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HYPOTHESIS

Minimal change disease: a "two-hit" podocyte immune disorder?

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Abstract Minimal change disease (MCD) is the most common nephrotic syndrome in children and is commonly thought to be a T-cell disorder mediated by a circulating factor that alters podocyte function resulting in massive proteinuria. We suggest that MCD is a "two-hit" disorder. As originally hypothesized by Reiser et al. in 2004, we propose that the initial hit is the induction of CD80 (also known as B7.1) on the podocyte, and that this results in an alteration in shape with actin rearrangement that alters glomerular permeability and causes proteinuria. We propose that CD80 expression may result from either direct binding of the podocyte by cytokines from activated T cells or by activation of podocyte toll-like receptors (TLR) by viral products or allergens. We further hypothesize that under normal circumstances, CD80 expression is only transiently expressed and proteinuria is minimal due to rapid autoregulatory response by circulating T regulatory cells or by the podocyte itself, probably due to the expression of factors [cytotoxic T-lymphocyte-associated (CTLA)-4, interleukin (IL)-10, and possibly transforming growth factor (TGF)-β] that downregulate the podocyte CD80 response. In MCD, however, there is a defect in CD80 podocyte autoregulation. This results in persistent CD80 expression and persistent proteinuria. If correct, this hypothesis may lead to both new diagnostic tests and potential therapeutics for this important renal disease.

Keywords Nephrotic syndrome · CD80 · CTLA-4 · T regulatory cell · Podocyte

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Introduction

In 1974, Shalhoub proposed that proteinuria in minimal change disease (MCD) was due to a circulating factor released by T cells [1]. His hypothesis was based on the observations that: (1) remission commonly occurs with measles infection, which causes cell-mediated immunosuppression; (2) MCD may occur with Hodgkin's disease, which is a known T-cell disorder; (3) MCD responds to steroids and cyclophosphamide, which are agents commonly used to suppress cell-mediated immunity; (4) unlike many glomerular disorders, there is an absence of humoral (immunoglobulin and complement) components in glomeruli; and (5) MCD is usually triggered by viral respiratory tract infections. Despite these insights, the precise mechanism of proteinuria in MCD remains unknown, although a key role of the podocyte has been suggested, as there are distinctive changes in the podocytes in MCD, including foot process fusion. Here, we expand our previous proposal that MCD is a podocyte disorder caused by a "two-hit" mechanism [2, 3]. First, we hypothesize, as has been previously proposed by Reiser et al. [4], that CD80 is induced in podocytes by circulating cytokines, microbial products, or allergens. Second, due to T-regulatory-cell (Treg) dysfunction and/or impaired autoregulatory function by the podocyte, elevated podocyte CD80 expression becomes persistent and induces proteinuria.

Minimal change disease: a CD80 disorder

CD80 (also known as B7.1) is a T-cell costimulatory molecule expressed on antigen-presenting dendritic cells, natural killer cells, and activated B lymphocytes. Binding of CD80 to its receptor CD28 on T cells has a key role in T-cell activation. The termination of the T-cell response also involves CD80 and is initiated by the binding of CD80 by cytotoxic T-lymphocyte-associated (CTLA)-4, the latter of which is on the cell membrane of Foxp3+ Treg. Treg may also inhibit the immune response by releasing soluble factors, such as soluble CTLA-4, interleukin (IL)-10, and transforming growth factor (TGF)-β. These factors inhibit T-cell effector (Teff) activation and cytokine release. Furthermore, CTLA-4 also suppresses CD80 expression on the antigen-presenting cells [5]. Thus, the immune response is initiated by an activation of CD80 on antigen-presenting cells and over time negatively regulated, with the Treg having a key role in this process.

It is known that podocytes and renal tubular epithelial cells can express CD80. Reiser et al. reported that lipopolysaccharide (LPS) injected into mice results in CD80 expression on podocytes by binding to toll-like receptor-4 (TLR-4) in association with the development of proteinuria and foot-process effacement [6]. LPS also induced CD80 expression in cultured podocytes, with actin reorganization and shape change [6]. LPS failed to induce proteinuria in CD80 knockout mice, whereas proteinuria was induced in immunodeficient severe combined immunodeficient (SCID) mice. These studies documented that direct activation of podocytes, independent of T-cell involvement, could induce CD80 expression and proteinuria. As the lesion induced by LPS resembled MCD, Reiser et al. proposed that glomerular CD80 expression might have a role in MCD [4]. However, proteinuria and CD80 expression by podocytes is transient in this experimental model and hence could not fully account for the pathogenesis of the proteinuria of MCD.

Minimal change disease and CD80

Consistent with this hypothesis, we found that in MCD, there is a pronounced expression of CD80 in podocytes, with increased urinary excretion of CD80 [2, 7]. Urinary CD80 levels correlated with disease activity, as it was significantly increased in MCD patients in relapse when compared with healthy controls and patients with MCD in remission or with other glomerular diseases, including focal segmental glomerulosclerosis (FSGS) [2, 7]. Biopsies of seven patients with corticosteroid-sensitive MCD confirmed the presence of CD80 in podocytes but not in renal tubules [7]. Finally, Western blotting documented that urinary CD80 was not the soluble, circulating, extracellular membrane moiety [molecular weight (MW) 23 kDa] but rather the entire cell-membrane molecule (MW 53 kDa), consistent with it being shed from the podocyte [7]. These studies provide compelling evidence that in MCD, the podocyte expresses CD80 during relapse.

Induction of CD80 in podocyte: the first hit

Previous studies therefore suggest that in MCD, the podocyte expresses CD80 during relapse, and this represents a dendritic-cell-like response to a circulating factor (cytokine, allergen, and/or microbial products) and is “the first hit” (Fig. 1). In experimental studies, we have found that CD80 expression can be induced in podocytes not only by activating TLR-4 by LPS, as reported by Reiser et al. [6], but by polyinosinic-polycytidylic acid [poly(I:C)] (viral RNA) that activates TLR-3 [8]. In this regard, transient proteinuria has been reported in 6.3% of healthy children when tested over a 6-year period, with some due to febrile illnesses (including upper respiratory infections), whereas others occur in asymptomatic individuals [9, 10]. We hypothesize that in some of these children proteinuria reflects exposure to a viral antigen and/or cytokine that causes transient CD80 expression. Indeed, in patients with MCD, relapse is usually heralded by an upper respiratory tract infection [11]. However, direct evidence that viral products are present in the circulation during the initiation of MCD has not been shown, neither has the pathogenic cytokine been isolated.

In addition to TLR activation, T-cell cytokines can also induce podocyte CD80 expression. IL-13 is elevated in the serum [12] as well as expressed by T cells [13] in patients with active MCD. In addition, Lai et al. reported that overexpression of IL-13 could induce nephrotic syndrome in rats in association with increased CD80 expression in podocytes [14]. The association of IL-13 with MCD may also account for the relationship of MCD with both Hodgkin’s disease [15, 16] and with allergies [3, 17], because Reed-Sternberg cells [16] and Teff cells in response to allergens [18] can secrete IL-13, which may have a role in inducing podocyte CD80 expression and subsequent proteinuria.

Ineffective censoring of CD80: the second hit

Proteinuria when it occurs with febrile illnesses is usually transient. Similarly, LPS injection in mice also results in transient proteinuria [6]. This leads to the hypothesis that a secondary system may normally intervene to turn off CD80 expression. Whereas Treg cannot directly access the podocyte, these cells can secrete soluble CTLA-4

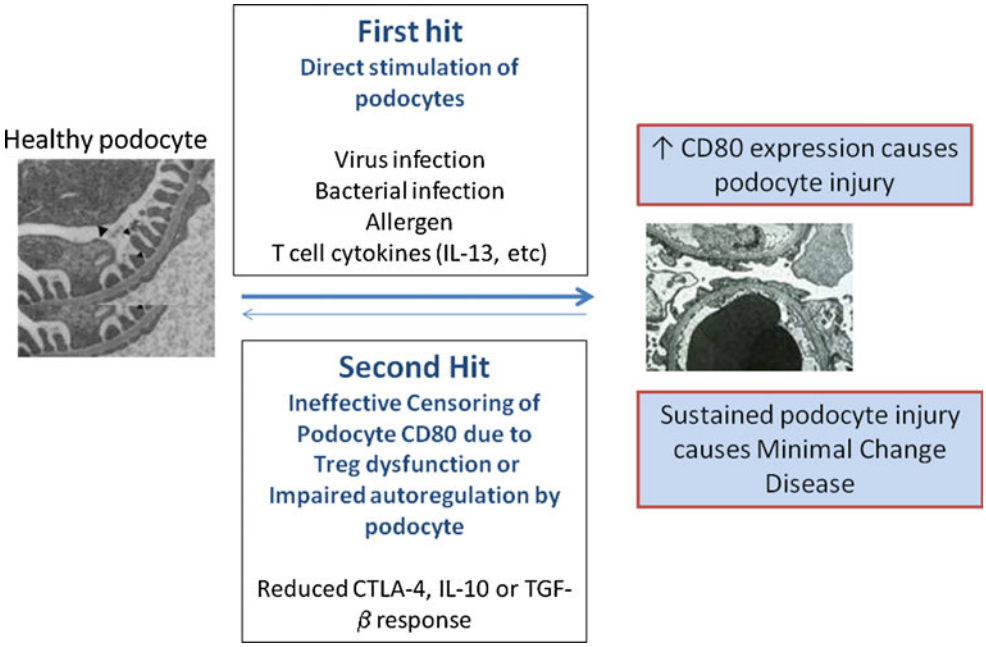

Fig. 1 Minimal change disease: a "two-hit" podocyte immune disorder. The first hit consists of the induction of CD80 in podocytes by microbial products, allergen, or T-cell cytokines such as interleukin (IL)-13. In normal settings, CD80 expression on podocytes is terminated by regulatory cytokines from T regulatory cells (Treg) and/or cytotoxic T-lymphocyte-associated (CTLA)-4 and interleukin (IL)-10 by podocytes, and as a consequence, proteinuria is transient and mild. However, we propose a second hit occurs in MCD and consists of abnormal censoring of podocyte CD80 expression due to a defective autoregulatory response by Tregs or by the podocyte itself. As a consequence, CD80 expression becomes persistent and nephrotic syndrome results

(sCTLA-4), IL-10, and TGF-β, which could cross the glomerular basement membrane (GBM) and bind to CD80 on the podocyte. Both CTLA-4 and IL-10 are known to inhibit CD80 expression on dendritic cells [5, 19]. This leads to our hypothesis that a dysfunctional Treg-cell population might not be able to turn off podocyte CD80 expression once it is induced (second hit) (Fig. 1). Consistent with this hypothesis, our group reported that serum and urinary CTLA-4 tends to be low in MCD in relapse but that urinary CTLA-4 returns to higher levels during remission [2]. In addition, we found that, whereas Tregs are not numerically reduced in MCD, their ability to suppress Teff in vitro is impaired and is associated with decreased production of IL-10 by the Treg cells [20].

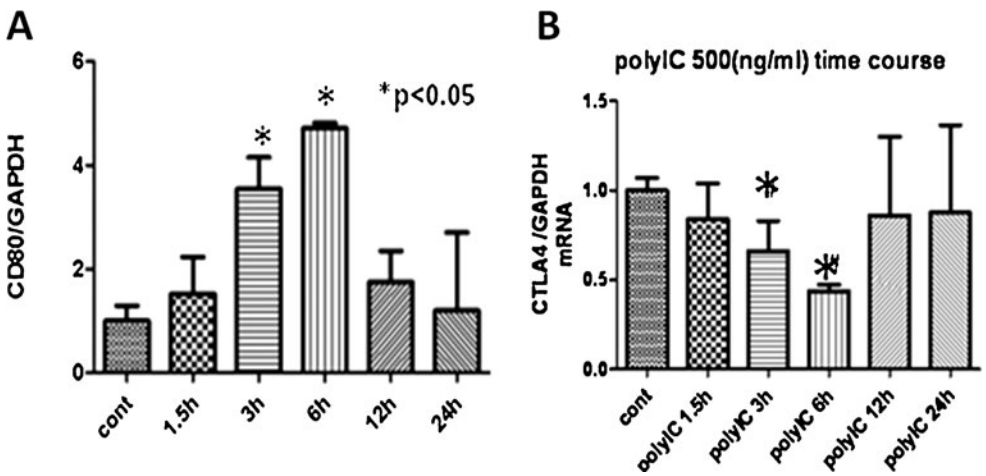

Fig. 2 Podocyte expression of CD80 and cytotoxic T-lymphocyte-associated (CTLA)-4: evidence for autoregulatory response. Cultured human podocytes (gift of M. Saleem and P. Mathieson) [33] show (**a**) increased expression of CD80 messenger RNA (mRNA) at 6 h in response to activation of toll-like receptor (TLR)-3 with polyinosinic-polycytidylic acid [poly(I:C)] (500 ng/ml), with (**b**) a mirror-like reduction in CTLA-4 mRNA. The increased expression of CD80 mRNA is transient and resolves simultaneously with an increase in CTLA-4 expression

Additional evidence supports a role for abnormal Tregs in mediating MCD. For example, Treg dysfunction from a *Foxp3* mutation causes the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome; and rarely, this syndrome has been associated with the development of MCD [21]. Nonsteroidal drugs can also cause idiopathic MCD with interstitial nephritis, and a side effect of COX-2 inhibitors is inhibition of Treg function [22].

In contrast, stimulation of Treg function can cause remission of proteinuria in the Buffalo/Mna rat, which develops both an MCD and FSGS-like lesion [23]. Measles infection has also been reported to induce remission in MCD [1] and also stimulates the Treg population and increases IL-10 levels [24]. IL-10 levels may persist following measles infection and be associated with prolonged impairment of cell-mediated immunity [25]. In addition, recent reports that rituximab (anti-CD20 antibody) can improve MCD could potentially be attributed to improving Treg function. Indeed, rituximab was shown to improve Treg function in some patients with idiopathic thrombocytopenic purpura and lupus nephritis [26–28].

Whereas Tregs might provide an exogenous source of CTLA-4, recent studies by our group found that the cultured human podocyte also produces CTLA-4 and IL-10. Interestingly, the induction of CD80 expression in podocytes by TLR-3 stimulation results in a loss of podocyte CTLA-4 expression, which returns to baseline with the termination of CD80 stimulation, suggesting an autoregulatory response (Fig. 2). Thus, it is possible that persistent CD80 expression could relate to persistent stimulation (such as from a cytokine, allergen, or microbial product) and inadequate censoring of podocyte CD80 expression due to either an impaired CTLA-4 response by Tregs and/or by the podocyte itself.

The reason children with MCD may have defective Treg or podocyte autoregulatory function is not known but could be due to delayed or ineffective maturation of the T-cell response, possibly due to environmental factors. One possibility is that children with MCD may have had less exposure to infections, with the inability to develop a balanced T-cell response [29].

Implications for treatment

The mode of action of corticosteroids and cyclosporine (which are known to be effective in MCD) may involve actions not only on the T-cell population (such as by suppressing IL-13) but also via direct effect on podocytes [30, 31]. In addition, our studies suggest that agents that suppress CD80 expression, such as IL-10 and CTLA-4, may be effective in the treatment of this disease. Interestingly, the benefits of cyclosporine and corticosteroids might be in part via their ability to induce IL-10 expression in various cell types [32].

Conclusions

The discovery that the podocyte can express both dendritic-like antigens (such as CD80) and Treg-cell factors (such as CTLA-4) markedly expands our knowledge of this key cell involved in glomerular filtration. Alterations in podocyte immune function may underlie some glomerular diseases, such as MCD. Clearly, the immune role of the podocyte in disease deserves increasing attention. If, indeed, MCD represents a podocyte immune disorder, then it could open new avenues for both diagnosis and treatment of this important disease.

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