Functional and immunological studies in children with chronic renal failure: the effects of uremia and dialysis treatment
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Chapter 1

General Introduction
CHRONIC DIALYSIS TREATMENT IN CHILDREN AND ITS COMPLICATIONS.

Children with end stage renal failure are treated with continuous ambulatory peritoneal dialysis (CAPD) or one of its modifications since 1978. CAPD is a dialysis treatment modality introduced by Popovich et al. [1,2]. Peritoneal dialysis (PD) is the most commonly used method of pediatric dialysis because of its safety and simplicity. The principle of peritoneal dialysis is the transfer of water and solutes from the circulation to the peritoneal cavity across the peritoneal membrane. This can be achieved by instilling a hyperosmolar, mostly glucose, containing fluid through a permanent catheter into the peritoneal cavity. The advantages of PD over hemodialysis (HD) treatment in children are the steady-state biochemical control, the absence of a risk for desequilibrium syndrome, greatly reduced dietary and fluid restrictions, and no repeated hemodialysis needle punctures. Furthermore PD can be performed at home or at school, offering children the opportunity to experience a more normal childhood life. This contrasts with HD treatment in children which is mostly performed in the hospital, at least three times a week for about three hours. The main chronic PD modality is continuous ambulatory peritoneal dialysis (CAPD), a technique in which the dialysis fluid is changed manually every 4-6 hours. At present most children are treated with automated nightly intermittent peritoneal dialysis (NIPD). The best treatment for end stage renal failure in children is a successful renal transplantation, but children can stay waiting on PD for many years. However, peritonitis remains one of the major complications of this treatment [3-7]. The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) reported that the most common reasons for dialysis modality termination were peritonitis or exit site/tunnel infections (45%), when the child was not transplanted [3]. Nearly 50% of the children have had at least one peritonitis episode at the end of the first year on PD. An overall mortality rate of 5.7% has been described in the pediatric PD population [3]. The younger the child the higher the mortality risk. The primary reported causes of death in PD children are cardiopulmonary disease and infection. The treatment and the prevention of peritonitis are important elements in the care of pediatric PD patients, both for reduction of mortality and morbidity, and also for the preservation of the peritoneal membrane function. Previous reports have shown that the incidence of peritonitis in children is higher than that found in adults [5,6]. Furthermore, it has been demonstrated that infants and children up to six years of age develop peritonitis more frequently than older children [3]. Through technical improvements of PD systems, such as disconnect systems and double cuff catheters, the incidence of peritonitis has decreased [8,9]. However, the NAPRTCS reported an increase of the peritonitis incidence in PD children since 1995 despite technical improvements [10]. Besides technical causes for the risk of developing peritonitis in PD children, disturbances in the local intraperitoneal, or systemic host defense might be involved in the individual infectious susceptibility [4]. This subject has not been investigated extensively in children with chronic renal failure, either treated conservatively or with dialysis treatment.
PERITONEAL MEMBRANE AND FUNCTION

Successful blood purification by peritoneal dialysis depends on the sustained ability of the peritoneal membrane tissues to function as a dialysis membrane allowing adequate removal of solutes and excess of fluid. The function of this peritoneal membrane can be negatively influenced by peritonitis episodes and the toxic effects of the commonly used dialysis solutions [11-15].

Transperitoneal transport is based on two processes: diffusion and convection (Figure 1). Diffusion is the movement of solutes down a concentration gradient, whereas convection is the movement of solutes along the direction of the fluid flux. Small molecules are predominantly removed by diffusion [16].

The average peritoneal surface area in adults ranges from 1.0 m² [17] to 1.3 m² [18]. A newborn has a peritoneal surface area of 0.11 m² [17]. The ratio between peritoneal surface area and body weight in newborns is twice that of the adult [17]. But, a difference between adults and infants is barely present when the peritoneal surface area is related to the body surface area [17]. Solute transport across the peritoneal membrane is a size selective process. The vascular wall is probably the most important size-selective barrier. Transport across it is through a system of pores [19,20]. These pores consist of a small number of large pores with radii exceeding 150Å, involved in the transport of macromolecules such as serum proteins, a large number of small pores with a radius of about 40Å, through which small molecular weight solutes and water pass, and the water-conductive ultrasmall pores with radii of 3-5Å, allowing the transport of water but not of solutes [21,22]. The magnitude of transport of low molecular weight solutes is therefore dependent on the vascular surface area of the peritoneum (i.e. the number of perfused peritoneal capillaries or the number of pores), whereas the transport of high molecular weight solutes or macromolecules, such as proteins, is dependent on the permeability or size-selectivity of the peritoneal membrane (the pore size) [23,24].
Characterization of the peritoneal membrane permeability in patients treated with chronic PD can be performed with a peritoneal equilibration test (PET), developed by Twardowski et al. [25]. Through standardization of this test, adult patients could be categorized into low, average and high transporters according to their peritoneal solute transport results [26]. The PET is performed during a 4-hour dwell with a glucose 2.27% dialysis solution. It measures low molecular weight solute transport and ultrafiltration. Solute transport is calculated by the dialysate over plasma ratio of creatinine (D/Pcr) after a 4-hour dwell, and the ratio of the dialysate glucose concentration at the end of the test and the initial glucose concentration (D/Do). The net ultrafiltration is calculated as the difference between the drained and the instilled volume. The D/P ratio is easily calculated, but it has some disadvantages. The PET is based on the principle that peritoneal transport is diffusive. The D/P ratio is also influenced by convective transport from the circulation to the peritoneal cavity, especially, when high glucose containing dialysis solutions are used. Furthermore, D/P ratios vary with different instilled dialysate volumes [27]. Thus, in the same patient a higher D/P ratio can be found as the resultant of a lower dwell volume. More recently, the standard peritoneal permeability analysis (SPA) has been described by Pannekeet et al. [28]. In this modification and extension of the PET, the transport of low molecular weight solutes is expressed as mass transfer area coefficients (MTAC) instead of D/P ratios. Furthermore, peritoneal fluid kinetics during the dwell are determined using intraperitoneally administered dextran, and the peritoneal clearances of various serum proteins are calculated. The MTAC of a solute is equal to the maximum clearance, before transport has actually started, thus at the theoretical point when the dialysate concentration for the solute is zero [29-31]. It has been demonstrated in adult CAPD patients that the assessment of the MTAC is more accurate than the D/P ratio, since the MTAC is more constant from bag exchange to bag exchange and the MTAC is independent of dialysate volume [28]. Furthermore, the D/P ratio overestimates the MTAC values in the lower ranges and underestimates them in the higher ranges [28]. However, the MTAC is a complicated calculation whereas the D/P ratio is easily calculated. The size selectivity of the peritoneal membrane to macromolecules, or the intrinsic peritoneal permeability, can be expressed as the restriction coefficient (RC). The RC is the slope of the linear relationship between the MTAC of various solutes and their free diffusion coefficients in water when plotted on a double logarithmic scale [32]. The higher the RC, the lower the permeability for high molecular weight molecules.

It has often been assumed that solute transport in children is different compared to that in adults [33-35]. Consequently, peritoneal loss of proteins such as immunoglobulins might also be higher. This could be one of the explanations for the higher peritonitis incidence in children when compared to adult PD patients. Therefore, we have used the SPA in children. The results are described and compared with those performed in adult CAPD patients in chapter 2.
The peritoneum is covered by a single layer of mesothelial cells. Peritoneal mesothelial cells are important for the local host defense and membrane integrity since they produce various substances, such as phospholipids, cytokines and chemokines and they can ingest bacteria [36-40]. During active peritonitis, mesothelial denudation is temporarily observed. The peritoneum is continuously exposed to unphysiological dialysis solutions which contain high concentrations of glucose and other toxic substances. Glucose was more toxic to mesothelial cells than the low pH, lactate or hyperosmolality, when studied in a chronic animal model [41,42]. Cancer antigen-125 (CA125) is a 220,000 Dalton glycoprotein, and it is produced by mesothelial cells. CA125 concentrations in serum are used as a marker for ovarian neoplasms [43]. In stable adult PD patients CA125 is a useful marker of mesothelial cell mass or mesothelial cell turnover [44-46]. No significant correlation could be found between the peritonitis incidence and dialysate CA125 concentration in adult PD patients [47]. In children on PD no information is present on dialysate CA125 levels. This has been investigated in chapter 3.

High glucose concentrations in dialysis solutions might not only cause loss of mesothelial cells, it also might induce diabetifor m vascular changes caused by the formation of advanced glycosylation end products (AGEs) [48,49]. These products are created by a reaction of the carbonyl group of a sugar with the amine group of a protein in the absence of enzymes. This is also called the Maillard reaction. Initially, reversible Amadori products are formed, followed by the irreversible AGEs [50]. Non-enzymatic glycation of proteins such as IgG might modify the ability of IgG to activate complement and promote phagocytosis [51]. This subject has been studied and described in chapter 5.

HOST DEFENCE IN HEALTHY CHILDREN

The body’s defense against infectious microorganisms can be divided into the innate immunity, which attacks an infectious agent from its very beginning and is effective against a wide range of potentially infectious agents, and the adaptive or specific immunity which amplifies the effector mechanisms and is antigen specific [52-54]. The adaptive immune response is the part of the immune defense that is enhanced by vaccination and provides long-term protection and memory against infections. In normal, healthy individuals most infectious agents that penetrate the mechanical barriers of the body are eliminated quickly by the innate immune response before causing any obvious symptoms of disease. One of the first components of the innate immunity to be activated is the complement system. Secondly, phagocytes such as macrophages and neutrophils, and natural killer cells provide the innate cell-mediated immunity by killing microorganisms. Blood monocytes differentiate into macrophages and leave the blood stream to reside in the tissues. Macrophages also serve as professional antigen-presenting cells for the development of the adaptive immunity [55]. Neutrophils are attracted to the site of infection by cytokines released from activated macrophages. Phagocytosis of microorganisms can occur after recognition of the bacterial constituents by surface molecules on macrophages and
neutrophils such as the mannose receptor, the lipopolysaccharide receptor, and the complement receptor [56].

The adaptive immunity is highly specific for a particular pathogen. The adaptive or specific immunity can be divided in the humoral and cell-mediated immunity, in which antibodies, B and T lymphocytes respectively play a central role. Initiation of the adaptive immune response is primary dependent on cytokines produced by CD4⁺ T lymphocytes, but CD8⁺ T cells also influence the immune response. The CD4⁺ T cells differentiate into T helper type 1 (Th1) or T helper type 2 (Th2) effector cells [57,58]. The mechanism behind which the differentiatation pathway is taken is not completely understood. Subsequently, the effector T cells are guided to the site of infection by newly expressed cell adhesion molecules such as the integrins. The B cells which come into contact with T cells in the T cell area of the lymphoid tissue, start to proliferate and differentiate into antibody producing cells [59]. This leads to the primary adaptive immune response. When the child is re-infected with the same pathogen a secondary immune response will be encountered, which is faster and stronger.

**IMMUNOGLOBULINS**

Immunoglobulins (Ig) are glycoproteins present in blood, free and on the cell surface of B cells, and in extravascular fluids. Ig are composed of two identical light and two identical heavy polypeptide chains, linked together by disulphide bonds. The light chains exist in two forms, the kappa (κ) and lambda (λ) light chains. Ig are produced by plasma cells and memory cells and can be classified into 5 distinct classes namely IgG, IgA, IgM, IgD and IgE. These immunoglobulins vary in their size, amino acid composition and carbohydrate structure [60,61]. IgG can be divided into four subclasses, IgG₁, IgG₂, IgG₃, and IgG₄ (Figure 2), based on amino acid differences in their heavy polypeptide chains [62]. IgG is the major Ig of serum, making up to 75% of the total. Functional, all antibody molecules are composed of the same basic unit structure with two Fab (antigen binding fragment) portions, containing the antigen-recognizing site and one Fc (crystalline fragment) region, carrying out the effector functions [63]. The Fab and Fc fragments are connected by the hinge-region. The length of the hinge region varies considerably among the classes and subclasses. The diversity in the Fc region between the various heavy chains is responsible for the different biological activities of the antibody response. However, the amino acid sequences of antibody molecules found within the domains responsible for the antigen binding (variable domains) exhibit also considerable diversity. The diversity of antibodies is not only determined by their isotypic differences but also by allotypic variations or polymorphisms. This is the unique genetic variation which exists among individuals. The Ig genes encoding for the heavy chain of Ig are located on chromosome 14, for the λ light chain on chromosome 22 and for the κ light chain on chromosome 2 [64].
Figure 2. Structure of IgG subclasses. Each IgG molecule consists of two identical heavy (H) chains and two identical light (L) chains. H chains and L chains have variable (resp. \( V_H \) and \( V_L \)) and constant (resp. \( C_H \) and \( C_L \)) domains. The antigen binding fragment (Fab) and the crystalline fragment (Fc; responsible for the effector functions) fragment are connected by the hinge-region (red). The carbohydrate units lie between the \( C_{\gamma 2} \) domains (blue).

The diversity in the heavy chain genes arises from rearrangement of the variable (\( V_H \)), joining (\( J_H \)) and diversity (\( D \)) gene segments [65]. The VDJ segment together with the constant gene is then transcribed into messenger RNA (mRNA). A very similar process occurs with the assembly of mRNA for light chains, except that D genes do not exist.

An important function of Ig is opsonization; this is the binding to antigens and to receptors (Fc-receptors) of phagocytic cells to facilitate the phagocytic process [66]. In any antibody response, IgM is the first antibody to be made. In contrast to IgG, phagocytes have no receptors for the IgM Fc region. However, the Fc region of IgM can bind complement and activate the complement system.

**Fetal and postnatal development of Ig levels.**

During fetal development of the immune system lymphoid tissue appears first in the thymus at about 8 weeks of gestation. T cell functions can be demonstrated by the tenth week of gestation [67]. Ig secreting cells appear in the spleen and lymph nodes at about 20 weeks. From this period onwards IgM and IgD are produced by the fetus [68]. The synthesis of IgG by the fetus is very little. At birth the IgG concentration of the neonate is similar to that of the mother because IgG crosses the placenta [69]. After birth, IgM levels in blood of the infant increases steadily whereas serum IgG levels display first a decrease because of infant growth and catabolism of IgG. The lowest levels are reached between 4-6 months, and increase thereafter, reaching adult levels at the age of about 10 years [70]. The slow onset of IgG production of the neonate is probably a reflection of the intrinsic B cell development rather than a maternal dependent process, because it has been demonstrated that children
born to a mother with agammaglobulinemia, show the same pattern of IgG levels after birth compared to children born to a mother with normal IgG levels [71]. The development of serum IgG subclass increments is not parallel: IgG₁ and IgG₃ reach adult levels at an earlier age than IgG₂ and IgG₄ [72].

**IgG subclass deficiencies**

IgG subclass deficiencies are based on either disturbances in the B cell maturation, T cell help or both. For example, IgG subclass deficiency is also a common finding in other diseases with a T cell defect such as Di-George syndrome, ataxia-teleangiectasia, or AIDS [73]. IgG₂ and IgG₄ synthesis require more T cell help than that of IgG₁ and IgG₃ [73]. In some cases complete deficiency of one or more IgG subclasses is the result of a gene defect [73,74]. IgG subclass deficiencies are more common in children with recurrent bacterial sinopulmonary infections [75,76]. However, other factors must also be involved in the infectious susceptibility, since some children have reduced IgG subclass levels without any symptoms, and some children have normal IgG subclass levels and suffer from recurrent sinopulmonary infections. The latter group showed reduced antibody responses to polysaccharide antigens [77].

**RECEPTORS FOR IgG**

The IgG Fc receptors (FcγR) on phagocytic cells serve as a link between the humoral and cellular immunity [78-81]. The FcγR are membrane glycoproteins belonging to the Ig gene superfamily [82,83]. The FcγR are encoded by different genes located on chromosome 1. Genetic variations or polymorphisms of the FcγR influence the function of the receptor [84]. The basic structure of FcγR is composed of three domains, the extracellular or binding domain, the transmembrane and the intracellular or cytoplasmatic domain [82]. Besides these three domains, all FcγR need a subunit, the γ-chain, for signal transduction into the cell, with exception of FcγRII. The signal transducing domain of this receptor is located in its cytoplasmatic tail. FcγR have two (FcγRII and FcγRIII) or three (FcγRI) immunoglobulin-like extracellular domains which bind to the Fc portion of IgG. Three classes of FcγR exist: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), based on the structure of their extracellular domain (Figure 3).

FcγRI (CD64) is a 72-kD glycosylated receptor, present on monocytes, macrophages and activated neutrophils. FcγRI has a high affinity for IgG and binds monomeric IgG.

FcγRII (CD32) is a 40-kD glycoprotein, present on monocytes, macrophages, neutrophils, B lymphocytes and platelets. This receptor has a lower affinity for IgG and binds only IgG complexes or aggregates. Two functional allelic forms of FcγRIIa have been
identified: the FcγRIIa-H131 and the FcγRIIa-R131 allotype. In humans, the allotype FcγRIIa-H131 of FcγRII, is the only receptor that can bind IgG2 [85].

Figure 3. A schematic representation of the three IgG-Fc-receptors (FcγR). The extracellular part of FcγRI, FcγRII and FcγRIII consists of two or three immunoglobulin-like domains which bind to the Fc portion of IgG. FcγRI and FcγRII are complexed with disulphide-linked subunits (γ-chains). FcγRIII has no transmembrane part and cytoplasmic tail, but is attached to the cellmembrane by a glycosylphosphatidylinositol glycan (GPI) anker.

FcγRIII (CD16) is an extensively glycosylated receptor with a molecular weight of 50-80kD. The FcγRIIIa is expressed on macrophages and natural killer cells. The FcγRIIib, is expressed on neutrophils with a glycanphosphatidylinositol (GPI) anchor. Both, the FcγRIIIa and FcγRIIib gene contain two allotypes: the FcγRIIIa-158V and FcγRIIa-158F, and the NA1- FcγRIIib and NA2- FcγRIIib [86,87]. FcγRIIib contains no intracellular domain, but it is capable of transducing signals into the neutrophil upon crosslinking. Both FcγRIIIa and FcγRIIib are the only receptors that can be cleaved from the membrane by proteases and their soluble forms can be detected in plasma [88].

FcγR and disease

Functional FcγR polymorphisms have been associated with disease, such as autoimmune and infectious disease. This subject has been reviewed by van der Pol et al. [89]. The FcγRIIa-H131 allotype is the only receptor which can bind IgG2 [85]. Therefore this receptor has been studied in relation to the susceptibility to infections with encapsulated bacteria, in which IgG2 is the predominant subclass induced. It has been shown that phagocytosis of IgG2 opsonized bacteria by polymorphonuclear cells of individuals with the homozygous H131 allotype was higher than that of the R131 allotype. [90,91]. A skewed distribution of the FcγRIIa genotypes in children with recurrent upper respiratory tract infections has been found [92]. Furthermore, the combination of FcγR polymorphisms and late complement component deficiencies in children with meningococcal septic shock has
been described [93,94]. Interestingly, recent data have shown that FcγR might be relevant for viral infections [95]. The FcγRIIa genotype has also been related to pneumococcal disease in patients with systemic lupus erythematosus [96]. The relevance of the FcγRIIa genotype for SLE or SLE associated complications is controversial [97-99].

RECEPTORS FOR COMPLEMENT

Complement has an important role in the defense against infections, in autoimmunity and in immune complex diseases [100]. C3 is an important component and is present in the blood circulation in similar concentrations as some immunoglobulins (1-2 mg/mL). Complement fragments that are produced during activation have an opsonic function and bind with antigen to specific receptors on the surface of immune cells. This process enhances the phagocytosis of microorganisms. Cleavage of C3 results in the formation of C3b, iC3b and C3dg fragments. These fragments can bind to four different complement receptors: type 1 to 4 (CR1-CR4). CR1 (CD35) is an opsonic receptor, present on neutrophils, monocytes and macrophages and mediates phagocytosis. CR1 binds to C3b and iC3b. CR2 (CD21) is expressed on B lymphocytes with ligands for iC3b and C3dg and is involved in B cell activation. CR3 (CD11b) mediates phagocytosis of iC3b opsonized particles. The ligand specificity and the tissue distribution of CR4 is very similar to that of CR3. CR1 and CR3 are most important for inducing phagocytosis of complement coated bacteria.

CR and disease

An inherited deficiency of complement receptors is very rare. Reduced numbers of CR1 (CD35) on B lymphocytes and neutrophils of patients with SLE have been found [101]. A deficiency or complete absence of CR3 (CD11b) on leukocytes leads to the disease leukocyte adhesion deficiency. In this disease, leukocytes are unable to migrate into sites of inflammation. This results in uncontrolled tissue infections and delayed clearance of debris and wound healing [102].

B-CELL DIFFERENTIATION

The bone marrow is the specific microenvironment where B cells can differentiate from lymphoid precursors to naive B cells [103]. The first phase of B cell development is without a contact with an antigen. There is an ordered rearrangement of immunoglobulin V-D-J gene segments during B cell development [104,105]. When this is successful a μ heavy chain is produced, followed by rearrangement of the κ or λ light chains. After this process the cell is capable of expressing IgM on the surface of the developing B cell. These immature B cells will be eliminated when they bind to molecules via the surface immunoglobulin to prevent autoreactivity. The last step of development in the absence of
antigen is the addition of surface IgD expression on the cell. Now, the B cell is called a mature naive B cell, leaves the bone marrow and enters the peripheral circulation and lymphoid tissues. When these B cells do not encounter antigen, they will die. Differentiation of B cells also characterizes the acquisition of other cell surface molecules such as the major histocompatibility complex (MHC) class II molecules, Fc receptors and complement receptors. The next step towards antibody forming plasma cells and memory cells is the antigen-driven maturation of these mature B cells. B cells first start to proliferate (clonal expansion) with help of activated CD4\(^+\) T-helper cells in the T cell areas of secondary lymphoid organs. These antigen-driven B cells first become short lived plasma cells that produce IgM. Some of the activated B cells migrate to the primary B cell follicles and start the germinal center (GC) reaction [106,107]. Within the GC, B cells undergo extensive proliferation along with somatic hypermutation, Ig isotype switching and affinity maturation. Essential in this process is the interaction between the CD40 molecule of the B cell receptor and CD40 ligand of T cells and the environment of cytokines [107,108]. B cells that survive this process differentiate into plasma cells which migrate to the bone marrow and produce high-affinity antibodies, or they become memory cells and enter the circulation where they can survive for months without antigenic stimulation. Fully mature plasma cells lose their surface immunoglobulin and MHC class II molecules. On re-exposure of memory cells to the antigen, they can become antibody producing plasma cells.

Several non-Ig antigens on the surface of B cells have also been identified [109]. Some of them are found during most stages of maturation, others are present only in a certain differentiation stage of the B cell. Based on functional and phenotypic characteristics, CD27 is a marker for memory type B cells [109-111]. CD5 is present on only some B cells and there is now strong evidence that this is a marker which identifies a subpopulation of B cells (B1-cells), especially present during fetal life and in the peritoneal cavity [112].

**T-CELL FUNCTION**

Precursor T cells, situated in the thymus do not express CD4 or CD8 molecules. They are also called double negative T cells. Later, T lymphocytes develop into double positive cells and express low levels of T cell receptor and CD3 on their surface. Mature T cells either express CD4 or CD8 [70]. T cells differ from B cells in the way they recognize antigens. The T cell receptor does not recognize antigens on intact molecules but they recognize a complex of peptide, derived by proteolysis of the antigen, bound to a class I or II MHC protein. CD4\(^+\) T cells recognize only peptides presented by MHC class II whereas CD8\(^+\) T cells recognize only peptides of MHC class I.

CD4\(^+\) T cells or T-helper cells are the major regulatory cells of the immune system. Their regulatory function depends both on a cell surface molecules such as CD40-ligand, and on the variety of cytokines they produce when activated. The CD4\(^+\) T cells differentiate into T-helper type-1 (T\(_{h1}\)) or T-helper type-2 (T\(_{h2}\)) cells. T\(_{h1}\) cells produce mainly IL-2 and IFN-\(\gamma\) and T\(_{h2}\) cells IL-4, and IL-5 [55]. T\(_{h1}\) cells are important for the cellular immunity, involving
enhancement of microbicidal activity of monocytes and macrophages, whereas $\text{T}_{\text{H}2}$ cells are important for the humoral immune response, by helping B cells to develop into antibody producing cells. $\text{CD}8^+$ T cells can develop into cytotoxic T lymphocytes (CTL). These cells can lyse target cells that express antigens recognized by the cytotoxic T cells. This is a major mechanism for the destruction of virally infected cells.

**PREVENTION OF INFECTIONS BY IMMUNIZATIONS**

Prevention of infectious diseases can be achieved by immunization. The principle of immunizations is to induce long-lasting memory. A second exposure to the same antigens induces a more rapid and strong antibody response which prevents disease. The antibody response to bacterial or viral antigens can be divided into T cell-dependent (TD) and T cell-independent (TI) responses [113]. The T cell-independent antigens can be further subdivided into T cell-independent type 1 (TI-1) and type 2 (TI-2) antigens [114]. The TI-1 antigens, such as lipopolysaccharide (LPS) on gram-negative bacteria and protein A on the surface of Staphylococcus aureus, have the property of inducing polyclonal B cell activation. TI-2 antigens, are typically repeating polymers, such as polysaccharide antigens, and do not induce a polyclonal B cell activation. T cell cytokines can enhance the antibody response to TI-2 antigens, but are not necessary for a TI-2 response. IL-2, IFN-$\gamma$, IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are especially important in promoting the TI-2 response. TI-2 antigens do not induce immunological memory [115]. Only children above the age of 2 years are capable of mounting a strong antibody response to TI-2 antigens. Conjugation of a T cell-independent antigen to an immunogenic carrier protein shifts the antibody response towards a T cell-dependent response. Conjugated vaccines are also suitable and effective for children under the age of 2 years. For example, since the introduction of the conjugated *Haemophilus Influenzae* type B vaccine for children under the age of 2 years, the incidence of *Haemophilus Influenzae* infections, especially meningitis, has fallen dramatically [116].

**HOST DEFENCE IN CHILDREN WITH CHRONIC RENAL FAILURE**

The development of immunity, both intraperitoneally and systemic, during chronic renal failure of childhood, with or without dialysis treatment has been studied very scarcely. Since the peritonitis incidence in children on PD is higher than in adult PD patients, the results obtained from adults cannot be directly extrapolated to children. Low levels of IgG and/or subclasses have been described in PD children [117-121]. This was ascribed to peritoneal loss in most of the studies [117,119,120]. However, one study reported that the Ig deficiency was already present before the dialysis treatment had started [121]. Our study was designed to clarify these conflicting results (chapter 4). Besides Ig deficiencies, low serum complement levels might also be present in PD children. However, this was not found
in a study performed by Reddingius et al. [122]. Furthermore, these authors found evidence for the occurrence of complement activation in the peritoneal cavity [123].

Information on the expression of complement receptor 1 and 2 and the three Fc\(\gamma\) receptors on monocytes/macrophages, neutrophils and lymphocytes in children with CRF and the effects of dialysis treatment on this are not available. One study has been performed by Wasik et al. on the CD16 (Fc\(\gamma\)RIII) and CD35 (CR1) expression in CAPD children [124]. They found a temporarily increase of the number of CD16 and CD35 positive neutrophils after one month of PD treatment. Furthermore, these authors reported an improvement of phagocytosis after CAPD treatment had started. We performed two studies on the Fc\(\gamma\) and CR expression. The first study analyzed the difference in CR and Fc\(\gamma\)R expression on white blood cells in peripheral blood and peritoneal dialysis effluent (chapter 7). The second study investigated the effects of uremia and dialysis treatment modalities on the receptor expression (chapter 8).

Three studies have been published on the lymphocyte subsets in peripheral blood of children with chronic- or end-stage renal failure [125-127]. Results from these studies are conflicting. Two of them concluded that children on CAPD had normal percentages of T lymphocytes and subsets, and of B lymphocytes [125,126]. In contrast, Aksu et al. reported an increased CD4/CD8 ratio and a reduced B lymphocyte and natural killer cell percentage in CAPD children when compared to healthy controls [127]. Valle et al. performed a study on the cellular populations in peritoneal effluent in PD children [128]. They found a trend towards a decrease of monocyte/macrophage to T lymphocyte ratio after initiation of CAPD. T-helper (CD4) T-suppressor (CD8) ratios were not different in blood and dialysate. However, these authors found more activated T lymphocytes in peritoneal effluent, defined by the presence of IL-2 receptor on T lymphocytes, than in peripheral blood. We performed a study on the classification of white blood cells and lymphocyte subsets in a large group of children with chronic renal failure, with or without dialysis treatment, and a control group of healthy children (chapter 9).

No studies have been published on B cell differentiation, either in children or in adult patients with chronic renal failure, to explore the etiology of low Ig levels. Such a study is described in chapter 10.

Immunity can also be studied by measuring the antibody response after vaccination. This has been studied in children with renal failure, but results are again conflicting [129,130]. Because pneumococcal vaccination is recommended in children with chronic renal disease, but not administered in many centers, we analyzed the antibody response to pneumococcal polysaccharide antigens (chapter 6).
AIM OF THE STUDY

The aim of this thesis is to explore the functional and immunological disturbances in uremic children which might lead to more infections such as peritonitis, by:
1. Analyzing the peritoneal transport function and peritoneal membrane characteristics in PD children (part one).
2. Analyzing parts of the specific and aspecific immunity in children with chronic renal failure, and the effects of uremia and dialysis treatment modalities on this (part two, three and four).

In Part One, the functional characteristics of the peritoneal membrane are described. In chapter 2, the results of investigations on peritoneal permeability are given in children treated with peritoneal dialysis by using a standardized test. The possible differences between adults and children are analyzed. In chapter 3, dialysate cancer-antigen-125 (CA125) levels are measured in PD children as marker for their mesothelial cell mass. The results are compared to those in adults and analyzed for relationships with other parameters.

In Part Two, the humoral immunity of children with chronic renal failure is studied. In chapter 4, the effects of chronic renal failure and dialysis treatment on the serum IgG, IgA, IgM and IgG subclasses are analyzed. In chapter 5, the effects of glucose containing dialysis solutions on the IgG glycation intraperitoneally and the effects on phagocytosis are investigated. In chapter 6, the IgG and subclass antibody response to T-cell independent pneumococcal vaccination is measured in children treated with dialysis and after renal transplantation.

In Part Three, the expression levels of membrane receptors that bind opsonins such as immunoglobulin and complement on white blood cells are analyzed. In chapter 7, the complement and Fcy-receptor expression on phagocytes in peripheral blood and in peritoneal dialysis effluent are compared. In chapter 8, the effects of uremia and dialysis treatment on the complement and Fcy-receptor expression on lymphocytes, monocytes and neutrophils are determined.

In Part Four, differentiation of white blood cells and peritoneal cells are characterized. In chapter 9, the white blood cell differentiation in peripheral blood and dialysate is investigated. Also lymphocyte subsets are studied. In chapter 10, B lymphocyte subsets are determined, T_H1 and T_H2 cytokine profiles and the in vitro Ig production by lymphocytes are measured.
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