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添付資料：研究報告書

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2. 研究テーマ

anti-ICOS antibody inhibited the recall response of long-term accepted heart following skin grafting

3. 成果の概要

The authors investigated the effect of blockade of the ICOS pathway on recall response in rat with cardiac allograft model. The authors report here that the treatment with blockade of ICOS can enhance cardiac allograft survival after following secondary donor type skin grafting, and Anti-ICOS antibody inhibited the immigration of activated memory T cells for target organ but not the function of memory T cells.

4. 研究業績

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anti-ICOS antibody inhibited the recall response of long-term accepted heart following skin grafting

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Abstract

Background: The inducible co-stimulatory molecule (ICOS), a member of CD28/CTLA4 family, is expressed on activated T-cells and effector/memory T cells.

Methods: Hearts from DA (RT1a) rats were transplanted into Lewis (RT1 l) recipients with intravenously single dose of AdCTLA-4Ig at 10^9 plaque-forming units (pfu) immediately after transplantation. The recipients with long-term accepted primary cardiac allografts (over 100 days) were received secondary donor-type skin grafting. Anti-ICOS antibody was injected (1mg/kg intravenously every other day for two weeks) after skin grafting. Immunohistochemistry, mixed lymphocyte reaction (MLR), and flow cytometry were performed.

Results: AdCTLA-4Ig treated recipients rejected primary accepted cardiac allografts following secondary donor type skin grafting. The rejected cardiac allografts appear to be high ICOS positive lymphocytes infiltration. In vitro secondary MLR showed that anti-ICOS antibody could not inhibit recall T cell proliferation response.

Conclusions: our findings suggest that the treatment with blockade of ICOS can enhance cardiac allograft survival, indicating costimulator molecular ICOS signaling plays an important role in the recall response of long-term accepted heart following skin grafting in vivo and vitro. Anti-ICOS antibody inhibited the immigration of activated memory T cells for target organ but not the function of memory T cells.

Keywords Costimulator molecular, memory T lymphocytes, recall, CTLA4Ig

INTRODUCTION

A novel costimulatory molecule, inducible costimulator (ICOS), was identified as the third member of the CD28 family and only express at very low levels on naïve T-cells. ICOS-mediated signal is thought to contribute mainly to regulation of activated T-cells and to effector T-cells functions similar to CD28 [5] [6]. The authors previously reported that administration of cytotoxic T-lymphocyte antigen 4(CTLA4) Ig combined with anti-ICOS antibody yielded long-term anti-specific allograft survival in a rat heart and liver transplantation model [3] [4]. When a previous antigen is encountered, memory T lymphocytes are rapidly mobilized to deliver a recall response that surpasses a primary response to new antigen in speed, magnitude and efficacy [11] [12] [13]. This enhanced memory response is often beneficial, and can provide protective immunity against recurrent pathogens; however, in transplantations the presence of memory immunity is potentially deleterious. Some studies

suggest that the presence of alloreactive memory T cells may impact survival of an allograft. Recent studies have provided evidence that certain newly recognized costimulatory pathways could be involved in the recall of memory T cells but have little effect on naïve T cells. ICOS costimulatory pathways could be a potential approach to inhibiting the recall of alloreactive memory T cells.

In present study, the authors investigated the effect of blockade of the ICOS pathway on recall response in rat with cardiac allograft model. The authors report here that the treatment with blockade of ICOS can enhance cardiac allograft survival after following secondary donor type skin grafting,and Anti-ICOS antibody inhibited the immigration of activated memory T cells for target organ but not the function of memory T cells.

MATERIALS AND METHODS

Hearts from DA (RT1a) rats were transplanted into Lewis (RT1 l) recipients with intravenously single dose of AdCTLA-4Ig at 10^9 plaque-forming units (pfu) immediately after transplantation. The recipients with long-term accepted primary cardiac allografts (over 100 days) were received secondary donor-type skin grafting. Anti-ICOS antibody was injected (1mg/kg intravenously every other day for two weeks) after skin grafting. Immunohistochemistry, mixed lymphocyte reaction (MLR), and flow cytometry were performed.

RESULTS

Prolongation of allograft survival after secondary donor type skin grafting

Treatment with anti-ICOS antibody prolonged cardiac allograft survival (median survival time [MST], >50 days; n=15) significantly in comparison with no-treatment (MST,12 days; n=12; $p<0.05$)(Fig.1).

Histological studies

No-treatment group of cardiac allograft at 10 days after secondary donor type skin grafting showed myocyte necrosis and infiltration of mononuclear in comparison with heart grafts treatment with anti-ICOS antibody (Fig.2).

DISCUSSION

Blockade of CD28-CD80 interaction by CTLA-4Ig prolonged survival of vascularized grafts and frequently led to permanent graft acceptance in animal model including kidney and islet allotransplantations. However, in rat heart transplantation model, an administration of CTLA-4Ig alone did not induce a stable tolerance, only 20% heart allograft survival more than 100 days. Costimulation blockade of the CD154/CD40 pathway in the presence of donor-specific transfusion (DST) has been remarkably successful in promoting permanent survival of heart and islet allograft [9]. However, this same strategy is wholly ineffective if the recipient has been previously primed with donor-specific antigen [10]. The other pathway is the interaction of inducible costimulator (ICOS) on the T cells with B7RP-1 on APC [6]. Blockade of the ICOS/B7RP-1 costimulatory pathway prolongs allograft survival in rodents at a delayed time point after transplantation, suggesting that it interferes with the recall of primed T cells [7]. Those findings suggest that targeting ICOS costimulatory pathways could be a potential approach to inhibiting the recall of alloreactive memory T cells. Recent studies have provided evidence that certain newly recognized costimulatory pathways could be involved in the recall of memory T cells but have little effect on naïve T cells.

Treatment with anti-ICOS antibody prolonged cardiac allograft survival significantly in comparison with no-treatment. Anti-ICOS antibody blocked primary accepted cardiac allograft rejection follow skin grafting. Flow cytometry analysis resealed that the majority of infiltrating T cells express ICOS in peripheral blood and spleen cells, which was also confirmed by immunohischemical staining after

secondary donor type skin grafting. The findings were consistent with those of a previous study showing that ICOS is express strongly on activated T cells at the site of inflammation. When skin transplants were performed, memory T lymphocytes changed to effector phase, but this proliferative response was not inhibited by blockade of the ICOS pathway. The findings suggest that ICOS which regulates immune responses independently as a costimulatory molecule have no function with memory T cells. Treatment with anti-ICOS antibody only inhibited naïve T cells expanding but not division. The blockade of anti-ICOS antibody prevents activated T cells associated with ICOS-Ligand. The activated T cells could not immigrate the target organ. Our results suggested that anti-ICOS antibody plays critical roles in the phase of recall response. In other experiment, we found that anti-ICOS antibody is might to be function with developing of memory T cells, but could not interfere with developed memory T cells function (data not published).

In summary, our findings suggest that the treatment with blockade of ICOS can enhance cardiac allograft survival, indicating costimulator molecular ICOS signaling plays an important role in the recall response of long-term accepted heart following skin grafting in vivo and vitro. Anti-ICOS antibody inhibited the immigration of activated memory T cells for target organ but not the function of memory T cells. In transplantation, it is very important to consider recall response of the memory T cells in understanding the pathogenesis of graft rejection and in designing new and more effective immunosuppression strategies.

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