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Full Length Research Paper

Effects of anti-CD25 monoclonal antibody on the corneal allograft rejection in a rat model

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This study aims to investigate the effects of anti-CD25 monoclonal antibody (mAb) on the effector T cells (Teff) and CD4+CD25+ regulatory T cells (CD4+CD25+Treg) in corneal allograft rejection. Wistar rats were used as donors and SD rats were used as recipients. Routine penetrating keratoplasty (PKP) was performed. 90 SD rats were randomly divided into 5 groups: A - E. Group A (n = 6) was normal group and rats in Groups B (n = 24), C (n = 18), D (n = 18) and E (n = 24) were transplanted and subconjunctivally treated with normal saline, 100 µg anti-CD25 mAb, 100 µg anti-CD25 mAb plus 50 µg dexamethasone and 100 µg dexamethasone, respectively, on day 0, 2, 4, 6 and 8 following transplantation. The average transplant survival time in the Group B was significantly shorter than that of Groups C, D and E ($P < 0.05$). The mRNA expression of IFN-γ, CD25 or FOXP3 was not detected in the Group A. Compared to the Groups C, D and E, the mRNA expression of IFN-γ and CD25 in the grafts of Group B was markedly increased ($P < 0.05$) on day 11 following PKP. Compared with the Groups D and E, the mRNA expression of FOXP3 in the grafts of Group C was markedly decreased ($P < 0.05$). When compared with Group B, the mean IFN-γ level in the aqueous humour was remarkably decreased in Groups C, D and E ($P < 0.05$) on day 6 and 11 following PKP 11 days after PKP, the mean IFN-γ level in the aqueous humour of Groups D and E was profoundly decreased compared to the Group C ($P < 0.05$). No significant difference was observed in Groups D and E. But high-dose dexamethasone monotherapy have a high risk of side effects. The results suggest Teff and CD4+CD25+Treg play important roles in the corneal allograft rejection.

Key words: Anti-CD25 monoclonal antibody, CD4+CD25+ regulatory T cell, effector T cell, corneal transplantation.

INTRODUCTION

Corneal transplantation is the predominant strategy in regaining sight in patients with corneal diseases. Although the success rate of surgery is relatively high, postoperative immunological rejection is still a main cause of transplant failure (Yamagami et al., 1996; Price et al., 2003). To date, glucocorticoids and cyclosporine (CSA) have been commonly applied in the prevention and treatment of corneal allograft rejection. But severe complications including infection, cataract, glaucoma and nephrotoxicity can be observed in patients after long term treatment with these drugs. Therefore, it is imperative to develop new immunosuppressive drugs with high effectiveness and low toxicity. Anti-CD25 monoclonal antibody (mAb) is a novel immuno-suppressive drug and has exerted protective effects on the immunological rejection following kidney transplantation. However, little is known about the effects of anti-CD25 mAb on corneal allograft rejection although occasional studies have reported the application of anti-CD25 mAb in the prevention and treatment of immunological rejection following corneal transplantation (Hoffmann et al., 1994; Zhong et al., 1997). The present study aimed to investigate the effects of anti-CD25 mAb on the effector T cells and regulatory T cells and to further explore the preventive effects of anti-CD25 mAb on the corneal allograft rejection, which may provide experimental basis for clinical application of anti-CD25 mAb in ophthalmology.

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MATERIALS AND METHODS

Materials

Animals

Wistar rats (male or female; specific pathogen free) weighing 180 ~ 220 g and male Sprague-Dawley (SD) rats (specific pathogen free) weighing 180 ~ 220 g were purchased from the Experimental Animal Center of the Southern Medical University. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Experimental Animal Center, Chinese PLA General Hospital. This study was approved by the ethics committee of the Chinese PLA General Hospital.

Reagents

ELISA kits (Bender MedSystems) for IFN-γ and IL-4, TRIzol, kits for RT-PCR (KeyGEN), mouse anti-rat CD25 monoclonal antibody (Tellides et al., 1989) (MCA494XZ, NDS61, Serotec), dexamethasone sodium phosphate injection and 0.9% sodium chloride injection were used in the present study.

Methods

Grouping and modeling

A total of 42 Wistar rats were used as donors and 90 SD rats as recipients. These SD rats were randomly divided into 5 groups: Groups A - E. Rats in Group A (n = 6) did not receive treatment and those in the other 4 groups received corneal transplantation. Rats in Group B (n = 24) were treated with normal saline in which 12 rats were used for observation of corneal rejection and survival time. Rats in Group C (n = 18) were treated with 100 μg of anti-CD25 mAb in which 6 rats were used for observation of corneal rejection and survival time. Rats in Group D (n = 18) were treated with 100 μg of anti-CD25 mAb plus 50 μg of dexamethasone in which 6 rats were used for observation of corneal rejection and survival time. A total of 24 rats were included in the Group E and were treated with 100 μg of dexamethasone and 12 rats were used for observation of corneal rejection and survival time. Subconjunctival injection was performed on day 0, 2, 4, 6 and 8 following transplantation.

The surgical technique used for penetrating keratoplasty was similar to that described by Williams and Coster (1985). The right eyes of recipient rats underwent transplantation and the donor rats provided bilateral corneas. Tropicamide drops and tetracaine 1% drops were administered thrice 15 min before surgery. A 3.5 mm diameter full thickness corneal disc was trephined from the center of the donor cornea and grafted into a 3.00 mm graft bed of the recipient cornea, which was then secured with 8 interrupted 10/0 nylon sutures. Sutures were cut as short as possible, but were not removed. Aseptic technique was used throughout the surgery and sterilized air was injected forming anterior chamber.

Observation of grafts

Recipient corneas were examined daily under a slit lamp microscope. Corneal allograft opacity, edema and vascularization were graded according to criteria described by Larkin et al. (1997) (Table 1). The rejection index (RI) was defined as the sum of scores for opacity, edema and vascularization. Corneal allograft rejection was defined as RI ≥ 5 or opacity ≥ 3. The time of rejection was recorded. The rats with transplant failure caused by infection, hyphema, absence of anterior chamber or severe edema within 5 days after surgery were excluded from the study.

Detection of IFN-γ and IL-4 by ELISA

Aqueous humour from 4 eyes of each group (Group A - E) was obtained on day 6, 11 and 24 following surgery. Briefly, rats were anesthetized and conjunctival sac was cleared. The maximum available volume of aqueous was removed from the graft recipient eye through a peripheral corneal incision. The aqueous humour from normal rats was used as control. Aqueous humour was centrifuged at 1,000 g for 5 min at 4℃ and the supernatant was removed, which was stored at -80℃ for use. The levels of IFN-γ and IL-4 were determined with ELISA. The concentration of standard IFN-γ was set at 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8, 3.9 and 0 (pg/ml) independently. The concentration of standard IL-4 was set at 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0. The remaining procedures were performed according to manufacturer's instructions. The optical density (OD) was obtained at 450 nm with a microplate reader (BIO-RAD) and standard curve was delineated. The concentrations of IFN-γ and IL-4 of samples were calculated according to the standard curve.

Expression of IFN-γ, CD25 and FOXP3 mRNA

The normal and transplanted corneas were removed and the mRNA expression of IFN-γ, CD25 and FOXP3 was determined by RT-PCR:

(1) Total RNA was extracted with TRIzol;
(2) RT-PCR was performed according to instructions;
(3) Amplification: For IFN-γ, CD25:

The cycling conditions for PCR were 30 cycles of denaturation (94℃ for 30 s), annealing (48℃ for 30 s) and extension (72℃ for 30 s). A preheating step at 94℃ for 3 min and a final extension step consisting of 5 min at 72℃ were also carried out. For FOXP3: The cycling conditions for PCR were 30 cycles of denaturation (94℃ for 30 s), annealing (52℃ for 30 s) and extension (72℃ for 30 s). A preheating step at 94℃ for 3 min and a final extension step consisting of 5 min at 72℃ were also carried out. For G3PDH: The cycling conditions for PCR were 35 cycles of denaturation (94℃ for 30 s), annealing (50℃ for 30 s) and extension (72℃ for 30 s). A preheating step at 94℃ for 3 min and a final extension step consisting of 5 min at 72℃ were also carried out. The product was separated by 1% agarose gel electrophoresis and stained with ethidium bromide.

Representative photographs were captured followed by analysis with a gel imaging analysis system. The density of bands was normalized by that of G3PDH. The specific primers were synthesized in the Jetway Biotech Co., Ltd. CD25: forward: 5'-TGTCTGTATGACCCACCG-3', reverse: 3'-CTCTATTCCACCTGC GTA-5', the anticipated size of amplified products was 453 bp; IFN-γ: forward: 5'-GTCTTGGTTTTGCAGCTC-3', reverse: 3'-CTTTCGGATCTTTCAGACT-5', the anticipated size of amplified products was 82 bp; FOXP3: forward: 5'-ACCCAGGAAA GACAGCAAC-3', reverse: 3'-AACAAACGACACGCCTCT-5', the anticipated size of amplified products was 403 bp; G3PDH: forward: 5'-TATCGGACGCCTGGTTAC-3', reverse: 3'-GTTCCGACTCTTA CCCTT-5', the anticipated size of amplified products was 159 bp.

Statistical analysis

The quantitative data were presented as mean ± standard deviation ($\bar{x}$ ± SD) and statistical analysis was performed with statistic

Table 1. Grading of clinical findings used in diagnosis of graft rejection.

Index score	Opacity	Oedema	Vascularization
0	Completely transparent	No oedema	No vascularization of graft
1	Minimal loss of transparency	Moderate oedema	Vessel growth to 25% of graft radius in any quadrant
2	Moderate loss of transparency, but iris vessels visible on retroillumination	Marked oedema with obvious graft thickening	Vessel growth to 50% of graft radius
3	Iris vessels not visible, but pupil outline visible		Vessel growth to 75% of graft radius
4	Pupil outline not visible		Vessel growth to centre of graft

software SPSS 11.5. One way ANOVA was used for comparisons between multiple groups. ANOVA with factorial design was conducted for comparisons between groups with multiple contributing factors. Multiple comparisons between means were performed with LSD method. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Corneal allograft rejection and time to rejection

Transplant failure caused by hyphema, absence of anterior chamber and infection was observed in 6 eyes, which were excluded from the study, and extra rats were supplemented. The time to corneal rejection was 10.583 ± 1.084 d, 13.167 ± 1.169 d, 17.333 ± 2.160 d, and 16.417 ± 1.379 d in the Groups B, C, D and E, respectively. Compared to Group B, the time to rejection in the Group C, D and E was significantly prolonged ($P<0.05$). The survival time of corneal graft in the Group D and E was longer than that in the Group C ($P<0.05$). However, no significant difference in the survival time of corneal graft was observed between Groups D and E ($P > 0.05$).

Expression of IFN-γ and IL-4 in the aqueous humour

(1) Delineation of standard curve and establishment of regression equation: Based on the concentrations of standard IFN-γ or IL-4 solution, as well as the OD values, the standard curves for IFN-γ and IL-4 were determined and regression equation was obtained as follows:

IFN-γ: =-0.0121+0.0010X (R2=0.995, F=1821.70, P=0.000)

IL-4: $\hat{Y}$ = 0.3556+0.0371X (R^2= 0.908, F= 69.49, P = 0.000)

(2) Levels of IFN-γ (Table 2 and Figure 1) and IL-4 (Table 3 and Figure 2) in the aqueous humour. Significant difference in the IFN-γ level was found among different groups (F = 54.021, P = 0.000), among different time points (F = 75.187, P = 0.000). Interactive effect between groups and time points was found (F = 13.721, P = 0.000). The level of IFN-γ in the aqueous humour of Group B was markedly higher than that of normal group on day 6, 11 and 24 following surgery ($^1P < 0.05$). In addition, the IFN-γ level in the aqueous humour of Group B 11 days after transplantation (155.947 ± 23.807 pg/ml) was higher than that at days 6 and 24 following surgery ($^2P < 0.01$). The IFN-γ level in the aqueous humour of Groups C, D, and E on day 6 and 11 following surgery was lower than that of Group B at the same time points ($^3P < 0.05$), but no profound difference in the IFN-γ level was found between Groups C, D, E and B 24 days after transplantation ($^4P > 0.05$). When compared with Group C, the IFN-γ level of Groups D and E on day 11 after surgery was markedly decreased ($^5P < 0.05$), however, no significant difference was found in the IFN-γ level between Groups D and E 11 days after transplantation ($^6P > 0.05$).

Significant difference in the IL-4 level was found among different groups (F = 282.217, P = 0.000), among different time points (F = 88.417, P = 0.000). Interactive effect between groups and time points was found (F = 57.975, P = 0.000). The level of IL-4 in the aqueous humour of Group B was markedly higher than that of normal group on day 6, 11 and 24 following surgery ($^1P < 0.05$). The IL-4 level of Group B on day 11 after surgery was only slightly increased compared to those 6 days after transplantation ($^2P > 0.05$). The IL-4 level of Group B 24 days after transplantation was markedly increased when compared with that on day 11 following surgery ($^3P < 0.05$). The IL-4 level in the aqueous humour of Group B on day 6 and 11 following surgery was lower than that of Group C at the same time points ($^4P < 0.05$), but higher than that of Groups D and E ($^5P < 0.05$). 6 and 11 days after transplantation, the IL-4 level of Groups D and E were dramatically decreased compared to Group C ($^6P < 0.05$). On day 24 following surgery, no significant difference in the IL-4 level was found between Groups C, D and B ($^7P > 0.05$), and between Groups D, E and C ($^8P > 0.05$). Nevertheless, in the Group C, the IL-4 level at day 11 after surgery was higher than that on day 6 and 24 following transplantation ($^9P < 0.05$).

Table 2. The level of IFN-γ in the aqueous humour at different time points after surgery ($\bar{x}$ ±SD, pg/ml).

Group	n	IFN-γ level			F	P
		6 d	11 d	24 d		
A	9	3.503 ± 1.789	3.473 ± 0.870	4.113 ± 1.280	0.210	0.816
B	9	55.180 ± 8.253[1]	155.947 ± 23.807[1,2]	44.303 ± 13.396[1]	41.881	0.000
C	9	27.657 ± 6.826[3]	103.407 ± 17.114[3]	45.373 ± 3.992[4]	39.756	0.000
D	9	26.307 ± 6.418[3]	64.407 ± 11.405[3,5,6]	53.377 ± 12.159[4]	10.840	0.010
E	9	23.903 ± 2.102[3]	67.440 ± 23.800[3,5]	54.070 ± 9.691[4]	6.734	0.029
F		31.104	30.270	14.630		
P		0.000	0.000	0.000		

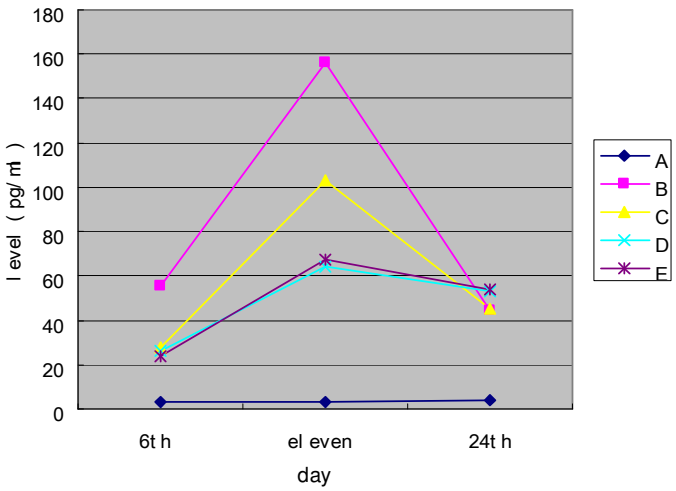

Figure 1. The IFN-γ level in the aqueous humour at different time points after surgery ($\bar{x}$ ±SD, pg/ml).

mRNA expression of CD25, IFN-γ and FOXP3

The mRNA expression of IFN-γ, CD25 and FOXP3 on day 11 after transplantation was presented in the Figures 3a - c, respectively. The expression of IFN-γ, CD25 and FOXP3 could not be detected in the normal cornea. The gray values of IFN-γ, CD25 and FOXP3 in each group were normalized by that of G3PDH.

The mRNA expression of IFN-γ in the corneas of Groups B, C, D and E was 0.695 ± 0.036, 0.498 ± 0.025, 0.359 ± 0.040 and 0.335 ± 0.059, respectively. Compared to Group B, the IFN-γ expression in the Groups C, D and E was markedly decreased ($P < 0.05$). The IFN-γ expression in the Groups D and E was lower than that of Group C ($P < 0.05$). However, no significant difference in the IFN-γ expression was found between Groups D and E ($P > 0.05$).

The expression of CD25 in the corneas of Groups B, C, D and E was 0.985 ± 0.150, 0.218 ± 0.095, 0.554 ± 0.059 and 0.537 ± 0.224, respectively. When compared with Group B, the CD25 expression in the Groups C, D and E was markedly decreased ($P < 0.05$). In addition, the CD25 expression in the Groups D and E was higher than that in the Group C ($P < 0.05$). However, no marked difference in the CD25 expression was found between Groups D and E ($P > 0.05$).

The expression of FOXP3 in the corneas of Groups B - E was 0.544 ± 0.117, 0.352 ± 0.043, 0.825 ± 0.028 and 0.933 ± 0.163, respectively. Compared to Group B, the FOXP3 expression was only slightly decreased in Group C ($P > 0.05$), but was significantly increased ($P < 0.05$) in Groups D and E. In addition, the FOXP3 expression in Groups D and E was higher than that in Group C ($P < 0.05$). However, no marked difference in the FOXP3 expression was found between Groups D and E ($P > 0.05$).

DISCUSSION

Establishment of corneal allograft rejection animal model and its importance

In studies on immunology of cornea transplantation, the allogeneic corneal transplantation rat model is an ideal model. Because the background of inbred rats is clear, the amount of monoclonal antibodies against rat cells and other immune molecules is abundant, the major histo-compatibility antigens of rats are similar to humans and especially the expression of major histocompatibility anti-gens in the cornea of rats is almost the same to humans (Treseler and Sanfilippo, 1986; Katami, 1991). In the present study, penetrating keratoplasty was performed between Wistar rats and SD rats, which express different major histocompatibility antigens. In the control group, corneal rejection was observed average 11 days after transplantation. Therefore, the corneal allograft rejection was investigated on day 6 (pre-rejection), 11 (rejection) and 24 (post-rejection) following transplantation. Our results showed the treatment with immune-suppressants significantly prolonged the survival time compared to control group. Therefore, the establishment of this corneal rejection model is important to investigate the immune mechanism underlying corneal transplantation

Table 3. The level of IL-4 in the aqueous humour at different time points after transplantation ($\bar{x}$ ±SD, pg/ml).

Group	*n*	IL-4 level			F	P
		6 d	11 d	24 d		
A	9	2.287±0.297	2.132±0.220	2.021±0.418	0.513	0.623
B	9	37.523±3.006[1]	40.577±4.086[1,2]	13.033±3.509[1,3]	53.928	0.000
C	9	46.460±3.847[4,9]	71.400±4.858[4]	19.130±4.456[7,9]	105.596	0.000
D	9	11.400±2.675[5,6]	16.150±1.800[5,6]	18.803±1.645[7,8]	9.665	0.013
E	9	10.207±1.738[5,6]	15.033±3.542[5,6]	19.930±2.841[8]	9.000	0.016
F		162.583	201.363	19.765		
P		0.000	0.000	0.000		

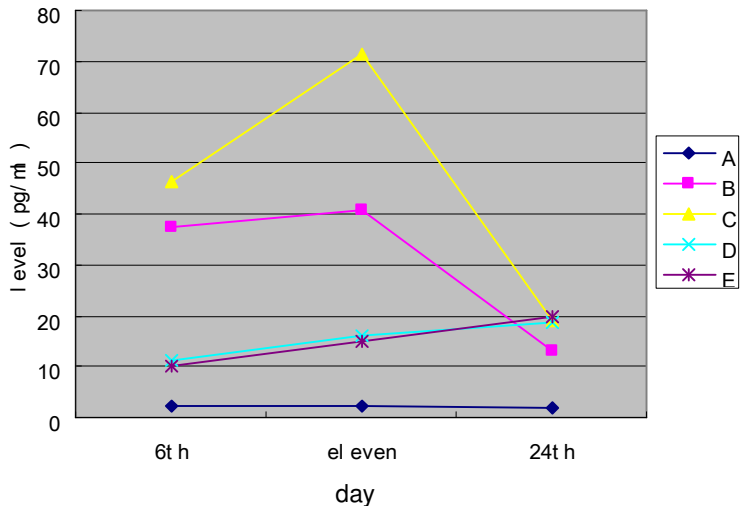

Figure 2. The IL-4 level in the aqueous humour at different time points after transplantation (±SD, pg/ml).

and immunosuppressive therapy of corneal allograft rejection.

Effects of CD25 monoclonal antibody on effector T cells

Immunological rejection following corneal transplantation is a complicated process involving multiple factors, but the exact mechanism is still poorly understood. Some studies have indicated that the rejection following allogeneic corneal transplantation is a delayed type hypersensitivity (DTH) which is mediated by effector T cell (Teff) (Niederkorn and Mellon, 1996; Yamada et al., 1998).

CD25 is the alpha chain of the IL-2 receptor (IL-2R) and an important marker of T cell activation as well as initiation of immune response. Under the stimulation of antigens, the secreted IL-2 by activated T cells binds to the own IL-2R resulting in the proliferation of activated cells via autocrine, subsequently leading to acute rejection. The CD25 monoclonal antibody can specifically bind to the IL-2R on the activated T cells blocking the binding between IL-2R and the secreted IL-2, which results in suppression of cell activation and proliferation. In addition, CD25 monoclonal antibody also down-regulates the expression of CD25 on the T cells (Lietz et al., 2001) exerting immunosuppressive effects. IFN-γ is the main cytokine secreted by CD4+Thl cells. IFN-γ predominantly activates macrophages, promotes the expression of MHC and antigen presentation and inhibits Th2 cells. IL-4 is mainly secreted by CD4+Th2 cells. The expression of IL-4 is related with Th2 reaction. IL-4 can promote Th2 cell differentiation, assist Th2 cell mediated humoral immunity, inhibit the proliferation of Thl cells and down-regulate the secretion of Th1 cytokines.

Cornea has unique anatomical and physiological characteristics and exists in a relatively independent immune micro-environment. The corneal allograft rejection is closely associated with anterior chamber. The corneal endothelial cells are the main target cells in the rejection and aqueous humour is the local micro-environment for endothelial cell survival. Studies have

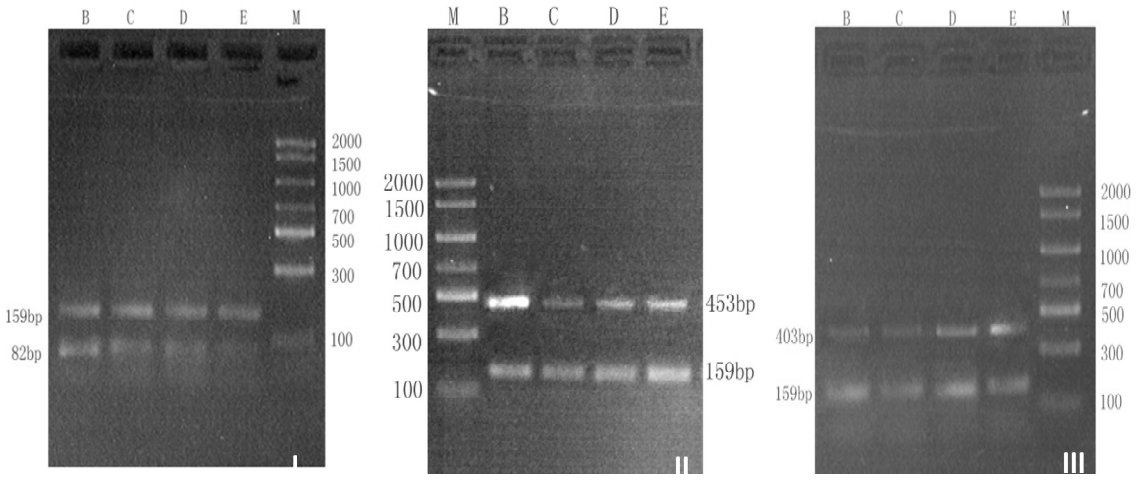

Figure 3. mRNA expression of IFN-γ, CD25 and FOXP3 in the corneal grafts of Groups B, C, D and E. IFN-γ expression; α: CD25 expression; β: FOXP3 expression.

indicated changes in certain components in the aqueous humour were strongly related with corneal rejection (Funding et al., 2005; Yamagami et al., 2003).

Our results showed only extremely small amount of IFN-γ and IL-4 was found in the aqueous humour of normal rats. Six days after transplantation, in Group B, the content of IFN-γ and IL-4 in the aqueous humour was markedly increased. The IFN-γ content reached a maximum on day 11 following transplantation, and the IL-4 content was not profoundly changed on day 6 and 11 after surgery. The eleventh day after transplantation was in an acute rejection period, and the IFN-γ (Th1 factor) level was significantly increased, while IL-4 (Th2 factor) level was also increased postoperatively but without significant difference. The reason was that corneal allograft rejection was a main Th1 (IFN-γ)-mediated immune response, so IFN-γ level was significantly increased. In the rats with CD25 mAb treatment, the IFN-γ level in the aqueous humour was decreased, but the IL-4 level was increased on day 6 following surgery compared to control group. On day 11 after transplantation, the IFN-γ level and IL-4 level in the aqueous humour was significantly decreased and markedly increased, respecttively, when compared with the control group. In Groups D and E, the levels of IFN-γ and IL-4 were markedly decreased on day 6 and 11 following surgery compared to the control group. The survival time of grafts in the rats treated with CD25 mAb alone was dramatically prolonged when compared to the control group. Furthermore, the survival time of grafts in the Groups D and E was longer than that in the Group C. Therefore, we postulated that the suppressive effects of CD25 mAb on the corneal rejection were mediated by down-regulating IFN-γ (Thl) expression and up-regulating IL-4 (Th2) expression. However, the protective effects of CD25 mAb plus dexamethasone were mediated by down-regulating IFN-γ (Thl) and IL-4 (Th2) expression.

Studies have indicated the interaction between Th1 cytokines and Th2 cytokines. Th1 cytokines are related with transplant rejection and Th2 cytokines with transplantation tolerance (Amirzargar et al., 2005; Furukawa et al., 2005). But other studies also showed the association between Th2 cytokines and transplant rejection and inhibitors of Th2 cytokines could prolong the survival time of grafts (Hsu et al., 2003; Wang et al., 2005). The relationship between Th1 to Th2 shift and corneal allograft rejection is complex. In the study of Gong et al. (2006), adenovirus carrying CTLA4Ig gene was intraperitoneally applied in the allogeneic corneal transplantation and protective effects on the transplant rejection were achieved. Additionally, the expression of Th1 cytokines and Th2 cytokines was markedly decreased in the corneas after this treatment. Pindjakova et al. (2005) found the expression of Th1 cytokines and Th2 cytokines was markedly increased after xenogenic corneal transplantation, which was significantly decreased after treatment with CD4 mAb accompanied by markedly prolonged survival time of the graft.

Based on the previous results and our findings, we postulated that the strategy aiming to block immune response with involvement of Th1 and Th2 cytokines may be more effective in suppressing corneal allograft rejection and prolonging graft survival time than that aiming to promote the shift from Th1 cell response to Th2 cell response.

Effects of CD25 mAb on regulatory T cells

CD4+CD25+ regulatory T cells (Treg) are a T cell subset with immunological regulation (immune anergy and immunosuppression) which is different from Th1 cells and Th2 cells. Treg can suppress the activation of autoimmune cells through direct interaction with target cells or

secreting regulatory cytokines (IL-10 and TGF-β), which play important roles in maintaining homeostasis of immunologic tolerance and immune response (Stephens et al., 2004; Chatila, 2009). The balance between Teff cells and Treg cells is crucial for the stability of autoimmune response. CD25 is a marker of not only Teff cell activation but Treg cells. Therefore, detection of CD25 is difficult to differentiate Teff cells and Treg cells. Numerous studies showed (Hori et al., 2003; Fontenot et al., 2003; Chauhan et al., 2009) the development and maintenance of immunologic function of Treg cells are related with Foxp3, which is a specific marker of CD4+CD25+Treg cells (Ramsdell, 2003) and plays pivotal roles in the regulation of Treg cell development. Foxp3 can be detected in CD4+CD25+ T cells with immunosuppressive properties but not in those without immunosuppressive characteristics. Therefore, Foxp3 expression was detected as a marker of CD4+CD25+ Treg cells.

Our results showed CD25 mAb not only inhibited effector T cells, but suppressed regulatory T cells. In the present study, the mRNA expression of IFN-γ, CD25 and FOXP3 was determined by RT-PCR. Results indicated normal cornea did not have mRNA expression of IFN-γ, CD25 or FOXP3. However, during corneal allograft rejection (11 days after transplantation), the mRNA expression of IFN-γ, CD25 and FOXP3 was detectable and significantly different from that in the groups treated with immune-suppressants. In the corneas of rats treated with CD25 mAb, the IFN-γ and CD25 expression were down-regulated suggesting the number of CD4+CD25+ Treg cells was reduced. However, CD25 mAb in combination with low dose glucocorticoid not only suppressed effector T cells but relatively promoted regulatory T cells, which further enhanced post-operative immunosuppression.

So the graft survival time (17.3 d) of rats treated with CD25 mAb in combination with low dose glucocorticoid was markedly prolonged, when compared with those treated with CD25 mAb alone (13.2 d). Although the survival time of rats treated with CD25 mAb in combination with low dose glucocorticoid was not profoundly different from that of rats treated with glucocorticoid alone, but side effects might be observed after long term administration with high dose glucocorticoid. Therefore, CD25 mAb in combination with low dose glucocorticoid is more clinically applicable.

The conclusion on relationship between FOXP3 expression and immunologic tolerance or immune is complex and controversial. Certain studies proposed increased FOXP3 expression could suppress post-transplantation rejection. Several studies (Li et al., 2006; Webster et al., 2009) showed, after transplantation, the over-expression of FOXP3 or increased number of CD4+CD25+Treg could induce immunologic tolerance. But other investigations also indicated high expression of FOXP3 in the grafts with post-translantation rejection (Dijke et al., 2007; Demirkiran et al., 2007; Steger et al., 2006; Hautz et al., 2009). Muthukumar et al. (2005) found that, in patients with kidney transplantation, the urinary level of FOXP3 was markedly increased during rejection. They also speculated that FOXP3 might be a marker of acute rejection and the lower the FOXP3 expression, the shorter the survival time of grafts. Dijke et al. (2007) proposed that the increased number of FOXP3 positive Treg cells during rejection was a response aiming to suppress Teff cell response.

Because CD25 mAb can specifically block CD25 resulting in functional suppression of CD25, the application of CD25 mAb not only inhibits activated T cells, but affects the functions of CD4 + CD25 + Treg cells. The effects of CD25 mAb on the CD4+CD25+ Treg cells are still controversial. It has been indicated that administration with CD25 mAb could down-regulate the expression of Foxp3 and CTLA4 in mice with allogeneic liver transplantation and reduce the proportion of CD4+CD25+ Treg cells in activated T cells which then disturbed the balance between regulatory T cells and activated T cells and prevented against immunologic tolerance (Li et al., 2006). But other researchers did not found the changes in the number of CD4+FoxP3+Treg cells after treatment with CD25 mAb (Kohm et al., 2006).

Conclusion

In the present study, results showed CD25 mAb could reduce the incidence of corneal allograft rejection via suppressing and promoting the expression of Th1 cytokines (IFN-γ) and Th2 cytokines (IL-4), respectively, resulting in prolonged survival time of grafts. However, the CD25 mAb in combination with dexamethasone or dexamethasone alone suppressed both Th1 cytokine (IFN-γ) and Th2 cytokine (IL-4) expression, and the survival time was markedly longer than that in the rats treated with CD25 mAb alone. Therefore, we postulated that the suppression of both Th1 cytokines and Th2 cytokines was beneficial for the graft survival. Effector T cells and regulatory T cells played important roles in the process of rejection.

Inhibition of effector T cells and promotion of regulatory T cells may be crucial for the prevention and treatment of corneal rejection. CD25 mAb not only suppressed the functions of effector T cells, but inhibited those of regulatory cells. Nevertheless, CD25 mAb in combination with dexamethasone or dexamethasone alone suppressed the functions of effector T cells and promoted those of regulatory cells which were beneficial for the graft survival.

The effects of CD25 mAb in combination with low dose dexamethasone and dexamethasone alone on the prevention and treatment of corneal rejection were comparable. Additionally, administration with CD25 mAb plus low dose dexamethasone avoid the side effects of long term treatment with high dose dexamethasone and more clinically applicable in the prevention and treatment of corneal rejection.

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