Original Paper

4-1BB-mediated Signals Confer Protection Against Folic Acid-induced Nephrotoxicity

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Key Words

FAN (Folic acid induced nephrotoxicity) • Agonistic anti-4-1BB monoclonal antibodies (4-1BB mAb) • Tubulointerstitial lesions

Abstract

Background: The role of co-stimulatory molecules in renal diseases has been previously examined, however, little is known about the role of 4-1BB in the context of renal diseases resulting from nonimmune-mediated tubulointerstitial fibrosis. Folic acid induced Nephrotoxicity (FAN) in mice was used to explore the role of 4-1BB in this setting. **Methods:** CD1 mice were treated with folic acid and kidneys subsequently examined using histochemistry, in addition to defining T cell profiles and evaluating renal function. Increased CD3+ and CD4+ T lymphocytes present in blood and spleen at day 3 suggested immunopathological reactions during the early stages of FAN and decreased CD3+ and CD4+ T lymphocytes on day 14 were characteristic of an immunocompromised state observed during the late stages of FAN. **Results:** After 14 days of co-treatment with agonistic anti-4-1BB monoclonal antibodies, renal tubulointerstitial lesions were reduced. Renal function was improved, with Bun scores decreasing ($p < 0.01$) and sCr levels decreasing ($p < 0.01$). CD3+ and CD4+ T lymphocytes levels were increased during the early stages of disease in FA treated mice and reduced to the normal level in the 4-1BB-treated mice. CD3+ and CD4+ T lymphocytes levels were decreased in FA treated mice and returned to baseline in the 4-1BB-treated mice during later stages. **Conclusions:** Data presented in this report demonstrated that 4-1BB signals had immunoregulatory effects that attenuated early immune-mediated pathology and reversed the immunocompromised state observed during the later stages of disease.

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Introduction

Nonimmune factor induced renal diseases are common and can result from a variety of stimuli, including drug toxicity, ischemia, protein overload, renal ablation, obstructive uropathy, hypertension, diabetes and aging [1]. Some of these factors, such as hypertension and hyperlipidemia not only have been shown to induce nephropathy, but to also have been shown to contribute to progressive chronic kidney diseases (CKD). To date, there are no specific treatments for renal diseases caused by nonimmune factors except for some nonspecific therapies *e.g.*, anti-RAS (renin angiotension system) and anti-ROS (reactive oxygen species) drugs [1].

A question surrounding the mechanisms of disease associated with these renal disorders is whether some degree of immuno-pathogenesis is involved. Recent studies have been carried out examining the role of immune-mediated inflammation in cases of nonimmune renal diseases. The model of ischemic/reperfusion injury was used in the work of Bloudickova et al [2], which demonstrated that the immunosuppressant, mycophenolate mofetil (MMF), diminished immune cell function that resulted in a reduction in the rate of disease progression, supporting the hypothesis that the immune system played a role in the progression of chronic nephropathies. Similar data were demonstrated in diabetics and a 3/4 renal ablation induced nephropathies [3, 4].

A key issue in choosing immunosuppressant drugs as therapeutic alternatives for the treatment of renal diseases is that many of these compounds (*e.g.*, cyclosporine) also have numerous side effects, including the induction of nonimmune renal disease [5]. Therefore, identifying drugs that confer a degree of immunosuppressant compatible with the amelioration of symptoms associated with renal disease will be critical in the development of a successful treatment regimen.

Modification of specific immune responses can be carried out by targeting a variety of different stimulatory molecules, including 4-1BB that plays important costimulatory roles between B and T cells and antigen presenting cells (APC). 4-1BB ligation on these cell types induces proliferation and cytokine production associated with the elicitation of specific immune responses [6, 7]. Numerous reports have described a role for 4-1BB-derived signals in various autoimmune diseases that have been shown to play a role in the immunopathologic process of autoimmune lupus nephritis and rheumatoid arthritis [8, 9]. Furthermore, evidence has accumulated demonstrating that 4-1BB signals have become targets for the treatment of these diseases *e.g.*, 4-1BB signaling ameliorated experimental autoimmune encephalomyelitis and experimental autoimmune uveoretinitis [10, 11].

In this report, we describe the use of a mouse model of folic acid (FA)-induced nephrotoxicity we have used before to mimic nonimmune renal disease in which acute renal damage followed by partial regeneration and patchy tubulointerstitial fibrosis within several weeks [12]. This study examined the role of signals delivered via 4-1BB in the treatment of FA-induced nephrotoxicity. Data presented in this report suggested that agonistic 4-1BB mAb interventions could be developed as potential therapeutic approaches for the treatment of nonimmune renal diseases.

Materials and Methods

Animal Model

Male CD1 mice (8 weeks old, 18-22 g) were obtained from the Experimental Animal Center, Soochow University, China). In the preliminary experiment, mice were injected with an intraperitoneal (i.p.) FA injection (240 mg/kg) in vehicle (0.2 ml 0.3 M NaHCO₃) or treated with vehicle only. Kidneys from three mice/group from were analyzed on days 1, 3, 7 and 14 days post treatment.

In the intervention experiment, to assess vaccine efficacy, FA-treated mice (n=35/group) received 4-1BB mAb (kindly provided by Chen Liping from Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington, 98121, USA.) at a dose of 10 mg/kg by intraperitoneal injection on days 0, 3, 6 and 10

(4-1BBmAb group). FA-treated mice (n=35/group) received IgG at a dose of 10 mg/kg by intraperitoneal injection on days 0, 3, 6 and 10 (FA+IgG group). Ten vehicle -treated mice were also injected with the same dose nonspecific IgG as a negative control (control group). On days 3 and 14, spleen CD3+, CD4+ and CD8+ T lymphocyte profiles were assessed and on day 14 mice were assessed for tubulointerstitial lesions and serum analyzed for signs renal function. Mortality rates were assessed throughout the course of the experiment.

Histological Analysis

In the preliminary experiment, histological tissue analysis was carried out using Masson's trichrome staining procedure as a means of distinguishing cells from surrounding connective tissues. Briefly, deparaffinized slides were rinsed in deionized water and preheated in mordant in Boulin's solution at 56°C. Slides were washed in running tap water to remove unbound yellow color from sections and then stained with working Weiger's iron hematoxylin solution for 5 min. Slides were rinsed with deionized water and then stained in Biebrich Scarlet Acid Fuchsin and then placed in a Working Phosphotungstic solution for 5 min followed by a 5 min incubation in aniline blue. Finally, slides were placed in 1% acetic acid for 2 min, rinsed, dehydrated using standard gradient ethanol rehydration steps, cleared with xylene and then mounted.

Assessment of Renal Tubulointerstitial Lesions

The severity of cortical tubulointerstitial lesions, including inflammation and fibrosis, was assessed following microscopic analysis of lengthwise sections stained with hematoxylin and eosin. Tubulointerstitial lesions were scored using a scale from 0 to 4. A score of 0 was used to define tissues devoid of infiltrating inflammatory cells, 1 <25% cellular infiltrates; 2, 25-50% cellular infiltrates; 3, 50-75% cellular infiltrates and 4, 75-100% inflammatory infiltrates.

In addition, renal function assays were carried out to determining plasma Bun and Cr levels.

Flow Cytometric Analysis

Following treatment with 4-1BB mAbs for 3 and 14 days, mouse spleens were harvested and used to generate single cell suspensions. After hemolysis, cells were stained with rat anti-mouse CD3-FITC, anti-mouse CD4-FITC anti- mouse CD8-FITC antibodies (Caltag Laboratories, San Francisco, USA) at 4°C for 30 min. Cells were washed with PBS, 1% FCS and 0.01% sodium azide and then analyzed by flow cytometry using a FACS Calibur (Becton Dickinson Co., Mountain View, CA) flow cytometer according to the manufacturer's instructions. Data were analyzed using the Cell Quest program (Becton Dickinson, Franklin Lakes, NJ).

Statistics

One-way analysis of variance (ANOVA) and Tukey's test were used to determine statistical difference between treatment groups. Data are expressed as the mean $\pm$ SE. A p value of less than 0.05 was considered statistically significant.

Results

Histological analysis of FA-induced lesions

Masson's trichrome staining was used in preliminary studies as means of assessing tubular dilation and necrosis on days 1-3 and pathy tubulointerstitial fibrosis, inflammation and tubular atrophy on days 7-14 post FA administration. No defined glomerular fibrosis was observed in association with FA induced nephropathy through day 3; however, patchy morphologic changes due to tubular atrophy and tubulointerstitial fibrosis were observed on days 7 and 14 (Fig. 1). No differences were seen between control groups at each time point examined (not shown).

Effect of 4-1BB mAB on FA-induced nephrotoxicity

FA-treated mice presented with kidneys that were significantly reduced in size and weight in addition to presenting with granular changes that were significantly reduced in animals treated with 4-1BB mAb on day 14 (Fig. 2, Table 1). In addition, following administration of 4-1BB mAb on days 0, 3 and 10 the tubulointerstitial lesions were significantly reduced by day 14 when the tissues were analyzed (renal tubulointerstitial lesion scores were reduced from 3.07 ± 0.83 to 1.44 ± 0.76 , $p < 0.01$; Fig. 3 and Table 1), mortality rates were decreased from 60.00% to 28.57% ($p < 0.05$) and plasma Bun and Cr levels were also significantly altered ($p < 0.05$) (Fig. 3 and Table 1).

Fig. 1. Analysis of FAN-induced nephrotoxicity. Renal tissues harvested from mice injected with FA or healthy controls were carried out at several time points post FA administration (days 1, 3, 7 and 14 days). Sections were stained with Masson's trichrome. Tissues stained red represent either cytoplasm or muscle; tissues stained blue represent collagen and tissues stained black represent stained nuclear material. Marked tubular enlargement was observed at day 1 followed by improvement on day 3; however, pronounced tubulointerstitial fibrosis and tubular atrophy presented on both days 7 and 14. No glomerular fibrosis was observed at any time point. (Magnification X400).

Fig. 2. Gross kidney morphology. Kidneys from FA+IgG-treated mice or from mice treated with FA but also receiving injections of 4-1BB mAb were examined 14 days after injection with FA. Kidneys from FA+IgG-treated mice were shrunken and granular (middle) compared to kidneys from mice injected with FA but treated with 4-1BB mAb (right) and from IgG+vehicle treated controls (left).

Fig. 3. Morphological changes after treatment with 4-1BB mAb. Kidneys from IgG+vehicle treated control mice (A), treated with FA +IgG (B) or treated with FA and injected with agonistic 4-1BB mAb (C) were harvested and analyzed histological following staining with hematoxylin. Tissues from FA+IgG-injected mice (B) presented with elevated fibrosis compared to kidneys harvested from FA-injected mice that were treated with 4-1BB mAb (C). No any tubulointerstitial fibrosis was seen in IgG+vehicle treated control mice (A). Magnification X100.

Fig. 4. The effect of 4-1BB mAb on T lymphocyte subtypes. Percentage positive CD3+, CD4+ and CD8+ T cells were assessed in blood from mice in the respective treatment of FA+IgG (FA+IgG group), FA+4-1BB mAb (4-1BB mAb group) and IgG+vehicle treated controls (control group) by flow cytometry on days 3 (A) and 14 (B) post FA treatment. * $p < 0.05$ compared to the control and FA+IgG group.

Table 1. Intervention effects of 4-1BBmAb on FAN

	Control	FA+IgG	4-1BBmAb
n	10	14	25
tubulointerstitial lesion score	0	3.07±0.83	1.44±0.76 ++
Bun (mmol/L)	6.71±1.08	22.02±2.25	8.75±2.26 +
Cr (μmol/L)	27.80±1.11	73.79±3.93	42.44±9.49 +
Mortality rate	0%	60.00% (21/35)	28.57% (10/35) ++
Kidney weight (g)	0.22±0.03	0.13±0.05	0.20±0.04 ++

+: compared with group FA, $P < 0.05$ ++; compared with group FA, $P < 0.01$

Flow cytometric analysis

The effects of FA treatment and 4-1BB mAb administration on T cell profiles were assessed by flow cytometry on days 3 and 7. Profiles were compared between IgG+vehicle treated control (control group) mice and mice that received FA + nonspecific IgG (FA+IgG group) or FA + 4-1BB mAb (4-1BBmAb group). FA+IgG treatment initially increased CD3+ and CD4+ T cell numbers ($p < 0.05$), compared with the IgG+vehicle treated controls at day 3 (Fig. 4A). However, 4-1BB mAb treatment significantly reduced CD3+ and CD8+ cell counts compared to FA + IgG-treated mice at this time point ($p < 0.05$) and were similar to the T cell profile observed in the IgG+vehicle treated controls. No effects on CD8+ cell counts at day 3 were seen following treatment with FA or following 4-1BB mAb administration (Fig. 4A).

On day 14, however, the T cell profiles significantly changed. Specifically, the CD3+, CD4+ and CD8+ T cell percentages were significantly reduced in the FA + IgG group compared to IgG+vehicle treated controls and FA + 4-1BB-treated mice ($p < 0.05$, Fig. 4B). Although the T cell profile of the FA + 4-1BB mAb-treated mice was not identical to that of the IgG+vehicle treated controls (as was observed on day 3), all the subtype T cell counts in 4-1BB mAb-treated mice were significantly higher than in mice treated with FA + IgG, suggesting that 4-1BB mAb treatment could prevent changes to the T cell compartment induced by FA treatment.

Discussion

Until recently, pathologies associated with nonimmune factor-induced renal diseases were believed to be independent of immune-mediated damage. This premise has been tested recently as a consequence of observations suggesting that drugs used in the treatment of autoimmune diseases successfully reduced inflammatory responses associated with nonimmune factor induced renal disease [2-4]. To date, little research has been carried out investigating the role of immune cells in the context of nonimmune renal disease *i.e.*, renal disease presenting without immune etiopathogenesis, such as nephrotoxic drugs, chronic ischemia, proteinuria, hypercholesterolemia, hypertension, renal ablation, obstructive uropathy, aging, diabetes and FA-induced nephropathy [1].

In the study described here, FA-induced nephropathy was associated with the development of tubular enlargement and necrosis shortly after administration and mainly affected renal tubules 1-3 day post administration and induced tubulointerstitial fibrosis and tubular atrophy 7 days post FA treatment.

Additional systemic effects resulting from FA treatment were determined by assessing changes to the T cell compartment *i.e.*, changes to CD3+, CD4+ and CD8+ T cell percentages in spleen. This analysis demonstrated that during the early stages of FAN, CD3+ and CD4+ counts were significantly elevated compared to either FA-treated mice receiving 4-1BB mAb or untreated controls (CD3+ and CD4+ levels in FA + 4-1BB mAb-treated mice were indistinguishable from those of untreated controls). Increases in CD3+ and CD4+ cells in

mice presenting with later stage FAN (d7) were not observed at this time. In fact, CD3+, CD4+ and CD8+ counts were significantly down regulated on day 14 compared to FA-treated mice receiving 4-1BB mAb or the healthy controls. Although the CD3+, CD4+ and CD8+ counts in FA mice treated with 4-1BB mAb were not equal to those of the untreated controls, these percentages were still significantly greater than the values recorded in FA-treated mice, suggesting that 4-1BB mAb-mediated signals not only attenuated disease severity but altered the profile of the T cell compartment systemically. This significant decrease in CD3+ and CD4+ T cell counts observed at day 14 in FA-treated mice might explain the reduction in immune function observed following progression into the chronic renal failure stage that was also associated with an increased chance of developing an opportunistic infection.

Our hypothesis that FAN-associated pathology possessed an immune component was tested by treating FA-treated mice with 4-1BB mAb. Since 4-1BB mAb delivers stimulatory signals, we had originally hypothesized that 4-1BB mAbs would exacerbate renal tubulointerstitial lesions by activating inflammatory pathways. However, data presented in this report demonstrated that administration of 4-1BB mAb reduced renal tubulopinterstitial injury and improved renal function (Table 1 and Fig. 3). In addition, as described above, 4-1BB mAb treatments decreased T cell counts on day 3 but increased CD3+, CD4+ and CD8+ T lymphocyte counts at day 14 compared to FA-treated mice. One explanation for these results is that 4-1BB (which is an activation-induced tumor necrosis factor receptor family member) is a molecule that acts primarily as a powerful T-cell co-stimulatory molecule that may also possess previously undefined immunosuppressive properties. Recently, it was demonstrated that 4-1BB-mediated signals could elicit seemingly contradictory responses in different disease models [13-15]. To date, agonistic 4-1BB mAb administrations have been shown to possess therapeutic potential against a variety of tumors, CD4+ T-cell-mediated autoimmune diseases and chronic graft-versus-host disease. Deletion of 4-1BB in *lpr* mice accelerated lacrimal gland lesions as a consequence of increased CD4+ T-cell infiltration and the production of immune modulators [11]. Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies directed against nuclear antigens, including nucleosomes and DNA. To determine the role of the T-cell costimulatory molecule 4-1BB in the regulation of SLE, MRL-Fas (*lpr/lpr*) mice deficient in 4-1BB [*lpr*/4-1BB (-/-)] were generated and their disease phenotype compared to that of control *lpr* mice. The primary observation from this study was that *lpr*/4-1BB (-/-) mice had more pronounced skin lesions (which developed more rapidly), presented with increased lymphadenopathy, had increased renal damage and had higher mortality rates than 4-1BB mAb-treated *lpr* mice. These data suggested the 4-1BB/4-1BB ligand signaling pathway played an important role in the induction of SLE and that deletion of 4-1BB increased susceptibility to SLE in *lpr* mice, leading to accelerated disease progression and early rates of mortality [14]. These authors proposed that the dual agonistic and antagonistic signals delivered by anti-4-1BB were mediated by a novel CD11c+CD8+ T-cell population. Agonistic 4-1BB mAb-mediated expansion of this novel CD11c+CD8+ T cell population represents an additional regulatory mechanism that likely plays a critical in mediating immunosuppression *in vivo*. These CD11c+CD8+ T cells expressed high levels of IFN- γ , developed into effective killers and mediated selective suppression of CD4+ T cells. Indoleamine 2,3-dioxygenase-expressing dendritic cells (activated by 4-1BB signals) were involved in the depletion of autoimmune CD4+ T cells by suppressing tryptophan, important in the maintenance and growth of autoimmune CD4+ T cells. Agonistic 4-1BB-mediated expansion of CD4+CD25+ regulatory T cells was also involved in mediating suppression [16-19].

Accurately, T cells subtypes were affected by renal function. Chronic renal failure is associated with severe alterations of the immune system [20]. In our paper, renal dysfunction was resulted from folic acid induced nephrotoxicity and followed immune inflammation through variable T cells subtypes. CD137, co-stimulated factors, play an important role between APC (antigen presenting cell) and T cells. CD137 signal have dual effects on immune responds that reduce over inflammation and improve down-regulated immune reaction. We

concluded that CD137 signal has therapeutic effects on the renal tubulointerstitial lesions through suppressed immune inflammation by decreased T cells subtypes at day 3. And improved renal function might be the reason of T cells subtypes recovery at day14. But CD137 signal can also restore the down regulated T cells at day 14. In current situation it was difficult to determine whether T cells subtypes recovery was induced by improved renal function or combined with CD137 signal at day 14. It needs more researches to confirm.

In conclusion, this study demonstrated that 4-1BB-mediated signals reduced the severity of tubulointerstitial lesions via a mechanism that may be related to decreasing CD3+ and CD4+ levels during the early stages of FAN. During later stages of FAN, the decreased CD3+, CD4+ and CD8+ levels observed in FA-treated mice may have resulted in reduced cellular immune responses associated with chronic kidney disease that were elevated following the administration of agonistic 4-1BB mAb. These observations suggested that 4-1BB signaling might impart both activating and suppressing signals in FAN via mechanisms yet to be described. This report demonstrated for the first time that immune mediated injury was involved in the pathogenesis of FA-induced nephropathy and that successive treatment with 4-1BB mAb not only confirmed the immune component in the disease process but also demonstrated that the 4-1BB mAb significantly reduced FA-induced nephrotoxicity. This further suggested that 4-1BB-mediated signals represent a potential means of attenuating renal immune-inflammatory-mediated injuries and effectively preventing renal tubulointerstitial injuries. Additional studies will be needed to define the 4-1BB mAb mechanism of action.

Conflict of interest

The authors declare that they have no conflict of interest

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References

- 1 Rodriguez-Iturbe B, Pons H, Herrera-Acosta J, Johnson RJ: Role of immunocompetent cells in nonimmune renal diseases. *Kidney Int* 2001;59:1626-1640.
- 2 Bloudícková S, Rajnoch J, Lodererová A, Honsová E, Viklický O: Mycophenolate mofetil ameliorates accelerated progressive nephropathy in rat. *Kidney Blood Press Res* 2006;29:60-66.
- 3 Rodríguez-Iturbe B, Quiroz Y, Shahkarami A, Li Z, Vaziri ND: Mycophenolate mofetil ameliorates nephropathy in the obese Zucker rat. *Kidney Int* 2005;68:1041-1047.
- 4 Tapia E, Franco M, Sánchez-Lozada LG, Soto V, Avila-Casado C, Santamaría J, Quiroz Y, Rodríguez-Iturbe B, Herrera-Acosta J: Mycophenolate mofetil prevents arteriolopathy and renal injury in subtotal ablation despite persistent hypertension. *Kidney Int* 2003;63:994-1002.
- 5 Koppelstaetter C, Aydin S, Abberger T, Wolf AM, Mayer G, Pfaller W: Cyclosporine A induces senescence in renal tubular epithelial cells. *Am J Physiol Renal Physiol* 2007;293:F831-838.
- 6 Vinay DS, Kwon BS: Immunotherapy targeting 4-1BB and its ligand. *Int J Hematol* 2006;83:23-28.
- 7 Lee EA, Kim JE, Seo JH, Kwon BS, Nam SH, Kwon B, Cho HR: 4-1BB (CD137) signals depend upon CD28 signals in all immune responses. *Exp Mol Med* 2006;38:606-615.

- 8 Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, Choi BK, Vinay DS, Kwon BS: 4-1BB-mediated immunotherapy of rheumatoid arthritis. *Nat Med* 2004;10:1088-1094.
- 9 Vinay DS, Choi JH, Kim JD, Choi BK, Kwon BS: Role of endogenous 4-1BB in the development of systemic lupus erythematosus. *Immunology* 2007;122:394-400.
- 10 Sun Y, Lin X, Chen HM, Wu Q, Subudhi SK, Chen L, Fu YX: Administration of agonistic anti-4-1BB monoclonal antibody leads to the amelioration of experimental autoimmune encephalomyelitis. *J Immunol* 2002;168:1457-1465.
- 11 Choi BK, Asai T, Vinay DS, Kim YH, Kwon BS: 4-1BB-mediated amelioration of experimental autoimmune uveoretinitis is caused by indoleamine 2,3-dioxygenase-dependent mechanisms. *Cytokine* 2006;34:233-242.
- 12 Yuan HT, Li XZ, Pitera JE, Long DA, Woolf AS: Peritubular capillary loss after mouse acute nephrotoxicity correlates with down regulation of vascular endothelial growth factor-A and hypoxia-inducible factor-1alpha. *Am J Pathol* 2003;163:2289-2301.
- 13 Vinay DS, Cha K, Kwon BS: Dual immunoregulatory pathways of 4-1BB signaling. *J Mol Med* 2006;84:726-736.
- 14 Vinay DS, Kim JD, Asai T, Choi BK, Kwon BS: Absence of 4-1BB gene function exacerbates lacrimal gland inflammation in autoimmune-prone MRL-Fas^{lpr} mice. *Invest Ophthalmol Vis Sci* 2007;48:4608-4615.
- 15 Vinay DS, Kwon BS: Immunotherapy targeting 4-1BB and its ligand. *Int J Hematol* 2006;83:23-28.
- 16 Ju SA, Park SM, Lee SC, Kwon BS, Kim BS: Marked expansion of CD11c+CD8+ T-cells in melanoma-bearing mice induced by anti-4-1BB monoclonal antibody. *Mol Cells* 2007;24:132-138.
- 17 Penberthy WT: Pharmacological targeting of IDO-mediated tolerance for treating autoimmune disease. *Curr Drug Metab* 2007;8:245-266.
- 18 Elpek KG, Yolcu ES, Franke DD, Lacelle C, Schabowsky RH, Shirwan H: Ex vivo expansion of CD4+CD25+FoxP3+ T regulatory cells based on synergy between IL-2 and 4-1BB signaling. *J Immunol* 2007;179:7295-7304.
- 19 Park MJ, Min SY, Park KS, Cho YG, Cho ML, Jung YO, Park HS, Chang SH, Cho SG, Min JK, Park SH, Kim HY: Indoleamine 2,3-dioxygenase-expressing dendritic cells are involved in the generation of CD4+CD25+ regulatory T cells in Peyer's patches in an orally tolerated, collagen-induced arthritis mouse model. *Arthritis Res Ther* 2008;10:R11.
- 20 Girndt M, Sester M, Sester U, Kaul H, Köhler H: Molecular aspects of T- and B-cell function in uremia. *Kidney Int Suppl* 2001;78:S206-11.