Immune dysregulation in minimal change nephropathy

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Abstract

Minimal change nephropathy (MCN) has been associated with a wide variety of abnormalities in the immune response of affected individuals. Many of these may be a consequence of the renal lesion, rather than a cause thereof. This article reviews some of these immunological phenomena and concentrates in particular on the imbalance between type 1 and type 2 cytokine responses, suggesting that there are plausible mechanisms whereby a type 2 cytokine bias could directly or indirectly cause MCN.

Keywords: immune dysregulation; minimal change disease; nephrotic syndrome

Introduction

Minimal change nephropathy (MCN) has been associated with a wide variety of abnormalities in the immune response of affected individuals. For many of these, there is uncertainty as to whether the abnormalities are merely a consequence of the renal lesion, or whether they reflect an underlying disturbance of the immune response which is responsible for causing the condition. For many years, it has been assumed that MCN is caused by an aberrant immune response, with most studies focusing on the T cell compartment. In his often-quoted hypothesis article in The Lancet in 1974, Shalhoub [1] postulated that MCN was caused by a disorder of T cell function. He supported this assertion with observations on remissions of MCN occurring after measles infection, which is known to suppress cell-mediated immunity; the occurrence of MCN in patients with lymphoma; and the response to treatment with corticosteroids and cyclophosphamide. As with other authors before and since, Shalhoub suspected the presence of one or more permeability factors, produced by T lymphocytes, in patients with MCN. More recently, other authors have reinforced the notion that MCN has an immune/autoimmune cause, quoting immunogenetic factors, especially associations with products of the major histocompatibility complex [2], and the response to drugs which have potent effects on the immune system, such as corticosteroids, cyclophosphamide and cyclosporin [3,4]. This last drug has a mode of action which is relatively specific for T lymphocytes, and its beneficial effects in MCN have been used by some authors to strongly support the importance of T cells in the pathogenesis [4]. However, as cyclosporin has numerous other effects, including haemodynamic effects and actions on numerous other cell types [5–7], and as its benefits in MCN tend to be short-lived [8], my own view is that it is overly simplistic to conclude that as cyclosporin influences MCN, this implies that T cells necessarily play a key role in the pathogenesis of the condition.

In this contribution, I will address two questions.

(i) Is there a pattern of immune dysregulation which is specific to MCN?

(ii) Can we learn anything about pathogenesis from studies of immune dysregulation?

Immunological abnormalities in patients with MCN

A wide variety of immunological abnormalities have been described in MCN, including observations in vivo and in vitro, affecting both humoral immunity and cell-mediated immunity. In considering these, it is important to ask whether the abnormalities are 'cause' or 'consequence' of the nephrotic state. Are they specific for MCN? Are they present only in the 'active' phase of the disease, i.e. when the patient has nephrotic syndrome, or do they represent some more fundamental abnormality which is present even when the disease is in remission? Are the abnormalities explained by the effects of drug treatments? Are any similar abnormalities found in the families of affected individuals, implying an immunogenetic basis?
Interferon (IFN) and type 2 responses are dominated by interleukins 4 and 13 (IL4 and IL13). Type 1 cytokines predominate in cell-mediated immunity and type 1 immune responses are dominated by IgG1 and IgG2 are profoundly depressed and synthesis of these subclasses in vitro is impaired, but IgG3 and IgG4 are relatively spared [12]. This is further evidence that urinary loss cannot explain the hypogammaglobulinaemia as the different IgG subclasses are proteins of similar size, and preferential loss of some subclasses is unlikely. Instead, these observations on the differential effects on IgG1 and 2 vs IgG3 and 4 may be very instructive in understanding the immune dysregulation in MCN, and I will return to this point later. Other immunoglobulin isotypes are not depressed: levels of IgM are normal or high [13] and levels of IgE are also markedly elevated [14,15]. The elevation of IgE is not absolutely specific for MCN but is more frequent and greater in magnitude in this condition compared with other causes of nephrotic syndrome [10]. The elevation of IgE is a feature during active disease but is also present during remission and when treatments have been stopped [14,15].

Type 1 and type 2 cytokines

In recent years, there has been a growing understanding of the cytokine mediators which govern communications between cells of the immune system, and one particularly attractive concept is the functional subdivision of immune responses according to the cytokine(s) that predominate. This subdivision was originally applied to the T helper cell (Th) compartment, leading to the description of Th1 and Th2 cells with different cytokine profiles and different functional effects [18]. It is now clear that other cellular compartments of the immune system can be similarly subdivided and the preferred terminology is now ‘type 1’ and ‘type 2’. As a gross over-simplification, type 1 immune responses are dominated by γ-interferon (γIFN) and type 2 responses are dominated by interleukins 4 and 13 (IL4 and IL13). Type 1 cytokines predominate in cell-mediated immunity and type 2 cytokines in some aspects of humoral (antibody-mediated) immunity. Of relevance to MCN, type 2 responses are particularly concerned with class-switching of B cells to the production of IgG4 and IgE [18].

Hypogammaglobulinaemia

Perhaps the most obvious immune defect in patients with nephrotic syndrome is hypogammaglobulinaemia. In some causes of nephrotic syndrome there may be substantial urinary losses of IgG, but as the proteinuria in MCN is typically ‘selective’ and urinary loss of IgG is slight or absent, it is clear that the hypogammaglobulinaemia must have other explanations. There is considerable evidence of impaired IgG production in MCN [9,10] as well as in other causes of nephrotic syndrome [10], and there is some evidence of increased IgG catabolism [11]. The abnormalities are substantially reversed by therapy, normalizing during remission [9,10]. Interestingly, the IgG subclasses are not equally affected: circulating levels of IgG1 and IgG2 are profoundly depressed and synthesis of these subclasses in vitro is impaired, but IgG3 and IgG4 are relatively spared [12]. This is further evidence that urinary loss cannot explain the hypogammaglobulinaemia as the different IgG subclasses are proteins of similar size, and preferential loss of some subclasses is unlikely. Instead, these observations on the differential effects on IgG1 and 2 vs IgG3 and 4 may be very instructive in understanding the immune dysregulation in MCN, and I will return to this point later. Other immunoglobulin isotypes are not depressed: levels of IgM are normal or high [13] and levels of IgE are also markedly elevated [14,15]. The elevation of IgE is not absolutely specific for MCN but is more frequent and greater in magnitude in this condition compared with other causes of nephrotic syndrome [10]. The elevation of IgE is a feature during active disease but is also present during remission and when treatments have been stopped [14,15].

Other immune disturbances in MCN

The other aspect of MCN which deserves a mention at this stage is the well-recognized association with atopy and allergy in patients with MCN and in their first-degree relatives [19]. Atopy is typically associated with a ‘type 2’ cytokine response (these cytokines being synthesized and secreted by mast cells as well as by lymphocytes [20]). Together with the elevation of IgE in MCN and the preservation of IgG4 when synthesis of IgG1 and 2 is suppressed, the association with atopy strongly supports the presence of a cytokine bias towards a ‘type 2’ pattern in MCN. The literature on cytokine production in patients with MCN is complex and inconsistent, but does include reports of preferential production of type 2 cytokines, especially interleukin 4 [21,22]. As the elevation of IgE is present during remission and when patients are off all drug treatments, this suggests that any bias is not simply a consequence of the disease or its treatments but instead reflects an underlying feature of the immune response in patients who develop MCN. The fact that atopy and allergy are more common in first-degree relatives as well as in the patients themselves suggests immunogenetic factors responsible for this type 2-biased immune response. There is a great deal of interest in genes which predispose to atopy, with genes encoding type 2 cytokines or their receptors being attractive candidate susceptibility genes [23]. We have tested several of these candidate genes for any association with MCN and obtained negative results [24,25]. However, analysis of candidate susceptibility gene loci in a complex disease such as MCN is inevitably speculative and many other genetic loci could be involved. It remains likely that there are immunogenetic factors underlying the tendency to produce a type 2-biased cytokine response. If it is correct that MCN arises in the context of a biased cytokine profile, identification of these genetic loci will be helpful in understanding the pathogenesis of MCN.

Association of MCN with atopy

There is an extensive literature describing various immunological phenomena in patients with MCN. Various abnormalities of T cell subsets and of T cell responses in vitro have been described, but I do not propose to review these further because the literature is contradictory and there are many reasons for believing that these abnormalities are secondary to the nephrotic state rather than primary. One striking example is the observation that the inhibitory effect of serum from nephrotic patients on lymphocyte proliferative responses in vitro could be reversed by removal of the lipoprotein fraction, implying that apparent defects in cellular immunity could be explained simply by the associated hyperlipidaemia [26]. Other more specific immune defects have been studied: there is impairment of antibody-dependent cellular cytotoxicity [27], presumably consequent...
upon the hypogammaglobulinaemia discussed above. Macrophage killing of Candida is impaired, although phagocytic function is normal [28]. Neutrophil and natural killer cell function seem to be normal [28]. Phenotypic analysis of lymphocyte subsets has given inconsistent results. Most of the described abnormalities are not specific for MCN, occurring in other causes of nephrotic syndrome; they are only present during ‘active’ disease, i.e. associated with the nephrotic state itself; and they are only found in the patients themselves and not in their relatives. For these reasons, it is impossible to know whether these abnormalities are a cause or a consequence of MCN. The evidence suggests the latter and I do not consider these immunological abnormalities to be instructive in understanding the pathogenesis of MCN. The elevation of IgE and the associations with atopy are the possible exceptions, as discussed earlier.

Lessons from the effect of levamisole

Levamisole is a drug with an unknown mode of action with useful effects in helminth infections, where it is believed to enhance the immune response to the parasite and favour parasite clearance. It has also shown useful effects in certain forms of leprosy [29] and in cancer, especially colon carcinoma [30]. In childhood MCN, levamisole treatment significantly reduces the rate of relapse [31], and my own experience in adult MCN suggests that this drug is also useful in maintenance of remission in a subset of patients (author’s unpublished observations). As mentioned earlier, the belief that MCN is an immune-mediated disorder leads to the use of drugs with immunosuppressive activity in order to attempt to control it. Levamisole apparently has immunostimulatory rather than immunosuppressive activity, how can we explain its beneficial effects in MCN? Our own data in an experimental model in which there is a bias towards type 2 cytokines show that levamisole acts by augmenting the type 1 response and reciprocally down-regulating the type 2 response [32]. It achieves this by selective induction of gene transcription of a key cytokine, IL18. These results support the hypothesis that MCN is associated with a biased type 2 cytokine response and suggest that levamisole acts by resetting the balance.

Could a type 2 cytokine directly cause MCN?

If there is a type 2 cytokine bias in MCN, the next obvious question is whether this is directly causally related to the renal lesion. The most direct link would be provided by an effect of a type 2 cytokine on glomerular permeability: there is some evidence to support this mechanism. As will be discussed by other contributors to this supplement, the key target cell in the glomerulus in MCN seems to be the glomerular epithelial cell (GEC) or podocyte. Effects of type 2 cytokines on podocytes in vitro have been studied extensively in recent years. Coers et al. [33] studied rat GECs and showed that exogenous IL4 disrupted intercellular junctions between the cells without affecting cell viability. Data from van den Berg et al. [34] and our own group [35] show that human GECs express receptors for IL4 and IL13. Administration of IL4 or IL13 to GECs in vitro causes phosphorylation of the signalling molecule STAT6, suggesting that there is a receptor-mediated signal delivered to the cells by these cytokines, and decreases the electrical resistance across GEC monolayers [34]. These observations provide plausible mechanisms whereby excess production of type 2 cytokines could result in increased glomerular permeability, but this hypothesis has not been directly tested in vivo.

What about an indirect mechanism?

The other possibility is that any association between MCN and immune dysregulation is indirect and some recent experiments in my own laboratory have been designed to address this possibility. GECs/podocytes are complex cells with intriguing gene regulatory capabilities. For example, there are some podocyte-specific proteins not expressed in any other mature tissue (e.g. nephrin [36]). Some proteins (e.g. vascular endothelial growth factor, VEGF [37]) are produced in several different isoforms as a result of differential splicing of RNA, and for some of the mediators the podocyte seems to express both an active form and a natural inhibitor. Thus the podocyte is capable of self-regulation by autocrine pathways as well as by receptor-mediated interactions with exogenous mediators. The transcription factors responsible for the complex patterns of gene regulation in podocytes are largely unknown: one interesting example is the transcription factor GATA3, thought previously to be important mainly in T lymphocytes and especially in the induction of type 2 cytokines [38]. We have shown that human podocytes express GATA3 (unpublished), and it is of considerable interest that the promoter of the human nephrin gene contains GATA3 recognition sequences. Current experiments are addressing the role of GATA3 in regulation of nephrin gene expression. The possibility that T cells and podocytes share regulatory mechanisms illustrates one possible indirect link between immune responses and the podocyte. This may tie in with another possible link: immunotherapeutic agents may have direct effects on podocytes, so that although the drugs are used in MCN because it is believed that they exert their effects via the immune response, their efficacy may be partly or wholly due to direct effects on the podocyte. For example, podocytes express glucocorticoid receptors and incubation with dexamethasone enhances podocyte survival in vitro by down-regulation of cell cycle inhibitors; dexamethasone also down-regulates VEGF expression and may
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enhance nephrin expression (Xing et al., manuscript in submission).

Conclusions

Complex immune dysfunction is present in patients with MCN, but many of the abnormalities are non-specific and/or consequences rather than causes of the nephrotic state.

Type 2 cytokines predominate in patients with MCN and in their first-degree relatives, implying an immunogenetic basis for the biased immune response.

There are plausible mechanisms whereby a type 2 cytokine bias could directly or indirectly cause MCN.

References