Research Article

Development and Evaluation of a Novel Radiotracer $^{125}$I-rIL-27 to Monitor Allotransplant Rejection by Specifically Targeting IL-27Rα

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Noninvasive monitoring of allograft rejection is beneficial for the prognosis of patients with organ transplantation. Recently, IL-27/IL-27Rα was proved in close relation with inflammatory diseases, and $^{125}$I-anti-IL-27Rα mAb our group developed demonstrated high accumulation in the rejection of the allograft. However, antibody imaging has limitations in the imaging background due to its large molecular weight. Therefore, we developed a novel radiotracer (iodine-125-labeled recombinant IL-27) to evaluate the advantage in the targeting and imaging of allograft rejection. In vitro specific binding of $^{125}$I-rIL-27 was determined by saturation and competitive assay. Blood clearance, biodistribution, phosphor autoradioimaging, and IL-27Rα expression were studied on day 10 after transplantation (top period of allorejection). Our results indicated that $^{125}$I-rIL-27 could bind with IL-27Rα specifically and selectively in vitro. The blood clearance assay demonstrated fast blood clearance with 13.20 μl/h of $^{125}$I-rIL-27 staying in the blood after 24 h. The whole-body phosphor autoradiography and biodistribution assay indicated a higher specific uptake of $^{125}$I-rIL-27 and a clear radioimage in allograft than in syngraft at 24 h, while a similar result was obtained at 48 h in the group of $^{125}$I-anti-IL-27Rα mAb injection. Meanwhile, a higher expression of IL-27Rα was found in the allograft by Western blot. The accumulation of radioactivity of $^{125}$I-rIL-27 was highly correlated with the expression of IL-27Rα in the allograft. In conclusion, $^{125}$I-rIL-27 could be a promising probe for acutely monitoring 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 patients with end-stage organ failure [1, 2]. However, the appearance of acute rejection is strongly related to the loss of allograft and a poor prognosis [3]. Therefore, early detection of acute rejection with the noninvasive method could greatly improve the prognosis after organ transplantation [4].

Recently, IL-27Rα (interleukin-27 receptor α), along with its ligand (IL-27), has been shown to trigger the immune response, including cancer, abdominal aortic aneurysm, Sjögren syndrome, virus infection, and transplantation [5–9]. IL-27Rα is the specific subunit of the IL-27 receptor and is restricted primarily to lymphocytes and monocytes [10]. The IL-27 pathway has been shown to inhibit tumor growth by enhancing the response of T cells and decreasing the proportion of Treg cells (T regulator cells) [11]. Furthermore, IL-27...
showed a proinflammatory effect by enhancing the IL-1β (interleukin-1 β) secretion from monocytes and macrophages [12]. Moreover, IL-27 could also promote the function of NK cells (natural killer cells) by secreting more IFN-γ (interferon-γ) during influenza infection [13]. All of this suggested that IL-27 could activate IL-27Ra and enhance the proinflammation response.

Acute rejection of the allograft was a severe proinflammatory response participated by T cells and macrophages, and IL-27 has been shown to be closely related to allograft rejection [8, 14, 15]. IL-27Ra (IL-27 receptor α) expression in T cells exacerbated GVHD (graft-versus-host disease) by improving the effector function of Th1 cells (T helper 1 cells) and inhibiting subsets of Th2 and Treg cells [8], while IL-27Ra was apparently upregulated in alloreactive splenetic CD4+ T cells, T cells, and macrophage when acute rejection occurred [16–18]. In our previous study with the allografted mouse model, we found that a large amount of IL-27Ra-positive T cells and macrophage infiltrated in the rejection of the allograft and anti-IL27Ra mAb labeled with iodine-125 could obviously accumulate in the allograft noninvasively when rejection occurred [19].

Target tissue could be accurately and noninvasively diagnosed by molecular nuclear imaging with a specific probe, which was much more favorable than histopathological biopsies and traditional imaging examination [20–24]. Although histopathological biopsies were the “gold standard” of acute graft rejection, they still were an invasive examination and can induce complications, including pain, bleeding, and death [25, 26]. Noninvasive examinations, such as magnetic resonance imaging and ultrasound, reflected decreased graft function and were limited in targeting allograft [22, 24]. Targeted molecular imaging has advantages in tracking specific cells and monitoring the function of the target organ with probes that have detection signals [27–29]. Among them, radionuclide imaging was a noninvasive method by which a disease could be diagnosed effectively and quickly, and the therapeutic effect could be monitored with the help of a radio probe. Radionuclide imaging with radiolabeled macromolecular objects such as proteins and antibodies usually had the disadvantages of long time to reach the target tissue and the high background, resulting in poor image quality. However, small molecules could quickly accumulate in the target tissue and thus improve imaging. Therefore, the small-sized radio probe is a much more promising radiotracer in radionuclide imaging than the full-length antibody. Radiolabeled cytokines have been applied to track targeted immunocytes due to high contrast imaging, fast clearance, low background, and weak inflammation response [30–32]. Hartimath et al. developed [18F] FB-IL-2 to monitor activated T lymphocyte infiltration induced by cancer therapy in tumors [33]. Di Gialleonardo et al. demonstrated that 18F-FB-IL-2 could trace IL-2 receptor-positive cells [34]. [124I] I-F8-IL10 could accumulate in a patient with arthritic joints in rheumatoid arthritis. Meanwhile, fast clearance of [124I] I-F8-IL10 and [125I] I-F8-IL10 in nonspecific target tissues was found in a 24-hour span [35]. Consequently, imaging with radiolabeled cytokines had the advantage of specific recognition of target tissue with a 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-27Ra and evaluated its potential application in monitoring acute allograft rejection.

2. Materials and Methods

2.1. Chemicals, Reagents, and Equipment. IL-27Ra mAb was obtained from the R&D system (Minnesota, USA). Recombination IL-27 (rIL-27) was purchased from PeproTech (New Jersey, USA). Na125I was provided by the China Institute of Atomic Energy (Beijing, China). The Sephadex G-25M PD10 column was purchased from GE Healthcare (Pennsylvania, USA). RPMI-1640 medium was obtained from Biological Industries (Kibbutz Beit Haemek, Israel). Bromophenol orchid, HEPES buffer, BSA (bovine albumin) and red blood cell lysis buffer were obtained from Solarbio (China, Beijing). SDS (sodium dodecyl sulfate) loading buffer, antibody dilution buffer, and blocking buffer were obtained from Beyotime (Shanghai, China). PBS (phosphate-buffered saline), TBST (tris-buffered saline and Tween) buffer, H&E (hematoxylin and eosin) staining, and immunofluorescence (IF) staining reagent were purchased from Servicebio (Wuhan, China). The GAPDH solution was obtained from Bios (Beijing, China) and Bioworld (Illinois, USA). The HRP- (horseradish peroxidase-) labeled goat anti-Rat IgG solution and the HRP-labeled goat anti-rabbit IgG solution were obtained from Epizyme (Shanghai, China). The ECL (enhanced chemiluminescence) substrate and PVDF (polyvinylidene fluoride) membrane were purchased from Merck Millpore (Darmstadt, Germany). The 0.05 M PB (phosphate buffer) solution (250 ml) consisted of 0.6555 g NaH2PO4·2H2O and 7.2495 g Na2HPO4·12H2O dissolved in distilled water, with a constant volume of 250 ml. NaH2PO4·2H2O and Na2HPO4·12H2O were obtained from Solarbio (China, Beijing).

Radioactive counts were measured using the Gamma counter from Capintec Inc. (USA). The phosphor autoradiography images were captured and analyzed using Cyclone Plus Scanner (PerkinElmer, Life Sciences, USA). The membrane was scanned with the Tanon 5200 imaging system scanner (Tanon, Shanghai, Beijing).

2.2. Radiochemistry

2.2.1. Preparation of the Radio Probe. The preparation of the 125I-labeled probe was performed according to reference (36). Briefly, 0.05 M PB solution (100 μl), IL-27Ra mAb (12 μg) or rIL-27 (8 μg), and Na125I (11.9 MBq) were mixed in the tube with iodogen at room temperature for 15 minutes. The tube was gently shaken every 3–4 minutes. 150 μl 0.05 M PB was added to the tube and allowed to stand at room temperature for 10 minutes. 200 μl 5% BSA reagent (containing bromophenol blue) was added to the tube and gently shaken. The mixture was then added to the Sephadex G-25M PD10 column, followed by elution with 0.01 M PB solution. The eluent was collected in a tube (0.5 ml for each
tube), and the radioactive count of 10 μl eluent of each tube was measured by the Gamma counter.

Radiochemical yield
\[
\text{Radiochemical yield} = \frac{\text{(radioactivity of the first peak)}}{\text{(summed radioactivity counts for each tube)}}.
\]

Radiochemical purity was detected following the protocol [19]. Briefly, 2 μl of the radio probe was added to the filter paper (2 cm to the bottom). The bottom of the paper was then immersed in 0.9% saline and methanol solution (1:2, v/v). After 40 min, the paper was cut into slices (1 cm) and the radioactive count was measured using a Gamma counter. Radiochemical purity = (radioactivity near the sample)/(total radioactivity of every slice).

2.2.2. In Vitro Stability Study. The radio probe (12.5 μl) was dissolved in saline (100 μl) or mouse serum (100 μl), and the mixture was kept at 37°C for a period of time. At 1, 12, and 24 h, 2 μl of the sample was taken and analyzed to observe the change in radiochemical purity.

2.2.3. Determination of Lipophilicity. 125I-rIL-27 (0.2 μl, 4.08 × 10^4 MBq) was diluted in 1 M HEPES buffer (500 μl) and mixed with n-octanol (500 μl) for 30 min, followed by centrifugation for 10 min with 14000 × g. Subsequently, aliquots of the n-octanol and water phases (400 μl) were taken out and then centrifuged again. Finally, the radioactive count of each phase (100 μl) was measured by the Gamma counter and the octanol/water partition coefficient (log \( D_{ow} \)) was calculated.

2.3. Cell Assays. Cell assays were performed using isolated mouse model spleen cells on day 10 after transplantation. Briefly, the mouse model spleen was isolated and pressed twice, then immersed in 0.9% saline and methanol solution (1 : 2, v/v) and radioactivity was measured after twice centrifugation at 10 min with 14000 × g. Then, remove the cells from.name on the right shoulder and transfer the cells to the recipients. Finally, petrolatum gauze was placed on the graft and covered with bandage. Acute rejection occurred on day 7 after transplantation when the bandage with the escharotic area was removed greater than 50%.

2.4. Animal Experiments In Vivo. All animal experiments were performed according to the ARRIVE guidelines. The protocol was approved by the Shandong University Animal Care and Use Committee with the corresponding ethical approval code (LL-2016020400, 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 in 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 used as allogeneic and syngeneic transplant skin graft donors, respectively. BALB/c mice were recipients. Briefly, surgery was performed under anesthesia with 0.6% pentobarbital sodium (0.1 ml/10 g body weight) under sterility conditions. The mucous membrane and blood vessel of the graft were removed, and then, the graft was cut into a circle with 1 cm diameter.

3. Molecular Imaging
radio probe (0.08 MBq in 200 μl of 0.01 M 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 a Gamma counter, and the uptake of the radio probe was expressed as the percentage of injected dose per gram (%ID/g). The 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 Staining and Immunofluorescence Staining. On day 10 after transplantation, the grafts were collected and histological sections were prepared. H&E staining and immunofluorescence staining were performed following the staining kit protocols. The image was obtained under an optical microscope. Briefly, in H&E staining, sections were covered with hematoxylin for 5 min. After applying 1% acid ethanol reagent for 5 seconds, sections were covered with a blue promotor solution for 5 seconds. The sections were then covered with eosin solution for 10 minutes. Between each step, distilled water was used to wash out excess buffer. In IF staining, sections were treated with EDTA antigen repair buffer (pH 9.0) and blocked with BSA for 30 min. Anti-IL-27Rα Ab was then diluted in PBS (1:200) and added to the section at 4°C, overnight. Sections were washed with PBS and covered with a second antibody for 1 h. Later, sections were washed with PBS and the FITC reagent (green) was added to the sections. The sections were then washed with TBST and covered with tissue autofluorescence quencher reagent for 5 min. The excess reagent was then washed with distilled water for 10 min. The sections were discarded excess liquid and incubated with DAPI reagent (blue) for 10 min at room temperature. Finally, the sections were washed with PBS and then enclosed with anti-fade mounting medium.

2.4.6. Western Blot. After 10 d of transplantation, the grafts were separated, lysed, and reacted with SDS loading buffer. Electrophoresis was performed, and the protein was transferred to the PVDF membrane. The target membrane was then treated with blocking buffer and then covered with anti-IL-27Ra mAb solution and a GAPDH solution overnight. The membrane was then washed with TBST buffer and covered with HRP-labeled goat anti-rat IgG solution and HRP-labeled goat anti-rabbit IgG solution, respectively. Finally, the membrane was washed with TBST buffer, followed by ECL substrate covering. The band was scanned with the Tanon 5200 imaging system scanner and analyzed with ImageJ software.

2.5. Statistical Analysis. All data were quoted as mean ± standard deviation (mean ± SD), and each data point emerged from 3 independent experiments. Comparisons between two groups were analyzed using the unpaired Student t-test. The correlation between DLU/mm² of ¹²⁵I·rIL-27 and the expression of IL-27Ra was calculated using a correlation assay. The statistically significant level was established at p < 0.05.

3. Results

3.1. Radiochemistry. The labeling yields of ¹²⁵I·rIL-27 and ¹²⁵I·anti-IL-27Ra mAb were 84.4% and 99.0%, respectively. The radiochemistry purity of these radio probes was 93.3% and 95.3%. The stabilities of the ¹²⁵I·rIL-27 and ¹²⁵I·anti-IL-27Ra mAb were more than 90% in saline and mouse serum even after 24 h (supplement Figure 1), respectively. The results showed that ¹²⁵I·rIL-27 and ¹²⁵I·anti-IL-27Ra mAb were quite stable. The log D_nw values for ¹²⁵I·rIL-27 were −1.18 ± 0.23, which means that ¹²⁵I·rIL-27 has a 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 are shown in Figures 1(a) and 1(b). The B_max values of ¹²⁵I·rIL-27 in allo-reactive and synreactive splenocytes were 2545 cpm/10⁶ cells and 1607 cpm/10⁶ cells, respectively. Furthermore, K_d values were found to be 48.59 nM and 49.04 nM for allo- and syn-reactive splenocytes, respectively.

3.2.2. Competition. Figure 1(c) shows that the binding of ¹²⁵I·rIL-27 decreased as anti-IL-27Ra mAb increased. Using the K_d value of ¹²⁵I·rIL-27 from the saturation assay, the determination of the K_i value was 769.9 nM using the Cheng-Prusoff equation.

3.3. Small Animal In Vivo Experiments

3.3.1. Blood Clearance Assay. To understand how fast ¹²⁵I·rIL-27 cleared in vivo, a blood clearance assay was performed. Blood clearance was represented as a clearance value. The allogroup showed a significantly lower concentration of ¹²⁵I·rIL-27 than ¹²⁵I·anti-IL-27Ra mAb in the blood (0.450 ± 0.095 vs. 0.808 ± 0.089 ng/μl, p < 0.01), and the syngroup showed the same pattern (0.342 ± 0.281 vs. 0.8967 ± 0.0753 ng/μl, p < 0.05) at 24 h after injection. The AUC was shown in Figure 2, and the retention of ¹²⁵I·rIL-27 in the blood was shorter than that of ¹²⁵I·anti-IL-27Ra mAb in the allogroup (31.53 vs. 52.65 h μl, p < 0.01). The stabilities of the ¹²⁵I·rIL-27 and ¹²⁵I·anti-IL-27Ra values for ¹²⁵I·rIL-27 were 34.2 ± 0.30 and 95.3%. The stabilities of the ¹²⁵I·rIL-27 and ¹²⁵I·anti-IL-27Ra mAb were quite stable. The log D_nw values for ¹²⁵I·rIL-27 were −1.18 ± 0.23, which means that ¹²⁵I·rIL-27 has a hydrophilic character.

3.3.2. Dynamic Whole-Body Phosphor Autoradiography Imaging. To investigate ¹²⁵I·rIL-27 imaging in vivo, we performed dynamic whole-body phosphor autoradiography imaging. As shown in Figure 3(a), the uptake of ¹²⁵I·rIL-27 was obtained in the allogeneic graft (DLU/mm²: 43421.58 ± 53524.20) at 6 h after intravenous injection and then reached a plateau up to 24 h (DLU/mm²: 163603.46 ± 56677.03), while lower uptake was obtained in the syngeneic graft (DLU/mm²: 66401.60 ± 29698.30).
The in vivo specificity of $^{125}$I-rIL-27 was confirmed by blocking studies using excess unlabeled anti-IL-27Rα mAb (DLU/mm²: 68252 ± 38373). Ex vivo autoradiography apparently showed a high accumulation of activity in the allograft. A similar result of $^{125}$I-anti-IL-27Rα mAb was observed at 48 h, and the uptake of $^{125}$I-anti-IL-27Rα mAb in the allogeneic graft was also higher than in the syngeneic graft (Figure 3(b)). However, the image using $^{125}$I-rIL-27 in the allogeneic graft exhibited a lower background compared to the image with that using $^{125}$I-anti-IL-27Rα mAb. These indicated that $^{125}$I-rIL-27 could target the allograft specifically and produce better images with high contrast and low background.

### 3.3.3. Biodistribution Assay

To gain first insight into the potential relevance of $^{125}$I-rIL-27 for transplantation imaging, a biodistribution assay was performed using skin transplantation mice. Biodistribution data for $^{125}$I-rIL-27 was shown in Figure 4(a). Higher uptake was observed in the allogeneic skin graft compared to that in the syngeneic group (Figure 4(b)).

$p < 0.01$). The in vivo specificity of $^{125}$I-rIL-27 was confirmed by blocking studies using excess unlabeled anti-IL-27Rα mAb (DLU/mm²: 68252 ± 38373). Ex vivo autoradiography apparently showed a high accumulation of activity in the allograft. A similar result of $^{125}$I-anti-IL-27Rα mAb was observed at 48 h, and the uptake of $^{125}$I-anti-IL-27Rα mAb in the allogeneic graft was also higher than in the syngeneic graft (Figure 3(b)). However, the image using $^{125}$I-rIL-27 in the allogeneic graft exhibited a lower background compared to the image with that using $^{125}$I-anti-IL-27Rα mAb. These indicated that $^{125}$I-rIL-27 could target the allograft specifically and produce better images with high contrast and low background.
The uptake of the activity of $^{125}$I-rIL-27 in the allograft was higher than that in the syngraft (%ID/g: $5.648 \pm 1.735$ vs. $1.751 \pm 0.967$, $p < 0.01$). The T/NT ratio and the T/B ratio increased significantly in the allogroup compared to the syngroup in Figures 4(b) and 4(c).

More interestingly, compared to $^{125}$I-anti-IL-27Ra mAb, fewer $^{125}$I-rIL-27 in the blood were obtained 24 h after injection not only in the allogroup (%ID/g: $6.960 \pm 0.754$ vs. $4.083 \pm 0.710$, $p < 0.01$) but also in the syngroup (%ID/g: $6.090 \pm 0.508$ vs. $3.230 \pm 1.835$, $p < 0.05$). Furthermore, the activity uptake of $^{125}$I-rIL-27 was also lower than that of $^{125}$I-anti-IL-27Ra mAb in the liver, lung, kidney, and spleen. These indicated that $^{125}$I-rIL-27 could specifically recognize IL-27Ra overexpressed in the allograft and have favorable imaging with low background.

3.3.4. IL-27Ra Expression in the Rejection of Allograft. To study the correlation between the accumulation of activity of $^{125}$I-rIL-27 and the expression of IL-27Ra in the rejection...
of the allograft, IF staining was performed on day 10 after transplantation to determine the IL-27Rα expression.

HE staining in Figure 5(a) confirmed that a severe rejection response occurred in allogeneic graft, while mild inflammation occurred in syngeneic graft. IL-27Rα expression was obviously higher in the allograft (Figure 5(b)). The accumulation of activity (DLU/mm²) in the graft had a positive correlation with IL-27Rα expression (Figure 5(c)). Fluorescence imaging also confirmed the higher expression of IL-27Rα on the surface of infiltrated cells of rejecting the allograft (Figure 5(d)). All of these suggested that 125I-rIL-27 could specifically bind the IL-27Rα in the allograft, monitoring acute rejection.

4. Discussion

Early acute alloreactive rejection is usually more responsive to allograft transplant therapy, and therefore, timely detection of acute rejection could benefit the prognosis [39]. Up to now, molecular imaging with specific radio probes was a promising method responsible for the detection of allograft rejection [23]. IL-27, a pleiotropic cytokine with proinflammatory properties, was reported with enhanced antitumor and antivirus activities and participated in the rejection response [40–42]. IL-27 could promote the infiltration of CD4+ T cells and CD8+ T cells in the tumor and upregulate IFN-γ, granzyme B, and perforin production, resulting in an improved antitumor effect of T cells [11]. Moreover, IL-27 could also boost NK cells proliferation and cytotoxic activity synergistically with IL-15/IL-18 [43]. All of these indicated that IL-27/IL-27Rα was a promising target in the proinflammatory immune response. IL-27Rα, the subunit of the IL-27 receptor, which was also expressed in T cells and macrophages, had the highest expression during the acute rejection period in the allograft [44–46]. In our previous study, 125I-anti-IL-27Rα mAb has been found with high specificity towards IL-27Rα [19]. However, it had limitations in nonspecific binding to Fc recognition, slow metabolism, and clearance, compared to a small antibody fragment or ligand [31, 47]. Therefore, a small radio probe could provide better imaging with a low background.

The cytokine was a small ligand of the cytokine receptor that was expressed on the surface of effector cells [47]. Many radio cytokine probes have already been applied in target imaging [48–51]. Radiolabeled IL-2 probes were used in clinics for the targeted detection of lymphocytic infiltration in transplantation and atherosclerotic plaque [48, 49]. Glau demans et al. found that symptomatic plaques with high CD3+ cell infiltration had a significant uptake of 99mTc-HYNIC-IL-2 and the lung of the rejection patient had
increased $^{99m}$Tc-HYNIC-IL-2 uptake. In their researches, no side effects were found with the administration of $^{99m}$Tc-HYNIC-IL-2. We also developed the $^{125}$I-rIL-27 targeted radio probe with high radiochemical purity. Because the Sephadex G-25M PD10 column allowed for rapid group separation of high-molecular-weight substances from low-molecular-weight substances, the radio probe which has high molecular weight would be eluted first. The radioactive probes were easier to observe because they contain proteins that could be stained blue with bromophenol blue. Meanwhile, the radio count assay of the elution would help to confirm the radio probe. We found that $^{125}$I-rIL-27 would keep stable for 24 hours after synthesis using the paper chromatography method. This is a traditional method to determine radiochemical purity and has reproducible and accurate radio counts [52]. When the radio compound is not dissociated, the radio compound swims slowly in the medium because of its large molecular weight. Therefore, the place where the sample was had the highest radioactivity count. And when the radio compound dissociated, free iodine 125 would produce a higher radioactivity count on the upper paper. This method will help to demonstrate the radiochemical purity and stability of the radio probe. Also, we also found that this radio probe had no side effects in the mouse model.

The in vitro experiment showed that our $^{125}$I-rIL-27 had a specific binding to IL-27Rα on the spleen cells. However, the binding ability and affinity of $^{125}$I-rIL-27 were lower than those of $^{125}$I-anti-IL-27Rα mAb. This could be due to the fact that $^{125}$I-anti-IL-27Rα mAb has nonspecific binding of the Fc fragment. Matsushima et al. developed $^{125}$I-labeled IL 1β in a human large granular lymphocyte cell line (YT cells), and this radio probe showed a higher affinity of 0.1 nM ($K_d$ value) compared to our probe [53]. It may be due to the different receptor expressions of the cells. Furthermore, the isolation process of spleen cells may also result in some loss of receptors [54].

In the imaging of $^{124}$I-F8-IL10, it was suggested that the target area had the highest uptake and target-to-background ratios at 24 h after injection of the radio probe [35]. Therefore, we carried out biodistribution and 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 could be due to different levels of cytokines and antibody glycosylation, which influence receptor recognition and blood clearance [55]. The blood clearance assay showed a shorter retention of $^{125}$I-anti-IL-27Rα in the blood compared to monoclonal antibody, which could be due to recognition of Fc [56]. The whole-body phosphor autoradiography imaging demonstrated that the allograft had more activity accumulation than the syngeneic graft, and this accumulation could be blocked by excess of anti-IL-27Rα mAb. A lower background was also observed at 24 h in the $^{125}$I-rIL-27 group compared with $^{125}$I-anti-IL-27Rα. The tumor necrosis factor superfamily (TNFSF) contains CD40L, FasL, TRAIL (TNF-related apoptosis-inducing ligand), LiGHT, VEGF (vascular endothelial growth factor), lymphotoxin alpha, lymphotoxin beta, and lymphotoxin alpha1/beta2, which could be fused with the F8 antibody for tumor targeting. In biodistribution,
it was suggested that the %ID/g of $^{125}$I-rIL-27 in the allograft was similar to that of F8-TRAILtrunc, lower than that of F8-CD40L, and higher than that of other TNFSF in the tumor [57]. The reason may be the different expressions and affinity of different receptors for the receptors. The %ID/g of $^{125}$I-rIL-27 (47.8 KDa) in the blood was higher than that of F8-TNFSF, F8-IL-10 (18.6 KDa), and $^{99m}$Tc-VEGF165 (16 KDa), probably due to the lower molecular weight of other cytokines [50]. However, the activity of $^{125}$I-rIL-27 in the blood was much lower compared to that of $^{125}$I-anti-IL-27Rα mAb. Imaging with this small radio probe could be a promising strategy for noninvasive monitoring of IL-27Ra-overexpressed allograft.

5. Conclusions

In this study, the acute rejection of allograft could be detected by targeting IL-27Ra in allograft specifically with $^{125}$I-rIL-27. The rejecting allograft had higher specific $^{125}$I-rIL-27 uptake than nonrejecting syngeneic graft, and the accumulation of activity was in close correlation with the expression of IL-27Ra of the graft. More importantly, low background and rapid clearance were obtained for $^{125}$I-rIL-27 compared with $^{125}$I-anti-IL-27Ra mAb. Imaging with this small radio probe could be a promising strategy for noninvasive monitoring of IL-27Ra-overexpressed rejecting allograft.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Additional Points

Statement. A preprint has previously been published [58].

Conflicts of Interest

The authors have no conflicts of interest to disclose.

Authors’ Contributions

Shanshan Zhao and Guihua Hou designed the studies. Shanshan Zhao and Qian Liu carried out the study, including data collection and data analysis. Shanshan Zhao, Qian Liu, Feng Gao, and Guihua Hou wrote the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

The figure showed the radiochemistry of $^{125}$I-rIL-27 and $^{125}$I-anti-IL-27Ra, including labeling yields and stabilities. The labeling yields were calculated by the radio counts of the elution. Stabilities were performed by the paper chromatography method. (Supplementary Materials)

References


