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Paired Immunoglobin-like Receptors A and B Are New Targets for Inducing Dendritic Cells Tolerance in Mice*

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Summary: The expression of paired immunoglobin-like receptors A (PIR-A) and B (PIR-B) and their relationship with tolerogenic dendritic cells (T-DC) in mice were investigated. The mouse DCs line, DC2.4 cells were cultured with the recombinant murine interleukin-10 (IL-10) and recombinant human transforming growth factor β1 (TGF-β1) respectively to develop the T-DC and stimulated with lipopolysaccharide (LPS) for 48 h to induce the mature dendritic cells (LPS-DC). Special small interfering RNAs (siRNA) molecule for PIR-B was chemically synthesized and transfected into DC2.4 cells (Si-DC) by lip2000. The expression of PIRs on DC2.4 cells were detected by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR), flow cytometry (FCM) and Western blot. Realtime reverse transcriptase-polymerase chain reaction (Realtime-PCR) was applied for measurement of PIR-A and CD80, CD86, MHC-Ⅱ mRNA expression. The allogeneic stimulating capacity of DCs was measured by mixed lymphocyte reaction (MLR) using ^{3}H-thymidine incorporation test. The concentration of IFN-γ in supernatants of MLR from distinct groups was analyzed by ELISA. The results showed that PIR positive rate was (28.65±8.12)% examined by FCM on DC2.4 cells. PIR positive rate was increased dramatically to (54.21±6.34)%, (58.78±4.70)%, (48.24±6.75)% respectively for IL-10, TGF-β1 and LPS induction (P<0.01), but there was no significantly different among the three groups (P>0.01). The semi-quantitative RT-PCR and Western blot revealed that IL-10 and TGF-β1 induced the higher PIR-B level and lower PIR-A level. On the contrary, the LPS down-regulated the PIR-B expression and up-regulated the PIR-A expression. Realtime PCR examination demonstrated that PIR-A and co-stimulating molecules such as CD80, CD86 and MHC-Ⅱ were increased significantly after stimulation with LPS. Compared with the DC2.4 cells and the LPS-DC, the T-DCs inhibited alloactived T cell proliferation and down-regulated the IFN-γ secretion in MLR supernatant. Si-DC promoted the T cell proliferation (P<0.01) and enhanced the IFN-γ secretion (P<0.01). It was concluded that up-regulating the PIR-B and down-regulating the PIR-A expression were the general feature of phenotype and constructed the new targets for dendritic cells to acquire immune tolerance in mice. Overexpression of PIR-B can inhibit the up-regulation of the PIR-A, CD80, CD86 and MHC-Ⅱ expression, which might be the molecular mechanism for the T-DC.
Key words: dendritic cell; tolerance; receptor, paired immunoglobin-like; siRNA

Dendritic cells (DCs) are crucial to maintain the peripheral immune activation or tolerance[1]. The tolerant DCs (T-DCs) can attenuate specific alloreactivity of T lymphocyte proliferation while maintaining graft-versus-tumor effect (GVT) and are still the ideal target cells for immune tolerance[2, 3]. Recently, studies have demonstrated that multiple pathways including IL-10 and TGF-β1 produce the T-DCs[4, 5], but the mechanism is not understood. The work in this area may provide new strategies for immune tolerance induction.

Paired immunoglobin-like receptor (PIR)-A and PIR-B are respectively active and inhibitive receptors belonging to typical murine pair of the immunoglobulin-like receptor family, expressed mainly on antigen presenting cells (APC), such as monocytes, macrophages and DCs[6]. The orthologue of PIR-B in human are immunoglobin-like transcript (ILT)3 and ILT4, the important molecules to acquire tolerance for DCs[7]. But no study about the expression of PIR-A/B on T-DCs and its function in mouse were reported. With the object of DC2.4 cells derived from the C57B L/6 mice, the aim of this study was to investigate the PIR-A/B expression and its relationship with T-DCs mechanism in mice. The work may help to provide new research orientation or molecular target for genetic therapy in immune tolerance area.

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1 MATERIALS AND METHODS

1.1 Cell Line Culture and Induction

The mouse DC2.4 cell line was bought from the De-

partment of Immunology, Tongji Medical College, HUST (China), cultured in RPMI1640 containing 10% new-born calf serum (GIBCOL, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Recombinant murine interleukin-10 (rmIL-10, 20 ng/mL, R&D, USA) and recombinant human TGF-β1 (rhTGF-β1, 20 ng/mL, R&D, USA) were used to induce the T-DCs for 6 days as IL-10 group and TGF-β1 group respectively. Lipopolysaccharide (LPS, 1 μg/mL, R&D, USA) was used to induce the mature DCs (LPS-DC) for 48 h as LPS-DC group. The DCs without any treatment served as control group.

1.2 Transfection of siRNA

Twenty-four hours before siRNA transfection, 2.5×10^4 cells/well were seeded in 24-well plates in OPTI-minimal essential medium (OptiMEM, Invitrogen, USA) containing 10% new-born calf serum without antibiotics. Cells were seeded per 24-well plate to give 30% to 50% confluence at the time of the transfection. The transfection of siRNA (Invitrogen, USA) molecule was performed using Silencer ™ siRNA construction kit (Invitrogen, USA) with a final concentration of 200 nmol/L using Oligofectamine (Invitrogen, USA) by Lip2000 (Invitrogen, USA) according to the manufacturer's instructions. DC2.4 cells transfected with FITC-OligOfluorence served as a positive control group to detect the transfection efficiency. The non-transfected DCs and DCs transfected with PBS were taken respectively as blank control and blank transfection groups. DC2.4 cells transfected with siRNA for 24 and 48 h served as si-DC-24 h group and si-DC-48 h group respectively. The forward specific sequence for PIR-B was 5'-AUU GAG AGC CUU CAG GUA CAU AUG C-3' and the downward sequence 5'-GCA UAUBGUA CCU GAA GGC UCU CAU U-3' respectively.

1.3 Semi-quantitative RT-PCR Assay for Detection of PIR-A/B mRNA Expression

Total mRNA extracted from the DC2.4 cells by Trizol (Promega, USA) was reversely transcribed to the first-strand cDNA with oligo (dT18) and AMV reverse transcriptase (Takara, Japan) and amplified with a common forward primer (5'-CCTGTGGAGCTCACAGTC TCAG-3')[2] and the PIR-A-specific primer (5'-CCCAG AGTGTAGAACATTGAAGATG-3') or PIR-B-specific primer (5'-GTGTTCAGTTGTTCCCTTGACATGA-3') (Invitrogen, USA), and fragments of 257 bp for PIR-A and 470 bp for PIR-B yielded. The amplification reaction underwent 30 cycles of: degeneration at 95ºC for 1 min, annealing at 58.5ºC for 1 min, and extension at 72ºC for 1 min. A final extension was performed at 72ºC for 10 min.

Amplification of β-actin transcription with the primers 5'-CTG GCA CCA CAC CTC C0TA CA-3' and 5'-AGT ACT TGC GCA CAG GAG GA-3' was also performed as a control. The purity and amount of RNA was determined using an ultraviolet (UV) spectrophotometer at 260 nm and 280 nm (*A*260/*A*280>1.8). The amplified product was electrophoresed in 2% agorose and stained with ethidium bromide. Photodensity scan was performed by UVItec GAS9500 system (British) assayed by the ratio of PIR-A/β-actin, PIR-B/β-actin.

1.4 Detection of DCs PIR-A and Co-stimulatory Molecules by SYBR Green I Real-time PCR

Total mRNA extraction and the first-strand cDNA conformation were same as that in the semi-quantitative RT-PCR assay. SYBR green 1 was diluted with a ratio of 1:10 000 and added to the reaction. The mRNA of PIR-A, CD80, CD86, MHC-Ⅱ were examined by fluorescent quantitation PCR meter (U7900, USA). The primers were designed with Primer 5.0 (table 1). The mRNA expression was detected by transcrition accumulative index ($TAI=2^{-\text{delta delta CT}}$). The black control group and the Si-DC-24h group stimulated with the LPS for 24h were used to detect the PIR-A, CD80, CD86, MHC-Ⅱ mRNA expression.

1.5 Assay of PIR by Flow Cytometry

10 000 cells for each sample were stained with PE-anti-mouse PIR-A/B (6C1, R&D, USA) and sorted by flow cytometry (Becton Dickinson, USA, EP-ICSXL-MCL Cell Analysis System) and analyzed by CellQust software for positive rate.

1.6 Western Blot Assay

1×10^6 cell of nomal DC2.4 cells, TGFβ-DC, IL-10-DC, LPS-DC cells were lysed in 100 μL lysis buffer (1% NP-40, 50 μmol/L Trish-HCl pH 7.4, 25% deoxycholic acid Na, 150 μmol/L NaCl, 1 μmol/L EDTAc, 1 μmol/L PMSF, 1 μg/mL aprotonin, 1 μg/mL $NaVo_3$ pH 10.0, 1 μmol/L NaF) on ice for 30 min. The supernatants were extracted by centrifuging at 4ºC with 14 000 r/min and stored in －80ºC. The protein concentration was determined with Bradford method. 50 μg protein for each sample was subjected to 10% SDS-PAGE, electro-transferred to nitrocellulose membrane (NC), blocked with 1% TBST/5% skimmed milk powder, incubated with goat-anti-mouse antibody (1:1000) (R&D, USA) diluted in 1% TBST/5% skimmed milk powder overnight, rinsed three times in 0.1%TBST and incubated with horseradish peroxide enzyme rabbit-anti-goat antibody (1:5000, Pierce, USA) for 2 h. After DAB colouration for 5－10 min, brown bands were taken as positive results. By using GAS9500 image analysis system (UVItecy Co., GB), the absorbance (*A*) values of hybridity bands on NC membrane and the ratio to β-actin representing the protein relative quantity were determined.

1.7 Assay of Allogenetic Mixed Lymphocyte Reaction

DC2.4 cells served as stimulators and the allogenetic spleen cells of BALB/c mice as reaction cells. BALB/c mouse spleen cells were separated and red cells was lysed with Tris.NH_4Cl. Spleen cells were passed through the nylon column to remove the B lymphocytes. The DC2.4, IL-10-DC, TGF-DC, LPS-DC cells were inactivated with mitomycin (MMC, Sigma–Aldrich, USA) at 37ºC, 5% CO_2 for 30 min. After being washed with PBS for 3 times, DCs and spleen cells were seeded in a 96-well plate with the ratio of 1:10 and incubated for 5 days. Each group had 3 duplicated wells. The cultures were pulsed with 1 μCi H.thymidine (TdR) in each well 18 h before finishing the incubation and ^{3}H-TdR incorporation was detected by liquid scintilation counter (1409, USA). The supernatant of MLR was collected for enzyme linked immunosorbent assay(ELISA).

1.8 Determination of Cytokines by ELISA

The supernatants were stored at －80ºC, and the levels of IFN-γ detected according to the instruction of IFN–γ ELISA kit (Jingmei, China).

1.9 Statistical Analysis

Data were expressed as $\bar{x}\pm s$. All analyses for statis-

tically significant differences were performed with the One-way ANOVA test. $P<0.01$ was considered significant.

2 RESULTS

2.1 Effect of LI-10, TGF-β1 and LPS on the Expression of PIRs mRNA in DCs

Gel imaging system revealed that there were two bands of 257 bp and 470 bp in each sample respectively for PIR-A and PIR-B with the dominant PIR-B expression other than PIR-A. The ratio of absorbance (*A*) values of PIR-B to β-actin was 0.702±0.108, 0.963±0.105, 0.917±0. 036, 0.396±0.111 (F=36.564, $P<0.01$, fig. 1) and that of PIR-A to β-actin was 0.384±0.038, 0.186±0.042, 0.200±0.047, 0.566±0.04 (F=88.945, $P<0.01$, fig. 1) respectively in control group, IL-10 group, TGF-β group and LPS-DC groups. IL-10 and TGF-β1 could up-regulate the expression of PIR-B and down-regulate the expression of PIR-A mRNA, while LPS could up-regulate the expression of PIR-A and down-regulate the expression of PIR-B mRNA. There was no significant difference in either PIR-A or PIR-B of DCs induced by IL-10 or TGF-β1 among the group ($P>0.05$).

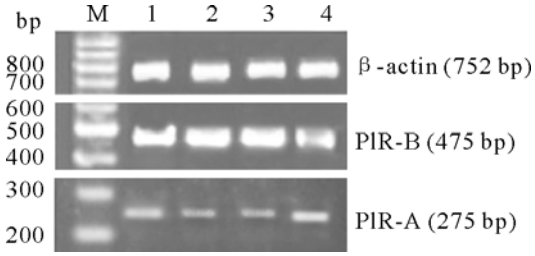

Fig. 1 IL-10-, TGF-β1- and LPS-induced PIR-A and PIR-B mRNA expression detected by RT-PCR
M: Marker; 1: DC2.4 group; 2: IL-10-DC group; 3: TGFβ-DC group; 4: LPS-DC group

2.2 Detection of the PIRs Expression by Flow Cytometry

The PIR positive rate was (28.65±8.12)% in control group, and that was increased to (54.21±6.34)%, (58.78±4.70)%, (48.24±6.75)% in IL-10, TGF-β1 and LPS-DC groups respectively (F=93.87, $P<0.01$). But no significant difference was found among IL-10, TGFβ and LPS groups ($P>0.01$).

2.3 Detection of PIRs Protein Expression by Western Blot

The bands of PIR-A and PIR-B proteins about 85 kD (1 kD=0.9921 ku) and 120 kD appeared respectively. The ratio of absorbance (*A*) values of PIR-B to β-actin was 0.581±0.18, 0.872±0.14, 0.853±0.12, 0.321±0.04 (F=42.53, $P< 0.01$) (fig. 2) and that of PIR-A to β-actin was 0.302±0.11, 0.153±0.08, 0.194±0.06, 0.471±0.17 (F=21.11, $P< 0.01$) (fig. 2) respectively in control group, IL-10 group, TGF-β group and LPS-DC group. IL-10 and TGF-β1 increased the expression of PIR-B and decreased the expression of PIR-A, while LPS decreased the expression of PIR-B and increased the expression of PIR-A.

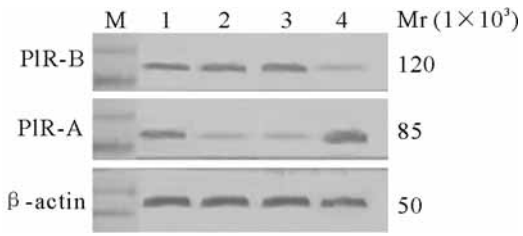

Fig. 2 IL-10-, TGF-β1- and LPS-induced PIR-A and PIR-B protein expression detected by Western blot
M: Marker; 1: DC2.4 group; 2: IL-10-DC group; 3: TGFβ-DC group; 4: LPS-DC group

2.4 Effect of siRNA on the Expression of PIR-B

2.4.1 Transfection Rate of siRNA Flow cytometry revealed that the transfection rate in the positive control group was 93.12%.

2.4.2 The Expression of PIR-B mRNA after siRNA Transfection Gel imaging system revealed that the ratio of absorbance (*A*) values of PIR-B to β-actin was 0.597±0.081, 0.609±0.040, 0.134±0.05, 0.101±0.036 in the blank control, blank transfection, and Si-DC-24 h and Si-DC-48 h groups respectively (F=241.44, $P< 0.01$) (fig. 3). PIR-B mRNA level was decreased by 78.0% and 83.4% at 24 and 48 h after transfection respectively.

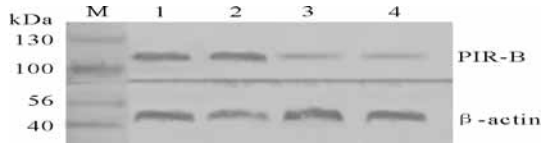

Fig. 3 The PIR-B mRNA expression after siRNA transfection
M: DNA marker; 1: Blank control group; 2: Transfection control group; 3: Si-DC (24 h); 4: Si-DC (48 h)

2.4.3 The Expression of PIR-B Protein after siRNA Transfection The ratio of absorbance (*A*) values of PIR-B to β-actin was 0.775±0.014, 0.749±0.041, 0.180±0.014, 0.110±0.081 respectively in the blank control, blank transfection, Si-DC-24 h, Si-DC-48 h groups (F=90.274, $P<0.01$) (fig. 4). The level of PIR-B protein was degraded by 76.0% and 85.3% at 24 and 48 h respectively after transfection.

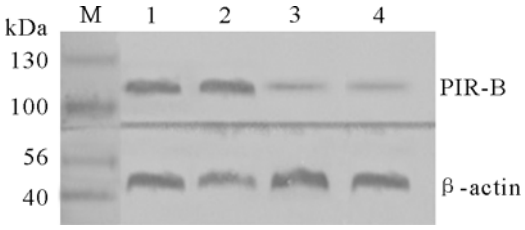

Fig. 4 The PIR-B protein expression after siRNA transfection
1: Blank control group; 2: Transfection control group; 3: Si-DC (24 h); 4: Si-DC (48 h); M: Marker

2.4.4 Detection of PIR-A and CD80, MHC-Ⅱ and CD86 Molecules Expression in DCs by Realtime PCR
After the DC2.4 and Si-DCs were stimulated with LPS for 48 h, the mRNA expression of MHC-Ⅱ, CD80 and CD86 was increased in blank control group and Si-DC-24 h group. TAI of MHC-Ⅱ, CD80, CD86 and PIR-A in blank control group was 5.02 ±1.09, 4.69±1.75, 5.46 ±1.79, 6.02±2.13 respectively; and that in Si-DC-24 h group 8.79 ±2.2, 11.03±1.96, 10.26± 2.55, 12.10±2.83 respectively. Compared with the blank control group, the expression of MHC-Ⅱ, CD80, CD86 and PIR-A mRNA in Si-DC-24 h group was increased by 3.72, 6.34, 4.8, 6.08 respectively. It was suggested that silencing the PIR-B made PIR-A and CD80, CD86 and MHC-Ⅱ mRNA overexpression.

2.5 Mixed Lymphocyte Reaction *In Vitro*

DC2.4 cells stimulated the proliferation of the allogenetic spleen cells derived from the BALB/c mice. ^{3}H-TdR incorporation test demonstrated that the CPM of the control group, LPS, IL-10, TGF-β1, Si-DC-24 h groups was 9870±780, 18750±1080, 4729±325, 4463±360, 21839±980 respectively. Compared with the control group, alloreactivity of IL-10 and TGF-β1 groups was inhibited, and that of LPS group was promoted. Specially silencing PIR-B expression (Si-DC) also promoted the alloreactivity proliferation (F=92.497, P<0.01, fig. 5).

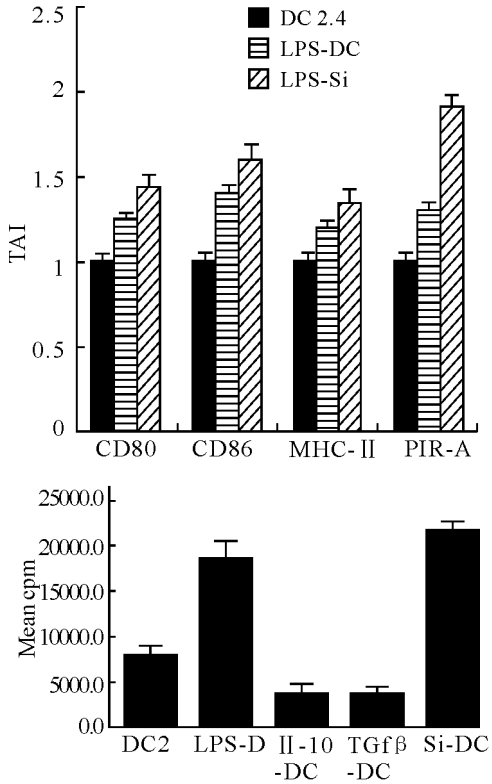

Fig. 5 ^{3}H-TdR detection of the allogenetic lymphocyte proliferation

2.6 Detection of IFN-γ Levels in MLR Supernatant by ELISA

IFN-γ concentrations in MLR supernatant were 15.63±1.78, 39.13±3.58, 4.09±2.72, 4.90±1.92 and 34.16±4.11 pg/mL respectively in the control group, LPS, IL-10, TGF-β1 and the Si-DC-24 h groups (F=38.210, P<0.01). The assay revealed that the IFN-γ concentration was decreased in IL-10 and TGF-β1 groups and increased in LPS and Si-DC-24 h groups as compared with control group.

Table 1 The primers of CD80, CD86, MHC-Ⅱ and β-actin examined by real-time PCR

Primers	PCR product size (bp)	Sequence of the primers
CD80	186	5′5′-TTACCTGGCATCAATACGACAAT-3′
		3′3′-AGTTCTTGCTATCAGGAGGGTC-5′
CD86	132	5′5′-GTGTTCTGGAAACGGAGTCAAT -3′
		3′3′- GAGCAGCATCACAAGGAGGAG-5′
MHC-II	149	5′5′-GCTGTGGTGGTGCTGATGGT-3′
		3′3′- TGCGTCCCGTTGGTGAAGTAG-5′
β-actin	287	5′5′-GCTACAGCAGCTTCACCACCACAG-3′
		3′3′-CTGCAACTGTAGGCATTTATGG-5′

3 DISCUSSION

The T-DCs are presumed as a group of heterogeneity DCs[8]. T-DCs induced by IL-10- and TGF-β1 could directly and indirectly inhibit the activation of allogenetic lymphocytes while retain the GVL[1, 2] even under the inflammatory conditions *in vivo*[9]. T-DC induad by CD8+CD28- T suppressor cells (T suppression cell, Ts) and had a distinct mRNA microarray profile which differed from that in mature DC and immature DC within the many respects involved in signal transcription, chemokines, cytokines, transcription factors, and apoptosis-related protein and cell growth regulators[10, 11]. Most importantly, T-DCs exhibited high expression of the inhibitory molecules such as ILT3 and ILT4, which was critical to the tolerogenic capacity acquired by DCs in human[7]. PIR-A and PIR-B are the orthologue of LT3 and ILT4 and respectively the active and inhibitor receptor which recognize the same ligand— histocompatibility complex (MHC) class 1 molecule. The PIR-A requires the Fc receptor common gamma chain for its efficient cell-surface expression and characteristic ITAM for delivering activation signal[12]. In contrast, PIR-B exerts their negative regulation signals in vitro by recruiting SH2-containing tyrosine phosphatase (SHP)-1 and(or) SHP-2 to their phosphorylated ITIM. For the matched MHC class I molecule between the donor and recipient is a critical determinant for outcome of allogenetic organ transplantation, PIR-B(-) mice showed an accelerated lethal GVHD[15]. It is concluded that recognition systems constituted by PIRs and MHC class I molecules are crucial for keeping the immune steady and preventing the GVHD.

This study found that both PIR-A and PIR-B were expressed on DC2.4 cells by RT- PCR and Western blot, which was consistent with that reported by Pereira *et al*[16]. The suppression of dominant PIR-B expression on DCs suggested a physiological importance such as maintaining their resting state and preventing extra activation upon continuous interaction with H-2 molecule[17]. Both IL-10 and TGF-β1 induced up-regulation of PIR-B and down-regulation of PIR-A. On the contrary, the LPS induced higher PIR-A expression and lower PIR-B ex-

pression. Because the FCM detected limitedly the PIR, which is the common extramembranous of PIR-A and PIR-B, either IL-10, TGF -β1 or LPS induced nearly equally high expression levels of PIR-A or PIR-B.

Moreover, the function of allogenetic stimulating capacity of different DCs was measured by MLR. DC2.4 cells derived from the C57B L/6 mice[18] stimulated the alloreactivity of BALB/c spleen cells[19]. The capacity was further alleviated by IL-10 and TGF-β1 induction and enhanced by LPS stimulation.

Though PIR-A and PIR-B are in paired-wise fashion, they work independently. PIR-B knockout has no effects on the expression of PIR-A. Our study further demonstrated that down-regulation of PIR-A and up-regulation of PIR-B at the same time was the general phonotype feature and the molecular mechanism for DCs to acquire tolerance in mice. PIR-B overexpression intensifies the inhibitory function and this may be the reason that PIR-B inhibits the PIR-A activity. But whether other related signal transduction pathways have alleviated the PIR-A activation during induction of DC tolerance needs to be further confirmed.

LPS is a known factor to stimulate CD80, CD86 and MHC-Ⅱ molecules expression or the DCs mature. After silencing the PIR-B expression, the up-regulation of PIR-A, CD80, CD86 and MHC-Ⅱ mRNA levels was even more significant than the normal groups and its capacity to stimulate allogenetic lymphocytes proliferation was also exaggerated when DC2.4 cells were stimulated by LPS. On the one hand, it was suggested that PIR-B receptor inhibited the PIR-A and CD80, CD86 and MHC-Ⅱ expression under the normal conditions and this is an important way to make the DCs tolerance. On the other hand, this might be associated with the changed balance between PIR-A, PIR-B and its co-ligands for the MHC- I. So, PIR-A was necessary for DCs activation and the inhibition of the PIR-A activity was another important way for PIR-B to modulate DCs function. The dominant expression of PIR-B may be important for maintaining the steady of DCs under physiological conditions. Specific RNAi fragment for PIR-B *in vitro* enhanced the alloreactivity in MLR and increased IFN-γ levels in MLR supernatants. The data proved the mechanism by which PIR-B induced T-DCs was associated with inhibition of PIR-A overaction and decreased IFN-γ production[15].

In a word, higher PIR-B and lower PIR-A expression are the general feature of T-DCs in mice. PIR-A and PIR-B constitute new targets for inducing the DCs tolerance and perhaps these will suggest new pathways for inducing DC tolerance by overexpressing PIR-B or silencing the PIR-A expression. Further studies about the relation of PIR-A and PIR-B expression or the signal transduction pathway may provide not only the rational basement, but also offer novel thinking or method for the success of organ transplantation for the treatment of malignant and viral diseases.

REFERENCES

1 Gad M, Claesson M H, Persen A E. Dendritic cells in peripheral tolerance and immunity. APMIS, 2003, 111(7–8):766-775

2 Sato K, Yamashita N, Baba M *et al*. Modified myeloid dendritic cells act as regulatory dendritic cells to induce anergic and regulatory T cells. Blood, 2003, 101(9): 3581-3589

3 Sato K, Yamashita N, Yamashita N *et al*. Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse. Immunity, 2003,18(3):367-379

4 Hirano A, Luke P P, Specht S M *et al*. Graft hyporeactivity induced by immature donor-derived dendritic cells. Transpl Immunol, 2000,8(3):161-168

5 Bellinghausen I, Brand U, Steinbrink K *et al*. Inhibition of human allergic T-cell responses by IL-10-treated dendritic cells: differences from hydrocortisone-treated dendritic cells. J Allergy Clin Immuno, 2001,108:242-249

6 Kubagawa H, Burrows P D, Cooper M D. A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. Proc Natl Acad Sci U S A, 1997, 94(10):5261-5266

7 Manavalan J S, Rossi P C, Vlad G *et al*. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. Transpl Immunol, 2003,11(3-4): 245-258

8 Banchereau J, Briere F, Caux C *et al*. Immunobiology of dendritic cells. Annu Rev Immunol, 2000,18:767-811

9 Rutella S, Danese S, Leone G. Tolerogenic dendritic cells: Cytokine modulation comes of age. Blood, 2006,108(5): 1435-1440

10 Chang C C, Ciubotariu R, Manavalan J S *et al*. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat Immunol, 2002, 3(3):237-243

11 Suciu-Foca C N, Piazza F, Ho E *et al*. Distinct mRNA microarray profiles of tolerogenic dendritic cells. Hum Immunol, 2001,62(10):1065-1072

12 Maeda A, Kurosaki M, Kurosaki T. Paired immunoglobulin-like receptor (PIR)-A is involved in activating mast cells through its association with Fc receptor gamma chain. J Exp Med, 1998,188(5):991-995

13 Yamashita Y, Ono M, Takai T. Inhibitory and stimulatory functions of paired Ig-like receptor (PIR) family in RBL-2H3 cells. J Immunol, 1998, 161(8): 4042-4047

14 Takai T. A novel recognition system for MHC class I molecules constituted by PIR. Adv Immunol, 2005, 88:161-192

15 Nakamura A, Kobayashi E, Takai T. Exacerbated graft-versus-host disease in Pirb-/- mice. Nat Immunol, 2004,5(6):623-629

16 Pereira S, Zhang H, Takai T *et al*. The inhibitory receptor PIR-B negatively regulates neutrophil and macrophage integrin signaling. J Immunol, 2004, 173(9): 5757-5765

17 Takai T. Paired immunoglobulin-like receptors and their MHC class I recognition. Immunology, 2005,115(4): 433-440

18 Okada N, Saito T, Masunaga Y *et al*. Efficient antigen gene transduction using Arg2Gly2Asp fiber-mutant adenovirus vectors can potentiate antitumor vaccine efficacy and maturation of murine dendritic cells. Cancer Res, 2001, 61(21):7913-7919

19 Ni H T, Spellman S R, Jean W C *et al*. Immunization with dendritic cells pulsed with tumor extract increases survival of mice bearing intracranial gliomas. J Neurooncol, 2001,51(1):1-9

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