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Adoptive Immunotherapy Against Allogeneic Kidney Grafts in Dogs with Stable Hematopoietic Trichimerism

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Dogs given nonmyeloablative conditioning and marrow grafts from 2 dog leukocyte antigen (DLA)-identical littermate donors developed stable trichimerism and stably accepted a subsequent kidney graft from one of the marrow donors without the need for immunosuppression. In this study, we used trichimeras to evaluate strategies for adoptive immunotherapy to solid tumors, using the kidney as a tumor surrogate. Three DLA-identical trichimeric recipients were established by simultaneously infusing marrow from 2 DLA-identical donor dogs into a DLA-identical recipient conditioned with 2 Gy of total body irradiation (TBI) and given a short course of postgraft immunosuppression. After stable hematopoietic engraftment was confirmed, a kidney was transplanted from 1 of the 2 marrow donors into each respective trichimeric recipient. Peripheral blood lymphocytes from each kidney donor were then used to sensitize the alternate marrow donor. The trichimeric recipients were given donor lymphocyte infusions (DLIs) from the sensitized dogs and monitored for chimerism, graft-versus-host disease (GVHD), and kidney rejection. After DLI, we observed both prompt rejection of the transplanted marrow and donor kidney and disappearance of corresponding hematopoietic chimerism. Presumably due to shared minor histocompatibility antigens, host chimerism also disappeared, and GVHD in skin, gut, and liver developed. The native kidneys, although exhibiting lymphocytic infiltration, remained functionally normal. This study demonstrates that under certain experimental conditions, the kidney—an organ ordinarily not involved in graft-versus-host reactions—can be targeted by sensitized donor lymphocytes.

INTRODUCTION

Graft-versus-tumor effects against hematologic malignancies after major histocompatibility complex–identical allogeneic hematopoietic cell transplantation (HCT) are the result of donor lymphocyte activity against minor histocompatibility (H) antigens expressed on hematopoietic cells [1,2]. Target minor H antigens include both ubiquitously expressed antigens and those specific for hematopoietic cells. Given these observations with blood cancers, clinicians have been quick to explore whether allogeneic graft-versus-tumor effects might exist and might be therapeutically exploited in various metastatic solid tumors. Malignancies evaluated include colon, breast, prostate, and renal cancers, among others [3-7]. Outcomes have been variable. Convincing graft-versus-tumor effects have been reported in a minority of patients with colon and renal cell cancer and possibly breast cancer, whereas patients with other types of malignancy have shown no response.

Previous work in our laboratory evaluated whether graft-versus-host (GVH) reactions, typically directed against hematopoietic cells, skin, gut, and liver, could be diverted to reliably include metastatic solid malignancies [8]. In these experiments, we used a canine HCT model in which the kidney served as a surrogate tumor target. The experiment was designed to determine whether adoptive immunotherapy could result in rejection of a specific organ not ordinarily involved in GVH reactions. Stable mixed chimerism in this model was maintained by regulatory T cells [9,10] and could not be dislodged by infusion of naïve donor lymphocytes [11]. To shift mixed chimerism to
all-donor chimerism and induce graft-versus-host disease (GVHD), donor lymphocytes were sensitized to host minor H antigens [8]. Accordingly, after mixed chimerism was established in donor lymphocyte antigen (DLA)-identical littermate recipients, the marrow donors underwent transplantation of kidneys from their respective mixed chimeric recipients, which they rejected within 3 to 5 weeks. When marrow donor lymphocytes harvested after kidney graft rejection were injected into the recipients, the mixed hematopoietic chimerism converted to full donor chimerism. But although 2 of 5 dogs developed GVHD, the residual native kidneys, which expressed the same minor antigens used to sensitize the donor, were not targeted by the adoptively transferred lymphocytes. Thus, although marrow donors readily rejected their recipients’ kidneys and thereby became sensitized to the mixed chimeras’ ubiquitously expressed minor H antigens, as evidenced by elimination of host hematopoiesis and GVHD, the level of sensitization apparently was insufficient to induce immunologic damage to the recipients’ remaining kidneys.

To investigate this question further, we developed a trichimeric model in which marrow from each of 2 DLA-identical littermate donors was transplanted simultaneously into a third littermate conditioned with 2 Gy of total body irradiation (TBI) and given a short course of postgraft immunosuppression [12]. A kidney heterotopically transplanted from 1 of the 2 marrow donors into the groin of each trichimeric recipient was stably accepted and then served as a solid organ surrogate for graft-versus-‘‘tumor’’ reactions. Toward this end, the other marrow donor was sensitized against H antigens of the marrow/kidney donor, and sensitized lymphocytes were subsequently infused into the respective trichimeras. The heterotopic location of the kidney grafts facilitated histological monitoring for lymphocyte infiltration and rejection. In addition, we postulated that the kidney transplantation would give rise to danger signals [13], thereby increasing the likelihood of becoming targets for lymphocytes sensitized to minor H antigens.

MATERIALS AND METHODS

Dogs

Litters of beagles and beagle/mini-mongrel mixes were raised at the Fred Hutchinson Cancer Research Center and assessed for disease. They received a preventive medicine program against worms, distemper, parvovirus, adenovirus (type 2), parainfluenza virus, corona virus, rabies, and canine papilloma virus. The dogs were aged 7 to 9 months (median, 8 months) and weighed 7.2 to 12.3 kg (median, 8.5 kg). The study design was approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center (which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International). Selection of donor/recipient triplets included typing of litters and parents to determine the identity of triplets for highly polymorphic microsatellite markers within DLA class I and class II regions [14], which was confirmed by DLA-DRBI gene sequencing [15].

Marrow Grafts

On day 0, recipients were treated with 2 Gy of TBI and subsequent i.v. infusion of marrow from donors 1 and 2, as described previously (Figure 1A) [12,16]. Bone marrow was aspirated from the humeri of anesthetized donors by vacuum pump aspiration. In brief, the skin over the humeral heads was surgically prepared. Using sterile technique, a long aspiration needle was inserted into the marrow cavity of the shoulder joint. The needle was connected by surgical tubing to a vacuum flask containing heparin. Approximately 150 mL of a blood–marrow mixture was collected per donor. The mixture was passed through 0.307- and 0.201-mm diameter stainless steel mesh screens. Nucleated marrow cell counts were corrected for white blood cell content. Marrow from both donors was infused into the recipients through the cephalic vein, with an approximate 2-hour interval between infusions. The marrow grafts from individual donors

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**Figure 1.** A, Schema for generating trichimeric marrow recipients and kidney transplant rejection. After 2 Gy TBI (1), marrow from 2 DLA-identical donors was simultaneously injected into the recipient (2). Postgrafting immunosuppression with CSP and MMF followed for 35 and 28 days, respectively (3). B, After stable chimerism was established, a kidney was transplanted from donor 2 into the recipient (4). Once stable kidney engraftment was verified, donor 1 was sensitized against minor H antigens of donor 2 with 3 PBMC injections (5). After DLI from sensitized donor 1 into the trichimeric recipient (6), the dogs were monitored for a shift in donor chimerism, GVHD, and kidney graft rejection (7).
contained a median of $4.6 \times 10^8$ nucleated cells/kg (range, 3.6 to $6.1 \times 10^8$). The dogs received supportive postgraft care. Immunosuppression consisted of oral cyclosporine (CsA), 15 mg/kg orally twice daily on days –1 to 35, and mycophenolate mofetil (MMF), 10 mg/kg s.c. twice daily on days 0 to 27 [17]. The MMF dosage was adjusted according to clinical toxicity, involving gastrointestinal distress.

**Chimerism Analysis**

Chimerism analyses were conducted on peripheral blood mononuclear cells (PBMCs) and granulocytes after separation of blood on Ficoll (density = 1.074), on marrow cells after buffered NH₄Cl lysis of red cells [12], and on sections of kidney allografts collected at necropsy. For the latter, infiltrating lymphocytes were collected from the minced sections of resected kidneys after 16 hours of incubation (37°C, 5% CO₂) through methods similar to those described previously [18], but without the use of enzymatic digestion. After the resected minced kidney tissue was incubated overnight in RPMI medium (Gibco/Invitrogen, Carlsbad, CA) plus 10% heat-inactivated dog serum, the cells were layered over Ficoll (density 1.074) and centrifuged at 1100 x g for 40 minutes. Cells at the interface were collected and washed in phosphate-buffered saline by centrifugation. The contributions of recipient and donor cells to peripheral blood and other hematopoietic tissues and kidney were quantified by fluorescent variable-number tandem repeat (VNTR) polymerase chain reaction (PCR) analysis, as described previously [12].

**Kidney Transplantation**

Kidney allografting from 1 of the marrow donors (donor 2) to the respective trichimeric marrow recipients was performed as described previously [19]. In brief, donor 2 was anesthetized, a midline laparotomy was performed, and the left kidney was exposed. The ureter, renal vein, and renal artery were secured and transected. The kidney was removed from the body cavity and perirenal vein, and renal artery were secured and transected. The ureter was anastomosed to the femoral artery and vein, respectively; and the ureter was implanted into the bladder [8]. The kidney graft recipients received no immunosuppressive therapy after day 35 after marrow grafting. Dog G513 required 15 weeks of CsA (7.5 mg/kg with taper to 2.5 mg/kg) before successful resolution of GVHD; in this dog, an additional 14 weeks elapsed between discontinuation of CsA and kidney transplantation. The kidneys were negative for lymphocytic infiltration for 17 to 23 weeks before DLI, as assessed by histological examination of needle biopsy specimens and measurement of kidney volume (Figure 2, left column). Trichimerism remained stable and unchanged between kidney transplantation and DLI.

**Donor Sensitization and Donor Lymphocyte Infusion (DLI)**

Marrow donor 1 was sensitized against marrow/kidney donor 2 with 3 injections of PBMCs administered 10 days apart. The kidney graft recipients received no immunosuppressive therapy except for CsA (7.5 mg/kg with taper to 2.5 mg/kg) before successful resolution of GVHD; in this dog, an additional 14 weeks elapsed between discontinuation of CsA and kidney transplantation. The kidneys were negative for lymphocytic infiltration for 17 to 23 weeks before DLI, as assessed by histological examination of needle biopsy specimens and measurement of kidney volume (Figure 2, left column). Trichimerism remained stable and unchanged between kidney transplantation and DLI.

**Effect of DLI on Kidney Allografts and Native Trichimeric Kidneys**

In each of the 3 sets of littermates, marrow donor 1 was sensitized against marrow/kidney donor 2 with 3 injections of PBMCs administered 10 days apart.
Ten days after the final injection, a mean of $4.2 \times 10^8$ PBMCs (range, 2.5 to $5.1 \times 10^8$) was harvested from sensitized donor 1 and injected into the respective tri-chimeric recipient.

Within a mean of 7 days after DLI (range, 3 to 15 days), the transplanted kidney volumes began to increase in the 3 trichimeric recipients; for example, kidney volume in one of the dogs increased by 162% in 28 days (data not shown). The biopsies of transplanted kidneys revealed lymphocytic infiltration at various time points after DLI (data not shown). Samples obtained at necropsy demonstrated extensive tubulitis and interstitial lymphocytic infiltration of the transplanted kidneys, consistent with acute severe rejection (Figure 2, middle column). Periglomerular lymphocytic infiltration was observed in the trichimeric recipients’ native kidneys, although these infiltrations were far less pronounced than those in the allogeneic kidneys (Figure 2, right column). In dog G643, 73% of the cells obtained from cultured transplanted kidney tissue were from donor 1 and 27% were from donor 2, possibly due to contaminating kidney cells (data not shown). Serum chemistry analysis after DLI found normal serum creatinine and blood urea nitrogen levels, indicating normal function of the recipients’ native kidneys (data not shown).

**Chimerism**

Before DLI, all 3 dogs had trichimerism that had been stable for more than 9 months (Table 1). After DLI, a rapid shift from mixed to nearly 100% donor 1 chimerism occurred in all 3 dogs within a median of 14 days (range, 9 to 28 days). This is illustrated for dog G643 in Figure 3. Before DLI, donor 1 (G641) contributed the least to the 3 hematopoietic systems in recipient dog G643, but after DLI, both granulocytes and PBMCs from donor 1 dominated the hematopoietic system. The shift from trichimerism to all donor 1 chimerism was also observed in nucleated cells collected from marrow and lymph node at necropsy (data not shown). Peripheral blood cell counts did not change significantly during the shift in chimerism (data not shown).

**GVHD after DLI**

All 3 dogs developed liver GVHD, as indicated by serum levels of alkaline phosphatase (1646, 2004, and 3165 U/L), aspartate aminotransferase (355, 862, and 1201 U/L), alanine aminotransferase (3380, 5440, and 5618 U/L), and bilirubin (5.0, 7.3, and 7.3 mg/dL). Furthermore, skin GVHD, characterized by inflammatory rashes of the skin of the ears, abdomen, nose, or oral mucosa, was observed within 30 days after DLI in dog G362, within 23 days after DLI in dog G513, and within 32 days after DLI in dog G643. All 3 dogs developed diarrhea and lost weight. The diagnosis of GVHD was confirmed by histopathology after the dogs were euthanized (Figure 4). The skin exhibited changes ranging from minimal infiltration of PBMCs to the presence of apoptotic bodies. The liver showed lymphocytic infiltration in central venous regions and bile duct lesions, confirming the diagnosis of GVHD. Atypical cell shapes within bile ducts of G513 indicated cell regeneration. The jejunum exhibited infiltration of PBMCs, and the ileum of dog G362 revealed multiple exploding crypts and crypt abscesses. Thus, all 3 dogs eventually developed 3-system acute GVHD (aGVHD) and were euthanized due to their worsening condition.

**DISCUSSION**

The current study was undertaken with the aim of targeting the kidney—an organ not among the typical targets for GVH reactions (i.e., hematopoietic system

| Trichimeric Recipient | Kidney Transplant | DLI | Euthanasia | GVHD After |
|-----------------------|-------------------|-----|------------|------------|
|                       | Granulocytes      | PBMCs | Granulocytes | PBMCs | Granulocytes | PBMCs | Granulocytes | PBMCs | Kidney Transplant | DLI |
| G 362                 |                   |       |            |            |            |       |            |            |            |     |
| Donor 1               | 33                | 28    | 30         | 28         | 100        | 99    |            |            |            |     |
| Donor 2               | 66                | 43    | 68         | 56         | 0          | 1     |            |            |            |     |
| Recipient             | 1                 | 29    | 2          | 16         | 0          | 0     |            |            |            |     |
| G 513                 |                   |       |            |            |            |       |            |            |            |     |
| Donor 1               | 96.9              | 53    | 87.4       | 39         | 91         | 86    |            |            |            |     |
| Donor 2               | 0.1               | 19    | 0.2        | 34         | 6          | 1     |            |            |            |     |
| Recipient             | 3                 | 28    | 12.4       | 27         | 3          | 13    |            |            |            |     |
| G 643                 |                   |       |            |            |            |       |            |            |            |     |
| Donor 1               | 28                | 5     | 18         | 17         | 95         | 94    |            |            |            |     |
| Donor 2               | 55                | 45    | 62         | 49         | 4          | 5     |            |            |            |     |
| Recipient             | 17                | 50    | 20         | 34         | 1          | 1     |            |            |            |     |

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*Donor 2 donated 1 kidney to the trichimeric recipient. Donor 1 underwent apheresis after 3 injections of peripheral blood donated by donor 2. The apheresis product was injected i.v. into the trichimeric recipient dog. Percent chimerism of each of the trichimeric dogs was determined before kidney transplantation, DLI, and at the time of euthanasia. Blood for serum chemistry analysis was collected before and after kidney transplantation and within 1 week before euthanasia of the trichimeric kidney recipients. GVHD was diagnosed for skin (S), gut (G), and liver (L) at necropsy.
and epithelial cells from skin, gut, liver, and mucous membranes)—for allogeneic immune reactions. The study was prompted by the desire to understand how graft-versus-tumor effects, typically observed for hematologic malignancies, could be extended to include metastatic solid tumor targets. Several clinical studies have used marrow transplantation to treat various metastatic solid tumors but with very limited success [3, 7]. In these studies, no tumor- or tissue-associated minor H antigens were used to sensitize donors against the patients’ tumors. Therefore, it would seem reasonable to explore adoptive immunotherapy with specifically sensitized lymphocytes to achieve successful treatment of metastatic solid malignancies. Because no transplantable tumors exist for random-bred dogs, a functioning kidney allograft with a unique set of minor antigens was chosen as a surrogate target, because impairment of kidney function could be easily assessed by serum creatinine levels and histopathologic examinations of percutaneous kidney biopsies.

In an earlier study, we sensitized canine marrow donors by transplanting kidneys from their respective mixed hematopoietic chimeric recipients, which they promptly rejected [8]. Subsequent lymphocyte infusions from marrow donors into chimeras caused conversion of mixed to all donor chimerism and, in some cases, GVHD in skin, gut, or liver; however, the remaining native kidneys did not come under immunologic attack. What explained this lack of graft-versus-kidney effect, a finding in striking contrast to the speed with which the marrow donors rejected kidneys from their chimeric recipients? We hypothesized that native kidneys lacked the appropriate “danger signals” [13] to attract lymphocytes, and that these signals were present in the transplanted kidneys. Under this model, danger signals may be either constitutively expressed or, in the case of transplantation, induced on stress, hypoxia, or trauma.

Here we attempted to address the hypothesis in stable trichimeras that had received marrow grafts...
from 2 DLA-identical littermates and a kidney graft from 1 of the marrow donors. The marrow donor kidney was grafted heterotopically as a putative target for lymphocytes that had been sensitized to minor H antigens. In all 3 cases, the kidneys were stably engrafted in the trichimeric recipients due to the induction of tolerance established by dual HCT. After immunizing the first marrow donors against minor H antigens of the second (marrow and kidney) donors and then infusing sensitized lymphocytes into the marrow recipients, an immunologic chain of events occurred that supported further and after receipt of sensitized DLI from donor 1. Stable long-term granulocyte chimerism and mononuclear cell trichimerism in the HCT recipient, donor 1 (■) and donor 2 (▲) was interrupted after an infusion of sensitized lymphocytes from donor 1 at week 44. Data points were obtained by VNTR PCR analysis.

Consistent with the kidney allograft rejection after DLI, the previously stable contribution of the marrow/kidney donor to the trichimeric hematopoiesis disappeared. Furthermore, and perhaps expected due to sharing of minor H antigens among recipients and kidney/marrow donors, host hematopoiesis also disappeared along with the appearance of GVHD in classical target organs, skin, gut, and liver. Somewhat in contrast with the widespread immunologic damage in allografted kidneys; host skin, gut, and liver; and host and donor 2 hematopoiesis, the hosts’ native kidneys showed mild to moderate lymphocytic infiltration (Figure 2), but renal function was not impaired. These findings are consistent with the “danger signal” hypothesis [13]. The basis of this model was that antigen-presenting cells (APC) are activated by signals from injured cells that had been exposed to toxins, pathogens, and mechanical damage [23]. Also falling into this category were transplanted organs in which surgical and or ischemic damage occurred, resulting in activation of antigen-presenting cells. Thomas et al. [24] reported that long-term renal allografts were established in histocompatibility complex-mismatched macaques treated with deoxyspergualin with or without immunotoxin (anti–CD3-CRM9, a mutant diphtheria toxin). The proposed mechanism of action of deoxyspergualin was the prevention of APC maturation, suppressed expression of costimulatory molecules, and establishment of a state of tolerance. Although in our trichimeric model the transplanted kidneys demonstrated stable engraftment, undetected molecular events may have been involved in the graft-versus-host reaction after DLI. In the transcriptional profiles of histologically normal living donor kidney allografts, Park et al. [25] found ongoing injury response and inflammation at 1-year posttransplantation, with up-regulated genes associated with inflammation, immunity, or response to injury.

Despite this evidence of susceptibility to rejection in the transplanted kidneys, we nonetheless found only limited infiltration of lymphocytes within the native kidneys. Perhaps more time was needed for renal function impairment to occur; however, the observation time was cut short by the development of life-threatening severe GVHD in the skin, gut, and liver. Nevertheless, it is clear that we successfully induced an immune response against a kidney tissue not normally targeted in GVHD. Further experiments with this model may shed light on immunologic mechanisms to avert GVHD in many normal tissues and may ultimately lead to methods for specifically targeting these responses against solid tumors. Next-generation sequencing studies are currently underway to identify coding of nonsynonymous single-nucleotide polymorphisms in breeders and their offspring that are unique to HCT recipients and expressed in tissues. Some of these polymorphisms may display ubiquitous tissue expression, whereas others may be restricted to hematopoietic cells or individual organs, such as kidneys, and thus define sets of H antigens that could be used to sensitize the marrow donor against a candidate target organ. Accordingly, subsequent DLI would result in immune reactions restricted to the desired target organ (e.g., marrow or kidney) without the development of GVHD.
ACKNOWLEDGMENTS

The authors thank Michele Spector, DVM, Alix Joslyn, Brian Steinmetz, and the research technicians in the canine facilities of the Fred Hutchinson Cancer Research Center for assistance with animal care; David Mathes, MD for performing kidney transplantations; Patrice Stroup and Stacy Zellmer for DLA typing; Drs Beard, Kiem, Milcerek, Nash, Parker, Thakar, Venkataraman, and Wang, who participated in weekend animal treatments; and Bonnie Larson and Helen Crawford for help with manuscript preparation. They also thank Dr Elizabeth Squires (SangStat Medical Corp, Fremont, CA) for the gift of cyclosporine and Dr Sabine Hadulco (Roche, Palo Alto, CA) for the gift of mycophenolate mofetil.

Financial disclosure: This work was supported by National Institutes of Health grants CA78902, CA15704, and DK56465-P30 (Core Center of Excellence in Hematology). The laboratory also was supported by an award from the Joseph Steiner Krebsstifung, Bern, Switzerland and a grant from the Lupin Foundation, Metairie, Louisiana (to R.S.).

REFERENCES

1. Frederik JH, van de Corput L, Marijt EWA, et al. Minor histocompatibility antigens in human stem cell transplantation. Exp Hematol. 2003;31:743-751.
2. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. Nat Rev Cancer. 2004;4:371-380.
3. Carnevale-Schianca F, Cignetti A, Capaldi A, et al. Allogeneic nonmyeloablative hematopoietic cell transplantation in metastatic colon cancer: tumor-specific T cells directed to a tumor-associated antigen are generated in vivo during GVHD. Blood. 2006;107:3795-3803.
4. Bishop MR. Allogeneic hematopoietic stem cell transplantation for metastatic breast cancer (review). Haematologica. 2004;89:599-605.
5. Ringden O, Le Blanc K. Allogeneic hematopoietic stem cell transplantation: state of the art and new perspectives (review). APMIS. 2005;113:813-830.
6. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med. 2000;343:750-758.
7. Tykodi SS, Warren EH, Thompson JA, et al. Allogeneic hematopoietic cell transplantation for metastatic renal cell carcinoma after nonmyeloablative conditioning: toxicity, clinical response, and immunological response to minor histocompatibility antigens. *Clin Cancer Res*. 2004;10:7799-7811.

8. Junghanss C, Takatu A, Little M-T, et al. Adoptive immunotherapy against kidney targets in dog leukocyte antigen-identical mixed hematopoietic canine chimeras. *Transplantation*. 2003;75:268-274.

9. Taranova AG, Georges GE, Yunusov M, et al. Breaking tolerance in stable mixed chimeric dogs with low-dose TBI and donor or recipient lymphocyte infusion [abstract]. *Blood*. 2003;102(Part 1):76a.

10. Lesnikova M, Nikitine A, Mason N, et al. Ex vivo expanded T regulatory (Treg) cells block conversion of mixed chimeras to complete donor chimerism [abstract]. *Blood*. 2006;108(Part 2):382b.

11. Georges GE, Storb R, Thompson JD, et al. Adoptive immunotherapy in canine mixed chimeras after nonmyeloablative hematopoietic cell transplantation. *Blood*. 2000;95:3262-3269.

12. Graves SS, Hogan W, Kuhr CS, et al. Stable trichimerism after marrow grafting from 2 DLA-identical canine donors and nonmyeloablative conditioning. *Blood*. 2007;110:418-423.

13. Fuchs EJ, Matzinger P. Is cancer dangerous to the immune system? (review). *Semin Immunol*. 1996;8:271-280.

14. Wagner JL, Burnett KC, DeRose SA, et al. Histocompatibility testing of dog families with highly polymorphic microsatellite markers. *Transplantation*. 1996;62:876-877.

15. Wagner JL, Works JD, Storb R. DLA-DRB1 and DLA-DQB1 histocompatibility typing by PCR-SSCP and sequencing. *Tissue Antigens*. 1998;52:397-401.

16. Ladiges WC, Storb R, Thomas ED. Canine models of bone marrow transplantation. *Lab Anim Sci*. 1990;40:11-15.

17. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood*. 1997;89:3048-3054.

18. Belldegrun A, Muul LM, Rosenberg SA. Interleukin 2 expanded tumor-infiltrating lymphocytes in human renal cell cancer: isolation, characterization, and antitumor activity. *Cancer Res*. 1988;48:206-214.

19. Kuhr CS, Allen MD, Junghanss C, et al. Tolerance to vascularized kidney grafts in canine mixed hematopoietic chimeras. *Transplantation*. 2002;73:1487-1493.

20. Li GP, Zhang H, Zhu CM, et al. Avidin-biotin system pretargeting radioimmunomaging and radioimmunotherapy and its application in mouse model of human colon carcinoma. *World J Gastroenterol*. 2005;11:6288-6294.

21. Sandmaier BM, Storb R, Santos EB, et al. Allogeneic transplants of canine peripheral blood stem cells mobilized by recombinant canine hematopoietic growth factors. *Blood*. 1996;87:3508-3513.

22. Lupu M, Gooley T, Zellmer E, et al. Principles of peripheral blood mononuclear cell apheresis in a preclinical canine model of hematopoietic cell transplantation. *J Vet Intern Med*. 2008;22:74-82.

23. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002;296:301-305.

24. Thomas JM, Contreras JL, Jiang XL, et al. Peritransplant tolerance induction in macaques: early events reflecting the unique synergy between immunotoxin and deoxyspergualin. *Transplantation*. 1999;68:1660-1673.

25. Park W, Griffin M, Grande JP, et al. Molecular evidence of injury and inflammation in normal and fibrotic renal allografts one year posttransplant. *Transplantation*. 2007;83:1466-1476.