been seen in most rodent models of T-cell-mediated disease). Can the enzyme therapy compete with this?

Treatments with monoclonal antibodies frequently fail in the long run because the immune system produces neutralizing antibodies against the injected antibody. Even human or humanized monoclonal antibodies injected into humans are likely to induce the production of neutralizing antibodies, jeopardizing the therapeutic effect and occasionally causing severe side-effects. Perhaps the most exciting perspective of enzyme therapy is that trypsin, because of its active resorption and enteropancreatic recirculation, can be administered orally; monoclonal antibodies and most other therapeutic proteins given this way would not be resorbed in significant quantities. Most importantly, antigens that the immune system encounters after oral exposure induce a specific state of immune tolerance, called oral tolerance [12]. In contrast, systemic exposure to the same antigen, after injection, usually induces an immune response. Thus, when injected, both monoclonal antibodies and trypsin should be immunogenic in the long run. However, oral administration of trypsin entails induction of specific immune tolerance, preventing the generation of neutralizing antibodies. With the increasing need for tolerogenic delivery of therapeutic proteins like monoclonal antibodies, efforts should be made to engineer those molecules to mimic the trick that trypsin employs.

In conclusion, studies such as Dr Gaciong's and Dr Heidland's suggest that enzyme therapy is a promising approach to the treatment of T-cell-dependent diseases in humans. The evaluation of its efficacy awaits randomized, controlled therapeutic trials, which should be facilitated by the fact that the enzymes are already on the market, are relatively inexpensive, and have proven to be virtually free of side-effects.

References

The management of the failed renal allograft

Y. Vanrenterghem and S. Khamis
Department of Nephrology and the Leuven Collaborative Group for Transplantation, University Hospital Gasthuisberg, Leuven, Belgium

Introduction

Patients returning to dialysis after a failed renal transplantation represent about 10% of the population starting dialysis each year [1]. Although a significantly higher mortality has been described for these patients compared to age- and disease-matched stable dialysis patients [2], the optimal management of the failed allograft has been poorly studied.

Mortality and morbidity of the allograft nephrectomy

In the pre-cyclosporin era, graft nephrectomy was considered a risky intervention. Mortality was reported to range between 7.3% [3] and 16.3% [4]. A paper from India by Sharma and co-workers even reported a mortality rate as high as 38.7% [5]. At that time only the Maastricht group reported on a mortality of no more than 0.9% [6]. Death was mainly due to septic complications. Severe wound infection was also a frequently reported complication [7].

Since the more widespread use of cyclosporin and the lower doses of steroids, graft nephrectomy has
become a safe procedure with almost no mortality and a low incidence of severe morbidity. In contrast to their experience in the pre-cyclosporin period, Hansen reported more recently no mortality in a consecutive series of 19 patients treated with cyclosporin [8]. Glicklich in 1993 reported two deaths in 97 patients [9]. In our own experience out of a total group of 960 first cadaveric allograft recipients transplanted between January 1985 and December 1994, 90 patients had to undergo graft nephrectomy. No postoperative mortality was seen. In parallel with the decrease of mortality, the incidence of severe complications decreased as well, i.e. from more than 20% to less than 10%. Serious side-effects occurred in 8.9% of our cases.

Timing of allograft nephrectomy

When graft failure occurs early after transplantation, the graft will be removed immediately in most cases. Delay of nephrectomy will not only increase cost of hospitalisation [3] but also expose the patients to further complications. When the cause of graft failure is a technical problem such as an acute arterial or venous thrombosis, immediate allograft nephrectomy is almost always mandatory. In case of therapy-resistant rejection, most authors will also prefer to remove the rejecting kidney in order to avoid serious complications such as graft rupture and persisting fever. In our experience 64 or 94% of the 68 grafts which failed within the first 6 months were also immediately removed. In the study of Glicklich 63 or 74% of the 85 patients with graft failure within 6 months of transplantation required allograft nephrectomy for unremitting acute rejection [9].

When graft failure occurs later, the policy is much less uniform. A reason for not removing the allograft may be its possible contribution to haemopoietic and other metabolic functions as suggested by Shapiro et al. [10]. Since the availability of erythropoietin this argument, however, holds no longer true. In our experience in some cases the presence of the rejected graft may even be the cause of persistent anaemia, despite erythropoietin therapy. The graft was ultimately removed in 25 or 58% of our 43 patients with graft failure beyond the 6th month after transplantation. In the study of Glicklich 34 or 35% of the 96 patients who returned to dialysis more than 6 months after transplantation required graft nephrectomy [9].

Immunosuppressive therapy after graft failure

In case of early graft failure and nephrectomy, immunosuppressive therapy will be interrupted immediately. In case of late graft failure when the graft is left in place, several policies are possible. Some authors will maintain these patients on a low dose of immunosuppressive drugs in order to avoid acute rejection [11,12]. This policy, however, exposes the patient to the side-effects of continued immunosuppression, with the risk of haemorrhage from graft rupture. In our opinion the potential risks of continued immunosuppression often outweigh the benefits of keeping the graft in place. In agreement with other authors [9] we progressively taper and eventually stop all immunosuppressive therapy. In the latter case, however, careful follow-up of the patients is needed as acute rejection of the graft may occur. The need for elective nephrectomy after withdrawal from all immunosuppressive drugs varies from one author to the other. The need for nephrectomy appears to be the higher the later the graft fails [4,11]. Two authors reported that the need for graft nephrectomy in patients treated with cyclosporin was higher than in patients treated with azathioprine [13,14]. In our analysis the need for nephrectomy in the patients with graft failure after the first 6 post-transplant months was 36.6%. This figure is comparable to 35% found by Glicklich [9]. In the analysis of Madore nephrectomy was required in 63% of the patients [14]. Lower figures were described by Thomas [11] and by Gallo [12], but as mentioned above in both studies patients were maintained on low dose immunosuppression. Low percentages of nephrectomies (7%) were also reported by Kibel and Belitsky [13]. In half of their 32 studied patients cyclosporin was tapered for more than 6 months (mean 14 months). High morbidity due to infections was noted. Patients tapered over a shorter period of time had less infections.

The reasons for performing elective nephrectomy include hypertension, acute rejection, massive proteinuria, haematuria, therapy resistant anaemia and fever of unknown origin. The latter may be related to adrenal insufficiency in patients under long-term therapy with corticosteroids, in which case withdrawal from steroids may cause an Addisonian crisis. As most of these patients have no residual kidney function, the clinical presentation is often atypical and misleading [15].

Effect of graft nephrectomy on the outcome of a second graft

In 1976 Freier et al. reported a significant effect of graft nephrectomy on the outcome of the second graft [16]. Removal at the time of retransplantation resulted in a 88% one year graft survival of the second graft (n = 9) versus only 25% in the 25 patients with earlier graft nephrectomy. These single centre data were compared with the data from the NIH Organ Transplant Registry and again a significant difference in favour of the group removal of the graft at the time of the second transplantation was found. In the same year an analysis of the UCLA data by Opelz could not confirm these findings [17]. More recently Sumrani et al. summarized their experience in 95 retransplants performed with cyclosporin as basic immunosuppressant [18]. Nephrectomy before retransplantation was associated with a significantly higher incidence
of initially nonfunctioning and a trend towards lower one year graft survival. A similar observation was also made by So et al. in a group of azathioprine-treated children [19]. It must be stressed that graft nephrectomy was done on a randomized basis in neither series. It is therefore likely that differences in survival are more related to the indications for graft nephrectomy (e.g. signs of acute rejection) rather than to nephrectomy per se. In our own patients no deleterious effect of pretransplant graft nephrectomy on the survival rate of the subsequent transplant was noted (one year graft survival 83.3% in the nine patients with their first graft still in place at retransplantation versus 83.1% in the patients with pre-emptive nephrectomy).

Effect of graft nephrectomy on HLA-antibody formation

One of the major causes of sensitization is the failure of an allograft, particularly when the graft fails early. Even after an extensive literature search, virtually no studies were found addressing the effect of graft nephrectomy on HLA antibody formation. In the study of Sumrani [19] analysing the effect of allograft nephrectomy on the survival rate of a subsequent transplantation, nephrectomy of the failed graft prior to retransplantation was associated with a subsequent increase in preformed antibody level (57% of the patients with graft nephrectomy had panel reactive antibodies [PRA] >30% compared to 33% in those without graft nephrectomy). In our experience allograft nephrectomy is followed by a transient increase of the HLA-antibody level. At the time of nephrectomy no patients were highly sensitized. After nephrectomy 8.3% of the patients had developed HLA antibodies with more than 80% PRA. This effect was however transient as at the time of last follow-up the percentage of highly sensitized patients (PRA >80%) was again decreased from 8.3% to 4.1%. At the time of last follow-up 11% of the patients in whom the graft was still in place were highly sensitized.

Conclusions

Although a substantial number of renal allografts continue to fail, especially in the late post-transplant period, the management of the failed graft has been poorly investigated. Data in the literature are mostly anecdotal. No well planned randomized studies are available to answer such issues as the effect of nephrectomy on subsequent graft survival and HLA antibody formation or whether immunosuppressive therapy can be stopped safely while leaving the failed graft in place. Taking into account these shortcomings and based on our own uncontrolled experience we come to the following conclusions.

Since the use of cyclosporin and lower doses of steroids, graft nephrectomy has become a safe procedure with limited morbidity. In case of early graft failure we recommend early nephrectomy and complete interruption of immunosuppressive therapy. When graft failure occurs later, the graft can be left in place, while immunosuppressive therapy is tapered and stopped. Patients must be followed carefully as in at least one third of the patients side-effects will occur necessitating subsequent graft nephrectomy. Randomized studies are needed to analyse the safety of this approach in terms of HLA sensitization and its effect on subsequent graft survival.

References

Cytotoxicity of peritoneal dialysis fluid—is it related to glucose breakdown products?

Anders P. Wieslander

Gambro AB, Lund, Sweden

In the winter of 1962–63 bee-keepers in England lost many colonies fed with commercial food candies. These food candies were composed of sucrose hydrolyzed by heat and acid instead of enzymes. It was later demonstrated that caged bees fed on the same type of commercial candy died within a few days. A higher percent of those bees fed on the darkest varieties of the candy died more quickly. It was concluded that the bees had died because of some unknown substance related to the heat induced decomposition of the glucose [1]. This report is only one of many, demonstrating toxicity due to break-down products of carbohydrates. Actually, one of the first reports, published in 1887 demonstrates that a bacterial medium exposed to sunlight kills bacterial spores as a consequence of changes in the carbohydrate fraction [2]. A later report, from 1935, demonstrates that after exposure to UV radiation several different carbohydrates prevent the growth of bacteria due to the formation of non-volatile and thermostable substances [3]. Among the carbohydrates investigated in this study were glucose and dextrin, two carbohydrates which are used as osmotic agents in fluids for peritoneal dialysis. The side-effects of carbohydrate degradation products have also been described for many mammalian cellular systems.

A patient on peritoneal dialysis (PD) uses between 8 and 20 l of dialysis fluid every day depending on the treatment regime. This results in the consumption of 3–7 tons of fluid with 1.5–4.0% glucose (50–175 kg pure glucose) every year. In spite of the notably high local exposure of the cells within the peritoneal cavity to these fluids, the presence of contaminating substances such as glucose degradation products has met with remarkably little interest over the years.

The breakdown substances in PD fluids are mainly formed during the heat-sterilization but some further glucose degradation occurs during storage. Despite this, biocompatibility studies of PD fluids have instead focused on the low pH, especially in combination with lactate, and the high osmolality of the fluids. This may be because low pH, until it is neutralized, has such a dramatic effect on cultured cells that it conceals all other problems connected with PD fluids. However, a few in vitro reports discuss the presence of other toxic properties, in addition to pH and osmolality, which might relate to glucose degradation products. Reports from the middle of the 1980’s discuss the role of glucose degradation products on infusion pain and loss of ultrafiltration [4]. A few years ago our laboratory started to analyse ‘basal cytotoxicity’ in material toxicology tests. Basal cytotoxicity is considered as injury to basic cellular processes and can therefore be measured using very simple in vitro assays, such as proliferation of cell lines. The findings of large multicenter studies are that these simple in vitro assays correlate well with known human toxic blood concentrations, if a sufficiently long in vitro exposure period is used.

When testing the basal toxicity of PD fluids we found that all major brands of commercial PD fluids were cytotoxic in contrast to PD fluids sterilized by filtration [5]. The PD fluids were tested after dilution with cell growth media, in order to eliminate pH and osmolality as the cause of cytotoxicity. We also demonstrated that the cytotoxicity of laboratory-made sterile-filtered PD fluid increased significantly after heat sterilization in glass bottles. Thus the cytotoxicity was not due to interference with the PVC plastic of the bag but instead to the degradation of glucose. Slightly different cytotoxicity levels were noticed in the different brands of commercial PD fluids, ranging from 53% to 75% inhibition of growth. The same results were also observed using cultured human neuroblastoma cells or cultured mouse macrophages [6].

Basal cellular functions are known to support organ-specific cell functions. One important function of the cells in the peritoneal cavity is to release inflammatory signals upon stimulation and thus defend the cavity against invading micro-organisms. In an in vitro model mimicking this situation we found that heat sterilised PD fluids impaired the stimulated release of the pro-inflammatory cytokines TNF-α [6] and IL1-β [7]. A reduction of oxygen radical release after exposure to commercial PD fluids was also demonstrated in human neutrophils [6]. In anaesthetized rats the superfusion of the peritoneal mesentery with commercial heat-

Correspondence and offprint requests to: A. P. Wieslander, Gambro AB, Box 101 01, S-220 10 Lund, Sweden.