Development and Evaluation of a Novel Radiotracer 125I-rIL-27 to Monitor Allotransplantation Rejection by Specifically Targeting IL-27Ra

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Abstract

Non-invasive monitoring of allograft rejection is benefit for the prognosis of patients with organ transplantation. Recently, IL-27/IL-27Rα was proved in close relation with inflammatory diseases, and 125I-anti-IL-27Rα mAb our group developed demonstrated high accumulation in rejecting allograft. However, antibody imaging has limitation in the imaging background due to its large molecule weight. Therefore, we developed a novel radio tracer (iodine-125 labeled recombinant IL-27) to evaluate the advantage on the targeting and imaging of allograft rejection. In vitro specific binding of 125I-rIL-27 was determined by saturation and competitive assay. Blood clearance, biodistribution, autoradio-imaging and IL-27Rα expression were studied on day 10 post transplantation (top period of allorejection). Our results indicated that 125I-rIL-27 could bind with IL-27Rα specifically and selectively in vitro. Blood clearance assay demonstrated a fast blood clearance with 13.20 µl/h of 125I-rIL-27 staying in blood after 24 h. Whole-body phosphor-autoradiography and biodistribution assay indicated that higher specific uptake of 125I-rIL-27 and clear radio-image in allograft than syngraft at 24 h, while similar result was obtained at 48 h in group of 125I-anti-IL-27Rα mAb injection. Meanwhile, higher IL-27Rα expression was found in allograft by the western blot study. The activity accumulation of 125I-rIL-27 was highly correlated with IL-27Rα expression on allograft. In conclusion, 125I-rIL-27 could be a promising probe to acutely monitor the allograft rejection with high specific binding towards IL-27Rα on allograft and low imaging background.

1. Introduction

Solid organ allotransplantation has been the most effective therapeutic strategy for the patient with end-stage organ failure[1]. However, the occurrence of acute rejection is strongly related with the allograft loss and the poor prognosis[2]. Therefore, early detection of acute rejection with non-invasive method could greatly benefit for the prognosis after organ transplantation[3].

Recently, IL-27Rα (IL-27 receptor α), along with its ligand IL-27, is evidenced to trigger the immune response, including cancer, Abdominal aortic aneurysm, Sjögren's syndrome, virus infection and transplantation[4][5][6][7][8]. IL-27Rα is the specific subunit of IL-27 receptor and restricted mainly to lymphocytes and monocytes[9]. IL-27 pathway has been proved to inhibit tumor growth by enhanced T cells response and decreased Treg cells (T regulator cells) proportion[10]. Besides, IL-27 displayed a pro-inflammatory effect by enhancing the IL-1β secretion from monocytes and macrophages[11]. Moreover, IL-27 could also promote NK cell function by secreting more IFN-γ during influenza infection[12]. All these suggested that IL-27 could activate IL-27Rα and enhance pro-inflammation response.

Acute allograft rejection is a severe pro-inflammation participated by T cells and macrophage[13] and IL-27 has been proved in close relation with allograft rejection[7][14]. IL-27Rα (IL-27 receptor α) expression on T cells exacerbated GVHD by enhancing effector function of Th1 cells (T helper 1 cells) and inhibiting Th2 and Treg cell subsets[7], while IL-27Rα was found apparently up-regulated on alloreactive splenetic CD4+T cells, T cell and macrophage when acute rejection happened[15][16][17]. In our previous study with
allografted mice model, we found a great amount of IL-27Rα positive T cells and macrophage infiltrated in rejecting allograft and iodine-125 labeled anti-IL-27Rα mAb could obviously accumulate in allograft non-invasively when rejection occurred[18].

Target tissue could be diagnosed precisely and non-invasively by nuclear molecular imaging with a specific probe, which is much more favorable than histopathological biopsies and traditional imaging examination[19][20][21][22][23]. Although histopathological biopsies was the “gold-standard” of acute graft rejection, it still was an invasive examine and may induce complication including pain, bleeding and death[24][25]. The non-invasive examines such like MRI and ultrasound reflected the decrease graft function and were limited in targeting allograft[21][23]. Targeted molecular imaging has advantages in tracking specific cells and monitoring the function of the target organ with the probes which have the detection signals [26][27][28]. Among them, radionuclide imaging is a non-invasive method by which the disease could be diagnosed effectively and timely, and the therapeutic effect could be monitored with the help of radio-probe. Radionuclide imaging with radiolabeled macromolecular such as protein, antibody and so on usually had the disadvantages in the long time to reach the target tissue and the high background, resulting in the poor imaging quality. However, small molecular could accumulate in the target tissue quickly, and thus make a better imaging. Therefore, radio-probe with small size is the much more promising radio tracer in radionuclide imaging compared with full length antibody. Radio-labeled cytokine has been applied to track targeted immune cells due to the high-contrast imaging, fast clearance, low background and the weak inflammation response. [29][30][31]. Hartimath et al developed [18F]FB-IL-2 to monitor cancer therapy-induced activated T lymphocyte infiltration in tumor[32]. Di Gialleonardo et al demonstrated that 18F-FB-IL-2 could trace IL-2 receptor–positive cells[33]. Ding et al suggested [124I] I-F8-IL-10 could accumulate in arthritic joints in rheumatoid arthritis patient. Meanwhile, fast clearance of [124I] I-F8-IL10 and [131I] I-F8-IL-10 in non-specific target tissues was found within the a span of 24h[34]. Accordingly, imaging with radiolabeled cytokine had advantage in specific recognition of target tissue with low background, and could be a promising strategy for allorejection detection.

In this study, we prepared a novel radio-probe (125I labeled recombinant IL-27, 125I-rIL-27) to specifically target IL-27Rα, and evaluated its possibility for the potential application in acute allograft rejection monitoring.

2. Materials And Methods

2.1 Chemicals, reagents, and equipment

IL-27Rα mAb was obtained from R&D system (Minnesota, USA). Recombination IL-27 (rIL-27) was purchased from peprotech (New Jersey, USA). Na125I was provided by China Institute of Atomic Energy (Beijing, China). SephadexG-25M PD10 column was purchased from GE Healthcare (Pennsylvania, USA). RPMI 1640 medium was get from Biological Industries (Kibbutz Beit Haemek, Israel). HEPES buffer and Red Blood Cell lysis buffer were got from Solarbio (China, Beijing). SDS loading buffer, antibody dilution buffer and blocking buffer were obtained from Beyotime (Shanghai, China). PBS TBST buffer, H&E
staining and immunofluorescence (IF) staining regent were purchased from Servicebio (Wuhan, China). GAPDH solution was obtained from Bioss (Beijing, China) and Bioworld (Illinois, USA). HRP-labeled Goat Anti-Rat IgG solution and HRP-labeled Goat Anti-Rabbit IgG solution was get from EpiZyme (Shanghai, China). ECL substrate was purchased from Merck Millipore (Darmstadt, Germany).

The radioactive counts were measured by Gamma Counter from Capintec Inc (USA). The phosphor-autoradiography imaging was captured and analyzed by Cyclone Plus Scanner (PerkinElmer, Life Sciences, USA). The membrane was scanned by Tanon 5200 imaging system scanner (Tanno, Shanghai, Beijing).

2.2 Radiochemistry

2.2.1 Preparation of the Radio-probe

The preparation of $^{125}$I labeled probe was performed according to the reference[35]. Briefly, 0.05M PB solution (100µL), IL-27Rα mAb (12µg) or rIL-27 (8µg) and Na$^{125}$I (11.9 MBq) was mix in the tube with Iodogen. Then the mixture was added into the SephadexG-25M PD10 column, following by the elution with 0.01M PB solution. The eluent was collected in tube (0.5ml for each tube) and the radioactive count of 10µL eluent from each tube was measured by Gamma Counter.

The radiochemical purity were detected following the protocol. Briefly, 2µL of the radio-probe was added into the filter paper (2 cm to the bottom). Then bottom of the paper was immersed in the solution of 0.9% saline and methanol (1:2, v/v). After 40min, the paper was cut into slice (1cm) and radioactive count was measured by Gamma Counter..

2.2.2 In vitro stability study

Radio-probe (12.5µL) was dissolved in saline (100µL) or mouse serum (100µL), and the mixture was kept at 37°C for a period of time. At 1, 12, 24h, 2µL of the sample was taken out and analyzed so as to observe the change of radiochemical purity

2.2.3 Determination of lipophilicity

$^{125}$I-rIL-27 (0.2µL, 4.08×10⁻⁴MBq) was diluted in 1M HEPES buffer (500µL) and mixed with n-octanol (500µL) for 30min, following by the centrifugation for 10min with 14000 x g. Subsequently, Aliquots of n-octanol and water phases (400µL) was taken out and then centrifuged again. Finally, the radioactive count of each phase (100µL) was measured by Gamma Counter and the octanol/water partition coefficient (Log D$_{o/w}$) was calculated.

2.3 Cell Assays

Cell assays were performed using spleen cells isolated from the mouse model on day 10 post transplantation. Briefly, spleen of mouse model was isolated and pressed on mesh 200. Then, cells were
treated with Red Blood Cell lysis buffer, washed with PBS and final suspended in RPMI 1640 medium. Cells were cultured in 48-well plates for 2h with each well $1 \times 10^6$ cells in 200 µL RPMI 1640 medium, and used for further studies after attachment.

2.3.1 Competition study

For competition binding assay, non-labeled anti-IL-27Rα mAb (0 to 71.4 µM) was incubated with alloreactive spleen cells for 1h before 147.09 nM $^{125}$I-rIL-27 was added. Wash the cells with cold PBS buffer twice and discard the supernatant. The activity bound in the cells was measured by Gamma Counter. B/B₀ was described as the ratio of radioactive counts with non-labeled anti-IL-27Rα mAb to the radioactive counts without non-labeled anti-IL-27Rα mAb. The inhibition constant ($K_i$ value) was calculated in GraphPad Prism software.

2.3.2 Saturation study

$^{125}$I-rIL-27 (3.68 to 147.09 nM) was incubated with spleen cells for 2 h at 37 °C to obtain the total activity binding of $^{125}$I-rIL-27. In order to test the non-specific binding, cells were pre-treated with 10.46µM non-labeled rIL-27 for 1 hour.

After incubation with $^{125}$I-rIL-27, cells were washed with cold PBS buffer twice and radioactive counts were measured in Gamma Counter. The maximum binding capacity ($B_{max}$) and dissociation constant ($K_d$) were calculated in GraphPad Prism software. The specific binding was the value of total binding minus non-specific binding.

2.4 Small animal in vivo experiments

All animal experiments were performed in agreement with the ARRIVE guidelines. The protocol was approved by the Animal Care and Use Committee of the University with the corresponding ethical approval code (LL-201602040, 2016-2022). Female BALB/c mice (H-2d) and C57BL/6 mice (H-2b) were purchased from Vital River Laboratory Animal Technology (Beijing, China) and housed under standard conditions with free access to water and standard food.

2.4.1 Animal models

To establish the skin transplantation model, C57BL/6 mice and BALB/c mice were employed as the skin graft donor of allogeneic and syngeneic transplantation, respectively. BALB/c mice were the recipients. Briefly, surgery was performed under anesthesia with 0.6% pentobarbital sodium (0.1mL/10g body weight) in sterility condition. The mucous membrane and blood vessel of graft was removed and then cut the graft into circle with 1cm in diameter. Then, remove the skin of recipients in right shoulder and transfer the graft to recipients. Finally, petrolatum gauze was put on the graft and covered with bandage. Acute rejection occurred on day 7 post transplantation when removing the bandage with escharotics area over 50%.
2.4.2 Blood clearance assay

At 1h, 2h, 6h, 12h and 24h post injection of radio-probe, mice were anaesthetized with 0.6% pentobarbital sodium solution. Then 5µL blood was collected from the tail vein. The activity of radio-probe stayed in the blood was counted by Gamma Counter. Each mouse was weighted and the concentration of radio-probe in blood (ng/µL) was calculated using 78 mL/kg as blood factor. AUCs (Area Under Curves) of 125I-rIL-27 in 24h and 125I-anti-IL-27Rα mAb in 48h were obtained using GraphPad Prism software. Blood clearance (CL, µl/h) was calculated as dose/AUC with the study referred[36].

2.4.3 Dynamic phosphor-autoradiography

Mice were divided into allo-group, syn-group and blocking group (n=5 for each group) according allogeneic, syngeneic transplantation and allogeneic transplantation model with specific antibody blocking. After fed with 3% NaI solution for 24h, 60µg non-labeled IL-27Rα mAb was injected to the blocking group. One hour later, all mice were injected with 125I-rIL-27 (0.37 MBq) and 125I-anti-IL-27Rα mAb (0.37 MBq) on day 9 post transplantation, respectively. Mice were anesthetized and scanned by Cyclone Plus Scanner. Regions of interest (ROIs) were quantied using the OptiQuant Image Analysis Software and presented as Digital Light Units per square millimeter (DLU/mm²).

2.4.4 Ex vivo Biodistribution

Three groups of mice (allo-group, syn-group, and blocking group, n = 3 for each group) were sacrificed with at 24 h after intravenous injection of radio-probe (0.08MBq in 200µL of 0.01M PB). Organs or tissues of interest including blood, liver, lung, kidney, spleen, control skin and graft were excised and weighed. The activity was measured by Gamma counter and the uptake of radio-probe was expressed as the percentage of injected dose per gram (%ID/g). T/NT (Target/non-Target) ratio was calculated by dividing the %ID/g of the target graft to that of the control skin (opposite site), while T/B (Target/Blood) was %ID/g of the target graft to that of the blood.

2.4.5 H&E (hematoxylin and eosin) staining and immunofluorescence staining

On day 10 post transplantation, grafts were collected and histological sections were prepared. H&E staining and immunofluorescence (IF) staining were performed following the protocols of staining kit. The image was obtained under the optical microscope. Briefly, in H&E staining, the sections were covered haematoxylin for 5min. After 1% acid ethanol regent for 5 second, sections were covered with blue promoting solution for 5 seconds. Next, the sections were covered with eosin solution for 10 minutes. Between every step, the distilled water was used to wash out the excess buffer. In IF staining, the sections were treated with EDTA antigen repair buffer (pH 9.0) and blocked with BSA for 30min. Then, anti-IL-27Rα Ab was diluted in PBS (1:200) and added on the section in 4°C, overnight. The sections were washed with PBS and covered with second antibody for 1 hour. Later, wash the sections with PBS and add the FITC regent (Green) on the sections. Next, the sections were washed with TBST and covered with tissue autofluorescence quencher regent for 5min. Then, excess regent was washed with distilled water for
10min. The sections were discarded excess liquid and incubated with DAPI regent (Blue) for 10min at room temperature. Finally, the sections were washed with PBS and then enclosed with antifade mounting medium.

2.4.6 Western Blot

After transplantation for 10 d, grafts were separated, lysed and reacted with SDS loading buffer. Electrophoresis was performed and protein was transferred to PVDF membrane. The target membrane was then treated with blocking buffer and then covered with anti-IL-27Rα mAb solution and GAPDH solution overnight. Later, membrane was washed with TBST buffer and covered with HRP-labeled Goat Anti-Rat IgG solution and HRP-labeled Goat Anti-Rabbit IgG solution, respectively. Finally, membrane was washed with TBST buffer, following by ECL substrate covering. Band was scanned using Tanon 5200 imaging system scanner and analyzed with Image J software.

2.5 Statistical analysis

All data were quoted as mean ± standard deviation (mean ± SD) and each data point arised from 3 independent experiments. Comparisons between two groups were analyzed using the unpaired student’s t-test. Correlation between DLU/mm² of 125I-rIL-27 and IL-27Rα expression was calculated by correlation assay. Statistically significant level was set at P<0.05.

3. Results

3.1. Radiochemistry

The labeling yields of 125I-rIL-27 and 125I-anti-IL-27Rα mAb were 84.4% and 99.0%, respectively. The radiochemistry purity of these radio-probes was 93.3% and 95.3%. The stabilities of 125I-rIL-27 and 125I-anti-IL-27Rα mAb were over 90% in saline and mouse serum even after 24h, respectively. The results showed that 125I-rIL-27 and 125I-anti-IL-27Rα mAb were quite stable. The log D_o/w values for 125I-rIL-27 was -1.18 ± 0.23, which means 125I-rIL-27 possess hydrophilic character.

3.2. Cell binding assays

3.2.1 Saturation

Typical saturation graphs obtained after incubation of 1 × 10⁶ cells with 125I-rIL-27 was shown in Figure 1A-B. B_max values of 125I-rIL-27 on allo-reactive and syn-reactive splenocytes were 2545 cpm/10⁶ cells and 1607 cpm/10⁶ cells, respectively. Moreover, K_d values were found 48.59nM and 49.04nM for allo- and syn-reactive splenocytes, respectively.

3.2.2 Competition
Figure 1C showed the binding of $^{125}$I-rIL-27 decreased as anti-IL-27Rα mAb increased. Using the $K_d$ value of $^{125}$I-rIL-27 from saturation assay, the determination the $K_i$ value was 769.9nM by Cheng-Prusoff equation.

3.3. Small animal in vivo experiments

3.3.1 Blood clearance assay.

To understand how fast $^{125}$I-rIL-27 cleared in vivo, blood clearance assay was performed. The blood clearance was represented as Clearance Value (CL). The allo-group displayed significantly lower concentration of $^{125}$I-rIL-27 than $^{125}$I-anti-IL-27Rα mAb in blood (0.450 ± 0.095 vs 0.808 ± 0.089 ng/µL, p<0.01), and syn-group showed same pattern (0.342 ± 0.281 vs 0.8967 ± 0.0753 ng/µL, p<0.05) at 24h post injection. AUC (Area under curve) was shown in Figure 2, and the retention of $^{125}$I-rIL-27 in blood was shorter than that of $^{125}$I-anti-IL-27Rα mAb in allo-group (35.52 vs 53.17 h·ng/µL) at 24h. The syn-group showed the shorter retention of $^{125}$I-rIL-27 than $^{125}$I-anti-IL-27Rα mAb (31.53 vs 52.65 h·ng/µL) at 24h. $^{125}$I-rIL-27 demonstrated faster blood clearance compared with $^{125}$I-anti-IL-27Rα mAb in allo-group (13.20 vs 9.43 µL/h) and syn-group (14.87 vs 9.53 µL/h) at 24h. All these suggested a significantly faster blood clearance of $^{125}$I-rIL-27 than $^{125}$I-anti-IL-27Rα mAb in allograft rejection model mice.

3.3.2 Dynamic whole-body phosphor-autoradiography imaging.

To investigate $^{125}$I-rIL-27 imaging in vivo, we performed dynamic whole-body phosphor-autoradiography imaging. As shown in Figure 3A, high uptake of $^{125}$I-rIL-27 in allogeneic graft (DLU/mm$^2$: 434241.58 ± 53524.20) was obtained at 6h after intravenous injection and then reached a plateau up to 24h (DLU/mm$^2$: 163603.46 ± 56677.03), while lower uptake was obtained in syngeneic graft (DLU/mm$^2$: 66401.60 ± 29698.30) (p<0.01). In vivo specificity of $^{125}$I-rIL-27 was confirmed by blocking studies using excess unlabeled anti-IL-27Rα mAb (DLU/mm$^2$: 68252.033 ± 38373.75). Ex vivo autoradiography showed apparently high activity accumulation in allograft. Similar result of $^{125}$I-anti-IL-27Rα mAb was observed at 48h, and the uptake of $^{125}$I-anti-IL-27Rα mAb in allograft was also higher than that in syngeneic graft (Figure 3B). However, imaging using $^{125}$I-rIL-27 in allogeneic graft exhibited lower background in comparison with that using $^{125}$I-anti-IL-27Rα mAb. These indicated that $^{125}$I-rIL-27 could target allograft specifically and yield a better imaging with high contrast and low background.

3.3.3 Biodistribution assay

In order to have a first insight into the potential relevance of $^{125}$I-rIL-27 for transplantation imaging, biodistribution assay was performed using skin transplantation mice. The biodistribution data of $^{125}$I-rIL-27 was shown in Figure 4A. Higher uptake was observed in allogeneic skin graft compared with that in syngeneic group (Figure 4B). Activity uptake of $^{125}$I-rIL-27 in allograft was higher than that in syngraft
(%ID/g: 5.648 ± 1.735 vs 1.751 ± 0.967, p<0.01). T/NT ratio and T/B ratio significantly increased in allo-group compared with syn-group in Figure 4B-C.

More interestingly, compared with $^{125}$I-anti-IL-27Rα mAb, fewer $^{125}$I-rIL-27 in blood was obtained at 24h post injection not only in allo-group (%ID/g: 6.960± 0.754 vs 4.083 ± 0.710, p<0.01), but also in syn-group (%ID/g: 6.090± 0.508 vs 3.230 ± 1.835, p<0.05). Additionally, the activity uptake of $^{125}$I-rIL-27 was also lower than that of $^{125}$I-anti-IL-27Rα mAb in liver, lung, kidney, spleen. These indicated $^{125}$I-rIL-27 could specifically recognize IL-27Rα over-expressed in the allograft and have favorable imaging with low background.

### 3.3.4 IL-27Rα expression in rejecting allograft.

To study the correlation between activity accumulation of $^{125}$I-rIL-27 and IL-27Rα expression in rejecting allograft, IF staining was performed on day 10 post transplantation, so as to determine the IL-27Rα expression.

The HE staining in Figure 5A confirmed that severe rejection response was occurred in allogeneic graft, while mild inflammation in syngeneic graft. IL-27Rα expression was obviously higher in allograft (Figure 5B). The activity accumulation (DLU/mm$^2$) in graft had a positive correlation with IL-27Rα expression (Figure 5C). Fluorescence imaging also confirmed the higher IL-27Rα expression on the surface of infiltrated cells in rejecting allograft (Figure 5D). All these suggested that $^{125}$I-rIL-27 could specifically bind the IL-27Rα in the allograft, monitoring the acute rejection.

### Discussion

Early acute allorejection is usually more responsive to the therapy of allograft transplantation, and thus detection of acute rejection detection timely could benefit for the prognosis[37]. To date, molecular imaging with specific radio-probes was a promising method responsible for the detection of allograft rejection[22]. Recently, IL-27, a pleiotropic cytokine with pro-inflammation properties, was reported with enhanced antivirus and antitumor activities, and it participated in the rejection response[38][39][40][41]. IL-27 could promote infiltration of CD4$^+$T cell and CD8$^+$T cell in tumor, up-regulate IFN-γ, Granzyme B and Perforin production, resulting in improved antitumor effect of T cell[10]. Moreover, IL-27 could also boost NK cell proliferation and cytotoxic activity synergistically with IL-15/IL-18[42]. All these indicated that IL-27/IL-27R was a promising target in pro-inflammation immune response. IL-27Rα, the subunit of IL-27 receptor which is also expressed on the T cell and macrophage, had highest expression on the top acute rejection period in the allograft[43][44][45]. In our previous study, $^{125}$I-anti-IL-27Rα mAb has been found with high specificity towards IL-27Rα[18]. However, it had limitations in non-specific binding to Fc recognition, slow metabolism and clearance, as compared with small-sized antibody fragment or ligand[46][30]. Therefore, small-sized radio-probe could provide a better imaging with low background.
Cytokine was a small-sized ligand of the cytokine receptor which is expressed on surface of effector cells[47]. Many radio-probes of cytokine have already been applied in targeting imaging[48][49][50][51]. Radiolabelled IL-2 probes were used in clinics for in targeted detection of the lymphocytic infiltration in transplantation and atherosclerotic plaque[48][49]. Glaudemans et al found symptomatic plaques with high CD3 + cells infiltration had significant uptake of $^{99m}$Tc-HYNIC-IL-2 and the lung of rejection patient had increased $^{99m}$Tc-HYNIC-IL-2 uptake. However, in their research, no side effect was found when administration of $^{99m}$Tc-HYNIC-IL-2. We also developed the targeted radio-probe $^{125}$I-rIL-27 and also found no side effect in the mouse model.

In vitro experiment showed our $^{125}$I-rIL-27 had a specific binding to the IL-27Rα on the spleen cells. However, binding ability and affinity of $^{125}$I-rIL-27 was lower than that of $^{125}$I-anti-IL-27Rα mAb. This might be due to that $^{125}$I-anti-IL-27Rα mAb have non-specific Fc fragment binding. Matsushima et al developed $^{125}$I-labeled IL 1β in a human large granular lymphocyte cell line (YT cells) and this radio-probe showed higher affinity of 0.1 nM ($K_d$ value) compared with our probe[52]. It maybe was due to the different receptor expression of the cells. Besides, the isolation process of spleen cells may also result in some loss of receptors[53].

In the imaging of $^{[124]}$I-F8-IL10, it was suggested that targeted area had highest uptake and target-to-background ratios at 24 h post injection of the radio-probe[34]. Therefore, we carried out the biodistribution, blood clearance of $^{125}$I-rIL-27 within 24 h after radio-probe injection. In the blood clearance assay, $^{125}$I-rIL-27 showed faster blood clearance than $^{125}$I-anti-IL-27Rα, which was might due to the different cytokine and antibody glycosylation level, influencing the receptor recognition and blood clearance[54]. Blood clearance assay showed shorter retention of $^{125}$I-anti-IL-27Rα in blood compared with monoclonal antibody, which might be due to Fc recognition[55]. Whole-body phosphor-autoradiography imaging demonstrated that the allograft had more activity accumulation than syngeneic graft, and this accumulation could be blocked by the excess of anti-IL-27Rα mAb. Lower background was also observed at 24 h in $^{125}$I-rIL-27 group compared with $^{125}$I-anti-IL-27Rα. Tumor necrosis factor superfamily (TNFSF) contains CD40L, FasL, TRAIL, LIGHT, VEGI, lymphotoxin alpha, lymphotoxin beta and lymphotoxin alpha1/beta2, which could be fused with F8 antibody for tumor targeting. in the biodistribution suggested that the %ID/g of $^{125}$I-rIL-27 in allograft was similar to that of F8-TRAILtrunc, lower than that of F8-CD40L and higher than that of other TNFSF in tumor[56]. The reason may be the different receptor expression and affinity of different cytokine to the receptors. The %ID/g of $^{125}$I-rIL-27(47.8 KDa) in blood was higher than that of F8-TNFSF, F8-IL-10 (18.6 KDa), $^{99m}$Tc-VEGF$_{165}$ (16 KDa), which was probably due to the lower molecular weight of other cytokine[50]. However, the activity of $^{125}$I-rIL-27 in blood was much lower compared with that of $^{125}$I-anti-IL-27Rα (155KDa), perhaps caused by the non-specific Fc binding of $^{125}$I-anti-IL-27Rα. The uptake of $^{125}$I-rIL-27 in lung was higher than that in other organs except graft and blood. This might result from the enrichment of IL-27Rα-overexpressed immune cells in lung. Meanwhile, blood pollution should also be considered. The activity accumulation in kidney was found higher than liver, which may be because of the hydrophilic character of $^{125}$I-rIL-27. Moreover,
125I-rlIL-27 accumulation was also found to have close correlation with IL-27Rα expression of the graft. All these indicated that 125I-rlIL-27 was a promising radiotracer which could specifically target IL-27Rα for the imaging of acute rejecting allograft with faster blood clearance and low background, compared with 125I-anti-IL-27Rα mAb.

**Conclusion**

In this study, the acute allograft rejection could be detected by targeting IL-27Rα in allograft specifically with 125I-rlIL-27. The rejecting allograft had higher specific 125I-rlIL-27 uptake than non-rejecting syngeneic graft and the activity accumulation was in close correlation with IL-27Rα expression of the graft. More importantly, low background and fast clearance was obtained for 125I-rlIL-27 compared with 125I-anti-IL-27Rα mAb. Imaging with this small-sized radio-probe might be a promising strategy for non-invasive monitoring of IL-27Rα-overexpressed rejecting allograft.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| 125I-rlIL-27 | Iodine-125 labeled recombinant IL-27 |
| Treg cell    | T regulatory cell |
| IL-27Rα      | IL-27 receptor α |
| rlIL-27      | recombinant IL-27 |
| Bmax         | maximum binding ability |
| Kd           | dissociation constant |
| Ki           | inhibition constant |
| AUC          | Area Under Curve |
| CL           | Blood clearance |
| DLU/mm²      | Digital Light Units per square millimeter |
| %ID/g        | the percentage injected dose per gram |
| IF           | immunofluorescence |
| T/NT         | Target/non-Target |
| T/B          | Target/Blood |
| TNFSF        | tumor necrosis factor superfamily |

**Declarations**
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Conflicts of interest

The authors have no conflicts of interest to disclose.

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Figures

Figure 1

Binding assay of 125I-rIL-27 in vitro. Allogeneic and syngeneic skin transplantation mice were established and represented as Allo and Syn group. Spleen cells were isolated on day 10 post transplantation and competition binding assay was performed. A-B. The Saturation assay of alloreactive spleen cells (A) and synreactive spleen cells (B). C. The competitive binding of 125I-rIL-27 in alloreactive spleen cells.
Blood clearance assay. Time- Radio probe concentration in blood curves after administration of 125I-IL-27 and 125I-anti-IL-27Rα mAb in allogeneic and syngeneic transplantation mice. The inset table was the AUC from 1–24h of 125I-rIL-27 and 125I-anti-IL-27Rα mAb and blood clearance assay.
Dynamic whole-body phosphor-autoradiography imaging assay. Allogeneic and syngeneic skin transplantation mice were established and represented as Allo and Syn group. Mice was injected with 125I-rIL-27 and 125I-anti-IL-27Rα mAb on day 9 post transplantation and scanned at different time. Graft and opposite control skin were isolated on day 10 (24h after radio probe injection). ARG means the autoradiography. A. Imaging at 1, 6, 12, 24h post 125I-rIL-27 injection and DLU/mm² assay. The circle
indicated the position of the graft. B. Imaging at 24, 48h post 125I-anti-IL-27Rα mAb injection. **p < 0.01 was used in Allo vs Syn group. #p < 0.01 was used in Allo vs Blocking group.

Figure 4

Bio-distribution study on day 10 post transplantation. The organ was separated from allografted and syngrafted mouse model on day 10 and biodistribution assay at 24h post radio probe injection. T/NT and T/B assay was calculated. A. Biodistribution assay of 125I-rIL-27 injection. B-C. T/NT ratio (B) and T/B ratio (C) by 125I-rIL-27 injection. **p < 0.01 was used in Allo vs Syn group. #p < 0.05 was used in Allo vs Blocking group.
H&E staining and IL-27Rα expression. The graft was isolated on day 10 post transplantation. A. H&E staining of allogeneic and syngeneic graft. B. IL-27Rα expression was detected by Western blot assay. C. Correlation assay between 125I-rIL-27 accumulation and IL-27Rα expression in graft. D. IL-27Rα expression was detected by immunofluorescence staining.