Interleukin 1 Receptor Antagonist and (IL-1Ra) IL-Ra Producing Mesenchymal Stem Cell in Therapy of Diabetes Mellitus

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Introduction
Diabetes mellitus type 1 is an autoimmune disorder in which damage to beta cells are proposed to be due to abnormal T cell immune response.1 Diabetes mellitus type 2 is an endocrine, metabolic disease in which the key metabolic abnormality is chronic hyperglycemia due to an imbalance between insulin production and insulin action.2,3 However, several lines of evidence suggest that there are shared mechanisms of beta cell dysfunction in both types of diabetes mellitus.4 Different initial events characterize pathogenesis of diabetes mellitus type 1 and diabetes mellitus type.1,2,4 Nevertheless, oxidative stress and increase of pro-inflammatory cytokines, induced by glucotoxicity and lipotoxicity, lead to beta cell damage and promote beta cells apoptosis, regardless of the type of diabetes.4 Thus, blocking of pro-inflammatory cytokines should be an effective way for the treatment of diabetes mellitus. One possible therapeutic agent for diabetes mellitus treatment appears to be Interleukin 1 receptor antagonist (IL-1Ra), a member of the IL-1 cytokine family.4

It has been shown that a subset of mesenchymal stem cells (MSCs) is an excellent source of IL-1 Ra, which in addition to their regenerative capacity makes these cells a potential therapeutic agent in the treatment of diabetes mellitus.5 We review here the present understanding of the potential use of IL-1Ra and mesenchymal stem cells as modulators of diabetogenesis.

IL-1Ra in Type 1 Diabetes
IL-1Ra is a naturally occurring cytokine. It is an inhibitor of IL-1α and IL-1β. It binds to IL-1 receptors and by that it antagonizes the inflammatory effects of IL-1α and IL-1β.6 When IL-1 occupies its receptor, various pro-inflammatory events are initiated. Activated cells release highly inflammatory substances such as nitric oxide.6 In addition, binding of IL-1 to its receptor induces the synthesis and release of chemokines that attracts neutrophils, macrophages and lymphocytes resulting in tissue inflammation.7

When IL-1Ra binds to the IL-1 receptor, no such events are initiated, and binding of IL-1 is blocked by IL-1Ra which does not transduce signals.8 IL-1Ra is produced by immune cells,9 stromal cells,10,11 adipocytes,13 epithelial cells, keratinocytes14,15 and hepatocytes.16 It appears that deregulation in the balance between IL-1 and IL-1Ra is important in the pathogenesis of diabetes mellitus type 1 and diabetes mellitus type 2 [7].7 The IL-1Ra/IL-1 ratio is significantly different in newly diag-
nosed and long-standing diabetes mellitus type 1 patients. The IL-1Ra/IL-1 ratio is strongly reduced in newly diagnosed diabetes mellitus type 1 patients compared with healthy population. At the onset of disease there is an imbalance in favor of pro-inflammatory IL-1 which induces apoptosis in insulin-producing β-cells. During the evolution of disease, after all beta cells are damaged, the inflammatory process in pancreas ceases with decreased production of IL-1 resulting in an increase in IL-1Ra/IL-1 ratio. Also, the serum concentration of IL-1Ra increases during the course of the disease. While in newly diagnosed diabetes mellitus type 1 patients serum concentration of IL-1Ra is similar to that of healthy individuals, the level of IL-1Ra in serum of long-standing diabetes mellitus type 1 patients is significantly higher compared with the serum of healthy individuals and compared with that of newly diagnosed diabetes mellitus type 1 patients.17

IL-1 has a lytic effect on the islet cells which was clearly demonstrated in vitro.18 In addition, intraperitoneal administration of IL-1 accelerates the onset of diabetes mellitus type 1 in the BB rats19 and induces hyperglycemia and hypoinsulinemia in normal Wistar Kyoto Rats.20 In vitro IL-1Ra can protect insulin-producing cells from the deleterious effects of IL-1.21 Exposure (1-2 h) of rat and mouse pancreatic islets to 10 ng/ml recombinant IL-1 beta induced a 70-80% inhibition of insulin response to glucose after 12 h. Co-incubation with 100 ng/ml IL-1Ra efficiently counteracts these effects of IL-1 beta. The importance of these results can be seen not only in the suggestion that IL-1Ra can protect insulin-producing beta cells from damaging effects of IL-1, but also in showing that insulin-producing beta cells possess type 1 interleukin-1 receptors that indicates potential therapeutic use of IL-1Ra in the treatment of diabetes mellitus.21

In vivo blocking of IL-1 receptors by IL-1Ra modulates the deleterious effect of IL-1 on pancreatic beta cells.22 After the induction of diabetes mellitus by using streptozotocin (STZ), mice were injected repeatedly (10 daily injections) with either rat IL-1 inhibitor (IL-1 INH) derived from glucocorticoid-treated macrophages,23 or with recombinant DNA produced human IL-1Ra. Mice treated with either of these agents remained normoglycemic. In comparison with rat IL-1 INH, human IL-1Ra had milder but still significant protective effect.22

In humans there is positive correlation between IL-1Ra and C-peptide secretion and IL-1Ra was found to be elevated in patients with increased C-peptide secretion.24 There is also a positive association of IL-1Ra with body mass index (BMI) confirming adipose tissue as an important source of IL-1Ra.25 However, IL-1Ra showed positive correlation with C-peptide secretion without adjustment for BMI percentiles, suggesting other sources of IL-1Ra.24 These data suggested that anti-inflammatory IL-1Ra, as the specific receptor antagonist of IL-1β, by binding to IL-1 receptors, antagonizes the inflammatory effects of IL-1β and preserves β-cell function in type 1 diabetes. Therefore, IL-1Ra might be a new therapeutic agent in diabetes mellitus type 1.23 A high molar excess of IL-1Ra over IL-1 seems to be necessary to prevent IL-1 mediated β-cell toxicity.26

Sustained administration of IL-1Ra can prevent experimental diabetes mellitus elicited by multiple low-dose STZ treatment.27 After 5 days of STZ treatment (40mg/kg body weight), mice were injected with IL-1Ra (8 mg/kg body weight). The IL-1Ra was delivered with a subcutaneous implanted osmotic pump. The IL-1Ra administration (8 mg/kg body weight every day) started on the last day of STZ treatment and injected continuously for 12-14 days. Mice were normoglycemic up to the 19th day of the experiment. However, the mice became hyperglycemic in the following 10 days after cessation of the delivery of IL-1Ra. Morphological examination showed significantly lower degree of islet mononuclear cell infiltration on day 19 compared with day 29. On day 29, the majority of the pancreatic islets
were infiltrated with mononuclear cells.\textsuperscript{27} Thus continuous administration of IL-1Ra prevents pancreatic mononuclear cell infiltration, islet destruction and hyperglycemia in STZ-induced diabetes mellitus.

Several lines of evidence have shown that IL-1β impair glucose-stimulated insulin production in mouse, rat and human islets\textsuperscript{28-30} and there is evidence that IL-1 beta has a deleterious action on beta-cell replication resulting in abnormal pancreatic function and hyperglycemia.\textsuperscript{31} Apparently this can be prevented by IL-1Ra adenoviral overexpression. In the \textit{in vitro} experiment which used human pancreatic islets,\textsuperscript{31} adenoviral gene delivery of the cDNA encoding the IL-1Ra to cultured islets resulted in islet protection.\textsuperscript{31}

These results further support the idea that \textit{in vivo} gene therapy can be used to prevent insulinitis and the consequent diabetes mellitus.

\textbf{IL-1Ra in Type 2 Diabetes}

Besides the role in the evolution of diabetes mellitus type 1, IL-1 and IL-1Ra appear to play an important role in the pathogenesis of diabetes mellitus type 2.\textsuperscript{2,3,8,33} Although the precise mechanism of beta cell failure in diabetes mellitus type 2 is still unknown, metabolic stress caused by repetitive glucose excursions, dyslipidemia and increased levels of adipokines can induce an inflammatory response characterized by local cytokine secretion, islet immune cell infiltration and β-cell apoptotic death which can be important in the pathogenesis of diabetes mellitus type 2.\textsuperscript{33-36}

Beta cells were identified as the cellular source of glucose-induced IL-1.\textsuperscript{35} High glucose levels increase beta-cell production and release of IL-1β and isfollowed by beta cell apoptosis and insulin resistance.\textsuperscript{37} The IL-1 beta mRNA expression is increased in β-cells of patients with type 2 diabetes.\textsuperscript{38} Based on these findings, several lines of evidence indicate that IL-1Ra can be used as a therapeutic agent in the treatment of diabetes mellitus type 2.\textsuperscript{39-41}

When IL-1Ra was injected in C57BL/6J mice, previously fed a high-fat/high-sucrose diet (HFD) for 12 weeks, glucose tolerance and insulin secretion were improved.\textsuperscript{39} IL-1Ra was used in the clinical trial as a therapeutic agent for the treatment of diabetes mellitus type 2.\textsuperscript{40} Patients received either placebo or a subcutaneous injection of 100 mg "anakinra"-recombinant human IL-1Ra once daily for 13 weeks. At the end of the trial, the HbA1c level was lower in the IL-1Ra -treated group than in the control group. Further, the decrease of serum IL-6 and C-reactive protein levels, as markers of systemic inflammation, was noticed after IL-1Ra therapy.\textsuperscript{40} It was assumed that recombinant human IL-1Ra improved glycemic control in patients with diabetes mellitus type 2 probably through enhanced secretory function of pancreatic beta cells.\textsuperscript{40} The body mass index (BMI) of patients remained stable during the experiment implying that recombinant human IL-1Ra is not an anorexigenic agent.\textsuperscript{40}

Also, systemic hypoglycemia was not reported by any of IL-1Ra treated patient.\textsuperscript{40} A concern with the use of IL-1Ra is the potential increase in the incidence of infectious disease because of IL-1 blockade. However, this unwanted effect was not seen in more than 100,000 patients with rheumatoid arthritis who underwent long-term treatment with recombinant human IL-1Ra.\textsuperscript{41}

Importantly insulin resistance, insulin-regulated gene expression in skeletal muscle and serum adipokine levels were all unaffected by therapeutic use of IL-1Ra.\textsuperscript{40} It is therefore possible that long-term and higher doses of IL-1Ra therapy or treatment with other recombinant IL-1Ra that has a longer half-life than anakinra (anakinra has a short half-life, 6-8 hours) should prevent beta cell destruction and promote beta cell regeneration in patients with diabetes mellitus type 2.

\textbf{Mesenchymal Stem Cell Therapy of Diabetes Mellitus}

MSCs are self-renewable multipotent stromal cells that can be found in almost all postnatal
tissues. The most often used source tissues, for isolation of MSCs are bone-marrow, adipose tissue and umbilical cord blood, but MSCs can also be derived from virtually all tissues. There are two main MSC characteristics currently used for their isolation and identification: capacity for self-renewal and differentiation into tissues of mesodermal origin (for example bone, cartilage, and adipose tissue) and lack of expression of surface markers characteristic of hematopoietic cells (CD14, CD34, CD11a/LFA-1, and CD45), erythrocytes (glycophorin A), and platelet and endothelial cell (CD31). MSCs show variable levels of expression of several molecules: CD105 (SH2), CD73 (SH3/4), CD44, stromal antigen 1, CD166 (vascular cell adhesion molecule), CD54/CD102 (intracellular adhesion molecule), and CD49. 

It had been suggested that MSCs have immunomodulatory capacity. MSCs inhibit T cell proliferation by several pathways: by engagement of the inhibitory molecule programmed death 1 (PD-1) to its ligands PD-L1 and PD-L2, by producing factors which suppress their proliferation (such as TGF beta or IL-10) and through “tolerogenic” interaction with dendritic cells (DCs). There also findings indicating that MSCs can render T-cells anergic. This probably depends on their affect on differentiation of monocytes to DCs or on inhibiting DCs maturation. Immature DCs can render T cells anergic. MSCs can also alter the cytokine secretion profile of dendritic cells resulting in increased production of anti-inflammatory cytokine IL-10 and decreased production of inflammatory cytokines IFN-γ and IL-12. Furthermore, MSCs may regulate immune response by increasing number of CD4+CD25+FoxP3+ downregulatory T cells. Bone marrow-derived MSCs also have inhibitory effects on the proliferation and IgG secretion of β-cells as shown in BXSB mice experimental model for systemic lupus erythematosus.

One of the possible pathway of MSCs effects on inflammation is the expression of IL-1Ra. Recently a well characterized subpopulation of MSC which express IL-1Ra has been described. IL-1Ra expressed by MSCs is biologically active, antagonizes IL-1α and blocks release of TNF-α from activated macrophages in vitro. Considering that TNF-α and IL-1α are very important cytokines in inflammatory reactions, blocking their function, by MSCs which express IL-1Ra, protects tissues from inflammation-induced injuries.

IL-1Ra-expressing subpopulation of MSCs can also modulate the inflammatory response in vivo. This was shown in the experiment in which IL-1Ra-expressing subpopulation of MSCs inhibited bleomycin (BLM)-induced inflammation and fibrosis in the lungs of mice. MSCs were more effective than recombinant IL-1Ra, delivered systemically or by viral infection, in inhibiting immune cell trafficking and cytokine expression levels.

Although there are significant differences in the number of MSCs in different species (IL-1Ra-expressing subpopulation of MSCs is more abundant in murine than in human population), IL-1Ra expression by MSCs is conserved across species. These findings implied that IL-1Ra-expressing subpopulation of MSCs, independently of their origin, may be used as a cellular vector for down regulated diabetogenesis. It has been shown that infusion of human MSC (hMSCs) in multiple low dose STZ-induced diabetic, immunodeficient mice (NOD/SCID) reduced glycemic levels and increased peripheral insulin levels. Similarly, 5 x 10^5 mouse mesenchymal stem cells given at the end of diabetes induction with multiple low doses of streptozotocin prevented or delayed onset of glycemia and glycosuria in male C57BL/6 mice (our work in progress).

Systemic MSC administration also resulted in beta-cells regeneration and prevented renal damage in this model of diabetes. Bone-marrow derived mice MSCs were injected intravenously, as a single dose, in diabetic mice when hyperglycemia, glycosuria, massive beta-pancreatic islets destruction, and mild albuminuria were evident without renal hist-
pathologic changes. Thus, MSCs were injected when mice had symptoms similar to symptoms of human patients diagnosed with type 1 diabetes mellitus. Glycemic levels were significantly reduced one week after MSCs injection. Euglycemic values in mice that received MSCs were seen about one month after MSCs treatment. Normal values of glycemia in MSCs-treated mice lasted at least for two months (till the end of experiment) and during that period correlated with glycosuria reversion.67

Pancreatic islets in mice that received MSCs were architecturally organized as in normal mice.67 On the contrary, pancreatic islets were destroyed by inflammation in control diabetic mice. Although with normal structure, islets in MSCs-treated diabetic mice were smaller than islets in normal mice, probably because MSCs didn’t completely regenerate pancreatic islets. Morphologic analysis of these islets showed that MSC administration increased the number of insulin-producing cells and restricted expansion of glucagon-producing cells.67 These results suggest that corrections of blood glucose level happened due to regeneration of the pancreatic islets.

Systemic MSCs administration also recovered and prevented further impairment in renal function.67 Two months after MSC administration, histologic kidney analyses showed normal architecture of glomeruli and reduced albuminuria was observed in MSC-treated diabetic mice. Glomeruli in control diabetic mice showed hyalinosis, mesangial expansion and increased albuminuria.67

MSC transplantation can reduce cardiovascular complications and improve cardiac function in STZ-induced diabetic rats.68 Bone marrow derived MSCs administered systemically or locally at the wound site accelerate wound healing in diabetic rats.69 Through paracrine actions of growth factors secreted by MSCs diabetic polyneuropathy was improved by MSCs treatment, which was also shown in diabetic rats.70 These results suggest that bone marrow-derived MSC therapy could be considered for the treatment of diabetes mellitus type 1 and for the complications of diabetes mellitus: nephropathy, cardiovascular complications, polyneuropathy, delayed and poor wound healing.67-70

Besides these promising results, shown in animal models of diabetes mellitus, there are several problems which limit the therapeutic use of MSCs in humans.71,72 There is a potential risk for malignant transformation of MSCs because of their chromosomal instability, registered in vitro.71 Nevertheless, malignant transformation of human MSCs has not been noted till now.54 MSCs have a potential to differentiate into other, unwanted mesenchymal lineages which could also be problematic for their therapeutic use in humans.72 To prevent this unwanted MSCs differentiation, several studies were done in vitro and in vivo to define factors which influence differentiation pathways.73 However, the roles of these factors are not completely understood and differentiation of MSCs to unwanted mesenchymal lineages is still unsolved problem.

Concluding Comment
Experimental evidence indicates that IL-1Ra and MSCs are potential therapeutic agents in the treatment of diabetes mellitus type 1 and diabetes mellitus type 2. Initial clinical trials of the effect of IL-1Ra in type 2 diabetes are encouraging. Furthermore, experimental data suggest that MSCs could be used for prevention and treatment of complications of diabetes mellitus. However, there are still problems in the broader use of IL-1Ra and MSCs in humans such as: short-half life of human recombinant IL-1Ra, potential increase in the incidence of infectious disease because of long-lasting IL-1 blockade or potential risk for malignant transformation of MSCs and their unwanted mesenchymal lineages differentiation. Solving these problems could improve efficacy of IL-1Ra and MSCs that would recommend them as novel therapeutic agents in diabetes mellitus.
References


