Fabry Disease: New Therapeutic Approach

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Fabry's disease is an X-linked recessive lysosomal storage disorder that is caused by the deficient activity of the lysosomal α-galactosidase (α-GAL A). This results in the accumulation of globotriaosylceramide (GL3 or ceramide trihexoside, CTH) and related glycosphingolipids. In hemizygous male patients with the classic phenotype, levels of α-GAL A activity are very low or undetectable. Patients with detectable α-GAL A activity have a milder, variant phenotype. Diagnosis is therefore based on the measurement of α-GAL A activity in leukocytes or serum. In classically affected males, the progressive GL3 accumulation, particularly in the vascular endothelium, leads to the visceral complications of the disease involving kidneys, heart and central nervous system (CNS), responsible for early death. Estimates of prevalence range from about 1 in 50,000 to 1 in 100,000 males (1, 2).

Carrier (heterozygous) females have, on the average, a much milder disease than males. However some of them may develop visceral complications, affecting mainly the heart (left ventricular hypertrophy, atrioventricular block) and CNS (transient or persistent ischemic cerebrovascular accidents) and more rarely the kidneys. The clinical heterogeneity in heterozygous women is explained by random X-chromosomal inactivation.

1. Diagnosis of Fabry disease

Recent studies have shown that the diagnosis of Fabry disease is made too late in life. The median age at diagnosis is about 28-29 years. Diagnosis should be easy in families where a family member has already received a diagnosis of Fabry disease. Complete investigation of the at-risk subjects should be offered to these families. In contrast, the diagnosis may be difficult in kindreds where no affected member has been previously identified.

The first symptom of the disease is usually acroparesthesias and pain crises involving the extremities. These symptoms first appear between 5 and 12 years of age. They are triggered by heat exposure or fever. Pain may be excruciating. These symptoms are mistakenly attributed to other disorders, such as rheumatic fever, erythromelalgia etc…

Skin angiokeratomas appear later, predominating on the hips, back, thighs, buttock, penis and scrotum. However skin lesions may be very limited or even absent in some patients.

Other manifestations of Fabry disease are corneal deposits ("cornea verticillata"), decreased sweating, lymphoedema, and lens opacities. Among the visceral complications, cerebrovascular accidents occur often early in life, between 20 and 30 years of age: transient ischemic attacks, vertigo/dizziness deafness (found in 30% of the patients, which can be due to cochlear deposits or to ischemic defects in the vertebrobasilar circulation. Cardiac abnormalities include left ventricular enlargement, valvular involvement and conduction defects.

Progressive deposition of GL3 in intrarenal vessels is responsible for progression of renal disease. GL3 also accumulate in glomerular cells (predominantly in podocytes) and in tubular and interstitial cells. Histopathologic changes are characteristic for an experienced renal pathologist (3). Proteinuria is the first abnormality; hematuria is often absent or mild; the nephrotic syndrome is rare. Typically renal failure begins at around 30 years of age and progresses to ESRD in approximately 10 years (7). The age at ESRD is between 35 and 55 years but the end-stage may be reached earlier or later. Among the ESRD Fabry patients, 12% are females. Survival rate in dialysis is worse in Fabry patients than in non-Fabry patients. In contrast kidney transplantation is an excellent mode of treatment of ESRD. The graft and patient survival rate is similar to that of the general renal transplanted population.

It should be clear however that two types of renal lesions are present in hemizygous Fabry patients: 1) Some lesions are directly related to GL3 deposition in vascular and glomerular endothelial cells. 2) Progressively, renal ischemic damage develops, and "unspecific" changes appear, as in any ischemic nephropathy. This should be kept in mind to appreciate the effects of enzyme substitution. In addition "unspecific" changes will benefit of symptomatic management, such as blood pressure control and reduction of proteinuria.

The diversity of clinical involvement may explain why diagnosis of Fabry disease is made so late in life. Better training of nephrologists, cardiologists, neurologists and ophtalmologists should lead to earlier diagnosis in a time when enzyme replacement therapy (ERT) is available. Measurement of α-GAL A activity is a simple and reliable test of diagnosis in males.

2. Treatment of Fabry disease

In the past decades, only symptomatic management was available. Carbamazepine and related drugs have been shown to be effective in preventing pain in affected patients. Dialysis and transplantation are indicated in ESRD patients. Symptomatic treatment of cardiac and cerebrovascular accidents is also available.

Recent progress in molecular medicine has made possible to conceive and to design enzyme replacement with human α-GAL A in Fabry disease. The α-GAL A gene has been identified; knock-out mice have been generated, mimicking human Fabry disease; and human α-GAL A has been pro-

duced by two groups: agalsidase α (Replagal®, TKT) and agalsidase β (Fabrazyme®, Genzyme). Both drugs have been approved by the European Agency of the Evaluation of Medicinal Products whereas only Fabrazyme has been registered by the US Food and Drug Administration. Both drugs are administered intravenously, every two weeks, at a dose of 0.2 mg/kg of body weight for Replagal and 1 mg/kg for Fabrazyme. The two proteins are structurally and functionally very similar (5, 6).

To date, 109 patients with classic Fabry disease have been evaluated in 2 randomized, placebo-controlled trials. These trials demonstrated that patients receiving human α-GAL A had a 50% decrease (agalsidase α) or a drop to non detectable levels (agalsidase β) of plasma GL3. GL3 accumulation in the skin, heart and kidneys was significantly cleared. Subsequent studies showed an improved quality of life with ERT, and most particularly the disappearance of gastrointestinal symptoms, frequently found in untreated Fabry patients. Treatment with both α-Gal products has been well tolerated, except for mild to moderate infusion-associated reactions. These reactions were associated with the formation of non-neutralizing IgG antibodies but more detailed information on these antibodies and their evolution is needed.

The evolution of renal histopathological changes has been described in detail by Thurberg et al (7). After 20 weeks of Fabrazyme administration, renal endothelial cells (peritubular and glomerular capillaries, and vascular endothelium) are completely cleared of GL 3 deposits. In contrast vascular smooth muscle and tubular cells are incompletely cleared. Lysosomal accumulation of GL3 in podocytes is decreased only marginally. This is not surprising: such podocyte changes are frequently found in heterozygous females who have no urinary abnormalities; these changes persist despite a 50% decrease in α-GAL A activity on the average; podocytes are probably less accessible to infused enzyme than endothelial cells and their turn-over rate is very low, if there is any. These factors explain why podocytes changes are very little influenced by ERT. Most probably these changes are not involved in the progression of the renal disease.

These 2 short-term trials do not demonstrate that ERT modifies the clinical course of Fabry disease. This remains to be established in the future. No firm conclusion regarding renal progression can be drawn: Fabry patients enrolled in the trials had normal or slightly impaired renal function at entry. Renal function remained stable during ERT. Beneficial clinical effect on renal function was demonstrated in the patient studied by de Schoenmakere et al (8) in whom the slope of progression of renal failure was slowed by ERT. This effect depends on the balance between specific and unspecific kidney changes at initiation of ERT. If ERT is started too late in a patient with extensive ischemic and fibrotic changes, no beneficial effect on renal function will be observed. In these early years of ERT, I suggest to perform a renal biopsy before initiating this treatment to evaluate the severity of the unspecific changes which cannot be reversed by ERT. This is probably useful in patients older than 30 years; renal biopsy has to be discussed in younger patients since unspecific changes may develop early in males with Fabry disease.

In addition to ERT, other therapeutic perspectives in Fabry disease are open: substrate deprivation, enzyme stabilisation by chaperone molecules, or even gene therapy which has been successfully used in mice.

In conclusion, enzyme replacement therapy by human α-GAL A modified the therapeutic perspectives in Fabry disease. Many questions are still unanswered: Does it modify the natural history of the disease? Are all glycolipid deposits accessible to therapy? When to start treatment?

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Lymphatic Neoangiogenesis in Transplanted Kidneys

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Transplantation and chronic dialysis are life saving renal replacement therapies, that, however, are not only associated with human suffering, but also with substantial financial burdens (1). The function of grafted kidneys is imperiled by rejection reactions, that are mediated by massive invasion of alloreactive mononuclear recipient cells into the cortical stroma, and destruction of tubules and/or endothelial cells. Aggressive, unselective immunosuppressive therapy usually results in rapid clinical improvement of the transplant function, however, each acute rejection episode leaves a permanent mark on the graft's function (2). Poorly defined, persistent low-grade alloimmune responses are thought to continue during the entire life span of the graft, and eventually contribute to the multifaceted process of chronic transplant rejection that is currently the major cause for long term graft failure, and is out of reach of therapy (3). Thus, detailed knowledge of the molecular mechanisms that determine influx and disappearance of the rejection infiltrate and the site(s) and mechanisms of continuous immune responses are required to design therapeutic strategies specifically targeted at the recurrence of acute rejection and development of chronic rejection.

Acute interstitial rejection is accurately defined as a special form of chronic inflammation, with a mixed infiltrate of mononuclear inflammatory cells ($CD4^+$ and $CD8^+$ T-lymphocytes, macrophages) that invade the cortical tubulo-interstitial spaces (4). Despite many years of experience with transplant biopsies little is known about the kinetics of infiltrate clearing after immunosupressive therapy, and it is not certain that the nodular infiltrates of mononuclear cells in the cortical stroma are residues of the diffuse infiltrate found in acute rejection. Disappearance of the rejection infiltrate is in part due to apoptosis that occurs in the renal cortex at a rate similar to that in the thymus (5). However, even this relatively high apoptotic rate is obviously too low to account for the disappearance of inflammatory infiltrate cells after immunosuppressive treatment, thus raising the necessity for additional mechanisms of elimination. This has raised the possibility that, similar to other inflamed tissues, cortical lymphatic vessels could serve as exit routes (6), as suggested by previous studies in experimental renal transplantat rejection in which lymphatic vessels drained large amounts of fluid and mononuclear cells (7). Therefore we have investigated here the distribution, density, and function of lymphatic vessels in acute rejection, taking advantage of recently developed specific markers for lymphatic endothelial cells (8). We have chosen to examine biopsies and explants from human renal transplants rather than experimental models that only imperfectly mirror the course of rejection and its corresponding morphological features in humans.

Angiogenesis of renal lymphatic vessels under pathological conditions is an uncharted territory, primarily due to the lack of reliable markers for lymphatic endothelial cells. Here we have compared the distribution of lymphatic vessels in normal kidneys and grafts with transplant rejection by immunohistochemistry, and have discovered the occurrence of massive lymphatic neoangiogenesis in transplants, as well as a novel function of lymphatic vessels in association with immunologically active, intrarenal nodular lymphatic infiltrates.

Critical for investigations in the nascent field of lymphangiogenesis is the reliability of the immunocytochemical markers used (10). Currently, four markers distinguish lymphatic from blood vessel endothelial cells: (i) The membrane mucoprotein podoplanin that qualifies as highly specific for lymphatic endothelial cells (9), and was instrumental for the first isolation of pure lymphatic endothelial cell lineages from human dermis (11, 12); (ii) VEGFR-3 that is also expressed in endothelial cells of newly formed blood vessels (13); (iii) LYVE-1, a CD44-related hyaluronate receptor (14); (iv) Prox-1, a transcription factor that controls the lymphatic phenotype of endothelial cells (15). In this study, the immunohistochemical results were obtained primarily with antibodies to podoplanin, and identical results were obtained by doublelabeling with antibodies against LYVE-1 and Prox-1.

In the cortex of normal human kidney, podoplanin/LYVE-1/Prox-1-expressing lymphatic vessels were confined to the adventitia of large and middle sized arteries, as described previously (16), and this pattern of distribution persisted in acute phases of transplant rejection with intense interstitial mononuclear infiltration. However, in biopsies containing nodular infiltrates there was about < 100 fold amplification of the lymphatic vessel density over controls, with lymphatic microvessels reaching deep into the tubulo-interstitial space. These lymphatic vessels were formed by lymphangiogenesis, as many of them expressed the nuclear proliferation marker Ki-67, and presumably sprouted from preexistent perivascular lymphatics. In the lumina of the newly formed lymphatics $CD2^+$ and fewer $CD2^-$ cells were frequently encountered, indicating that the lymphatic vessels contribute to the clearing of the rejection infiltrate from the renal cortex. Lymphatic neoangiogenesis apparently involved the appearance of $CD68^+CD23^+$ macrophages that produce VEGF-C and VEGF-D, similar to a recently discovered subset of tumor-associated macrophages that were related to peritumoral lymphatic vessel proliferation (11),

and to tubulo-interstitial mononuclear cells in the rat remnant kidney model of cortical fibrosis (20). These data provide further support for the hypothesis that VEGF-C-producing macrophages contribute to regionalized lymphatic neoangiogenesis.

A potentially important observation in all biopsies with nodular infiltrates is the colocalization of lymphatic vessels with the nodular mononuclear infiltrates that apparently are immunologically highly active organoid structures, with sometimes massive $Ki67^+$ T- and B-lymphocyte activation. Immunohistochemical typing of the nodular mononuclear infiltrates revealed clusters of $CD4^+$ and $CD8^+$ cells, as well as $CD20^+$ B-lymphocytes, and λ and κ chain-expressing plasmacytoid cells. $S\text{-}100^+$ dendritic cells were observed in association with lymphatic vessels. These results provide evidence that within the peri-lymphovascular nodular infiltrates, activation and maturation of T-lymphocytes occurs by antigen presentation by dendritic cells, and that $CD\text{-}20^+$ B-cells mature to immunoglobulin-producing plasmacytoid cells. Thus, nodular infiltrates resemble lymphatic organs in autoimmune diseases that locally perpetuate autoimmune reactions, and support autoantigenic epitope spreading (18). However, the precise positioning of nodular infiltrates and lymphatic neoangiogenesis in the chronology of resolution of acute transplant rejection remains to be determined, and a large scale systematic analysis of protocol biopsies is underways to clarify this issue (our unpublished observations).

The close association of nodular infiltrates with lymphatic vessels raises the possibility that the lymphatic endothelial cells actively recuit lymphocytes. A good candidate chemokine for this purpose is SLC/CCL21 that organizes lymphatic follicles when expressed ectopically in □-cells of mouse pancreas (19), and attracts mononuclear cells in inflammatory diseases (20), while mice with deletion of the SLC/CCL21 gene fail to develop certain lymphnodes (21). We have previously discovered that SLC/CCL21 is produced and polarly secreted by isolated lymphatic endothelial cells (11). Here we show that lymphatic vessels in transplants produce SLC/CCL21 in situ, and are surrounded by $CCR7^+$ cells, providing direct evidence for SLC/CCL21-mediated lymphocyte, and presumably also dendritic cell chemoattraction by lymphatic vessels. These findings assign a novel, active role to lymphatic vessels in the organization of the peri-lymphovascular nodular infiltrates in renal transplants, and perhaps in lymphoid organogenesis in general.

In tissues SLC/CCL21 gradients are required for the directed migration of $CCR7^+$ cells (22), and are established by charge interactions with proteoglycans in basement membranes (23), that, however, are not produced by lymphatic vessels. Here we provide evidence by double labeling immunoelectron microscopy that podoplanin contributes to the peri-lymphovascular SLC/CCL21 gradient formation, as SLC/CCL21-podoplanin complexes appear on the basal membrane of lymphatic endothelia, and are shed into the perivascular stroma. Also, surface plasmon resonance binding indicated a high affinity charge and carbohydrate side chain-dependent binding of podoplanin to SLC/CCL21. Collectively, these data provide evidence that SLC/CCL21-podoplanin complexes contribute to the perivascular gradient formation, and assign a novel function to podoplanin, as a binder of cationic chemokines in lymphatic endothelial cells.

In conclusion, the novel results of this study put newly formed lymphatic vessels and peri- and para-lymphovascular infiltrates center stage as foci of immunological activity in human renal transplants. By virtue of their cellular composition, nodular infiltrates have the potential to launch and perpetuate specific immune responses to graft alloantigens, and could thus contribute to recurrent episodes of acute rejection, support humoral rejection (24), pave the way for chronic rejection and eventual loss of transplant function, and thus could provide a novel therapeutic target.

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