Study proposal:

Investigation of pathogenic molecules in etiology of nephrotic syndrome in children

Background

Immunosuppression with corticosteroids is the cornerstone of nephrotic syndrome (NS) therapy, therefore, it seems logical to suspect immune dysregulation as a pathogenic factor in disease development (1). Minimal change nephrotic syndrome (MCNS) has been proposed as a disorder of T-cell function, resulting in increased plasma levels of lymphocyte-derived permeability factor (2). This hypothesis was based on several clinical observations, such as follows: remission can be accompanied by measles infection whereby cell-mediated immunity is suppressed (3); MCNS is associated with Hodgkin's disease, which is a known T-cell disorder (4); patients show good response to corticosteroids and cyclophosphamide, known inhibitors of T-cell function (5); humoral component deposition (immunoglobulins) is absent in glomeruli, unlike in other glomerular disorders (6). As a consequence, proteinuria and hypoalbuminemia, associated with NS were thought to result from increased glomerular capillary wall permeability due to T-cell activation triggered by several stimuli, such as viral infection or allergens (1).

Investigators have, therefore, made attempts to identify the circulating factors released from T-cells that increase glomerular permeability to serum proteins and the most likely pathogenic factors are considered to be cytokines (1). Patients with relapses were found to have elevated serum or urine levels of various cytokines, including interleukin (IL)-2, soluble IL-2 receptor, interferon-gamma, IL-8, IL-13, tumor necrosis factor-α and vascular endothelial growth factor (7-16). Among them, IL-8 and IL-13 have been proposed to be most likely circulating factors. IL-8 may play a role in proteinuria by affecting the metabolism of glomerular basement membrane (GBM) components (10). IL-13 has been shown to stimulate intracellular podocyte protein trafficking and proteolysis in vitro (12). In addition, IL-13-transfected rats developed severe proteinuria and showed MCNS like nephropathy (13). Increased IL-13 also induced the overexpression of CD80 in podocytes, recently identified as a possible molecular mechanism underlying proteinuria in NS (17). However, not all patients with MCNS have elevated serum IL-13 during relapse, and serum IL-13 is also known to be increased in diseases not associated with proteinuria, such as asthma (18). Studies done over

the last four decades have reported conflicting results regarding the role of cytokines in MCNS (1).

MCNS has been traditionally considered a T-cell disease but results of the immunological studies have contributed to a more sophisticated understanding of its pathogenesis taking into account regulatory T-cells (Tregs) and B-cells. Cytokine release by T-cells is usually transient due to the activation of Tregs that interact with T effector cells to suppress cytokine production (1). Tregs have been suggested to constitute a second step in an MCNS cascade of a »two-hit model«, of which the first remains unclear. In addition, abnormal censoring of podocyte CD80 expression could underlie Treg dysfunction or impaired autoregulation by podocytes. Treg dysfunction could lead to transient massive proteinuria becoming persistent, following which podocyte injury, and eventually, MCNS, occur (19). The induction of Treg led to a marked reduction in proteinuria in animal models, and most patients with MCNS showed decreased levels of Treg (20). Unlike role of T-cells in MCNS, which has been extensively studied, the role of B-cells is currently not well understood. Clinical trials have been conducted that demonstrated MCNS remission after B-cell depletion using the anti-CD20 monoclonal antibody rituximab (21). The recent successful use of anti-CD20 monoclonal antibodies for the treatment of steroid sensitive NS raises the possibility of B-cells either influencing T-cells or themselves being primary players in NS. CD80 is expressed by both activated B- and T- cells, and increased nitric oxide production by B-cells observed in NS patients with relapse further supports the possibility of B-cell involvement (14).

CD80, also known as B7-1, is a transmembrane molecule present on the surface of both antigen presenting cells and activated B-cells, and acts as a co-stimulatory signal for T-cell activation (22). CD80, present on the surface of antigen presenting cells, binds CD28 on effector T-cells or cytotoxic T lymphocyte-associated protein 4 (CTLA4) in regulator T-cells, determining T-cell activation (CD28) or inhibition (CTLA4) (23). Under certain conditions, podocytes can express CD80, and its expression results in the development of a proteinuric condition. Proteinuria was not induced in CD80 knockout mice by lipopolysaccharides administration, but it was induced in SCID mice, which are deficient in T- and B-cell functions, showing that CD80 plays a key role independent of T- and B-cells (17). Increased CD80 levels in urine are observed in patients with MCNS with relapse compared to those in remission and with other glomerular diseases (lupus, FSGS)(24). A recent study has reported that high urinary CD80 excretion might be a biomarker for steroid responsiveness and a

predictor for good prognosis in NS (25). In addition, polyinosinic:polycytidylic acid (polyI:C), a ligand of Toll-like receptor 3 which mimics viral infection, promotes podocyte CD80 expression (26). This offers a possible reasoning to explain the frequent relapse of MCNS after upper respiratory virus infection. Because PolyI:C induces only transient proteinuria, impaired regulatory mechanisms after CD80 induction were postulated as a second hit cause of MCNS (19). Suppression of CD80 expression could be a novel therapeutic strategy for MCNS; however, more evidence is required to support this idea (1).

Another important aspect in the pathogenesis of MCNS is perturbation of normal anionic charge barrier. Charge selectivity has been proposed to be due to highly sulfated sialoglycoproteins in the glomerular basement membrane (GBM) and on the surface of endothelial and visceral epithelial cells. Alterations in GBM charge may be due to enzymes elaborated by mononuclear cells, or it may be due to circulating highly cationic substances that have not been discovered (27).

In asthma, common chronic obstructive airway disease, presentation of a selected antigen peptide to the T-cell receptor initiates sensitization and the subsequent immune response to the specific allergen (28). For efficient antigen-dependent T-cell activation engagement of either CD80 (B7.1) or CD86 (B7.2) on the dendritic cells with CD28 on T cells leads to sensitization, whereas lack of, or inefficient, engagement of these costimulatory molecules may lead to anergy (29). An alternative method of preventing sensitization and rendering T cells anergic is engagement of a second costimulatory molecule, cytotoxic T-lymphocyte antigen (CTLA)4, which has a higher affinity than either CD80 or CD86 for CD28 and can therefore prevent CD80/CD86 costimulation (30). This is the basis of the successful clinical application of the CTLA4–immunoglobulin fusion protein abatacept, used as an immunomodulatory agent in such diseases as rheumatoid arthritis and in an animal model of allergen-induced airway inflammation (31).

An important cell in the inflammation of allergic asthma is the eosinophil leukocyte, which is present in the airway wall as well as in the sputum and bronchoalveolar lavage fluid (32). These cells are in large part initially recruited from the bone marrow as CD34 precursors, following the release of PGD_2, cysteinyl leukotrienes, cytokines and chemokines from the asthmatic airway. The developing eosinophils then pass from the circulation via the microvascular compartment into the airway wall. IL-3 and GM-CSF and eotaxins 1–3 are

crucial to the early derivation of eosinophils from $CD34^+$ bone marrow precursor cells, with IL-5 being responsible for their maturation and recruitment into the airways (33). Eosinophils are a rich source of granule basic proteins, such as major basic protein, eosinophil peroxidase, and eosinophil cationic protein (ECP), and also have the capacity to generate eicosanoids such as prostacyclin (PGI_2) and cysteinyl leukotrienes and release potentially tissue-damaging superoxide and a range of cytokines and chemokines (34). The dramatic reduction in sputum and tissue eosinophils that occurs on treatment of asthma with inhaled or oral corticosteroids associated with clinical improvement has led to the idea that eosinophils are fundamental to airway dysfunction in asthma and are the principal target for this drug class (35). Corticosteroids have also been shown to increase clearance of airway eosinophils through egression into the airway lumen as well as by inhibiting chemokine production (36). In an animal model, activation of apoptosis through the Fas receptor has been shown to magnify rather than resolve asthmatic type inflammation (37). Thus, while much has been learnt about eosinophils in asthma, there still remain many unanswered questions (38).

Methods

According to above mentioned pathogenesis, it seems reasonable to study CD80 expression in children with NS and to compare this expression in patients with relapse to patients in remission as well as in patients with steroid-sensitive and steroid-resistant NS. This could potentially present a basis for further treatment with abatacept, CTLA4–immunoglobulin fusion protein, with CD80 as a therapeutic target, already used as an immunomodulatory agent in rheumatoid arthritis and in an animal model of asthma.

In addition, according to above mentioned similarities in pathogenesis and treatment options (such as glucocorticoids) of NS and asthma, it would seem reasonable to study eosinophil cationic protein - ECP (and perhaps some other substances, mentioned above) in blood of patients with NS and to compare these values in patients with relapse to patients in remission as well as in patients with steroid-sensitive and steroid-resistant NS. Namely, ECP may theoretically be involved in disruption of normal anion charge barrier in the GBM, that may be due to circulating highly cationic substances that have not been discovered yet, as mentioned above.

The study, if recognized as useful and relevant by appropriate board (such as ESPN glomerular diseases working group), is planned to be multicentric, international, in order to obtain a sample, large enough in order to get more firm results. Children with idiopathic nephrotic syndrome would be included, as early as possible in the disease course in order to avoid possible influence of immunosuppresive treatment on the results of investigations. Informed consent from patients and their parents / caregivers is planned to be obtained as well as ethical approval from ethical commitee.

Conclusions

On the basis of above described pathogenic mechanisms, CD80 expression in children with NS, as a therapeutic target for abatacept, and measurement of substances like ECP (candidate for circulating factor) in blood of patients with NS may contribute to better understanding of NS pathogenesis, with possible therapeutic implications.

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