Published in final edited form as:

Nucl Med Commun. 2009 February ; 30(2): 126–133. doi:10.1097/MNM.0b013e32831e89cd.

Molecular pathway-specific ^{99m}Tc-*N*-(3-bromo-2,4,6-trimethyacetanilide) iminodiacetic acid liver imaging to assess innate immune responses induced by cell transplantation

Brigid Joseph^a, Kuldeep K. Bhargava^b, Gene G. Tronco^b, Christopher J. Palestro^b, and Sanjeev Gupta^a

^aDepartments of Medicine and Pathology, Marion Bessin Liver Research Center, Cancer Research Center, Diabetes Center, Jack and Pearl Resnick Campus, Albert Einstein College of Medicine, Bronx, New York, USA

^bDivision of Nuclear Medicine and Molecular Imaging, North Shore-Long Island Jewish Health System Campus, Albert Einstein College of Medicine, Bronx, New York, USA

Abstract

Objectives—Inflammatory responses after cell transplantation impair engraftment of transplanted cells. We studied whether perturbations in specific molecular pathways after inflammation in a syngeneic cell transplantation model could be identified by noninvasive imaging.

Methods—After transplanting hepatocytes into the liver of dipeptidyl peptidase IV-deficient Fischer 344 rats, we imaged hepatobiliary excretion of ^{99m}Tc-*N*-(3-bromo-2,4,6-trimethyacetanilide) iminodiacetic acid (^{99m}Tc-mebrofenin). Fractional retention of peak hepatic mebrofenin activity over 60-min periods was correlated with parameters of hepatic inflammation.

Results—In healthy animals, 28 ± 6% ^{99m}Tc-mebrofenin activity was in the liver after 60 min, whereas cell transplantation dose-dependently inhibited excretion of ^{99m}Tc-mebrofenin, *P* value of less than 0.001. Resolution of this abnormality in ^{99m}Tc-mebrofenin transport required 2 weeks in the setting of prolonged activation of Kupffer cells with increased TNF- α and IL-6 expression. Hepatic transport of ^{99m}Tc-mebrofenin was promptly restored by anti-inflammatory treatments, including inhibition of cyclooxygenase activity, depletion of neutrophils, or blocking of inflammatory cytokines before cell transplantation. Moreover, these treatments improved transplanted cell engraftment.

Conclusion—Molecular pathway-based imaging offers appropriate noninvasive means to address activation of innate immune responses. This will help in developing suitable strategies for characterizing and overcoming immune responses for cell and gene therapy.

Keywords

cell transplantation; cytokine; inflammation; liver; mebrofenin

Introduction

Organ repopulation, for example, of the liver, is of intense interest for cell therapy. Engraftment of transplanted cells is critical in organ repopulation, although only a small fraction of

transplanted cells actually survives because of multiple barriers [1], including activation of inflammatory responses by cell transplantation [2,3]. The mechanisms of this tissue inflammation are incompletely understood and are dependent, in part, on the immunogenicity of the cell type transplanted, for example, syngeneic versus allogeneic cells, native versus genetically modified cells, and perhaps stem/progenitor cells versus mature cells. Of course, host immunocompetence governs the variable extent and duration of immune responses and tissue injury after cell or organ transplantation. Consequently, characterizing inflammation after cell transplantation will be highly significant for managing immunological responses.

To assess anatomical changes, accumulation of inflammatory cells, or gene expression during tissue inflammation, approaches with exogenous imaging ligands, substrates, or transgenes have been developed for magnetic resonance, positron emission tomography, and fluorescent tomography [4–9]. A different approach should concern applications of endogenous molecular pathway-specific processes regulated by inflammation. For instance, the radiotracer, ^{99m}Tc -*N*-(3-bromo-2,4,6-trimethoxyacetanilide) iminodiacetic acid (^{99m}Tc -mebrofenin), is exclusively incorporated in the liver and transported into bile [10]. Remarkably, biliary transport of ^{99m}Tc -mebrofenin is exquisitely sensitive to inflammatory cytokines, that is, IL-6 or TNF- α [11], suggesting that if cell transplantation activated responses involving inflammatory cytokines, ^{99m}Tc -mebrofenin transport will be impaired. In turn, this will offer insights into the cell and molecular basis of inflammation after cell transplantation while offering opportunities to develop ways for improving cell engraftment in the liver. Here, to examine the potential of hepatic transport-based changes after cell transplantation, we carried out studies in dipeptidyl peptidase IV (DPPIV)-deficient rats, where healthy cells with DPPIV can be conveniently identified [2,3].

Materials and methods

Animals and surgical procedures

A total of 120 DPPIV-Fischer 344 (F344) rats were studied. Animals were 6–8 weeks old, weighed 120–180 g, and were from the Special Animal Core of Marion Bessin Liver Research Center. Healthy F344 rats ($n = 20$) serving as cell donors were from National Cancer Institute (Bethesda, Maryland, USA). For surgical procedures, animals were anesthetized with ketamine and xylazine (Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Animal Care and Use Committees at Albert Einstein College of Medicine and Long Island Jewish Medical Center approved experimental protocols.

Primary hepatocytes were isolated from donor F344 rats by two-step collagenase perfusion of liver and viability by trypan blue exclusion was 85–90%. Hepatocytes in RPMI 1640 medium (Gibco Invitrogen Cell Culture, Invitrogen Corp., Carlsbad, California, USA) were transplanted over 9–12 s into the splenic pulp, as before [1–3]. For imaging studies, mebrofenin was mixed with 185–222 MBq sodium [^{99m}Tc] pertechnetate in 3 ml of physiological saline (Choletec, Bracco Diagnostics, Princeton, New Jersey, USA), and 7.4 MBq of ^{99m}Tc -mebrofenin was injected in 1 ml into splenic pulp [11].

Drugs and chemicals

Dexamethasone (D1159), naproxen (N8280), thalidomide (T144), and chemicals were from Sigma Chemical Co. (St. Louis, Missouri, USA), polyclonal rat neutrophil antiserum (51140 AD) from Intercell Technologies (Jupiter, Florida, USA), and etanercept from Amgen Inc. (Thousand Oaks, California, USA). Dexamethasone, naproxen, and etanercept were dissolved in physiological saline; thalidomide in dimethylsulfoxide followed by dilution in physiological saline. Drugs were given as follows: 6 mg/kg dexamethasone intraperitoneally (i.p.) daily for 3 days, 6 mg/kg naproxen i.p. once, 10 mg/kg thalidomide i.p. once, 5 mg/kg etanercept through

the tail vein once, and 1 ml antineutrophil antiserum through the tail vein once, based on earlier experience or pilot studies of cell engraftment [12–15]. Naproxen and thalidomide were given 2–4 h before cell transplantation, and etanercept and antineutrophil antiserum were given 16–18 h before cell transplantation.

Imaging

Ten-second dorsal images were acquired for 60 min beginning immediately after ^{99m}Tc -mebrofenin injection using a $64 \times 64 \times 16$ matrix at zoom factor of 2 with a γ -camera (Argus, ADAC Laboratories, Milpitas, California, USA), as described previously [11]. Data were analyzed on a commercially available nuclear medicine workstation by drawing regions of interest over the liver and generating time–activity curves. The time to maximal accumulation of ^{99m}Tc -mebrofenin (Tpeak) was determined. The fraction of Tpeak ^{99m}Tc -mebrofenin activity remaining at 60 min after injection was also determined.

Reverse-transcription polymerase chain reaction

Total RNA was isolated with TRIzol (Invitrogen Corporation, Carlsbad, California, USA). After treatment with RNase-free DNase (Qiagen Inc., Valencia, California, USA), cDNA was prepared from 2 to 4 μg total RNA with Omniscript RT Kit (Qiagen). cDNAs were subjected to polymerase chain reaction (PCR) with DNA Polymerase (Qiagen) at 95°C for initial denaturation followed by 35 cycles: $94^\circ\text{C} \times 30\text{ s}$, annealing at various temperatures $\times 30$ – 60 s , extension at $72^\circ\text{C} \times 1\text{ min}$, and final extension at $72^\circ\text{C} \times 7\text{ min}$. Products were resolved in 1% agarose with ethidium bromide. PCR primers, annealing temperatures, and product sizes and sources are listed in (Table 1) [16–19].

Kupffer cell activation

Liver samples were obtained from animals treated with 60 min pulse of carbon contained in 0.5 ml India ink (Pelican #17, Hannover, Germany), which was injected into the tail vein and prepared as described earlier [2]. Five-micrometer-thick cryosections were fixed with 4% paraformaldehyde in phosphate-buffered saline. Kupffer cells containing carbon were counted in 50 consecutive high-power fields (hpf) per tissue under $\times 200$ magnification with centering on portal areas. At least three to four rats were analyzed per condition.

Identification of transplanted cells

Liver samples were cooled to -80°C in methylbutane and stored. Five-micrometer-thick cryosections were prepared and stained histochemically to visualize transplanted cells with DPPIV activity, as described previously [1–3]. Transplanted cell numbers were determined in each animal with scoring of 100 consecutive areas centered on portal areas under $\times 200$ magnification. At least three to four animals were analyzed per condition.

Experimental design

Typically, each animal group contained six rats. Initially, 2×10^7 hepatocytes were transplanted; in subsequent dose–response studies, 1×10^6 , 5×10^6 , 1×10^7 , and 2×10^7 cells were transplanted ($n = 6$ each) (Fig. 1a). Transport of ^{99m}Tc -mebrofenin was analyzed 1 day after cell transplantation (Fig. 1a). In further studies, 1×10^7 cells were transplanted followed by ^{99m}Tc -mebrofenin transport analysis at 6 h, and 1, 3, 7, 14, 21, and 28 days after cell transplantation ($n = 6$ each). Hepatic inflammation in animals was analyzed by reverse-transcription PCR for inflammatory cytokines and Kupffer cell activation with carbon incorporation ($n = 3$ – 6 rats each). To demonstrate whether anti-inflammatory manipulations altered cell transplantation-induced changes in ^{99m}Tc -mebrofenin transport, animals were studied 1 day after cell transplantation ($n = 3$ – 6 each) (Fig. 1b). Changes in cell engraftment

were analyzed after 7 days ($n = 3-6$ each). Studies were repeated two to three times to verify key findings.

Statistical analysis

Data are shown as means $\pm$ SEM. Significances were analyzed by SigmaStat 3.0 software (Jandel Scientific, Carpenteria, California, USA) with Student's *t*-tests and analysis of variance (ANOVA), along with Holm-Sidak pairwise multiple comparisons as appropriate. A *P* value of less than 0.05 was taken as significant.

Results

Cell transplantation rapidly and persistently inhibited hepatobiliary transport of ^{99m}Tc -mebrofenin

In healthy control rats, ^{99m}Tc -mebrofenin rapidly accumulated in the liver and was promptly transported into bile, as shown by time-activity curve analysis (Fig. 2a), and as expected from previous experience [11]. Mean *T*_{peak} of hepatic ^{99m}Tc -mebrofenin accumulation was 250 ± 35 s and only $28 \pm 6\%$ of *T*_{peak} ^{99m}Tc -mebrofenin activity remained in liver after 60 min.

In recipients of cells, time to *T*_{peak} ^{99m}Tc -mebrofenin did not change and this was 270 ± 80 s 24 h after transplantation of 2×10^7 cells. After cell transplantation, time-activity curves, however, showed that ^{99m}Tc -mebrofenin transport was markedly impaired (Fig. 2b). This was related to the number of cells transplanted, as ^{99m}Tc -mebrofenin transport was unchanged 1 day after transplantation of 1×10^6 cells ($25 \pm 6\%$, *P* = NS), whereas 1 day after transplantation of 5×10^6 , 1×10^7 , or 2×10^7 cells, retention of ^{99m}Tc -mebrofenin progressively increased to 49 ± 1 , 70 ± 3 , and $90 \pm 5\%$ of *T*_{peak} values, respectively, *P* value of less than 0.001, ANOVA with the Holm-Sidak test (Fig. 2c).

To determine the duration over which ^{99m}Tc -mebrofenin transport was impaired, we studied animals for up to 4 weeks after transplantation of 1×10^7 cells. This showed that ^{99m}Tc -mebrofenin transport was abnormal for 2 weeks after cell transplantation (Fig. 2d). ^{99m}Tc -mebrofenin retention 6 h, and 1 and 2 weeks after cell transplantation was 765, 543, and 531%, respectively, *P* value of less than 0.001 versus healthy controls, ANOVA with the Holm-Sidak test.

After cell transplantation, changes in ^{99m}Tc -mebrofenin transport were associated with hepatic inflammation

Transplanted cells were in liver sinusoids at early times, for example, after 6 h and 1 day (Fig. 3a). Subsequently, cells engrafted in liver parenchyma (see below). In cell recipients, interspersed areas of liver ischemia were noted on histology, although 3 days after cell transplantation, and at all subsequent times, tissues were morphologically normal. After transplantation of 1×10^7 cells, Kupffer cells incorporated more carbon compared with healthy controls (Fig. 3b and c). Morphometric analysis established that this Kupffer cell activation persisted for 2 weeks (Fig. 3d). The kinetics of Kupffer cell activation matched the duration over which ^{99m}Tc -mebrofenin excretion was abnormal. As cytokines released by Kupffer cells serve key roles in inflammation, we examined hepatic expression of TNF- α , IL-6, IL-1 β , and IL-2. After cell transplantation, TNF- α and IL-6 mRNA expression increased over prolonged periods (Fig. 3e), although IL-1 β and IL-2 expression did not change (not shown). Altogether, these findings were in agreement with induction of hepatic inflammation after cell transplantation with activation of Kupffer cells and inflammatory cytokines.

Inhibition of cell transplantation-induced hepatic inflammation restored ^{99m}Tc -mebrofenin transport

To determine whether cell transplantation-induced inflammatory perturbations were directly responsible for altering ^{99m}Tc -mebrofenin transport, we treated rats with drugs to prevent inflammation before transplanting 1×10^7 cells (Table 2). Administration of naproxen, an inhibitor of cyclooxygenase-1 and cyclooxygenase-2, prevented cell transplantation-induced inhibition of ^{99m}Tc -mebrofenin transport, and hepatic ^{99m}Tc -mebrofenin retention after 60 min was 32 ± 6 versus $73 \pm 4\%$ in recipients of cells alone, P value of less than 0.001, t -test. When animals were treated with dexamethasone to deplete lymphocytes, ^{99m}Tc -mebrofenin transport remained abnormal after cell transplantation, 78 ± 8 versus $73 \pm 4\%$, $P = \text{NS}$. ^{99m}Tc -mebrofenin transport reverted to normal after cell transplantation subsequent to treatment with antineutrophil serum, 23 ± 3 versus $73 \pm 4\%$, P value of less than 0.001, t -test. Etanercept, which selectively blocks $\text{TNF-}\alpha$, did not prevent inhibition of ^{99m}Tc -mebrofenin transport, 78 ± 8 versus $73 \pm 4\%$, $P = \text{NS}$, t -test. Thalidomide, which interferes with the activity of $\text{TNF-}\alpha$ and other cytokines, however, prevented cell transplantation-induced inhibition of ^{99m}Tc -mebrofenin transport, 28 ± 4 versus $73 \pm 4\%$, P value of less than 0.001, t -test.

Normalization of ^{99m}Tc -mebrofenin transport was associated with superior cell engraftment

We considered that decrease in hepatic inflammation identified by ^{99m}Tc -mebrofenin transport should have benefited cell engraftment. Indeed, inhibition of inflammation by the cyclooxygenase mechanism through naproxen, depletion of neutrophils, or anticytokine treatments with etanercept and thalidomide produced engraftment of more cells in the liver compared with control animals (Fig. 4). Morphometric analysis showed 230 ± 35 transplanted cells per 100 hpf in control animals versus 506 ± 76 , 486 ± 58 , 720 ± 120 , and 980 ± 82 transplanted cells per 100 hpf in animals treated with naproxen, antineutrophil serum, etanercept, or thalidomide, respectively, P value of less than 0.05, ANOVA with the Holm–Sidak test.

Discussion

These studies show that changes in an endogenous molecular pathway in the liver facilitated identification of cell transplantation-induced inflammation. First, we found that cell transplantation activated hepatic inflammation, which was rapidly reflected by profound and prolonged perturbations in the biliary transport of ^{99m}Tc -mebrofenin, as long as inflammation persisted. Second, anti-inflammatory mechanisms that were capable of restoring hepatic transport of ^{99m}Tc -mebrofenin to normal were also successful in enhancing survival and engraftment of transplanted cells in the liver.

Although the process by which ^{99m}Tc -mebrofenin enters hepatocytes has not yet been identified, liver injury or inflammation does not block this mechanism [11], which was consistent with our finding that the net accumulation of ^{99m}Tc -mebrofenin after cell transplantation was unaltered. The measure of T_{peak} activity is not to be equated with extraction of ^{99m}Tc -mebrofenin, as T_{peak} reflects only the net balance between hepatic accumulation and transport of ^{99m}Tc -mebrofenin over time. The parameter of fractional retention in the liver of ^{99m}Tc -mebrofenin over 60 min has been validated in our earlier studies as an effective measure of the hepatobiliary transport of ^{99m}Tc -mebrofenin [11]. In recent studies, we identified that ^{99m}Tc -mebrofenin is transported from the liver by multidrug-resistance protein-2 (unpublished), which assigns this mechanism to a discrete biological pathway. We found that ^{99m}Tc -mebrofenin transport was extremely sensitive to the onset of hepatic inflammation and that this abnormality persisted throughout the period of inflammatory cell activity. This finding was in agreement with the effect on ^{99m}Tc -mebrofenin transport of hepatic inflammation after discrete exposure to the toxin, carbon tetrachloride [11].

Activation of IL-6 and TNF- α after cell transplantation should be in agreement with the central role of these inflammatory mediators in neutrophil and macrophage responses, which represent components of the innate immune system [20]. Earlier, we found that even a few nanograms of IL-6, producing blood concentrations of less than 100 pg/ml, were sufficient for inhibiting ^{99m}Tc -mebrofenin transport [11]. This evidence indicates that imaging for the integrity of ^{99m}Tc -mebrofenin transport mechanism will be highly sensitive and effective for identifying the onset and persistence of even minimal liver inflammation. This consideration was substantiated by inhibition of ^{99m}Tc -mebrofenin transport after transplantation of only 5 million hepatocytes, which constituted less than 1% of the liver cell mass, given that the liver of young adult rats contains over 1 billion cells.

We transplanted syngeneic cells in our studies to avoid changes induced by rejection of allogeneic cells, which is associated with additional recruitment of the acquired immune system. The lack of an effect of dexamethasone on ^{99m}Tc -mebrofenin transport suggests that lymphocytes were not involved in the inflammatory response after cell transplantation, which should be appropriate, as transplantation of syngeneic cells should have activated only innate immune system, exemplified by neutrophil and macrophage responses [20].

We found that ^{99m}Tc -mebrofenin transport was restored by targeting of neutrophil and Kupffer cell compartments with the nonsteroidal anti-inflammatory drug, naproxen, antineutrophil serum, and thalidomide, which has TNF- α cytokine-specificity and works through multiple additional mechanisms, including inhibition of nuclear factor- κB -mediated intracellular signaling [21]. This latter mechanism should be of interest in the context of the regulation of biliary function, which has been shown to be dependent, in part, on nuclear factor- κB -related intracellular signaling [22]. The inability of etanercept to normalize ^{99m}Tc -mebrofenin transport after cell transplantation suggests that inhibition of TNF- α alone was insufficient for this purpose. This finding will be in agreement with greater potency of IL-6, as well as synergistic activity of IL-6 and TNF- α in inhibiting ^{99m}Tc -mebrofenin transport [11]. The ability of drugs that restored ^{99m}Tc -mebrofenin transport after cell transplantation, that is, naproxen, antineutrophil serum, and thalidomide, to improve cell engraftment, however, strengthened the value of this transport mechanism. Improvement in cell engraftment with etanercept indicated a significant role for TNF- α in transplanted cell clearance, although superior cell engraftment after thalidomide was in agreement with the role of additional mechanisms in cell clearance.

In summary, the success of noninvasive imaging of ^{99m}Tc -mebrofenin transport after cell transplantation offers opportunities to assess the onset and persistence of hepatic inflammation. This will help optimize strategies for cell and gene therapy by permitting applications of anti-inflammatory interventions for improving survival and engraftment of transplanted cells. Such molecular pathway-centric imaging approaches should be helpful for establishing relevant mechanisms for assessing inflammation in other solid organs.

Acknowledgements

This study was supported in part by National Institutes of Health Grants R01 DK46952, P30 DK41296, P30 CA13330, and M01 RR12248. Ms Chaoying Zhang provided technical assistance.

References

1. Slehria S, Rajvanshi P, Ito Y, Sokhi R, Bhargava KK, Palestro CJ, et al. Hepatic sinusoidal vasodilators improve transplanted cell engraftment and ameliorate microcirculatory perturbations in the liver. *Hepatology* 2002;35:1320–1328. [PubMed: 12029617]

2. Joseph B, Malhi H, Bhargava KK, Palestro CJ, McCuskey RS, Gupta S. Kupffer cells participate in early clearance of syngeneic hepatocytes transplanted in the rat liver. *Gastroenterology* 2002;123:1677–1685. [PubMed: 12404242]
3. Wu YM, Joseph B, Gupta S. Immunosuppression using the mTOR inhibition mechanism affects replacement of the rat liver with transplanted cells. *Hepatology* 2006;44:410–419. [PubMed: 16871590]
4. Blé FX, Cannet C, Zurbrugg S, Karmouty-Quintana H, Bergmann R, Frossard N, et al. Allergen-induced lung inflammation in actively sensitized mice assessed with MR imaging. *Radiology*. 2008 [Epub ahead of print]
5. Behm CZ, Kaufmann BA, Carr C, Lankford M, Sanders JM, Rose CE, et al. Molecular imaging of endothelial vascular cell adhesion molecule-1 expression and inflammatory cell recruitment during vasculogenesis and ischemia-mediated arteriogenesis. *Circulation* 2008;117:2902–2911. [PubMed: 18506006]
6. Stelljes M, Hermann S, Albring J, Köhler G, Löffler M, Franzius C, et al. Clinical molecular imaging in intestinal graft-versus-host disease: mapping of disease activity, prediction, and monitoring of treatment efficiency by positron emission tomography. *Blood* 2008;111:2909–2918. [PubMed: 18057227]
7. Medarova Z, Tsai S, Evgenov N, Santamaria P, Moore A. In vivo imaging of a diabetogenic CD8 + T cell response during type 1 diabetes progression. *Magn Reson Med* 2008;59:712–720. [PubMed: 18302224]
8. Haller J, Hyde D, Deliolanis N, de Kleine R, Niedre M, Ntziachristos V. Visualization of pulmonary inflammation using noninvasive fluorescence molecular imaging. *J Appl Physiol* 2008;104:795–802. [PubMed: 18202169]
9. Vykhovanets EV, Shukla S, MacLennan GT, Resnick MI, Carlsen H, Blomhoff R, Gupta S. Molecular imaging of NF-kappaB in prostate tissue after systemic administration of IL-1 beta. *Prostate* 2008;68:34–41. [PubMed: 18004768]
10. Krishnamurthy GT, Krishnamurthy S. Cholescintigraphic measurement of liver function: how is it different from other methods? *Eur J Nucl Med Mol Imaging* 2006;33:1103–1106. [PubMed: 16858567]
11. Joseph B, Bhargava KK, Tronco GG, Palestro CJ, Gupta S. Systemic and local release of inflammatory cytokines regulates hepatobiliary excretion of ^{99m}Tc-mebrofenin. *Nucl Med Commun* 2008;29:336–344. [PubMed: 18317297]
12. Kroin JS, Schaefer RB, Penn RD. Chronic intrathecal administration of dexamethasone sodium phosphate: pharmacokinetics and neurotoxicity in an animal model. *Neurosurgery* 2000;46:178–182. [PubMed: 10626948]
13. Enomoto N, Takei Y, Hirose M, Ikejima K, Miwa H, Kitamura T, Sato N. Thalidomide prevents alcoholic liver injury in rats through suppression of Kupffer cell sensitization and TNF-alpha production. *Gastroenterology* 2002;123:291–300. [PubMed: 12105857]
14. Yee SB, Hanumegowda UM, Hotchkiss JA, Ganey PE, Roth RA. Role of neutrophils in the synergistic liver injury from monocrotaline and bacterial lipopolysaccharide exposure. *Toxicol Sci* 2003;72:43–56. [PubMed: 12604833]
15. Benten D, Kumaran V, Joseph B, Gupta S. Hepatocyte transplantation activates hepatic stellate cells with beneficial modulation of cell engraftment. *Hepatology* 2005;42:1072–1081. [PubMed: 16250034]
16. Haddad, el-B.; McCluskie, K.; Birrell, MA.; Dabrowski, D.; Pecoraro, M.; Underwood, S., et al. Differential effects of ebselen on neutrophil recruitment, chemokine, and inflammatory mediator expression in a rat model of lipopolysaccharide-induced pulmonary inflammation. *J Immunol* 2002;169:974–982. [PubMed: 12097404]
17. Kern S, Robertson SA, Mau VJ, Maddocks S. Cytokine secretion by macrophages in the rat testis. *Biol Reprod* 1995;53:1407–1416. [PubMed: 8562698]
18. Siegling A, Lehmann M, Platzer C, Emmrich F, Volk HD. A novel multispecific competitor fragment for quantitative PCR analysis of cytokine gene expression in rats. *J Immunol Methods* 1994;177:23–28. [PubMed: 7822831]

19. Irani AN, Malhi H, Slehria S, Gorla GR, Volenberg I, Schilsky ML, Gupta S. Correction of liver disease following transplantation of normal hepatocytes in LEC rats modeling Wilson's disease. *Mol Ther* 2001;3:302–309. [PubMed: 11273771]
20. Xia Z, Taylor PR, Locklin RM, Gordon S, Cui Z, Triffitt JT. Innate immune response to human bone marrow fibroblastic cell implantation in CB17 scid/beige mice. *J Cell Biochem* 2006;98:966–980. [PubMed: 16795075]
21. Ge Y, Montano I, Rustici G, Freebern WJ, Haggerty CM, Cui W, et al. Selective leukemic-cell killing by a novel functional class of thalidomide analogs. *Blood* 2006;108:4126–4135. [PubMed: 16940421]
22. Luedde T, Heinrichsdorff J, de Lorenzi R, De Vos R, Roskams T, Pasparakis M. IKK1 and IKK2 cooperate to maintain bile duct integrity in the liver. *Proc Natl Acad Sci U S A* 2008;105:9733–9738. [PubMed: 18606991]

Fig. 1.

General experimental design. The experiments were aimed at initially analyzing perturbations in ^{99m}Tc -mebrofenin handling after cell transplantation. (a) Shows that cells were transplanted at various times before analysis of ^{99m}Tc -mebrofenin excretion by image analysis protocols, as well as determination of the onset of hepatic inflammation by changes in cytokine expression and Kupffer cell activation. (b) Shows the plan for testing whether treatment with specific drugs prevented perturbations of ^{99m}Tc -mebrofenin handling and improved transplanted cell engraftment in short-term assays.

Fig. 2.

Scintigraphy showing hepatic uptake and excretion of ^{99m}Tc -mebrofenin. (a) Shows hepatic time-activity curves from several healthy control rats to demonstrate rapid accumulation and loss of ^{99m}Tc -mebrofenin. (b) Shows time-activity curves of hepatic ^{99m}Tc -mebrofenin accumulation and significant retention in animals after 2×10^7 cells were transplanted 1 day previously. Note that the peak accumulation of ^{99m}Tc -mebrofenin was similar under these two circumstances. (c) Shows dose-dependent abnormality in hepatic retention of ^{99m}Tc -mebrofenin 1 day after transplantation of cell numbers as indicated. (d) Shows that excretion of ^{99m}Tc -mebrofenin was perturbed over the long-term after transplantation of 1×10^7 cells.

Fig. 3.

Inflammatory changes in the liver after cell transplantation. (a) Shows liver from an animal 1 day after cell transplantation with histochemical staining for dipeptidyl peptidase IV. Several transplanted cells are noted within liver sinusoids or parenchyma (red color, arrows), consistent with the initial depositions of transplanted cells after injection into the splenic pulp. (b) Shows liver from an animal without cell transplantation with limited carbon incorporation in Kupffer cells (arrows). (c) Shows liver from an animal 1 day after cell transplantation with greater incorporation of carbon in activated Kupffer cells (arrows). (d) Shows cumulative morphometric data to indicate increased Kupffer cell activation within 6 h followed by its long-term persistence over 2 weeks after cell transplantation. (e) Shows reverse transcription PCR analysis of cytokine expression. PCR products are shown for TNF- α , IL-6, and the housekeeping gene, β -actin. Lane 1, molecular weight (MW) marker; lane 2, control untreated rat liver; lanes 3–6, liver from animals various times after transplantation of 2×10^7 cells; and lane 7, PCR mix alone as negative control. Note increased expression of TNF- α and IL-6 after cell transplantation. (a–c) Toluidine blue counterstain; Original magnification, $\times 200$.

Fig. 4. Changes in transplanted cell engraftment in response to earlier anti-inflammatory treatments. (a and b) Show transplanted cells in liver with dipeptidyl peptidase IV histochemistry 7 days after cell transplantation. In comparison with few transplanted cells in the control animal shown (a), far more transplanted cells are present in the animal treated with thalidomide before cell transplantation (b). Pa, portal area. (c) Shows cumulative morphometric analysis of transplanted cell numbers to indicate that transplanted cell survival improved after various anti-inflammatory treatments. Original magnification, (a and b), $\times 100$; toluidine blue counterstain.

Table 1

PCR primers

Gene	Forward primer	Reverse primer	Temperature (°C)	Product size (bp)	Reference number
TNF- α	5'-TACTGAACTTCGGGGTGATTGGTCC-3'	5'-CAGCCTTGTCCCTTGAAGAGAAACC-3'	60	295	[16]
IL-1 β	5'-GAGCAAGCTTTACCTATCCTGTGTAA-3'	5'-GAGAGAAATCCGTACTGTATGTACCAGT-3'	58	170	[17]
IL-2	5'-GCGCACCCCACTTCAAGCCCT-3'	5'-CCACCACAGTTGCTGGCTCA-3'	63	351	[18]
IL-6	5'-GTCTGAAATTCTCTCTGTGGTCTTCTGG-3'	5'-GTCTATCGATGGTCTTTGGTCTTTAGC-3'	58	228	[17]
β -Actin	5'-AGGCATACAGGGACAACAC-3'	5'-GGAGAAGATTTGGCACCCAC-3'	60	200	[19]

Table 2Effects of anti-inflammatory manipulations on ^{99m}Tc -mebrofenin excretion and engraftment of transplanted cells

Drug administered before cell transplantation	Mechanism of drug action	Hepatic excretion of ^{99m}Tc -mebrofenin	<i>P</i> values
Naproxen	Nonspecific cyclooxygenase inhibitor	Became normal	<0.001
Dexamethasone	Lymphocyte depletion	No change	Not significant
Rat antineutrophil serum	Neutrophil depletion	Became normal	<0.001
Etanercept	TNF- α blocker	No change	Not significant
Thalidomide	TNF- α and IL-6 blocker	Became normal	<0.001