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K : Diabetes mellitus; Insulin; Autoimmunity; Regulatory T cells; Immunetherapy

A : NOD: Nonobese Diabetic Mice; Treg: Regulatory T cell; mAb: Monoclonal Antibody; wks: Weeks; LN: Lymph Node

I

Type 1 diabetes mellitus is an immune-mediated metabolic disorder characterized by the destruction of the pancreatic beta cells by both humoral and cell-mediated immune mechanisms. This results in hyperglycemia and metabolic derangements requiring lifelong insulin therapy to sustain life. A hallmark of this chronic autoimmune disease is the lymphocytic infiltration of the islets that develops prior to the clinical onset of disease and is present both in man [1] as well as in animal models of diabetes [2]. Additional proof that this is an immune mediated disorder is the observation that treatment after clinical onset of diabetes with immunosuppressive agents can induce an insulin-free remission of the disease [3]. However, the toxicity associated with the use of immunosuppressive agents has limited this approached as an immune therapy of type 1 diabetes. This has resulted in increased interest in the use of more selective therapies such as the administration of autoantigens to induce protective immune responses and prevent the development of diabetes. Insulin is an important autoantigen in type 1 diabetes. Autoantibodies to insulin are detected prior to initiating insulin therapy [4] and are an important immunological marker of the disease [5].

The nonobese diabetic (NOD) mouse, an animal model of spontaneous diabetes, shares many features of human type 1 diabetes including the abrupt onset of overt diabetes, the dependence on exogenous insulin to sustain life, the presence of lymphocytic infiltration of the pancreatic islet cells before the onset of hyperglycemia, and the prevention of disease by immunotherapy. Insulin specific T cell clones have been isolated from NOD mice and have been shown to be capable of adoptively transferring diabetes in NOD mice [6]. A number of studies have demonstrated that insulin is capable of generating protective immune responses. In preclinical studies, subcutaneous administration of insulin to prediabetic animals has prevented or delayed the development of clinical disease [7,8]. Zekzer and colleagues generated an insulin-specific T cell clone from the pancreatic lymph node of an NOD mouse and demonstrated the ability of this clone to block the spontaneous development of diabetes in NOD mice [9]. However, even with the increasing amount of preclinical data, the translation of insulin-specific therapy for the prevention of diabetes has been difficult. In an NIH multicenter, randomized controlled

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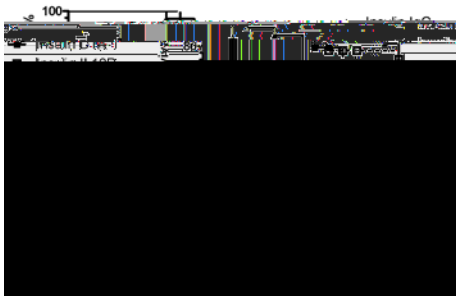
To determine if there was an increase in antigen-specific Treg



Anti-GITR antibody DTA-1 disrupts insulin mediated protection
Simultaneous subcutaneous insulin therapy (ýve days per week) and the the agonist monoclonal anti-GITR antibody DTA-1 or rat IgG was administer weekly for three weeks by intraperitoneal injection beginning at 12 weeks of age. As in Figure 1, mice were monitored weekly for the development of diabetes. Administration of the anti-GITR antibody disrupted the ability of insulin to prevent the development of diabetes (log rank P=0.0436), while mice treated with insulin with rat IgG or insulin with anti-IL10R (log rank P=0.8940 compared to IgG) have signiýcant protection from diabetes.

to modulate the development of diabetes in the NOD mouse model system [8]. Results were presented that addressed the mechanisms by which insulin therapy alters the development of diabetes. Prevention of disease by insulin therapy was accompanied by increased production of insulin-specific regulatory T cells in the lymphoid tissues of treated animals. Inactivation of the regulatory cells by treatment with anti-GITR antibody resulted in enhanced diabetes, demonstrating the functional significance of the insulin-therapy-induced increased production of Treg.

In unimmunized, normal adult mice, approximate 10% of peripheral $CD4^+$ cells and less than 1% of $CD8^+$ in normal adult mice express $CD25^+$ [13]. Numerous studies document the important role of regulatory $CD4^+CD25^+$ T cells (Tregs) that express the transcription factor FoxP3 to maintain self-tolerance and control immune responses (reviewed [14]). Transgenic mice with the FoxP3 scurfy mutation and a diabetogenic T cell receptor BDC 2.5, have accelerated lymphocytic infiltration of their islets and dramatic progression to overt diabetes [15]. Thus, Tregs have an important role in protection from the development of diabetes. There appears to be two important subsets of these regulatory T cells including "natural" $CD4^+CD25^+$ from the thymus and "adaptive" $CD4^+CD25^+$ that are induce in the periphery derived from $CD4^+CD25^-$ T cells if the appropriate antigenic stimulation and cytokine environment are present. Insulin therapy to prediabetic mice appears to induce this peripheral protective immune response.

Our results demonstrate that daily subcutaneous insulin therapy started after the onset of severe insulitis can still prevent the onset of diabetes if given at high dose. Through our long-term follow-up, we demonstrate that diabetes onset is prevented and not just delayed. Lymphocytic infiltration of the islets persists even in mice protected from diabetes. Splenocytes from insulin-protected mice are still capable of transferring diabetes to NOD/scid hosts suggesting the presence of active immune suppression and not insulin-mediated deletion of effector T cells. However, long-term daily subcutaneous insulin therapy induces a protective regulatory response as demonstrated by the presence of CD4+FoxP3+ splenocytes in insulin-protected mice. Compared to mice that are naturally-protected from diabetes, there are equal numbers of $CD4^+FoxP3^+$ regulatory cells but development of diabetes is significantly reduced in mice with the insulin-induced regulatory cells (diabetes free survival of 17% vs 56%, respectively, P=0.0001).

In the insulin-protected mice there was a significant increase in the insulin specific $FoxP3^+$ T cells compared to mice that have natural protection from diabetes. Furthermore, these T cells are $CD103^+CD4^+$. In studies in graftversus-host disease the in vivo-activated $CD103^+CD4^+$ regulatory T cells are more effective at reducing inflammation than naturally-occurring Tregs [12]. Our data would suggest that these cells may also be important in insulin-protection from diabetes.

The daily subcutaneous administration of antigen without adjuvant appears to provide an optimal environment to induce protective immune responses that are mediated by $GITR^+$ regulatory cells. Our previous study [9] demonstrated that inactive insulin is equally effective at preventing type 1 diabetes as metabolically-active insulin. Thus, it is the immunological properties of insulin that are important for preventing type 1diabetes. The advantage of using an inactive insulin analog, is that it is possible to use higher doses of insulin without the risk of developing hypoglycemia in prediabetic individuals to induce this protective immune response and prevent type 1diabetes.

C

Our results demonstrate that in animal models of diabetes, long-term therapy with high dose insulin prevents diabetes, and induces an insulin specific peripheral regulatory T cell response. Induction of a regulatory subset of lymphocytes may be an important factor to prevent the development of type 1 diabetes. The fact that treated mice do not develop diabetes after long-term therapy provides evidence that clinical diabetes is prevented rather than simply being delayed. A key point about the model system presented herein is that we evaluated the ability of subcutaneous injections of insulin, without any adjuvants, to prevent spontaneous diabetes since this would have more relevance for translation into future clinical trials. Our data suggests that the prevention of diabetes by daily subcutaneous injection of insulin is mediated by the generation of insulin-specific regulatory cells. Thus, the subcutaneous delivery of antigen provides a favorable environment for induction of antigen-specific Treg and preventing the clinical onset of Type 1 diabetes.. In future clinical trials, monitoring the development of insulin-specific Treg may be a helpful parameter for improving efforts to use insulin to prevent the development of type 1 diabetes in man.

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incidence of bb wistar rats by early prophylactic insulin treatment of diabetes prone animals. Diabetologia 28: 933-935.

8. Karounos DG, Bryson JS, Cohen DA (1997) Metabolically inactive insulin analog prevents type i diabetes in prediabetic nod mice. J Clin Invest 100: 1344-1348.

9. Zekzer D, Wong FS, Wen L, Altieri M, Gurlo T, et al. (1997) Inhibition of diabetes by an insulin-reactive CD4 T-cell clone in the nonobese diabetic mouse. Diabetes 46: 1124-1132.

10. Diabetes Prevention Trial--Type 1 Diabetes Study Group (2002) Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 346: 1685-1691.

11. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, et al. (2005) Effects of oral insulin in relatives of patients with type 1 diabetes: The diabetes prevention trial-type 1. Diabetes Care 28: 1068-1076.

12. Zhao D, Zhang C, Yi T, Lin CL, Todorov I, et al. (2008) In vivo-activated $CD103^+CD4^+$ regulatory t cells ameliorate ongoing chronic graft-versus-host disease. Blood 112: 2129-2138.

13. Sakaguchi S, Sakaguchi N, Asano M, Itoh M and Toda M (1995) Immunologic self-tolerance maintained by activated t cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 155: 1151-1164.

14. Homann D, Von Herrath M (2004) Regulatory T cells and type 1 diabetes. Clin Immunol 112: 202-209.

15. Chen Z, Herman AE, Matos M, Mathis D, Benoist C (2005) Where $CD4^+CD25^+$ Treg cells impinge on autoimmune diabetes. J Exp Med 202: 1387-1397.