Interactions between infections and immune-inflammatory cells in type 1 diabetes mellitus and inflammatory bowel diseases: evidences from animal models

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ABSTRACT

Type 1 diabetes mellitus (T1D) and inflammatory bowel diseases (IBD) are multifactorial disorders of autoimmune origin.

Several microbial agents have been reported to be associated with the development of type 1 diabetes and inflammatory bowel diseases in animal models by different mechanisms.

These models which resemble the phenotype of the human disease they mimic, can be very useful to identify important pathogenetic mechanisms, as well as therapeutical targets to treat the disease.

This review is focused on the immune inflammatory pathways which are considered to be associated with the pathogenesis T1D and IBD in transgenic mice.

Introduction

Autoimmune diseases such as Type 1 (insulin dependent) diabetes mellitus (T1D) and inflammatory bowel diseases (IBD) can occur in genetically predisposed individuals exposed to different environmental factors and/or in the presence of immunoregulatory dysfunctions (1-5).

Autoimmunity is most likely initiated during the course of an infectious episode when inflammation is provoked in the target tissue (*i.e.*, insulin-producing β cells in the pancreatic islet or intestinal epithelial cells) either by direct cytotoxic effects of the microbial agent or by immunopathologic reactions against a persistent infection which causes chronic inflammation within the target tissue (6).

In T1D, viruses play a primary role as triggering agents (7, 8) because they can induce a strong immune response and can infect insulin-producing β cells leading to local inflammation (9).

Infection-associated inflammation may involve the release of cytokines and chemokines which can be sufficient to activate lymphocytes directed against self antigens leading to initiation and/or exacerbation of autoimmune diseases.

In IBD, lesions of the gastrointestinal tract have been associated with increased production of macrophage-derived proinflammatory cytokines such as IL-1β, IL-6, and TNF-α (10).

Mouse models for autoimmune diseases can be very useful to explore the different immune-inflammatory pathways which are potentially involved in the pathogenesis of a specific disease (11-13).

To investigate the aetiopathogenesis of T1D and IBD, several animal models have been developed (14, 15).

With these models, the role of functionally distinct factors, including cells of the innate and immune system, cytokines and chemokines, receptor and their ligands which represent a potential therapeutic target for the treatment of the disease (16-18), has been described.

Studies conducted in mice genetically deficient of macrophage migration inhibitory factor (MIF) have shown that this cytokine plays a major role in immunoinflammatory diabetogenic pathways both in mice made diabetics with multiple low doses of streptozotocin and in non-obese diabetic (NOD) mice with accelerated forms of autoimmune diabetes, because of its capacity to induce Th1 inflammatory cytokines (19).

Furthermore, data from animal models obtained by T cell transfer colitis in transgenic mice for genes encoding immunoglobulin and T cell receptor (TCR) recombination events have elucidated the central role of the adaptive immune response in the regulation of colitis development. These studies revealed that there are different factors which play important roles for the

development of T cell transfer colitis in severe combined immunodeficient (SCID) recipient mice including the lack of regulatory T cells (Treg) in the transferred T cell population and the expression of chemokine receptor CXCR3 by the colitogenic T cells.
All the molecules identified in these different pathways may represent potential therapeutic targets for the treatment of T1D and IBD.

Exploiting the crosstalk between infections, innate immunity and beta cell destruction in autoimmune diabetogenesis

T1D is a multifactorial disorder caused by the lack of endogenous insulin which is thought to be a consequence of an immune attack mediated by autoreactive T cells and macrophages against pancreatic β-cells. The key role played by the immune system in the pathogenesis of the disease has focused much attention on identifying immunotherapeutical approaches that may halt or delay β-cell destruction in prediabetic individuals or those patients with newly diagnosed disease (20).
Animal models of human T1D, such as the NOD mouse, the diabetes-prone BB rat, and the mouse made diabetic with multiple low doses of streptozotocin (MLD-STZ), have extensively been used as *in vivo* tools to study immunopathogenic pathways and to identify novel immunotherapeutical approaches (21, 22).
Both in these models and in humans, the diabetogenic potential of autoreactive T cells and macrophages appear to be related to their capacity to secrete type 1 proinflammatory cytokines such as IL-1β, interferon-gamma, TNF-alpha, IL-12, and IL-18 (23).
MIF is a pleiotropic cytokine produced during immune responses by activated T cells, macrophages, and a variety of non-immune cells (19, 24, 25). Constitutive expression of MIF mRNA and protein is found in various non-immune cells within normal tissue, such as anterior pituitary cells; cardiac myocytes; parenchymal cells within liver, brain, or kidneys; or pancreatic islet β-cells. Notably, in many of those tissues the expression and release of MIF are significantly up-regulated under various pathological conditions, such as atherosclerosis, glomerulonephritis, multiple sclerosis, colitis, type 2 diabetes, and pancreatitis, thus implicating a role for MIF in the disease process. Indeed, recent studies using neutralizing antibodies (Ab) or MIF-deficient animals demonstrated that MIF is a crucial mediator of several immunoinflammatory disorders in rodents, including Gram-negative and Gram-positive sepsis, delayed-type hypersensitivity, leishmaniasis, glomerulonephritis, arthritis, experimental autoimmune encephalomyelitis, experimental autoimmune myocarditis, and colitis (19, 24, 25).
In contrast to the possible pivotal role of MIF in autoimmune diseases, its role in the pathogenesis of human T1D is still unclear. Although elevated MIF gene expression has been detected in spontaneously diabetic NOD mice during development of the disease, and exogenously administered recombinant MIF exacerbated disease development, the circulating levels of MIF were found to be decreased in patients with recent-onset T1D. However, despite these conflicting data, MIF possesses biological characteristics that anticipate a role for this cytokine in autoimmune diabetogenesis. These include the capacity of MIF to stimulate delayed-type hypersensitivity responses (19, 24, 25) which mediate β-cell destruction during development of T1D and to up-regulate the production of other proinflammatory cytokines and soluble mediators involved in the pathogenesis of the disease, such as TNF-alpha, IL-1β, and nitric oxide (NO) (23).
MIF may also influence cell-mediated β-cell destruction through metabolic pathways, because it is constitutively expressed and secreted together with insulin from pancreatic β-cells and acts as an autocrine factor to stimulate insulin release. This might contribute to immunoinflammatory diabetogenesis by favoring the expression on β-cells and the presentation to immune cells of antigens (Ag) which are up-regulated when functional activity is augmented (20). Thus, MIF possesses both hormonal and immunological properties which qualify it as a potentially important mediator in the initial events of β-cell dysfunction and destruction.
These observations prompted us to undertake this study evaluating the behavior of endogenous MIF during the development of immunoinflammatory diabetes in preclinical models. By using anti-MIF monoclonal or polyclonal antibodies and mice with genetic MIF deficiency (26, 27) we have shown that this cytokine plays a major role in immunoinflammatory diabetogenic pathways both in mice made diabetics with multiple low doses of streptozotocin and in NOD mice with accelerated forms of autoimmune diabetes (26, 27).
Even more importantly for the translation of these findings to the clinical setting, the small molecule (S, R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) which is the leading compound obtained through a strategy aimed at designing MIF antagonist drugs by targeting the catalytic site of MIF and which acts as a selective pharmacological inhibitor of MIF was also found to prevent MLDS-induced diabetes (28).
In addition, our studies in mice made diabetics with MLDSZ show that the MIF protein is significantly elevated in islet cells during the development of diabetes and that negating the activity of endogenous MIF reduced clinical and histopathological features of MLD-STZ-induced diabetes, such as hyperglycemia and insulitis (26, 27). Protection from diabetes was associated with reduced islet Ag-specific proliferative response of lymphocytes and defective adhesive cell-cell interactions under *ex vivo* conditions. In addition, neutralization of MIF down-regulated the *ex vivo* local and peripheral secretion of the proinflammatory mediators TNF-alpha, IFN-gamma, and NO; simultaneously, the capacity of spleen mononuclear cells to produce the antiinflammatory cytokine IL-10 was significantly increased by *in vivo* abrogation of MIF activity (26, 27).
These data warrant studies aimed at evaluating the possible role of specific MIF inhibitors in human type 1 diabetes.

Switching from reductionistic to holistic thinking in the natural history of IBD: lessons from animal models

IBD comprises two distinctly different chronic diseases, ulcerative colitis (UC) and Crohn's disease (CD). Genetic susceptibility, environmental factors and immunoregulatory dysfunctions all play a role in etiology, development, course and prognosis of the disease. UC is a diffuse mucosal inflammation limited to the colon whereas CD, by contrast, is a patchy transmural inflammation that may affect any part of the gastrointestinal tract. The prevalence of IBD in Europe and the US is > 1:1000 (29).

The last decade has witnessed several new animal models for both acute and chronic colitis. T cell transfer colitis in immunocompromised SCID or RAG$^{-/-}$ mice is the animal model which most closely resembles or mimics the two human major disease categories of chronic colitis (30, 31). In T cell transfer colitis, the colon of the diseased mice shows a progressing diffuse mucosal inflammation, patchy transmural inflammation, diarrhea, progressive weight loss and finally death within 4-6 weeks after T cell transfer.

In the T cell transfer model, genetic and immunological susceptibility relates to the recipient's lack of an adaptive immune system due to the presence of mutations in genes encoding immunoglobulin and TCR recombination events (SCID or RAG$^{-/-}$ recipients). This allows for an uncontrolled expansion in the recipient of the transferred colitogenic CD4$^+$ T cell subset provided that regulatory T cells (Treg) have been depleted prior to T cell transfer. As in human counterparts, T cell proliferation is driven by antigens derived from the gut bacterial commensal flora environment. Thus, no proliferation of transferred T cells takes place and colitis development is totally absent in SCID recipients kept under germ free conditions (32).

Our laboratory has been involved in the characterization of a population of *in vitro* generated Tregs which are more efficient than conventional CD4$^+$CD25$^+$ Tregs in the prevention of colitis when coinjected into SCID mice together with effector CD4$^+$CD25-T cells. These Tregs were generated *in vitro* by coculture of immature dendritic cells (DC) with unfractionated CD4$^+$ T cells for several days (33). Table I shows their characteristics compared with traditional Tregs. The DC cocultured Tregs show ten times higher expression of CD25 and L selectin (CD62) and eight times higher inhibitory activity *in vitro* than prototype Tregs. They are functionally resistant to IL-2 and around 50% of their inhibitory activity *in vitro* is mediated by soluble substances which are not IL-10 or TGF β. Thus it appears that crosstalk between T cells and immature DCs results in subsets of Tregs with a particular phenotype and an increased functional activity.

Table I. Differences between dendritic cell (DC)-induced T-regulatory (Treg) cells and prototype CD4$^+$ CD25$^+$ Treg (modified from ref. 33).

DC/CD4$^+$ co-culture-induced Treg cells	Prototype CD25$^+$ Treg cells
Derived *in vitro* from CD4$^+$ T cells	Present in freshly obtained CD4$^+$ CD25$^+$ T cells
CD25 $^{very\ high}$	CD25 ^{high}
CD45RB ^{high}	CD45RB ^{intermediate}
CD69 low/absent	CD69 low/absent
CD62L (L selectin)^{high}	CD62L^{intermediate}
Secrete inhibitory molecules	Do not secrete inhibitory molecules
Activation uncertain uncertain	Activation dependent
Resistant to IL-2	Inhibited by IL-2
Inhibition at 2% Treg comixture	Inhibition at 16% Treg comixture

Our laboratory has recently been focusing on the involvement of chemokines, their receptors and other immune regulatory molecules in SCID mice transplanted with colitogenic CD4$^+$ T cells depleted of CD25$^+$ Treg and in mice protected from colitis by coinjected Tregs. In the protected mice the locally expressed inflammatory chemokine receptors CCR1 and CXCR3 and their corresponding chemokine ligands were found to be down modulated (34). In accordance with these results, deletion of CXCR3 in transferred and potentially colitogenic CD4$^+$CD25$^-$ T cells ameliorated colitis development suggesting that CXCR3 expression is of importance for enteroantigen priming of the effector T cells and/or their migrating into the gut wall as well as their inflammatory potential. In contrast, lack of CXCR3 expression by Tregs does not affect their protective capacity *in vivo* (35).

In our most recent attempt to identify pathways of importance for immune regulation in SCID mice protected from colitis by Tregs we studied genome-wide mRNA expression profiles of inflamed and non-inflamed gut. The mRNA expression profiles of inflammatory cytokines, chemokines and certain growth factors were significantly down regulated in Treg protected mice. In particular, transcription factors such as STAT3, GATA2 and NF-κB, the cytokine IL-1β and the chemokine receptors CXCR3 and CCR1 as well as their ligands appear to play a central role in the inflammatory process and might alone or in combination represent new targets for future therapeutical approaches (36).

Most recent data suggest that the enteric nerve system and in particular substance P neurokinin receptor 1 (NKR-1) signaling plays an important part in the control of disease development in the T cell transfer colitis model. Thus, selected NKR-1 antagonists appeared to be powerful inhibitors of enteroantigen-specific T cell reactivity *in vitro* and capable of ameliorating disease development *in vivo*.

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