The Inhibitory Effects of Mouse ICOS-Ig Gene-Modified Mouse Dendritic Cells on T Cells

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The main approach to reduce graft rejection has been focused on the development of immunosuppressive agents at present. Although these strategies have reportedly reduced graft rejection, there has been a reciprocal increase in more severe immunosuppression and lethal infections, as well as severe side effects. Blockade of costimulatory T cell response has been proved as one of useful strategies to reduce graft rejection. Furthermore, it has been shown that infusion of dendritic cells (DCs) with a potent negative regulatory ability for T cells could prolong allograft survival. In this study mouse DCs (mDCs) were transfected with the recombinant plasmid pcDNA3.0 containing mouse inducible costimulator-Ig (mICOS-Ig) cDNA by electroporation. The transient expression of mICOS-Ig in mDC could be detected by ELISA and SDS-PAGE. Mouse ICOS-Ig fusion protein expressed in mDC and mICOS-Ig gene-modified mDC could inhibit lymphocyte proliferation in mixed lymphocyte culture (MLC) *in vitro*. Furthermore, mICOS-Ig gene-modified mDC could inhibit lymphocyte proliferation in recipient mice. These results suggested that mICOS-Ig gene-modified mDC exerted inhibitory effects on T cells, and might be suitable for treatment or prevention of graft rejection and immunopathologic diseases. *Cellular & Molecular Immunology*. 2004;1(2):153-157.

Key Words: dendritic cell, inducible costimulator, mixed lymphocyte culture

Introduction

It has been shown that T cells play an important role in rejection. Activation of T cells needs costimulatory signal provided by costimulators, among which inducible costimulator (ICOS) cloned in 1999 is an important member and can costimulate T cells *in vitro* independently of CD28 (1-11). mICOS-Ig is made of extracelluar domain of mICOS and human IgGFc, which can bind mICOS ligands on the surface of antigen presenting cells (APCs) and block costimulatory signal of ICOS for mouse T cells (12-14). But it does not damage DC, the most effective or professional APCs (15). Furthermore, DC are unique among APCs because of the ability to circumvent the need to internalize exogenous antigens before presenting them (16). Intracellular proteases of DC may degrade the internalized external antigens, generating peptides that can be loaded onto empty MHC molecules on their surface. They initiate and regulate T- and B-cell responses by expressing lymphocyte costimulatory molecules, migrating to lymphoid organs and secreting biologically active molecules.

Recent phase I and II clinical studies have shown the promise in the use of antigen-pulsed autologous DCs for vaccination of cancer patients. DCs not only activate lymphocytes to induce the immune response, but also minimize autoimmune reactions by tolerizing T cells to self-antigens. DCs also have applications in preventing rejection after transplantation (17). They become involved immediately after transplantation. Donor DCs migrate to the draining lymph nodes of the recipient and initiate either graft rejection or graft tolerance. It has been shown that infusion of DCs lacking cell surface costimulatory molecules prolongs allograft survival, whereas *in vitro* manipulation of DC by exposure to a variety of factors (e.g. IL-10 and transforming growth factor-β) can confer tolerogenic properties on these cells (18, 19). Corticosteroids have also been shown *in vitro* to initiate an immunosuppressive role by inhibiting DC maturation and therefore strongly reducing their ability to induce a Th1 response while allowing the selective expansion of IL-10 expressing T cells (20). Tolerance has been associated with the inhibition of antidonor CTL activity and microchimerism (passenger DC associated with the inhibition of antidonor CTL persistence within allografts). The immunologic goal in transplantation is to achieve long-term

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Abbreviations: DC, dendritic cell; ICOS, inducible costimulator; MLC, mixed lymphocyte culture; CTLA-4, cytolytic T-lymphocyte associated antigen-4.

acceptance of an allogeneic organ graft as the result of a brief immunomodulatory procedure, thereby to minimize the risks and toxicity of nonspecific immunosuppression, costimulation blockades such as CTLA-4-Ig have been paid more and more attention to (21). We modified mDC with the plasmid pcDNA3.0 that could express mICOS-Ig fusion protein in eukaryotic expressing system by electroporation, which made mDC express mICOS-Ig fusion protein that has biological function. So mICOS-Ig gene-modified mDC change into DC with a potent negative regulatory ability for T cells and may be suitable for treatment or prevention of graft rejection and immunopathologic diseases.

Materials and Methods

Preparation and identification of plasmid pcDNA3.0

The plasmid pcDNA3.0 that could express mICOS-Ig fusion protein in eukaryotic expressing system was prepared with Wizard Plus Maxipreps DNA Purification System (Promega). It was identified by digestion with EspI and NotI (MBI) and DNA sequencing.

Modification of mDC with the plasmid pcDNA3.0 by electroporation

Mouse DC derived from Balb/C mouse were presented by Prof. Weifeng Chen in Beijing University (22). Mouse DC were transfected with the plasmid pcDNA3.0 by electroporation, then transferred into culture bottle containing 1640 medium including 10% fetal calf serum (FCS, Sigma). Hydromycin (25 μg/ml, Sigma) was added into 1640 medium containing 10% FCS after transfected mDC had been cultured for 60 hours at 37°C 5% CO_2.

Detection of expression of mICOS-Ig in mDC

The culture supernatants of mDC with transient expression of mICOS-Ig was respectively collected. Mouse ICOS-Ig in culture supernatants was detected by ELISA. The ELISA plate was coated with 1:200 dilution of goat anti-human IgG. The secondry antibody was 1:1000 dilution of HRP (horseradish peroxidase)-conjugated goat anti-human IgG. After HRP substrate was added into each well, the optical density (OD) value of each well at 490 nm wavelength was detected. The OD values of culture supernatants of mICOS-Ig gene-modified mDC were compared with negative control (PBS and the culture supernatants of non-modified mDC) and positive control (human IgG). Furthermore, mICOS-Ig fusion protein in culture supernatants of mICOS-Ig gene-modified mDC was detected by SDS-PAGE.

Detection of the effects of mICOS-Ig fusion protein on lymphocyte proliferation by single MLC

Spleen cells separated sterilely from Balb/C mouse were used as stimulating cells and those cells from C57 mouse were used as reactive cells. MLC was performed in 96-well plates, in which the numbers of reactive cells and simulating cells were respectively 2×10^5 and 1×10^5. Culture supernatants (0 μl, 25 μl, 50 μl, 100 μl) of modified mDC were respectively added to the wells of 96-well plate. After 5 days of culture at 37°C 5% CO_2, the activity of cell proliferation was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylerazolium bromide (MTT) (Gibco).

Detection of the effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation by single MLC

Spleen cells separated sterilely from C57 mouse were used as reactive cells and mICOS-Ig gene-modified mDC (A group) and non-modified mDC (B group) were respectively used as stimulating cells. MLC was performed in 96-well plates, in which the numbers of reactive cells and simulating cells were respectively 2×10^5 and 1×10^5. There were five wells in each group. After 5 days of culture at 37°C 5% CO2, the activity of cell proliferation was detected by MTT.

Detection of the effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation in recipient mice by single MLC

Non-modified and mICOS-Ig gene-modified mDCs were respectively input into different C57 mice (2.5×10^6 per mouse) to induce recipient mice. After a week of induction, spleen cells separated sterilely from C57 mice induced by mICOS-Ig gene-modified mDCs, C57 mice induced by non-modified mDCs and C57 mice without induction were respectively used as reactive cells. There were five mice in each group. Non-modified mDCs were used as stimulating cells. MLC was performed in 96-well plate, in which the numbers of reactive cells and simulating cells were respectively 2×10^5 and 1×10^5. After 5 days of culture at 37°C 5% CO_2, the activity of lymphocyte proliferation proliferation was detected by MTT.

Data analysis

When effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation in single MLC and that of recipient mice were detected, data were expressed as mean values ± standard deviation. Analysis for statistically significant differences was performed using *t* test and values of $p < 0.01$ were considered significant.

Results

Identification of the recombinant pcDNA3.0-mICOS-Ig

The plasmid pcDNA3.0 was digested with EspI and NotI. The digested DNA sample and the plasmid pcDNA3.0 were loaded into slots; the gel was run at 40V. As shown in Figure 1A, it proved that the plasmid pcDNA3.0 was successfully prepared and contained the gene fragment of human IgGFc (about 1.4 Kb). The result of sequencing showed that it was accordant with the sequence of extracellular domain of mICOS in Genebank, which proved that the plasmid pcDNA3.0 contained the gene of extracellular domain of mICOS.

Expression of mICOS-Ig in mDC

Expression of mICOS-Ig in mICOS-Ig gene-modified mDC was detected by ELISA. The result (Figure 1B) showed that the OD values of culture supernatants of mICOS-Ig gene-modified mDC and negative control (PBS and culture supernatants of non-modified mDC) were of significant difference, but there was of no significant

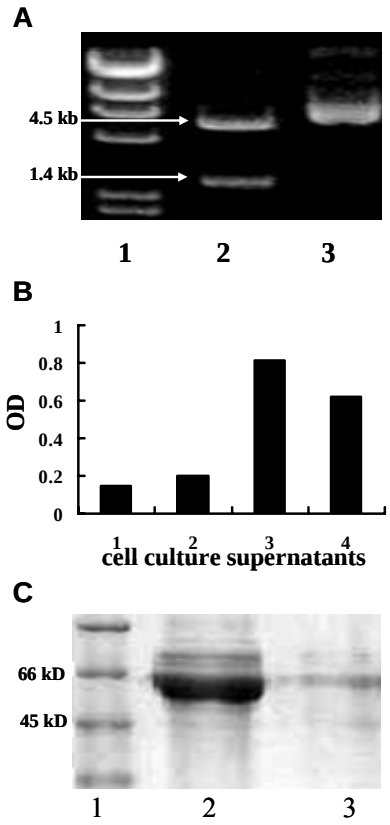

Figure 1. (A) Identification of the recombinant pcDNA3.0-mICOS-Ig. The recombinant pcDNA3.0-mICOS-Ig digested with EspI and NotI turned into two segments. (Lane 1, DNA marker; Lane 2, pcDNA3.0-mICOS-Ig digested with EspI and NotI; Lane 3, plasmid pcDNA3.0.) (B) Detection of mICOS-Ig in mDC by ELISA. The supernatants of modified and non-modified mDC were harvested and then assayed for the concentration of mICOS-Ig respectively. (Lane 1, PBS; Lane 2, supernatants of non-modified DC; Lane 3, human IgG; Lane 4, supernatants of mDC with transient expression.). (C) Detection mICOS-Ig in mDC by SDS-PAGE. The results showed that only the supernatants of modified mDC contained the mICOS-Ig fusion protein, suggesting that mICOS-Ig was successfully expressed in modified mDC. (Lane1, marker; Lane 2, culture supernatants of modified mDC; Lane 3, culture supernatants of non-modified mDC.).

difference between the OD values of culture supernatants of mICOS-Ig gene-modified mDC and that of positive control (human IgG), which proved that the culure supernatants of mICOS-Ig gene-modified mDC contained human IgG. So the plasmid was successfully transfected into mDC and mICOS-Ig was successfully expressed in mDC. Furthermore, the result of SDS-PAGE showed that the culture supernatants of mICOS-Ig gene-modified mDC contained mICOS-Ig fusion protein and the culture supernatants of non-modified mDCs did not contain the protein (Figure 1C). So mDCs were successfully modified by mICOS-Ig gene.

Effects of mICOS-Ig fusion protein on lymphocyte proliferation in single MLC

The results in Figure 2 showed that mICOS-Ig fusion

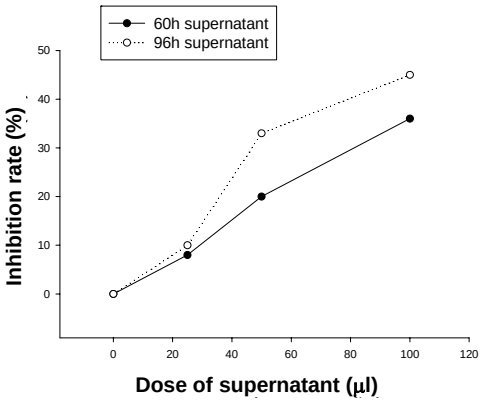

Figure 2 Effects of mICOS-Ig fusion protein on allo-spleen cells proliferation were tested by single mixed lymphocyte culture. The curve above was effects of 60h supernatant containing mICOS-Ig fusion protein on allo-spleen cells proliferation and the curve below was effects of 96h supernatant containing mICOS-Ig fusion protein on allo-spleen cells proliferation, which showed that with the increase of mICOS-Ig, the rate of inhibition enhanced.

protein expressed in mDC could inhibit lymphocyte proliferation in single mixed lymphocyte culture *in vitro* and with the increase of mICOS-Ig, the rate of inhibition was enhanced, indicating that mICOS-Ig gene-modified mDCs could express mICOS-Ig fusion protein which had biological function and changed into dendritic cells with a potent negative regulatory ability for T cells.

Effects of mICOS-Ig gene-modified mDC on lymphoproliferation in single MLC

The results in Figure 3A showed that there were statistically significant differences between the OD value of A group and that of B group, proving that mICOS-Ig gene-modified mDCs could inhibit lymphocyte proliferation in single MLC *in vitro*, indicating that mICOS-Ig gene-modified mDCs changed into dendritic cells with a potent negative regulatory ability for T cells.

Effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation in recipient mice

The results in Figure 3B showed that there were statistically significant differences among the OD values of A group, B group and C group, proving that mICOS-Ig gene-modified mDCs could inhibit lymphocyte proliferation of recipient mice, which indicated that mICOS-Ig gene-modified mDCs might be suitable for treatment or prevention of graft rejection and immunopathologic diseases.

Discussion

The main approach designed to reduce graft rejection has been focused on the development of immunosuppressive agents at present. If the graft is allogeneic bone marrow (BM), another important approach designed to reduce acute graft-versus-host disease (GVHD) is the *ex vivo* removal of the unfractionated donor T cell population from the marrow graft in addition to the approach above. Although these

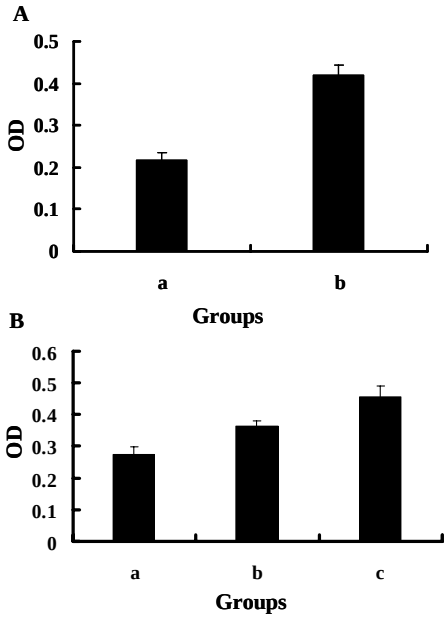

Figure 3. (A) Effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation *in vitro*. MLC was performed and the cell proliferation was detected after 5 day culture. It indicated that mICOS-Ig gene-modified mDCs could inhibit lymphocyte proliferation *in vitro*. Spleen cells sterilely separated from C57 was used as the reactive cells. Group a, mICOS-Ig gene modified mDCs as the stimulating cells; group b, non-modified mDCs as the stimulating cells. (B) Effects of mICOS-Ig gene-modified mDC on lymphocyte proliferation of recipient mice. C57 mice were given modified and non-modified mDCs respectively and lymphocytes were separated from those spleens as the reactive cells in the following lymphocyte proliferation detection. Non-modified mDCs were used as the stimulating cells. It suggested that mICOS-Ig gene-modified mDCs could inhibit lymphocyte proliferation of recipient mice. Group a, mice induced by modified mDCs; group b, mice induced by non-modified mDCs; group c, mice without induction.

strategies have reportedly reduced graft rejection and GVHD, there has been a reciprocal increase in the rate of more severe immunosuppression and lethal infections, as well as severe side effects (23). Mature DC are potent antigen-presenting cells (APCs) to induce T cell response. DC is involved in the induction of peripheral T cell tolerance under steady state conditions. However, the clinical application of immature DCs may not be suitable for the treatment of immunopathogenic diseases, because they likely change into mature DCs under inflammatory conditions (24, 25, 26). Therefore, further development of DCs with a potent negative regulatory ability for T cells is thought to facilitate their use for treatment or prevention of rejection and immuno-pathologic diseases (27).

We have transfected mDCs with CTLA4-Ig and found that CTLA-4-Ig expressed in mDCs could inihibit lympho-cyte proliferation in single MLC *in vitro* and lymphocyte proliferation of recipient mice. It has been reported that CTLA-4-Ig prolongs allograft survival. However chronic rejection follows, which suggests that additional key costimulatory pathways are active *in vivo*. ICOS plays a key role in acute and chronic rejection. It was shown that ICOS-Ig fusion protein suppressed intragraft T cell activation and cytokine expression and prolonged allograft survival (13). But the research about the biological function of mDC modified by mICOS-Ig has not been reported at present.

In this study, we modified mDC with the plasmid pcDNA3.0 that could express mICOS-Ig fusion protein in eukaryotic expressing systems by electroporation. The transient expression of mICOS-Ig in mDC was obtained. It was proved that mICOS-Ig fussion protein expressed in mDC and mICOS-Ig gene-modified mDC could inhibit lymphocyte proliferation in single MLC *in vitro*, indicating that mICOS-Ig gene-modified mDC could express mICOS-Ig fusion protein that had biological function and changed into DC with a potent negative regulatory ability for T cells. Further mICOS-Ig gene-modified mDC could inhibit lymphocyte proliferation of recipient mice, indicating that mICOS-Ig gene-modified mDCs could facilitate their use for treatment or prevention of graft rejection.

Collectively, our research and other research reported proved that the use of DCs with a potent negative regulatory ability for T cells may be therapeutically useful for the treatment or prevention of immunopathologic diseases, graft rejection and acute GVHD in allogeneic bone marrow transplantation (BMT) (27).

Acknowledgement

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