Modulation of human dendritic cell function by therapeutic agents
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Chapter 7

General Discussion

PART I
A possible role for SIgA in the regulation of mucosal immunity

The interaction between SIgA and DC at mucosal surfaces

The heavily glycosylated SIgA is the most abundant Ig isotype present at mucosal surfaces and is believed to be involved in the defence against incoming pathogens as well as in the prevention of responses against dietary and commensal antigens by adhering to microbes in the intestinal lumen, thus inhibiting them to penetrate the mucosae [1]. Another line of defence at mucosal surfaces is provided by DC that are present throughout the mucosal tissues (e.g. lamina propria, peyer’s patches, gut epithelium, respiratory tract) [2-6]. DC have the capacity to open tight junctions between epithelial cells and send their dendrites outside the epithelium to capture proteins in the gut lumen [7]. Moreover, it has been reported that DC present in the subendothelial dome of the peyer’s patches can capture antigens transported through specialized epithelial cells, called M cells [8]. In addition, M cells may also provide the transepithelial transport of SIgA [9]. These data are in line with the observation that immature DC can bind and take up SIgA (Chapter 3), and suggest that SIgA at mucosal surfaces may have another function in addition to simple neutralization of pathogenic organisms. There is evidence that IgA-mediated mucosal uptake of antigens can result in mucosal immune responses [10]. On the other hand, SIgA may be directed against autoantigens or commensal microorganisms [11, 12], against which responses may be harmful. Since binding and uptake of SIgA by immature DC is not accompanied by DC maturation (Chapter 3), it is tempting to speculate that SIgA-mediated antigen uptake may result in tolerance, in view of the current dogma stating that mature DC are immunogenic while immature DC are tolerogenic.

The concept that immature DC are tolerogenic is based on both in vitro and in vivo studies, although the mechanisms underlying the tolerance induction may vary. Human immature DC induce in vitro T cell anergy [13], whereas neoantigen-pulsed immature DC injected in vivo induce IL-10-producing T (regulatory) cells [14]. In contrast, peptide-pulsed mature human DC induce a strong immune response [15].

Several studies indicate that DC continuously capture nonpathogenic environmental proteins that are present in high amounts within our respiratory and digestive tract, such as commensal organisms or dietary proteins. In spite of the absence of migration-inducing signals, these DC spontaneously migrate and transport these proteins to the draining lymph nodes for presentation to T cells [16-19]. Although spontaneous maturation of migratory airway-derived mouse DC has been reported [17], it is reasonable to assume that there are gradual differences in DC maturation, depending on the environmental signals, and that part of the spontaneous migratory DC are
immature or partially mature. Indeed, it has been reported that circulating immature DC can traffic through tissues and pick up apoptotic cells, a high source of self-antigens [20, 21], which may not necessarily induce DC maturation [22, 23]. Moreover, a subpopulation of intestinal DC with weak T cell stimulatory potential has been reported to spontaneously migrate in the absence of inflammatory stimuli to the T cell areas of mesenteric lymph nodes. Interestingly, these cells are loaded with apoptotic bodies derived from intestinal epithelial cells [24].

Taken together these findings underscore the concept that, in the absence of inflammation (under steady-state conditions), immature DC efficiently capture and process self and dietary proteins or non-pathogenic organisms resulting in tolerance, mediated by T cell deletion or by the induction of anergic or regulatory T cells. Upon infection however, mature DC induce a specific T cell response against the pathogen. In that case, the self-antigens and harmless environmental antigens that are presented by the DC along with the pathogen are likely to be ignored as a consequence of T regulatory cells previously generated that prevent autoimmunity and chronic infection.

**Possible role for C-type lectin receptors in tolerance induction**

DC express various endocytic receptors including lectins such as MR and DEC-205 as well as FcR, that can capture antigens and deliver them to processing compartments [25, 26]. Recently, it has been demonstrated that MR ligation mediates negative intracellular signals, resulting in inhibition of IL-12 secretion by DC [27]. In addition, engagement of BDCA-2, which is a C-type lectin II expressed on the plasmacytoid DC subset, inhibits IFN-α production [28]. These observations implicate a more fundamental and diverse role for lectin receptors in the immune response besides their antigen-capturing function. An inhibitory role of endocytic receptors is further supported by experiments showing that mice immunized with mannosylated proteolipid protein (PLP), which is probably taken up via MR, and not mice immunized with non-mannosylated PLP, develop tolerance to PLP in an EAE model [29]. Furthermore, mice injected with hen egg lysozyme (HEL) fused to anti-DEC-205 antibodies, to specifically target the antigen to DC expressing DEC-205, developed antigen-specific tolerance to HEL. Peripheral tolerance in these mice, however, could be converted to immunity when the anti-DEC205/HEL antibody was given together with a DC maturation stimulus [30]. The binding and uptake of SLgA-bound antigens by immature DC is primarily mediated via C-type lectin receptors (Chapter 3). Preliminary data suggest that engagement of MR by SLgA mediates an inhibitory signal to DC, as the LPS-induced secretion of inflammatory cytokines could partially be inhibited in several donors (own unpublished observation), which is in line with previous observations [27]. Therefore, it can be hypothesized that lectin-dependent uptake of SLgA-bound antigens by DC may partially prevent their maturation and that the antigen is transported by these DC to the T cell areas of the draining lymph nodes and presented in a tolerogenic fashion.
PART 2

Therapeutic modulation of the immunoregulatory function of dendritic cells as a mechanism to reduce inflammatory responses

The Th1/Th2 polarizing function of DC can be affected directly by pathogens or indirectly by factors produced by cells of pathogen-infected tissues. In addition, pharmacological agents can modulate this DC function. Since this modulation of DC may have a clinical application in treating certain types of tumors or disease states with excessive Th1 responses (e.g. autoimmune disease, acute graft-versus-host disease) or Th2 responses (e.g. allergic diseases), great efforts have been made to explore the DC-derived factors that regulate Th1/Th2 cell development.

The cytokine IL-12, which is mainly produced by DC, plays a crucial role in cell-mediated immunity and is probably one of the most significant Th1-skewing factors [31, 32]. It has been demonstrated that the levels of IL-12 produced by DC are subject to regulation by inflammatory mediators as well as therapeutic agents [33, 34]. In the next paragraphs the current knowledge on the modulation of DC function by therapeutic agents used for the treatment of inflammatory disorders will be discussed.

The role of DC in autoimmune disease

Autoreactive T cells are deleted by negative selection in the thymus, but some may escape this process and respond to organ-specific self-antigens in the periphery [35, 36]. As their activation may lead to autoimmune disease, these autoreactive T cells are subject to peripheral regulation [37]. DC are thought to initiate not only protective immunity against pathogens by inducing antigen-specific Th cells but also tolerance to self-antigens by deleting or anergizing autoreactive T cells or inducing regulatory T cells [38]. However, the induction or maintenance of tolerance to self-antigens may be cross-regulated by ongoing inflammation. The onset of some autoimmune diseases has been associated with both bacterial and viral infections [39, 40]. In addition, cross-reactivity between self- and pathogen-derived antigens (molecular mimicry) has been described in autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM) and MS [41, 42].

As the activation of autoreactive T cells requires presentation of self-antigens by Th1-driving APC, DC probably play a role in the pathogenesis of autoimmune disease. Indeed, it has been hypothesized that persistent inflammation may lead to tissue destruction and prolonged presentation of self-antigens by DC. This may lead to persistent stimulation of autoreactive T cells and autoimmune disease [43, 44]. In humans, DC can be found in the synovial fluid of patients with RA [45] and in autoimmune lesions of psoriasis [46]. Also MS is associated with high numbers of circulating DC producing inflammatory mediators [47]. The finding that IFN-α-secreting plasmacytoid DC accumulate in cutaneous lupus erythematosus lesions suggests that they may play a role in the pathogenesis of systemic lupus erythematosus (SLE) [48]. Recently, it has been demonstrated that the exposure of monocytes to the serum of patients with SLE resulted in enhanced development of cells with DC morphology [49]. In nonobese diabetic (NOD) mice the first cells that infiltrate the target organ are DC and these cells are able to present autoantigen in the draining
lymph node [50]. Furthermore, transfer of DC that are pulsed in vitro with MBP-derived peptides induce severe EAE in mice [51].

The cytokine IL-12, which is mainly produced by DC, has been associated with the induction of pathology in several models of organ-specific autoimmune disease, including EAE [52], experimental colitis [53], IDDM [54], glomerulonephritis [55], and arthritis [56]. Also increased levels of IL-12 in the serum as well as the cerebrospinal fluid have been found in patients with MS [57, 58]. Therefore, the modulation of IL-12 secretion by DC has been a target for pharmacological intervention in autoimmune diseases as it may reduce the induction and prolongation of Th1-mediated responses and pathology. Indeed, it has been demonstrated that neutralization of IL-12 prevented the development of EAE whereas addition of IL-12 increased severity of the disease [59]. Furthermore, administration of antibodies to IL-12 could inhibit diabetes mellitus or colitis in mice [53, 60]. The therapeutic agent vitamin D3 has been demonstrated to prevent Th1-mediated autoimmune diseases in animal models for EAE, SLE, and type I diabetes [61-63]. This inhibition of Th1 development upon vitamin D3 treatment is thought to be primarily mediated via the reduction of IL-12 secretion by APC [64, 65].

**Modulation of DC function by therapeutic agents**

Various therapeutic agents have been described with a nonselective anti-inflammatory and immunosuppressive function in the treatment of inflammatory disorders, such as autoimmune and allergic diseases. Recently, it has been demonstrated that, in addition to their direct inhibitory effect on Th cells, these agents also suppress IL-12 secretion by DC and thus affect the outcome of the immune response (e.g. glucocorticoids [66, 67], vitamin D3 [65], and β2-agonists [68]). So far, the modulatory effects of PDE4 inhibitors (Chapter 4), type I IFN (Chapter 5, [69]), and GA (Chapter 6) on DC function have not been described in detail. Therefore, we will put these findings in perspective with previous observations.

**PDE4 inhibitors**

PDE4 is the main enzyme responsible for the degradation of cAMP and is predominantly expressed by immune cells (e.g. T cell, APC, mast cells, eosinophils). This implies that the use of a specific inhibitor of PDE4 will not be limited to a certain cell type. Indeed, the elevation of intracellular cAMP levels by PDE4 inhibitors has been reported to inhibit the function of a broad spectrum of immune cells including Th1 and Th2 cells, DC, monocytes/macrophages, mast cells, and eosinophils (Chapter 4, [70-72]). Therefore, these agents have been implicated to be effective in both autoimmune as well as allergic diseases.

PDE4 inhibitors have been demonstrated to suppress proliferation and cytokine production of both Th1 and Th2 cells [73, 74]. Although it has been reported that they preferentially inhibit proinflammatory cytokines in Th1-mediated autoimmune diseases such as IFN-γ and TNF-α [75-78], the opposite has also been found, namely preferential inhibition of Th2 responses in atopic individuals [73, 79, 80]. However, in allergic diseases the inhibitory effects of PDE4 inhibitors on the ability of mast cells and eosinophils to produce mediators and to relax the bronchial muscles, may play a
more important role to reduce disease severity than their inhibitory action on T cells [70, 71].

It has been demonstrated that PDE4 inhibitors strongly reduce the production of the proinflammatory cytokine TNF-α by DC, monocytes, and macrophages (Chapter 4, [81, 82]). Furthermore, selective inhibition of PDE enzymes suppresses IL-12 and TNF-α in various animal models of autoimmune disease and even reduces Th1 activity [83-86], which may be explained by their anti-inflammatory effect on DC (Chapter 4). These data are in accord with the observation that PDE4 inhibitor treatment reduces the number of TNF-α- and IFN-γ-secreting cells in patients with MS [87]. Furthermore, treatment with inhibitors of PDE enzymes suppresses the production of IL-12 and six out of eight patients with MS reported improved motor skills and less fatigue [76]. Taken together, these data suggest that PDE4 inhibitors are potential therapeutic agents for the treatment of Th1-associated diseases. It is likely that PDE4 inhibitors dampen inflammatory responses through prevention of the production of inflammatory cytokines (TNF-α, IL-12) by APC, resulting in a reduced induction of Th1 responses, while they simultaneously act directly on T cells reducing their proliferation and production of inflammatory cytokines (IFN-γ, TNF-α).

**Type I IFNs**

Despite the fact that type I IFN treatment has been shown to reduce the frequency of relapses and clinical exacerbations in patients with MS, the mechanism of action remains elusive [88, 89]. It has been reported that type I IFN treatment enhances the secretion of IL-10 and reduces the frequency of IFN-γ-secreting T cells in vivo [90-93]. This is in line with the observation that type I IFN inhibit IL-12 secretion by DC and thus reduce the development of IFN-γ-secreting (Th1) cells (Chapter 5, [69]). Even though IL-10 is a negative regulator of IL-12 production [94], this cytokine is probably not involved in the type I IFN-mediated inhibition of IL-12 production by DC [69, 95], but may exert its anti-inflammatory effects via other mechanisms. For instance, IL-10 may reduce inflammatory cytokine production by autoreactive T cells or affect the antigen-presenting capacity of APC [96]. It has been demonstrated that type I IFN interfere with IFN-γ-induced upregulation of MHC class II expression on human glioma cells [97]. Importantly, type I IFN interfere with the IL-12-enhancing effect of IFN-γ on DC independent of the maturation stage of the cells via a still unknown mechanism (Chapter 5). This indicates that the type I IFN-mediated inhibition of the positive feedback loop between IL-12 and IFN-γ may be beneficial for the course of the disease in MS patients. Besides their inhibitory effects on DC function, other inhibitory mechanisms may be involved in the clinical efficacy of type I IFN therapy in MS. For instance, type I IFN may affect the migratory potential of T cells to inflammatory sites by inhibiting matrix metalloproteinases (MMP) and VLA-4 expression [98-100] and inhibit Th cell proliferation [91, 101].

It has been reported that mature DC become resistant to further modulation by immunomodulatory molecules such as IL-10 or PGE_2 [102] as well as pathogenic compounds [103, 104]. In addition, PDE4 inhibitors are unable to modulate mature DC function (Chapter 4). This implicates that upon arrival in the lymph nodes the acquired cytokine profile of the effector DC is stable and allows them to adequately
polarize the antigen-specific Th cell response in relation to the conditions of the infected and/or inflamed tissue. This is supported by the finding that within one lymph node multiple antigen-specific T cells with distinct cytokine profiles can be detected [105]. In contrast with these observations is the finding that mature DC are highly susceptible to type I IFN-mediated inhibition of IL-12 production (Chapter 5, [69]). In addition, it has been demonstrated that mature DC are still sensitive to IFN-γ-mediated signalling, even though to a much lesser extent than immature DC [104]. These data implicate that under certain conditions mature DC can be sensitive to modulation.

Glatiramer acetate
The therapeutic agent GA has been reported to induce a shift in the balance from Th1 towards Th2 cells [106, 107] and to reduce the relapse frequency in relapsing-remitting MS patients [108]. Recently, GA was also shown to prevent murine graft-vs-host disease [109, 110] and transplant rejection [111], indicating its potential therapeutic use in several other inflammatory diseases.

The beneficial effects of GA may be explained by their ability to promiscuously bind to class II MHC on APC [112] and thereby directly competing with MBP-derived peptides for the MBP-specific TCR [113, 114]. There are several indications, however, suggesting that this antigen-nonspecific mechanism of action may not be the major basis of the therapeutic effects of GA in vivo. First, it is highly unlikely that GA reaches the site (e.g. CNS) where it could compete with autoantigens for binding since it is rapidly degraded into free amino acids and small oligopeptides after subcutaneous administration [115]. However, there are indications that APC interact with GA at the site of application and actively participate in mediating the effects of GA, as will be discussed below. Second, the fact that GA can act as a TCR antagonist exclusively against the MBP 82-100 peptide, but not MBP 1-11 or peptide from other protein, such as PLP 139-151 [114], is puzzling since its protective effects have been shown in EAE induced by various autoantigens [116-118]. Third, GA has been demonstrated to induce anergy in a MBP-specific T cell clone in the absence of MBP suggesting that specific TCR engagement takes place and that competition with MBP is not required for GA to be effective [119]. Fourth, although the stereoisomer of GA has been demonstrated to bind as effectively as GA to MHC class II [110], it failed to suppress EAE [120]. Overall these data indicate that merely competition for MHC binding is not sufficient to explain the beneficial effects of GA.

The observation that GA impairs the ability of DC to produce cytokines such as bioactive IL-12 and TNF-α (Chapter 6) support the hypothesis that GA induces anti-inflammatory Th cell responses by modulating the APC function of DC. Furthermore, GA reduced inflammatory cytokine production by cells from whole blood indicating its anti-inflammatory effects on other APC besides DC (own unpublished observations). Moreover, DC matured in the presence of GA polarize naive Th cells into effector IL-4-producing Th2 cells and this is accompanied by the enhanced secretion of the anti-inflammatory cytokine IL-10 (Chapter 6). These observations are in line with both in vitro and in vivo studies showing that GA induces a Th1 to Th2 shift accompanied by the production of IL-10 [106, 107, 121, 122]. Therefore, it can be speculated that GA modulates the APC function of sentinel DC that carry GA to the
draining lymph nodes and present it to GA-reactive T cells, which then migrate across the blood brain barrier to the site of infection [121]. In the CNS they may cross-react with myelin antigens and release anti-inflammatory (Th2-type) cytokines and induce bystander suppression of Th1-type cytokine production by autoreactive T cells [123, 124]. Whether these GA-reactive T cells possess so-called regulatory functions, as has been described for CD25+ CD4+ Th cells, needs to be elucidated.

Concluding remarks
It has become increasingly clear that DC play a decisive role in the outcome of the immune response. They are able to recognize, process and present pathogen and subsequently initiate naive Th cell responses, driving them into distinct classes of effector cells. In addition, DC have a strong inflammatory function in the periphery and are potent activators of memory T cell responses. The identification of the factors that modulate DC function and the way these cells subsequently promote Th1 or Th2 response can contribute to the design of novel therapeutic strategies.

Various therapeutic agents have been developed for the treatment of inflammatory disorders, such as autoimmune and allergic diseases. These anti-inflammatory and immunosuppressive agents have been described to nonselectively inhibit inflammatory processes. They have been shown to directly inhibit Th cell proliferation and reduce the inflammatory cytokine production by Th cells, monocytes and macrophages. Recently, it has become clear that therapeutic agents are also able to modulate DC function and thus affect the outcome of the immune response. The data discussed here indicate that several therapeutic agents including PDE4 inhibitors, type I IFN, and GA, are able to inhibit the IL-12-producing capacity of DC and, consequently, reduce the induction of Th1-mediated responses. These data improve our understanding of the mechanisms underlying the anti-inflammatory effects of therapeutic agents.

A critical step for the onset of specific immunity to pathogen is the activation of DC and their switch from an antigen-sampling mode into a T cell-stimulatory mode. However, how do DC decide to induce a tolerogenic or immunogenic reaction to a certain antigen? This decision may depend on the type of DC that presents the antigen, the maturation status of the DC which depends on the conditions (steady-state versus inflammation) under which the antigen is taken up (via lectin-like receptors?) and presented, or the character of the antigen. Finding the factors that regulate the balance between tolerance and immunity by DC are considered to be of utmost importance for the design of therapeutic strategies. The targeting of antigens to immature DC and their uptake via lectin-like receptors may be used as a novel strategy to dampen the autoimmune response.

References
General Discussion


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Chapter 7


