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ORIGINAL ARTICLE

Simultaneous blockade of the CD40/CD40L and NF-κB pathways prolonged islet allograft survival

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Conflicts of Interest
All the authors do not have any possible conflicts of interest.

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Summary

Activation of NF-κB pathway and co-stimulatory system CD40/CD40L promotes the inflammation, which plays a key role in the failure of islet graft. Therefore, the purpose of this study was to determine if simultaneous blockade of CD40/CD40L and IκB/NF-κB pathways could protect islet graft. Streptozocin-induced diabetic Wistar rats were transplanted intraportally with 2000 IEQ islets isolated from Sprague-Dawley rats. The rats were divided into five groups: nontreatment group, AdGFP-treated group, Ad-IκBα-treated group, Ad-sCD40LIg-treated group, and Ad-IκBα-IRES$_2$-sCD40L-treated group. The islet graft mean survival time (MST), insulin expression of islet grafts, and the levels of cytokines in peripheral blood, were measured for the animals in each group. Our study confirmed that islet cells transfected with low doses of adenovirus could achieve high transfection efficiency, and would not affect the function of islet cells ($P > 0.05$). Splenocytes cultured with Ad-IκBα-IRES2-CD40L-transfected islets resulted in homospecific hyporesponsiveness. The islet graft MST (>100 d) in the Ad-IκBα-IRES2-sCD40L-treated group was dramatically prolonged compared with that in the nontreatment group (7.1 ± 1.16 d). In addition, TNF-α, IL-1β, and IFN-γ were diminished in the Ad-IκBα-IRES2-sCD40L-treated group, which was commensurate with the reduced cellular infiltration ($P < 0.01$). Simultaneous blockade of the CD40/CD40L and IκB/NF-κB pathways could effectively extend the survival of islet grafts.

Introduction

The Edmonton Protocol is a procedure in which the islets are isolated from donor pancreas and implanted into the receipts for the treatment of type 1 diabetes The outcome of pancreatic islet transplantation were significantly improved by: using both immunosuppressants (sirolimus and tacrolimus) and monoclonal antibody (daclizumab), and improving isolation yield and transplantation sites [1–5]. However, the insulin independence rate after islet transplantation from one donor pancreas remains low. The barrier of the successful allogeneic transplantation is the rejection of islets by the host immune system and instant coagulation/inflammation reaction (IBMIR), which is characterized by the activation of the coagulation and complement systems, rapid binding and activation of platelets, and leukocyte infiltration into the islets [6,7]. In addition, the transplanted islets are exposed to a variety of stress conditions, e.g., proinflammatory cytokines, leading to the early graft failure [8–12].

It has been demonstrated that lack of co-stimulatory signals can lead to T-cell deletion or anergy and donor-specific tolerance [13,14]. The ligand of CD40 (CD40L) on the activated $CD4^+$ T-cells can interact with CD40, leading the proliferation and activation of B-lymphocytes [15]. Blockade of the CD40/CD40L interaction using CD40L-specific monoclonal antibody (mAb) has been applied in the heart, kidney, aorta, bone marrow, and

skin transplantations. Molano *et al.*, [16] and Jung *et al.*, [17] have also demonstrated that utilization of anti-CD40L mAb can protect the transplanted islets, and induces long-term survival of the islet allograft. Therefore, blockade of the CD40/CD40L pathway is a promising strategy to induce immune tolerance and dramatically prolong the allograft survival.

It has been demonstrated that NF-κB is a key ''switch regulator'' of transcription factors and gene networks, which controls cytokine-induced β-cell dysfunction and death [18]. In most of the cells, NF-κB is sequestered in the cytoplasm by the inhibitor IκB. Upon stimulation, IκB is phosphorylated by IKK, and subsequently polyubiquinated, which triggers its rapid degradation by proteasomes. Consequently, NF-κB is released and translocated into the nucleus, where it activates the expression of target genes, including those encode cytokines, adhesion molecules, chemokines, and acute phase proteins [19]. These molecules are important components of the innate immune response to invading microorganisms, and are required for migration of inflammatory and phagocytic cells to tissues where NF-κB has been activated in response to infection or injury. Giarratana *et al.*, [20] have shown that down-regulation of chemokine production by islet cells could inhibit T-cell recruitment into the pancreatic islets, which was achieved by up-regulation of IκBα, the most important inhibitor of the transcription factor NF-κB [21].

Furthermore, previous studies have shown that activation of CD40/CD40L could lead to the activation of IκB/NF-κB pathway, which provides a positive feedback to further increase CD40 expression [22]. We hypothesized that there is a synergistic effect between these two pathways on the immune tolerance and inhibition of inflammation. To test this hypothesis, we constructed a replication-defective recombinant adenovirus to co-express sCD40LIg and IκBα, and the effect of the co-expression on the survival of islet allograft was determined.

Materials and methods

Animals

Adult male Sprague-Dawley rats were used as donors, and Wistar rats were used as recipients. The animals (age: 6–8 weeks; weight: 200–250 g) were purchased from the Animal Center of the Xi'an Jiaotong University. All animal care and experimental procedures in this study were approved by the Institutional Animal Care and Use Committee of the Xi'an Jiaotong University.

Isolation of islets of langerhans

Pancreas was removed after Sprague-Dawely rats were anesthetized with Ketamine/xylazine (75/10 mg/kg) mixture and administrated with heparin (1000 IU) by intraperitoneal (i.p.) injection. Islets were isolated and purified by digesting pancreatic tissues with 1 mg/ml collagenase P (Roche, Indianapolis, IN, USA) followed by a discontinuous Ficoll (Type 400DL; Sigma, St. Louis, MO, USA) gradient purification [23]. The isolated islets were cultured free-floating in CMRL-1066 media (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS; Hyclone, Logan, UT, USA). The media were changed once every day. Individual islet was handpicked under an inverted microscope and quantified by Dithizone (DTZ; Sigma, St Louis, MO, USA) staining in duplicate using a standard islet diameter of 150 μm as one islet equivalent (IEQ).

Construction and identification of recombinant adenovirus vector expressing IκBα-IRES2-CD40L

Ad-IκBα-IRES2-CD40L, Ad-IκBα, and Ad-sCD40L were constructed in the previous studies to express IκBα and sCD40LIg simultaneously or separately using the pAd-Easy-1 Vector System according to the manufacturer's instruction (Stratagene, La Jolla, CA, USA) [24]. The virus containing only the green fluorescent protein (Ad-GFP) was used as a control. Briefly, the genes encoding CD40L with signal peptide, IκBα and IRES2, were amplified and inserted into pGEMT-easy vector for sequencing. The genes with correct sequences were inserted to shuttle-CMV vector, transduced into AdEasy adenovirus vector system, and the recombinant plasmid pAdv-IκBα-IRES2-CD40L was subsequently obtained. The recombinant vector was propagated and detected in 293 cells.

Glucose-stimulated insulin secretion (GSIS) assay

Islet cells were transfected with Ad-IκBα-IRES2-CD40L at multiplicity of infection (MOI) = 5, 10, and 30, respectively. After 72 h of transfection, the transfection efficiency was determined using fluorescence microscope. Transfected islets were exposed to 50 mg/dl glucose for 2 h, and then to 300 mg/dl glucose for 2 h. The insulin content was measured using an insulin RIA kit (Linco Research, St Louis, MO, USA). The stimulation index was calculated by the following formula: stimulation index = insulin response to high glucose/the insulin response to low glucose.

Mixed lymphocyte-islet reaction (MLIR)

The islets were divided into six groups: negative control group, positive control group (splenocytes co-cultured with untreated-islets), AdGFP-treated group, Ad-IκBα-treated group, Ad-sCD40LIg-treated group, and

Ad-IκBα-IRES2-sCD40L-treated group. The islets from each group were distributed into a 6-well culture plate, with 100IEQ (MOI = 10) in each well. PKH67 (Sigma) labeled allergenic naïve splenocytes from adult male Sprague-Dawley rats were prepared by tissue mincing and hypotonic lysis of red blood cells [25]. Subsequently, the labeled splenocytes were co-cultured with islets of each group for 3 days. PKH67-labeled splenocytes (1.0×10^6) that were not co-cultured with islets were used as negative control group. Cell Quest PRO software was used to obtain the data, and Modfit software was used to analyze the dynamic model of different cell subsets' proliferation. The increment of different cell subsets in different activators was measured. Proliferation index (PI) was obtained directly from the Modfit software. Results were expressed as the mean ± SEM [25].

Induction of diabetes

Wistar rats were fed standard rodent chow in a barrier animal facility under a condition with 12 h of light and 12 h of dark cycle. At the age of 8 weeks, hyperglycemia was induced by a single intraperitoneal injection of 220 mg/kg streptozotocin (STZ, Sigma) and confirmed by measurement of blood glucose using a glucometer (Fisher, Pittsburgh, PA, USA). Rats were considered hyperglycemic when their blood glucose levels were higher than 300 mg/dl and were used as recipients.

Islet transplantation and assessment of graft function

Diabetic Wistar rats were randomly divided into control or experimental groups with six animals in each group. Rats in the control group received nontransfected allogeneic islets. For experimental groups, rats received allogeneic islets transfected with AdGFP, Ad-sCD40L, Ad-IκBα, or Ad-IκBα-IRES2-sCD40L, respectively. Islets (2000 IEQ) were transplanted into the portal vein by gravity over a period of 5–10 min [26]. The nonfasting blood glucose levels were monitored three times per week for 30 days post transplantation. Normoglycaemia was defined when two consecutive blood glucose level measurements showed less than 200 mg/dl. Islet graft failure was considered as the recurrence of hyperglycemia (defined as serum glucose >300 mg/dl) for three consecutive days after transplantation [27].

Morphological and histochemical examination of islet grafts

The graft-bearing liver retrieved 1, 2, and 4 weeks after transplantation were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin. The sections (5 μm thick) were stained with hematoxylin and eosin. Immunohistochemical staining for insulin was performed using the avidin-biotin-peroxidase complex method. The immunoreaction was visualized with 3-amino-9-ethylcarbazol. All sections were slightly counterstained using Mayer hematoxylin. Rat pancreas was used as a positive control.

RT-PCR analysis of ICAM-1 and VCAM-1 expression

Transcription of ICAM-1 and VCAM-1 in islet graft was measured 7 days after islet transplantation using RT-PCR. Pure islet cells from tissue frozen sections were obtained by applying LCM, and then minimal RNA was extracted, purified, and concentrated. The primers were designed by Beijing Biological Academy (Beijing, China), and their mRNA probes were synthesized using PCR. Primer sequences for ICAM-1 were P1 5′-GGT CAG GAC GGT GCT GGT GAG GAG AGA TCA CCA TGG A-3′ and P2 5′-AGA AAT TGG CTC CAT GGT GA-3′. Primer sequences for VCAM-1 were P1 5′-CAC CTC CCC CAA GAA TAC AGA and P2 5′-GCT CAT CCT CAA CAC CCA CAG-3′. PCR products were electrophoresed on 2% agarose gel. The gel was stained with ethidium bromide, digitally photographed, and scanned using UVI Gel Analyzing System (UVI Tech, Cambridge, England).

Western blot analysis

Cell lysates (50 μg) of islet graft tissue were separated on 10% SDS polyacrylamide gel, and electrophoretically transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Nonspecific binding was blocked with 10% nonfat milk for 1 h. The membrane was then probed first with the primary antibodies that recognize CD40 at 4 °C overnight. After washing for three times with TBST buffer, the membrane was incubated with a horseradish peroxidase conjugated goat-anti-rabbit IgG secondary antibody (1:2000) for 1 h at room temperature. After three times of washing in TBST, membranes were detected using an ECL Western blot Substrate (PIERCE, Rockford, IL, USA).

Cytokine detection by ELISA

Peripheral blood of recipients rats were obtained 1 week after transplantation. Sera were separated by centrifugation at 258 ***g*** for 5 min. The concentration of TNF-α, IL-1β, and IFN-γ in the rat blood serum was measured using commercially available ELISA kits (Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions.

Statistical analysis

Data were expressed as the mean ± SEM. The statistical significance of differences was determined using one-way analysis of variance tests. Islet graft survival was evaluated using Kaplan–Meier analysis and log-rank tests. $P < 0.05$ were considered statistically significant (version 13.0, SPSS, Chicago, IL, USA).

Results

Separation, purification and identification of rat pancreatic islets

After retrograde infusion of collagenase and in situ perfusion, the pancreas expanded rapidly, and the surrounding tissue could be easily distinguished. DTZ staining was performed 10 min after the incubation and digestion in a 37 °C tank. The morphology of islet cells was intact with a scarlet color, whereas the exocrine tissue outside the pancreas almost showed no color. Islet cells (700 ± 120) were obtained with an average purity of 85% and an intact cellular morphology after purification.

Co-expression of sCD40LIg and IκBα in 293 cells

We first determined the co-expression of the sCD40LIg and IκBα in 293 cells. Immunofluorescense staining showed that IκBα was mostly expressed around the nucleus (Fig. 1a). In contrast, sCD40LIg was mainly localized in the cell membrane and cytoplasm. As a negative control, there was almost no expression of the sCD40LIg and slight expression of IκBα in the 293 cells transfected by AdGFP (data not shown).

Transfection efficiency of islet cells was directly related to the dose of adenoviral vectors

To block the function of NF-κB and CD40, islet cells were transfected with adenovirus containing the gene of IκBα and sCD40L. After 72 h of infection, EGFP was expressed in 57.12 ± 1.34%, 97.69 ± 0.94%, and 100 ± 0.00% of islet cells transfected with Ad-IκBα-IRES2-sCD40L at an MOI of 5, 10, and 30, respectively (Fig. 1b and c). These results suggested that the expression rate of EGFP in transfected islet cells was directly related to the dose of adenoviral vectors.

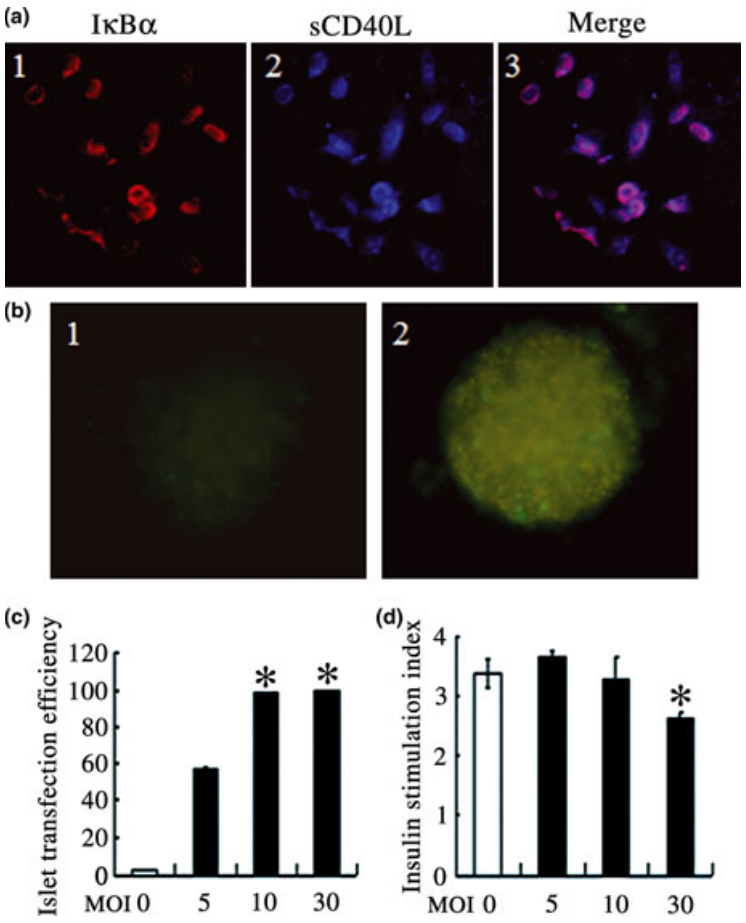

Figure 1 (a) Co-expression of sCD40LIg and IκBα in 293 cells was detected by the blue and red fluorescence, respectively (×400). The expression of IκBα was mostly around the nucleus (a1), and sCD40LIg was mainly in the cell membrane and cytoplasm (a2). The merged image was shown in a3. (b) Islet cells transfected with adenovirus vector *in vitro*. Islets cells transfected with empty adenovirus vector were used as control. b1: The negative control group; b2: Islet cells transfected with Ad-IκBα-IRES2-sCD40L at MOI of 10. (c): Islet cells transfected with Ad-IκBα-IRES2-sCD40L at MOI of 5, 10, and 30 (mean ± SEM, $n = 3$, $^*P < 0.05$ vs. control and MOI of 5). (d): The insulin stimulation index of islet cells transfected with adenovirus vector at different MOI (mean ± SEM, $n = 3$, $^*P < 0.05$ vs. control).

Identification of MOI that does not affect insulin secretion in the islet cells transfected with Ad-IκBα-IRES2-sCD40L

The glucose-stimulated insulin secretion assay was performed to detect if the function of insulin secretion in islet cells was affected by Ad-IκBα-IRES2-sCD40L transfection. Insulin stimulation index was not significantly different between the control and the islet cells transfected with Ad-IκBα-IRES2-sCD40L at an MOI of 5 and 10 ($P > 0.05$) (Fig. 1d). However, the stimulation index in the islet cells transfected with Ad-IκBα-IRES2-sCD40L at an MOI of 30 was 2.63 ± 0.11, which was significantly lower than that in other groups ($P < 0.05$). Therefore, islet cells transfected with adenovirus vector at an MOI of 10 was used in the following experiments.

Blockade of NF-κB and CD40 inhibited MLIR

To assess the role of IκBα and sCD40L expression in response to allogeneic stimulation, MLIR was performed before the transplantation of transfected islet cells to the diabetic Wistar rats. Significant proliferation was observed in the mononuclear cells co-cultured with each group of islet cells except the control and Ad-IκBα-IRES2-CD40L-transfected groups (Fig. 2). Co-culture with islet cells transfected with Ad-IκBα or Ad-sCD40L reduced the proliferation of spleen cells compared with the positive control or AdGFP group, but still higher than control group. Furthermore, inhibitory effect of Ad-sCD40LIg-treated group was stronger than that of Ad-IκBα-treated group, whereas the mononuclear cells were not proliferated in the Ad-IκBα-IRES2-CD40L-transfected group.

Effect of NF-κB and CD40 blockade on the survival of allogeneic islet graft

Transplantation of Wistar rats with 2000 IEQ islets, 3 days after Streptozotocin treatment consistently resulted in the restoration of blood glucose to normal levels. As shown in Fig. 3a, MST of the diabetic rats transplanted with nontreatment group of islet cells was 7.1 ± 1.16 days. MST of the diabetic rats transplanted with Ad-sCD40L-transfected and Ad-IκBα-transfected islet cells was 47.8 ± 9.02 days ($P < 0.01$) and 44.6 ± 8.84 days ($P < 0.01$), respectively. Blood glucose levels in the diabetic rats transplanted with Ad-IκBα-IRES2-CD40L-transfected islet cells were stable for as long as 100 days, at which time the experiment was terminated. We also determined insulin in islet grafts that were transplanted in the liver at 1, 2, and 4 weeks after transplantation using immunohistochemistry (Fig. 3b and c). The expression of insulin in the control group was undetectable, but the inflammatory cell infiltration was observed surrounding the graft. Less inflammatory cell infiltration and stronger insulin staining were detected in the Ad-sCD40L group and Ad-IκBα group. The Ad-IκBα-IRES2-sCD40L group showed the strongest insulin staining without inflammatory cell infiltration. We also found that Adenoviral-mediated gene transfer was capable of inducing sustained gene expression after 2 and 4 weeks of transplantation (Fig. 3c).

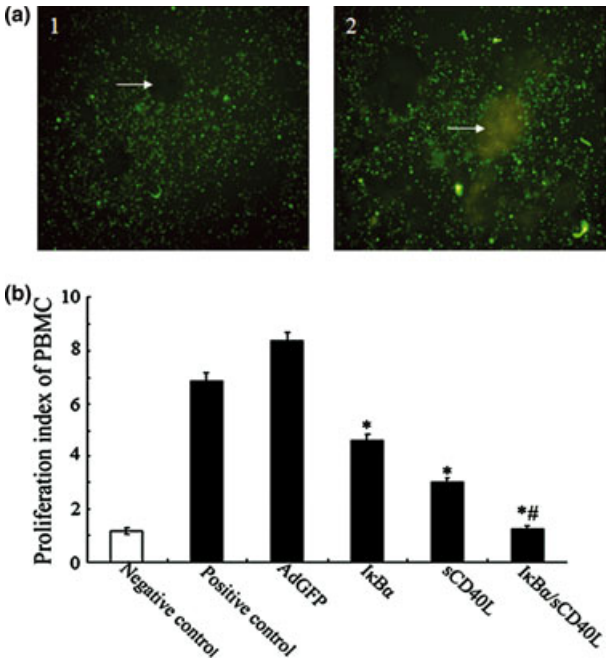

Figure 2 Inhibition of MLIR by NF-κB and CD40 blockade. (a) Mixed lymphocyte-islet reaction (MLIR): naive allogenic splenocyte cells were labeled by PKH67 (magnification×200). (b) Inhibition of lymphocyte proliferation by Ad-IκBα-IRES2-sCD40L transfection (MOI=10). Mean ± SEM, $n = 3$, $*P < 0.05$ vs. positive control, $\#P < 0.05$ vs. Ad-IκBα group and Ad-CD40L group.

Expression of ICAM-1 and VCAM-1 was down-regulated by blocking CD40/CD40L or/and IκB/NF-κB pathways

Transcription of ICAM-1 and VCAM-1 in the graft of Ad-sCD40L, Ad-IκBα, and Ad-IκBα-IRES2-sCD40L groups, was significantly lower than that in the nontreatment or EGFP control groups ($P < 0.05$). Interestingly, there were no significant difference for VCAM-1 expression between the Ad-sCD40L group and the Ad-IκBα group. However, ICAM-1 expression in Ad-IκBα group was significantly lower than that in the Ad-sCD40L group. The lowest mRNA level of ICAM and VCAM was observed in the Ad-IκBα-IRES2-sCD40L group (Fig 4a).

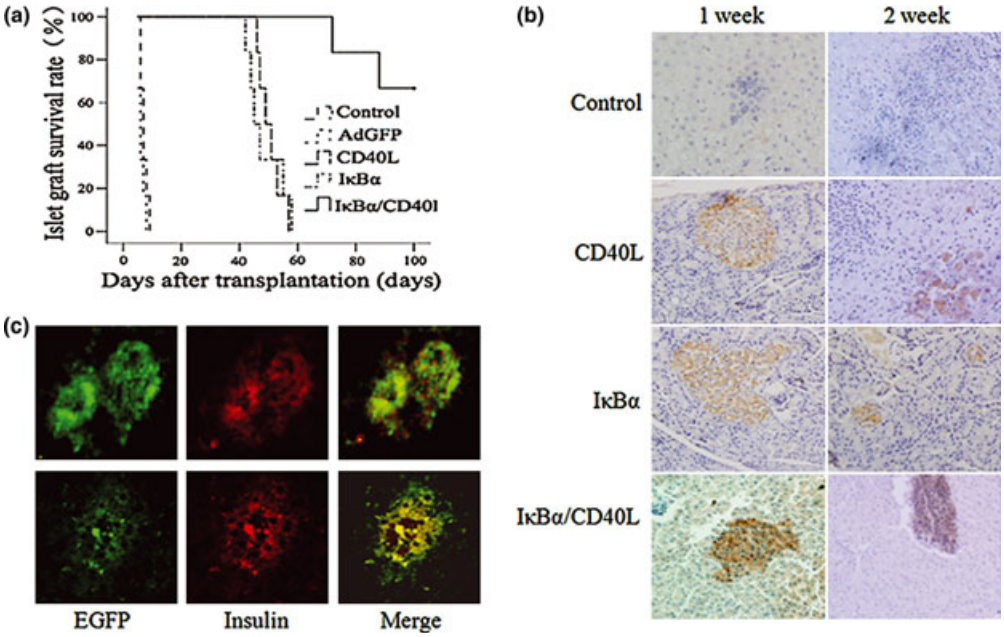

Figure 3 Effect of NF-κB and CD40 blockade on the survival of allogeneic islet graft. (a) Survival of islet allograft survival in diabetic Wistar rat recipients. (b) Detection of insulin in islet grafts transplanted into liver using immunohistochemistry (magnification×400). (c) The co-expression of the insulin and EGFP was examined in islet grafts using immunofluorescence staining, 2 and 4 weeks after transplantation (magnification ×400).

Down-regulation of CD40 expression

CD40 expression level in the graft was determined using Western blot after 7 days of islet transplantation. CD40 expression level was markedly reduced in the Ad-IκBα group, Ad-sCD40L group, and Ad-IκBα-IRES2-sCD40L group compared with that in the control group. Furthermore, the strongest inhibition of CD40 expression was found in the Ad-IκBα-IRES2-sCD40L group. These results indicated that blocking CD40/CD40L or/and IκB/NF-κB pathways could reduce CD40 expression around the graft, and also suggested that the induction of CD40 expression is at least partially mediated by NF-κB activation (Fig 4b).

Effect of NF-κB and CD40 blockade on cytokines in peripheral blood

As IκB and CD40 blockade could inhibit the expression of ICAM-1, VCAM-1, and CD40 in the graft (Fig. 4a and b), we further examined the production of proinflammatory cytokines in peripheral blood. The result showed that the levels of IL-1β, TNF-α ,and IFN-γ in the Ad-IκBα group, Ad-CD40L, and Ad-IκBα-IRES2-CD40L groups were significantly lower than those in the nontreatment group (Fig. 5). However, the level of these cytokines was not significantly different between the Ad-sCD40L group and the Ad-IκBα group ($P > 0.05$). Furthermore, IL-1β, IFN-γ, and TNF-α contents in the Ad-IκBα-

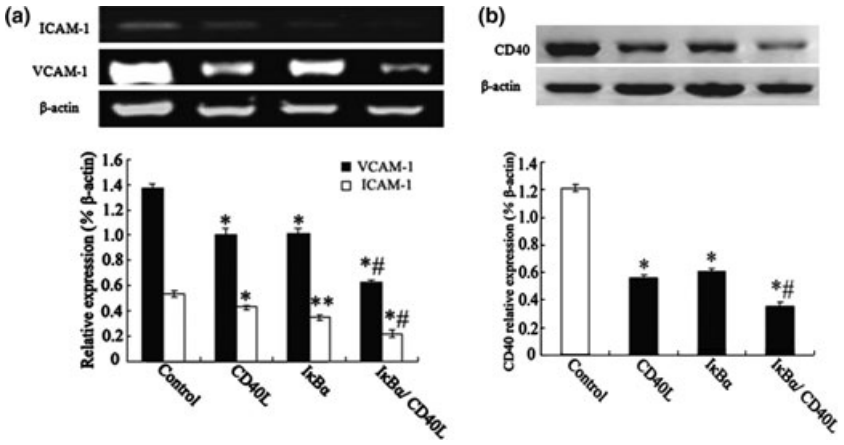

Figure 4 Down-regulation of ICAM-1 and VCAM-1 mRNA levels and CD40 expression by blocking CD40/CD40L or/and IκB/NF-κB pathways.

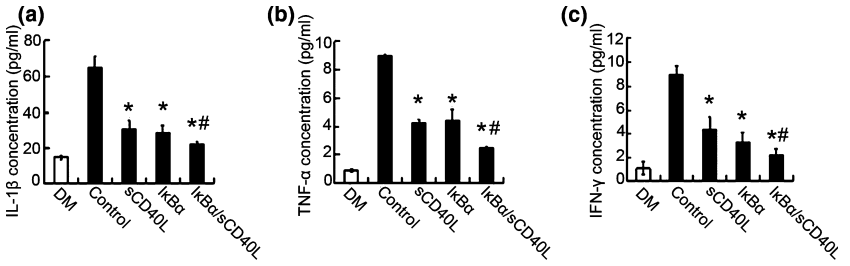

Figure 5 Detection of cytokines in peripheral blood after 1 week of islet transplantation using ELISA. TNF-α, IFN-γ, and IL-1β level of treatment groups were decreased significantly compared with the nontreatment group (mean ± SEM, $n = 3$, $^*P < 0.01$ vs. control group). IL-1β, TNF-α, and IFN-γ contents in the Ad-IκBα-IRES2-CD40L group were significantly lower than that in the Ad-IκBα group and Ad-CD40L group (mean ± SEM, $n = 3$, $\#P < 0.01$ vs. Ad-IκBα group and Ad-CD40L group).

IRES2-CD40L group were significantly lower than those in the Ad-IκBα and Ad-CD40L groups ($P < 0.01$).

Discussion

In this study, we showed that simultaneous interruption of CD40/CD40L and IκB/NF-κB pathways can extend the survival of grafts after allogeneic islet transplantation. We found that all the STZ-induced diabetic rats transplanted with Ad-IκBα-IRES2-sCD40L-transfected islet cells maintained blood glucose at normal level for more than 100 days. In contrast, none of the rats (zero of six) in the control group could maintain normal blood glucose level for more than 10 days. A previous study reported that normoglycemic condition in all STZ-induced diabetic rats was extended for 56 days in the Ad-sCD40LIg treated group [28], which might be because of the different injection route. *In vitro* transfected-islet cells can be targeted to the graft. Local immunosuppressive effect can be achieved without affecting the general immune status of the recipient as local revascularization by gene transfer strategies [29].

In vitro, splenocytes cultured with Ad-IκBα-IRES2-CD40L-transfected islets induced homospecific hyporesponsiveness, whereas the control adenovirus stimulated the proliferation. *In vivo*, the expression of insulin in the graft of control group was undetectable, whereas the inflammatory cell infiltration was observed around the graft. In the Ad-IκBα-IRES2-sCD40L group, the graft expressed high level of insulin without inflammatory cell infiltration. Furthermore, peripheral blood TNF-α, IL-1β, and IFN-γ levels were diminished, which was consistent with the reduced cellular infiltrate. These results indicated that simultaneous interruption of both CD40/CD40L and IκB/NF-κB pathways can effectively reduce inflammation and the proliferation and activation of lymphocytes, thereby reducing the rejection reaction and inflammatory damage to islet allograft [30,31]. We reasoned that there is a synergistic effect between these two pathways.

In this study, we found that ICAM-1 and VCAM-1 expressions in the graft tissue were decreased after simultaneous blockade of both CD40/CD40L and IκB/NF-κB pathways. Previous studys have demonstrated that higher level of VCAM-1 and ICAM-1 allows leukocytes and platelets to adhere to the vessel walls and disturb the graft microcirculation, leading to induction of inflammatory cytokines and instant coagulation/inflammation reaction [32,33].

Increasing body of evidence suggests that NF-κB is an important mediator in the pathophysiology of inflammatory or septic diseases characterized by elevated levels of cytokines and ROS [34]. Recent studies have shown that NF-κB plays an important role in the CD40 expression [35]. Both these mechanisms are able to disrupt the cell via the activations of inflammatory processes or via apoptosis. Our findings may add a further explanation of the beneficial effects of blocking IκB/NF-κB pathway on islet grafts.

Our results demonstrated that co-administration of both Ad-sCD40L with Ad-IκBα induced persistent expression of EGFP in islet cells and insulin on day 28, which is consistent with previous reports showing that adenoviral-mediated gene transfer is capable of inducing sustained gene expression 4 weeks after treatment in the pericardia [36,37].

In summary, we developed a relatively simple and safe method of adenovirus-mediated gene transfection to islet cells *in vitro* to induce systemic immune tolerance and reduce inflammation, which significantly prolonged the survival of islet grafts. We also found that there was a synergistic effect between the CD40/CD40L and NF-κB pathways on induction of immune tolerance and inhibition of inflammation. Utilization of Ad-IκBα-$IRES_2$-sCD40L provides a blueprint for better protection of islet mass and for improvement of islet engraftment during islet transplantation. The mechanisms by which these two pathways interact require further investigations.

Authorship

X-HW: designed, performed and analyzed the research, and wrote the paper. X-MD: designed and performed research. H-BL: designed research, contributed reagents, and helped write the paper. YL: performed research and collected data. W-JX: designed research. X-HT: performed research and analyzed data. X-SF: contributed important reagents. F-MJ: performed research. JZ: contributed substantially in writing the paper.

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