IL-1 and anti TNF-α therapies appeared to be most helpful in patients who had organ failure or were already in shock, whereas they were least beneficial (and potentially hazardous) in patients at risk of developing septic shock, but not as critically ill.

The results of these several clinical trials to unequivocally demonstrate efficacy of this novel approach does not indicate a failure of the underlying concept, but rather a failure in its implementation. Such results are not surprising given the fact that cytokines have both concurrent beneficial and pathologic roles. In fact, Echtenacher demonstrated that blocking an endogenous TNF-α response made a non lethal peritonitis model lethal (60). Similarly, van der Meer and Cyprinski demonstrated that administration of IL-1 improved outcome to a variety of gram negative bacterial infections and blocking an endogenous IL-1 response inhibited antimicrobial processes (61–64). Such results suggest that an endogenous proinflammatory cytokine response can have beneficial responses, and efforts to block an endogenous TNF-α or IL-1 response may have untoward negative effects. We believe that identifying the optimal patient population who can benefit from such therapies will only partially address the problems associated with the current approaches for delivering anticytokine therapies. A major difficulty with the current strategy of using systemically either inhibitors of IL-1 (IL-1ra) or TNF-α (monoclonal antibodies or soluble receptors), or infusing antiinflammatory mediators (such as glucocorticoids, IL-4, TGF-β, IL-10 or IL-13) is that systemic administration is an imprecise means of directing an anticytokine therapy to individual body compartments where exaggerated production is occurring. Similarly, because such therapies are inherently aimed at blocking cytokines primarily in the vasculature, but also in all organs of the body, they can be potentially hazardous to some patient populations where an organ specific cytokine production may be beneficial antimicrobial functions.

Systemic administration of cytokine inhibitors may in fact be an inappropriate means to block the paracrine actions of a cytokine. Only recently has a greater appreciation for the paracrine nature of TNF-α and IL-1 been recognized. Both IL-1 and TNF-α are known to exist in cell associated forms and retain some biologic activity (65, 66). Ginsberg et al. (67) reported in mice suffering adenovirus induced hemorrhagic pneumonia local, but not systemic, production of TNF-α and IL-1. TNF-α and IL-1 levels in the lung were often in excess of 10 ng/ml whereas plasma concentrations were less than 50 pg/ml and could not be detected by either immuno- or bioassays. Similarly, in rats expiring from a thermal injury and Pseudomonas infection, local but not systemic TNF-α production was reported (68, 69). Ulich has reported lung TNF-α levels exceeding 10 ng/ml in mice following intratracheal instillation of LPS, whereas levels in the plasma were less than 100 pg/ml (70). Similar findings have been seen with patients with ARDS (71, 72). In such patients, TNF-α was recovered from the lungs of patients with ARDS at levels as high as 15 ng/ml, whereas concentrations in the plasma were only 100 pg/ml. Thus, systemic administration of cytokine inhibitors must be given at levels sufficient to block the elevated concentrations in the tissues and not in the plasma compartment. This is exceedingly problematic since anti TNF-α monoclonal antibodies, soluble receptor fusion proteins and even IL-1ra are primarily sequestered in the plasma compartment (49, 56, 73).

Systemic administration of cytokine inhibitors is also problematic since the natural antagonists or inhibitors of TNF-α and IL-1 often have short biological half-lives, ranging from minutes to hours. For example, Fisher et al. reported that in the septic primate, IL-1ra has a biological half-life (beta phase) of approximately 21 min (49). To sustain therapeutic plasma concentrations of 10 to 15 mg/ml IL-1ra and soluble TNF receptors have to be given at concentrations of 1.5 to 2 mg/kg body weight/h or approximately 2.5 g/d for as long as the patient is septic. Such an approach may not be cost-effective. To some extent, these problems have been obviated by the use of antibodies or TNF receptors that are joined to the FC and hinge region of human IgG. This “Chimeric fusion” proteins have a biological half life between 20 to 60 h (57, 73).

Finally, exaggerated proinflammatory cytokine production may contribute to the pathology in one body compartment, while simultaneously, production in another compartment may not actually have beneficial effects. There has been little examination into the differential organ response to a variety of lethal and non lethal infections or inflammation. The implications of these findings are considerable. Systemic administration of cytokine inhibitors at
levels sufficient to exit the plasma pool in quantities sufficient to neutralize exaggerated TNF-α production in one tissue compartment may also block the presumably beneficial aspects of cytokine production in other tissue compartments. This latter point may explain some of the experimental observations where TNF-α inhibition is associated with adverse outcome.

In 1986, Beutler and Cerami characterized TNF-α’s actions as being 2 sides of the same coin (74). Even at that time it was understood that the biological actions of proinflammatory cytokines were in general beneficial to the host. Since then, considerable experimental data has arisen to suggest that an endogenous TNF-α or IL-1 response is critical to a normal non-specific host response that serves to reduce the amount of tissue damage and the likelihood of a secondary bacterial infection. It has been well recognized that endogenous TNF-α and IL-1 production contributes to the antimicrobial responses against several intracellular pathogens, such as listeria and pneumocystis (75). An endogenous TNF-α and IL-1 response, particularly in the liver and spleen, are essential to the anti-lisher response. In addition, there is also increasing appreciation for a beneficial role for TNF and IL-1 in the host response to gram negative bacterial infections (76). For example, Echtenacher et al. reported that passively immunizing mice against TNF-α converted an otherwise non lethal peritonitis (cecal ligation and puncture) into a uniformly lethal one (60). Similarly, Dinarello reported that some IL-1 production was critical in newborn rodents (76). He demonstrated that exaggerated IL-1 production could be lethal as an inadequate IL-1 production in a murine model of gram negative infection. The findings confirm that some IL-1 production is essential for eliciting an antimicrobial response, but either too much or too little is disadvantageous.

For the reasons described above, we propose that gene transfer of antiinflammatory cytokines or cytokine inhibitors represents a more efficient means to block proinflammatory cytokine action in tissue compartments than does the systemic administration of these same agents.

**Use of gene transfer to deliver anti-inflammatory cytokines or cytokine inhibitors**

We propose to employ gene transfer as a novel drug delivery system to transiently mitigate the inflammatory response in individual tissues and organs. We believe that coupling gene transfer technologies with surgical intervention and manipulation ultimately offers a unique means to modify the post-surgical and inflammatory response, by either down-regulating inflammatory processes in tissues where exaggerated production occurs, or in cases where up regulating the inflammatory response may stimulate antimicrobial processes. Based on this proposal, gene transfer technologies would be an integral component of the surgeon’s armamentarium, aimed at modulating wound healing, tissue regeneration and decreasing inflammatory cell-mediated injury.

The specific goals of gene therapy for sepsis and acute inflammation therefore differ in some important regards from efforts to correct germline disorders. Whereas the treatment of such germline disorders as ADA deficiency-induced SCID or cystic fibrosis seeks a stable integration of the foreign gene into the target tissue genome (77, 78, 79), the goal of gene transfer in sepsis or acute inflammation is a transient, non-stable transformation that results in maximal gene expression lasting days and at most weeks, in the case of down regulating an inflammatory response, stable integration of the gene for a antiinflammatory cytokine or cytokine inhibitor with a viral promoter-enhancer into the target cell genome could have adverse long lasting effects, including immune suppression and oncogenesis. Such stable transfactions are therefore not desirable. In addition to non-stable transfection, gene therapy approaches in sepsis are aimed at targeting several cell populations simultaneously in a single organ or tissue, such as pulmonary macrophages, or epithelial and endothelial cells in the lung. Under ideal conditions, the target cell population in sepsis is one in which excessive production of the proinflammatory cytokines IL-1 and TNF-α occurs, although it is recognized that current strategies are likely not to be that precise.

Our own experimental results underscore several advantages for the use of gene transfer as a treatment option for septic shock or other acute inflammatory episodes (80). In these studies, human gene transfer was used to deliver to organs of the reticuloendothelial system antagonists that either inhibit TNF-α synthesis or block its interaction with cellular receptors. Mice were treated intraperitoneally with cationic liposomes containing 200 μg of either a pCMV (cytomegalovirus)/p55 expression plasmid that contains the extracellular domain and transmembrane region of the human p55 TNF receptor, or a pCD-SR-a/hIL-10 expression plasmid containing the DNA for human IL-10. 48 h later, mice were challenged with lipopolysaccharide (LPS) and D-galactosamine. Pretreatment of mice with p55 or IL-10 cDNA-liposome complexes improved survival (p < 0.01) to LPS-D-galactosamine (Table 3). In additional studies, intratracheal LPS challenge reduced pulmonary TNF-α levels by 62% and decreased neutrophil infiltration in the lung by 55% as measured by myeloperoxidase activity (both p < 0.05).

### Application of gene therapy to organ transplantation

In 1945 Sir Peter Medawar, the English immunologist, appreciated that rejection in organ transplantation was an immune reaction – following recognition by the immune system that a graft is “foreign” and must be destroyed. If rejection was an immune event, what could be more logical than to try to protect grafts by altering the immune system? At the beginning of kidney transplantation total body X-ray of a kidney recipient was performed end to weaken the immune system (81). With the development of new “antilymphocytic agents” the results in organ transplantation including kidney, liver, heart, lung and pancreas were improving successfully (82-85). However, toxic side effects due to “systemic” immuno-suppression are still a valid concern in the post-operative management in these patients.

Therefore, the concept of local immuno-suppression by organ targeted therapies is also of most interest in organ transplantation. Gene transfer to organ transplantation offers the potential for modulation of immunity directly within an allograft without systemic side effects. Qin et al. (86, 87) demonstrated already in a series of experiments that gene transfer for heart transplantation can induce transient expression of a immunologically relevant cytokine (TGFB-1) within allografts that impede immune activation while avoiding the systemic toxicity of conventional immuno-suppression. With their murine experiments Qin et al. demonstrated firstly that syngeneic grafts injected with pRSVβ-gal displayed β-gal activity between 9 and 14 days after transplantation. These experiments demonstrate that purified, naked plasmid DNA can transfer into and express exogenous nucleic acids in cardiac isografts. Next, it is demonstrated that transfer and expression of an immuno-suppressive cytokine (TGFB-1) prolongs graft survival to 26.3 ± 2.5 days (p < 0.02).

The encouraging findings in these experiments is the fact that recipients of transfected allografts showed no apparent toxic effects and no mortality associated with the plasmid transfer. The issue of whether simple injection of recombinant, purified TGFB...
β1 protein also could prolong allograft survival also was addressed. Injection of excess amount of rTGF-β1 into the allograft at the time of grafting showed no effect on allograft survival. This suggests that gene transfer results in extended temporal expression of the gene product and provides for prolonged immunomodulatory effects within the graft.

The authors also investigated a possible toxicity of the gene product, since TGF-β1 has been demonstrated to be associated with exuberant fibrosis in a variety of systems. Thus, expression of TGF-β1 may also cause toxicity. However, histology could not show any evidence of acute or chronic rejection or other evidence of graft destruction. The authors conclusion include that transient expression of TGF-β1 may be advantageous for allografting. Such expression at the time of initial grafting could provide the appropriate signals to negatively regulate the initiation of an immune response, which could prevent or delay graft rejection. With IL-10, another immuno-suppressive cytokine, the authors already demonstrated a marked prolongation of survival. To achieve further immuno-suppression or induce tolerance, it will be necessary to enhance gene transfection efficiency and transgene expression.

Another conceptionally promising perspective is to irreversibly induce allotagant-specific T cell non-responsiveness (i.e., T cell anergy). The receptor ligand pairs CD28-B7 (expressed on T cells and Antigen Presenting Cells [APC]) and CD40-CD40L (expressed on T cells) induce apoptosis of T cell-dependent immune responses. Importantly, antigen recognition by T cells in the absence of CD28 co-stimulation leads to the induction of antigen-specific anergy in many systems. Nevertheless, newer data suggest that the elicitation of alloreactive T cell responses in vivo not only depend on B7-CD28 interactions but concomitantly require interaction of CD40 and CD40 ligand on APC and T cells, respectively. In fact, fascinating experiments by Lanzavecchia et al. (88) demonstrate that the simultaneous but not independent blockade of the CD28/B7 and CD40/CD40 ligand pathways effectively ablates clonal T cell expansion in vitro and in vivo, promotes long-term survival of allogeneic skin grafts, and inhibits the development of chronic vascular rejection of primarily vascularized cardiac allografts. It remains to be evaluated whether gene transfer, e.g., of CD40L, which reportedly inhibits the activation of B7 expression in vivo (89), can be used to inactivate the CD28/B7 and CD40/CD40 co-stimulatory pathways in vivo.

Conclusions

The successful application of gene therapy will continue to require the efforts of investigators in basic science, since basic science issues (e.g., transfection efficacy) underlie many of the problems that need to be overcome in order for gene therapy to succeed. Such consideration have already been discussed to become a subspecialty in medicine with physicians in this field performing gene therapy like us performing surgery. Although the medical potential of gene therapy is bright, the possibility for misuse of genetic engineering technology looms large, so society must ensure that gene therapy is used only for the treatment of disease. With both sides of this possibility in mind, somatic gene therapy will become a new and exciting therapeutic option for disease. With both sides of this possibility in mind, somatic gene therapy will become a new and exciting therapeutic option for disease.

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Invited Commentary to: “Gene Therapy in Surgery” J. Seifert

The therapy of surgical patients does not end with a successful operation but has to be continued in intensive care wards, and by postoperative therapies to achieve a most complete cure of the patient. Thus it is important to stay informed about the latest therapeutic developments, and to apply new and promising techniques to the benefit of their surgical patients.

In a generally intelligible way, Rogy et al. (1) describe which surgical patients could be considered to receive human gene therapy in the near future, which methods may be applied, and which chances of success they have if cautiously weighed. Gene technology is a relatively new tool, thus we cannot decide presently if it would not benefit a series of other surgical diseases as well. The use of this technique might be considered for improving healing disorders of wound and bone, for inducing cartilage regeneration, and, it might bring us further ahead in the treatment of chronic pancreatitis, progressive cirrhosis of the liver, emphysema, and many other diseases. As is the case with every new method, the limits of gene technology have to be first defined. Yet, the method is developed with breathtaking speed, and surgeons must take care that they will not again be faced with a new specialty splitting off and alienating surgery from a part of its tasks. Thus we are obliged to watch the potentials of gene technology very closely as they emerge, and to incorporate them in the field of surgery in a sensible way.

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