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Role of ICOS pathway in autoimmune and alloimmune responses in NOD mice

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Abstract Islet allografts are subject to alloimmune and autoimmune destruction when transplanted into autoimmune prone animals or humans. The ICOS-B7h pathway plays a role in alloimmune responses, but its function in autoimmunity against islet cells is controversial. We investigated the role of ICOS signaling in autoimmune and alloimmune responses in NOD mice. ICOS blockade prevents development of spontaneous disease in pre-diabetic NOD mice. Furthermore, while ICOS blockade prolongs graft survival in a fully mismatched non-autoimmune islet allograft model in C57BL/6 recipients, it has no beneficial effect in reversing diabetes in models of islet transplantation in NOD mice involving autoimmunity alone or both allo- and autoimmunity. Interestingly, ICOS blockade is effective in prolonging heart allograft (not subject to tissue-specific autoimmunity) survival in NOD mice. We conclude that in islet transplantation and autoimmune diabetes, ICOS blockade can be effective in inhibiting alloimmunity and preventing autoimmunity but is ineffective in inhibiting recurrence of autoimmunity.

Introduction

Type-1 diabetes (T1D) is a chronic autoimmune disease that is increasing in incidence worldwide [1]. Islet transplantation offers a potential cure for T1D [2]. Recurrent autoimmune damage, however, poses a serious challenge to successful long-term islet transplantation for T1D [3–5]. The non-obese diabetic (NOD) mouse is a very relevant and the best available model of T1D [6]. Indeed, the recent report of efficacy of non-mitogenic anti-CD3 mAb in humans with T1D is a direct translation of the original studies in the NOD mice

Abbreviations: ICOS, inducible costimulator molecule; mAb, monoclonal antibody; Ig, immunoglobulin; NOD, non-obese diabetic; T1D, type 1 diabetes; SRL, Sirolimus; DM, diabetes mellitus.

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[7]. In order for islet transplantation to be widely applicable and successful clinically in TID, the immunomodulatory therapeutic strategies should target both autoimmune as well as alloimmune responses.

Blockade of T cell costimulatory pathways provides clinically useful strategies to prevent allograft rejection, including islet transplantation [8]. In recent years, a CD28 homolog termed inducible costimulator (ICOS) expressed on activated T cells has been described [9,10]. ICOS binds to its ligand B7h (also described as ICOS-L, B7RP-1, B7H-2 and GL-50) expressed on B cells, macrophages and dendritic cells [11]. ICOS signaling stimulates both Th_1 and Th_2 cytokines during initial priming and effector T-cell responses [9,10]. In models of transplantation, ICOS blockade, especially in combination with CTLA-4Ig or anti-CD154 mAb therapy prevents acute and chronic allograft rejection [12,13]. We have previously demonstrated that the timing of ICOS blockade, however, is critical, with prolonged allograft survival on delaying the initiation of ICOS blockade until 4 days after transplantation [14]. In a model of islet transplantation utilizing chemically induced diabetic non-autoimmune strain of mice, Nanji et al. recently reported increased ICOS expression in rejected islet allografts and prolongation of graft survival with combination of anti-ICOS mAb and Sirolimus [15]. In autoimmunity, however, ICOS blockade has differential effects in different models and during different phases of the autoimmune response in the same model [16,17]. More recently, in a TCR transgenic model (BDC2.5) of autoimmune diabetes, Herman et al. reported that T regulatory (T_{reg}) cells were dependent on ICOS signaling in the regulation of effector cells in the pre-diabetic lesion [18]. In contrast, Shapiro's group has reported that combined ICOS and CD40-ligand blockade in pre-diabetic NOD mice prevents the development of spontaneous diabetes and emphasized the potential this therapy holds in clinical islet transplantation [19]. Furthermore, a variety of strategies have been reported to achieve stable tolerance to islet allografts involving non-autoimmune strains of mice with chemically induced diabetes mellitus (DM). However, these strategies have proven to be unsuccessful in the NOD mouse, which exhibits an unusually strong resistance to tolerance induction [20,21]. Here, we report the effects of ICOS blockade in the prevention of T1D and islet allograft survival in the NOD mouse models involving autoimmunity alone or both allo- and autoimmunity.

Materials and methods

Mice

Female NOD, NOD.SCID, BALB/c and C57BL/6 mice of various ages were obtained from The Jackson laboratory, housed in specific pathogen free conditions and cared for in accordance with institutional guidelines.

Monitoring for diabetes mellitus

Clinical DM was defined as blood glucose levels >250 mg/dl for three consecutive days. Blood glucose was measured by Accu-Chek Advantage glucometers (Roche Diagnostics). Ten-week-old mice were monitored daily by measuring tail vein blood glucose for the first 3 weeks followed by three times a week until the mice were killed. The neonatal mice were monitored initially for urine glucose on alternate days until 3 weeks of age, followed by blood glucose measurement according to the above schedule.

Murine models of islet transplantation

We have previously described models, utilizing non-autoimmune strains of chemically induced diabetic mice and spontaneously diabetic NOD mice, to study alloimmune, autoimmune and both allo- and autoimmune responses involved in islet allograft rejection [22]. Female C57BL/6 mice were made diabetic by treatment with streptozotocin (225 mg/kg, administered intraperitoneally), and 5 to 7 days after the injection, 800 islets from female BALB/c donors were transplanted under the renal capsule. Female NOD mice were allowed to develop T1D spontaneously, and 1000 islets from female BALB/c or NOD.SCID donors were transplanted under the renal capsule as described before [22]. Islet graft function was assessed with blood glucose measurements initially daily for 1 week and then three times a week until the mice were killed. Reversal of DM was defined as blood glucose levels of <200 mg/dl on 2 consecutive days. Graft rejection was defined as blood glucose levels of >250 mg/dl on 2 consecutive days.

Murine model of heterotopic heart transplantation

To compare the rejection of islet allograft with that of an organ not susceptible to tissue-specific autoimmune destruction, heterotopic heart transplantation (HHT) from female BALB/c donors was performed in pre-diabetic female NOD mice as described previously [5]. Cardiac graft survival was assessed by graft palpation; rejection was confirmed by histology.

Antibodies and treatment protocol

All monoclonal antibodies (mAbs) were administered intraperitoneally: (i) *Diabetes prevention studies*: Pre-diabetic 1- and 10-week-old female NOD mice were treated with anti-ICOS mAb (7E.17G9) or polyclonal isotype control Ig (0.5 mg on day 0 and 0.25 mg on days 2, 4, 6, 8 and 10). (ii) *Treatment of new-onset diabetes*: Anti-ICOS mAb or polyclonal isotype control Ig (0.5 mg on day 0 and 0.25 mg on days 2, 4, 6, 8 and 10) was administered to NOD mice with new-onset diabetes on the third consecutive day of blood glucose levels >250 mg/dl. (iii) *Islet and heart transplantation studies*: Anti-ICOS mAb or polyclonal isotype control Ig was administered utilizing the following treatment protocols: *early ICOS blockade* (0.5 mg on day 0, 0.25 mg on day 2, 4, 6, 8), *late ICOS blockade* (0.5 mg on day 4, 0.25 mg on day 6, 8, 10, 12), *late-prolonged ICOS blockade* (0.5 mg on day 4, 0.25 mg on days 6, 8, 10, 12, 14, 17, 21, 24, 28) with or without Sirolimus (3 mg/kg daily until day 7 followed by alternative days until day 28). Anti-CD154 (MR1) or CTLA-4Ig was administered in a dose of 0.25 mg on days 0, 2, 4 and 6.