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Original Article

PD-L1 partially protects renal tubular epithelial cells from the attack of CD8+ cytotoxic T cells

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Abstract

Background. Activated infiltrating T cells play a crucial role in nephritic inflammation via the direct interaction with proximal tubular epithelial cells (TEC). Under inflammatory conditions, major histocompatibility complex class I and II molecules are upregulated on the surface of renal TEC, enabling them to function as 'non-professional' antigen-presenting cells (APC) to activate T cells, and, in turn to be targeted by cytotoxic T lymphocytes (CTL) to cause tissue damage. It is known that co-stimulatory (e.g. B7/CD28) and co-inhibitory (e.g. PD-L1/PD-1) signals regulate and determine the magnitude of T cell responses. In this study, we examined the expression of co-stimulatory molecule PD-L1 by renal TEC and the functional role of renal PD-L1/PD-1 pathway in regulating CD8+ T cell responses induced by antigen-presenting renal TEC.

Methods. Renal TEC were treated with type I and type II interferons (IFN-α, IFN-β or IFN-γ). PD-L1 expression was then determined with flow cytometry and RT-PCR. To investigate the functional role of renal epithelial PD-L1 on CD8+ CTL responses, H-2K^b-restricted, OVA$_{257-264}$ peptide-specific CD8+ T cells isolated from OT-1 T cell receptor transgenic mice were co-incubated with IFN-stimulated, OVA$_{257-264}$ peptide-pulsed congeneic TEC. The activation of OT-1 CD8+ CTL was estimated either by IFN-γ production in the supernatants of co-cultures or by CTL activity.

Results. TECs do not constitutively express PD-L1 on their surface. However, a strong and dose-dependent upregulation of PD-L1 was observed on TEC after stimulation with IFN-β or IFN-γ, but not with IFN-α. OVA$_{257-264}$ peptide pulsed-TEC were able to activate OT-1 CD8+ T cells, indicated by the high amount of IFN-γ production and cytolysis of TEC. Blockade of epithelial PD-L1 with specific mAb significantly increased OT-1 CD8+ T cell activity, indicating that the PD-L1 pathway has a negative effect on CD8+ T cell responses. Moreover, IFN-β- or IFN-γ-stimulated TEC with high surface PD-L1 expression were more resistant to the cytolysis by OT-1 CTL.

Conclusion. Together our data reveal that the renal PD-L1/PD-1 pathway has a negative effect on CD8+ CTL activation. PD-L1 might, therefore, act as a protective molecule on TEC, downregulating the cytotoxic renal parenchymal immune response.

Keywords: CD8+ T cells; interferon; PD-L1; renal tubular epithelial cells

Introduction

The proximal renal tubular epithelium is an important target in tubulointerstitial immune-mediated kidney diseases [1]. Activated infiltrating T cells play a crucial role in nephritic inflammation via the direct interaction with renal proximal tubular epithelial cells (TEC). Thus, infiltrating CD4+ and CD8+ T cells surrounding the tubular epithelium are found in abundance in most tubulointerstitial disease processes, including tubulointerstitial nephritis and renal allograft rejection [2,3]. The ability of renal TEC to promote T cell activation is attributable to their expression of major histocompatibility complex (MHC) class I and class II molecules and co-stimulatory molecules. Renal TECs act as non-professional antigen-presenting cells (APC) to trigger specific T cell responses, and can in turn be targeted by antigen-specific cytotoxic T cells (CTL) during immune-mediated kidney diseases. Although it is well known that CD8+ T cells are the predominant effector cells in tubulointerstitial renal injury and allogeneic kidney transplantation, the regulatory mechanisms of CD8+ T cell responses to TEC have not been fully elucidated yet [4–8]. In particular, studies examining the role played by B7-related

co-stimulatory molecules on CD8$^+$ T cell responses to TEC are lacking.

Co-stimulatory pathways effectively regulate T cell activation and tolerance [9,10]. The classical co-stimulatory molecules expressed on professional APC include B7.1, B7.2 and CD40. Interactions of B7/CD28 and CD40/CD40L provide positive signals to upregulate T cell responses, whereas the B7/CTLA-4 pathway leads to suppression of T cell responses. Recently, several novel co-stimulatory and co-inhibitory molecules have been discovered. A negative T cell regulatory pathway which could potentially be important for immune-mediated tubulointerstitial injury is represented by the interaction between programmed death-1 (PD-1) expressed on activated T cells, and its two ligands PD-L1 and PD-L2 which are expressed on APC [9–11]. Recently, we and others have described that PD-L1 is strongly upregulated by TEC in rejected kidney transplants as well as in inflamed kidneys [2,3,12], suggesting that it may play an important regulatory role in immune-mediated renal injury.

We and others have previously shown that IFN-γ treatment rapidly and strongly upregulated PD-L1 but not PD-L2 on cultured murine TEC, and that blockade with mAbs of PD-L1 on TEC significantly increased antigen-specific CD4$^+$ T cell responses [2]. Thus far the functional role of the renal epithelial PD-L1/PD-1 pathway in CD8$^+$ CTL responses has not been investigated. In this study we first examined in detail the differential effect of type I and type II interferons (IFNs) on PD-L1 and MHC class I expression in renal TEC, then examined the functional role of the PD-L1/PD-1 pathway on the antigen-specific activation of CD8$^+$ T cells by TEC, and the cytolytic effect to TEC by activated CD8$^+$ CTL.

Materials and methods

General reagents

Cell culture reagents were obtained from Invitrogen (Gaithersburg, MD) and Sigma (St. Louis, MO). Recombinant mouse IFN-α, IFN-β and IFN-γ were purchased from R&D Systems (Oxford, UK). Anti-mouse PD-L1, PD-1 and MHC class I monoclonal antibodies (mAbs) were purchased from eBioscience (San Diego, CA). Biotin-conjugated anti-mouse H-2K^k was obtained from BD Biosciences (San Jose, CA). Chicken ovalbumin peptide 257-264 (OVA$_{257-264}$, amino acid sequence SIINFEKL) was purchased from Proimmune (London, UK). A control H-2K^b restricted β-galactosidase peptide 497–504 (β-Gal$_{497-504}$, amino acid sequence ICPMYARV) was a kind gift from Prof. Groettrup of Constance University, Germany. Anti-mouse CD8a (Ly-2) MACS microbeads were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany).

Animals, cell lines and renal proximal TEC cultures

T cell receptor (TCR) transgenic OT-1 mice [13] were obtained from Jackson Laboratories (Bar Harbor, ME). C57BL/6 (B6) and C3H/HeN (C3H) mice were purchased from Harlan (Horst, Netherlands). All animals were used at 8–16 weeks of age. The study protocol was approved by the regulatory commission for animal studies of the Canton Zurich, Switzerland. Primary cultures of murine renal proximal TEC were prepared as described previously [14]. Primary renal TEC and SV40-transformed murine renal proximal TEC line C1.1 were cultured on collagen-coated cell culture plates in modified K1 medium as previously described [14]. A murine macrophage cell line RAW 264.7 and EL-4 cells were grown in complete Dulbecco's modified Eagle's medium (DMEM) with Glutamax®, supplemented with 1000 U/ml penicillin, 100 μg/ml streptomycin and 10% FBS. All cells were maintained at 37°C with 5% CO_2.

RNA extraction and RT-PCR analysis

Total RNA from cell cultures was extracted using the RNeasy® mini kit (Qiagen, Valencia, CA). All samples were quantified by the measurement of the optical density at 260 nm, and equal amounts were amplified by reverse transcription-polymerase chain reaction (RT-PCR) (Qiagen® OneStep PCR kit, Qiagen). Primer sequences of PD-L1 were determined as previously described [2]. To ensure even amounts of template, the rat housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was co-amplified as described previously [15]. Reaction mixtures were separated on 1.5% agarose gels containing ethidium bromide, and bands were detected under UV light and analysed with the Bio-Image System Chemidoc^{TM} XRS (Bio-Rad, Hercules, CA).

Flow cytometric analysis

RAW264.7, the renal TEC line C1.1 and primary cultures of renal TEC were stimulated with IFN-α, IFN-β or IFN-γ for 48 h. Cells were harvested by light trypsinization, washed twice with Hanks' Balanced Salt Solution (HBSS, Invitrogen) and suspended in PBS containing 2% FBS and 0.1% sodium azide. Before staining with the primary antibody, macrophages were pre-incubated with anti-mouse CD16/CD32 Ab for 15 min to block FcR binding. After incubating with primary mAbs for 45 min on ice, cells were washed twice and incubated with the appropriate FITC-conjugated secondary Ab for 30 min. Cells were then washed and analysed using an FACScan flow cytometer and the Cell Quest^{TM} software (Becton Dickinson).

Isolation of CD8$^+$ T cells from OT-1 mice

Spleen and lymph nodes (LN) were harvested from 8–16-week-old naïve OT-1 mice. CD8$^+$ cells were isolated using anti-mouse CD8a (Ly-2) MACS microbeads according to the manufacturer's protocol. The freshly isolated OT-1 CD8$^+$ T cells were either used immediately for antigen presentation assays, or were activated in vitro. To activate OT-1 CTL, OT-1 CD8$^+$ T cells were stimulated with B6 splenocytes pulsed with 0.1 μg/ml of OVA$_{257-264}$ peptide and were incubated in DMEM medium supplemented with 5 μM 2-mercaptomethanol, 1% HEPES, 1000 U/ml penicillin, 100 μg/ml streptomycin and 10% FBS at a T cell to APC ratio of 1:5. After 6 days of stimulation, activated OT-1 CTL were used for CTL assays.

Antigen presentation of renal B6 TEC to resting OT-1 CD8$^+$ T cells

To study the antigen presentation of the H-2K^b-restricted peptide OVA$_{257–264}$ by congeneic primary TEC to OT-1 CD8$^+$ T cells, B6 primary TEC were pre-treated with IFNs for 48 h. Cells were washed to remove excess cytokines and were harvested by light trypsinization. The cells were then pulsed with 0.1 μg/ml of OVA$_{257–264}$ peptide or the control H-2K^b-restricted β-Gal$_{497–504}$ peptide for 1 h at 37°C. After washing, TEC were counted and seeded in 96-well U-bottom cell culture plates (Costar) in DMEM medium supplemented with 5% FBS, 1000 U/ml of penicillin and 100 μg/ml of streptomycin. The resting OT-1 CD8$^+$ T cells were added to TEC cultures and were co-incubated at 37°C. Anti-mouse PD-L1 mAb (10 μg/ml) were added to TEC 1 h prior to the addition of OT-1 CD8$^+$ T cells as indicated. After 24 h, supernatants from co-cultures were collected and analysed for IFN-γ content using a mouse IFN-γ-specific ELISA kit (OptEIA^{TM} mouse IFN-γ kit, BD Pharmingen). In control experiments, OVA$_{257–264}$ or β-Gal$_{497–504}$ peptide-pulsed EL-4 cells were used.

IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assays (Diaclone, Besançon, France) were also used to determine the IFN-γ production induced by resting OT-1 CD8$^+$ T cells after activation by OVA$_{257–264}$-presenting congeneic TEC. Briefly, untreated B6 TEC or EL-4 cells were pulsed with 0.1 μg/ml of OVA$_{257–264}$ or β-Gal$_{497–504}$ peptide for 1 h at 37°C. The cells were then washed and seeded into the ELISPOT plate. Resting OT-1 CD8$^+$ T cells were added to the TEC cultures and were co-incubated for 18 h at 37°C. ELISPOT plates were developed according to the manufacturer's protocol.

Cytotoxicity by activated OT-1 CD8$^+$ CTL to B6 TEC

To measure antigen-specific cytolysis of renal TEC by CTL, activated OT-1 CD8$^+$ CTL were added to IFN-pre-treated, OVA$_{257–264}$ peptide-loaded B6 TEC in 96-U bottom cell culture plates at various effector/target (E/T) ratios, in the presence or absence of anti-mouse PD-L1 mAb (10 μg/ml). After 4 h of incubation, 50 μl of supernatants were collected from each well for measuring the amount of lactate dehydrogenase (LDH) released upon cell lysis by using the CytoTox 96$^®$ non-radioactive cytotoxicity assay kit (Promega) according to the manufacturer's protocol. The percentage of target lysis was calculated as follows:

$$\% \text{ lysis} = 100 \times (\text{experimental LDH release} - \text{spontaneous LDH release of targets} - \text{spontaneous LDH release from T cells})/(\text{maximum LDH release} - \text{spontaneous LDH release of targets}).$$

Statistics

Results from IFN-γ ELISA, ELISPOT or CTL assays are expressed as means ± SD of triplicate or quadruplicate determinations from the representative experiments that gave rise to similar results. Statistical analysis was performed using unpaired Student's *t*-test. Significance was accepted at $P \leq 0.05$.

Results

Stimulation of renal proximal TEC with IFN-β and IFN-γ upregulated PD-L1

Murine macrophage RAW246.7 cells and murine primary proximal TEC cultures prepared from C57BL/6J (B6 TEC) mice were stimulated with 200 U/ml of IFN-β or 100 U/ml of IFN-γ for 1–4 h for determining changes in mRNA expression of PD-L1. As shown in Figure 1, PD-L1 mRNA was constitutively detected in macrophages and renal TEC. A strong increase of steady-state PD-L1 mRNA levels was observed in macrophages after 1 and 4 h of stimulation with either IFN-β or IFN-γ (Figure 1A), in renal B6 TEC after 1 and 4 h of stimulation with IFN-β, and slightly delayed after 4 h with IFN-γ (Figure 1B). Stimulation of TEC with IFN-α even at a higher concentration (up to 1000 U/ml) did not increase PD-L1 mRNA expression in TEC (data not shown).

The surface expression of PD-L1 and MHC class I on TEC was examined after stimulation with IFN-β or IFN-γ for 48 h. Expression of PD-L1 on untreated C1.1 or primary C3H renal TEC cultures (the original of C1.1 cells) was negligible, contrasting with RAW 264.7 macrophages which constitutively express surface PD-L1 (Figure 2A). Stimulation with IFN-β or IFN-γ strongly upregulated PD-L1 (Figure 2A) and MHC class I molecules (Figure 2B) on all cells tested. The upregulation of both PD-L1 and MHC class I molecules on IFN-β or IFN-γ-stimulated renal TEC was dose-dependent (our previous results [2] and Figure 3). Although stimulation of TEC with IFN-α did not increase PD-L1 surface expression even when a high dose (1000 U/ml) was used, a dose-dependent, strong increase of MHC class I expression was observed (Figure 3B), suggesting that IFN-α and IFN-β use different pathways to activate
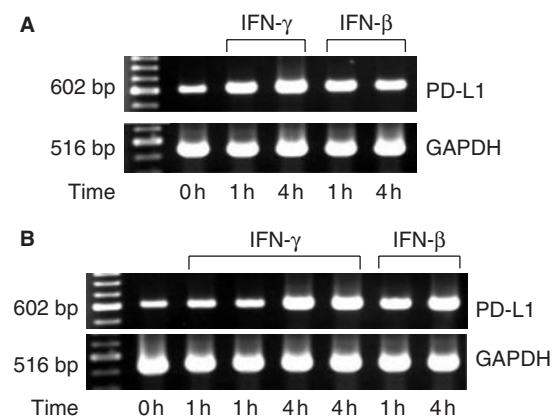

Fig. 1. RT-PCR analysis of PD-L1 mRNA expression in renal TEC treated with IFNs. Murine macrophage RAW264.7 cells (positive control cell line) (**A**) and primary renal TEC cultures generated from B6 mice (**B**) were treated with IFN-β (200 U/ml) or IFN-γ (100 U/ml) for 1 or 4 h. Results are representative of two independent experiments which gave similar results.

antigen-presenting cells. Similar results were also obtained by IFN-stimulation of primary TEC that were generated from different mouse strains, including C57BL/6, C3H, BALB/c and AKR/J (data not shown), indicating that this phenomenon is not limited to a specific cell line or mouse strain.

Activation of resting OT-1 CD8^{+} T cells by TEC

We used CD8^{+} T cells isolated from OT-1 TCR transgenic mice to examine the ability of renal TEC to present MHC class I-restricted antigenic peptide. OT-1 mice contain transgenic TCR genes in CD8^{+} T cells, which are designed to recognize the chicken ovalbumin protein residues 257–264 in the context of H-2K^b (OVA$_{257-264}$) [13]. FACS analysis showed that resting OT-1 CD8^{+} T cells express PD-L1 but not PD-1, whereas both molecules were strongly upregulated on *in vitro*-activated OT-1 CTL (Figure 4).

Antigen titration experiments were then performed to examine the capacity of presenting OVA$_{257-264}$

peptide to the resting OT-1 CD8^{+} T cells by renal B6 TEC. Similar amounts of IFN-γ were detected from supernatants of OT-1 CD8^{+} T cell cultures sensitized either by OVA$_{257-264}$ pulsed congeneic TEC (from C57BL/6 mice) or by EL-4 cells (Figure 5A). These results were further confirmed by IFN-γ ELISPOT assays. As shown in Figure 5B and C, resting OT-1 CD8^{+} T cells activated by either OVA peptide-presenting B6 TEC or EL-4 cells gave rise to a comparable level of IFN-γ production when captured and visualized by IFN-γ ELISPOTs. These results demonstrate that renal TEC have a strong capacity of presenting MHC class I-restricted antigenic peptides to activate antigen-specific CD8^{+} T cells *in vitro*.

PD-L1 on renal TEC inhibited antigen presentation to OT-1 CD8^{+} T cells

We studied next whether PD-L1 was able to regulate CD8^{+} T cell responses. For this purpose, TEC were first stimulated with IFN-β or IFN-γ for 48 h to
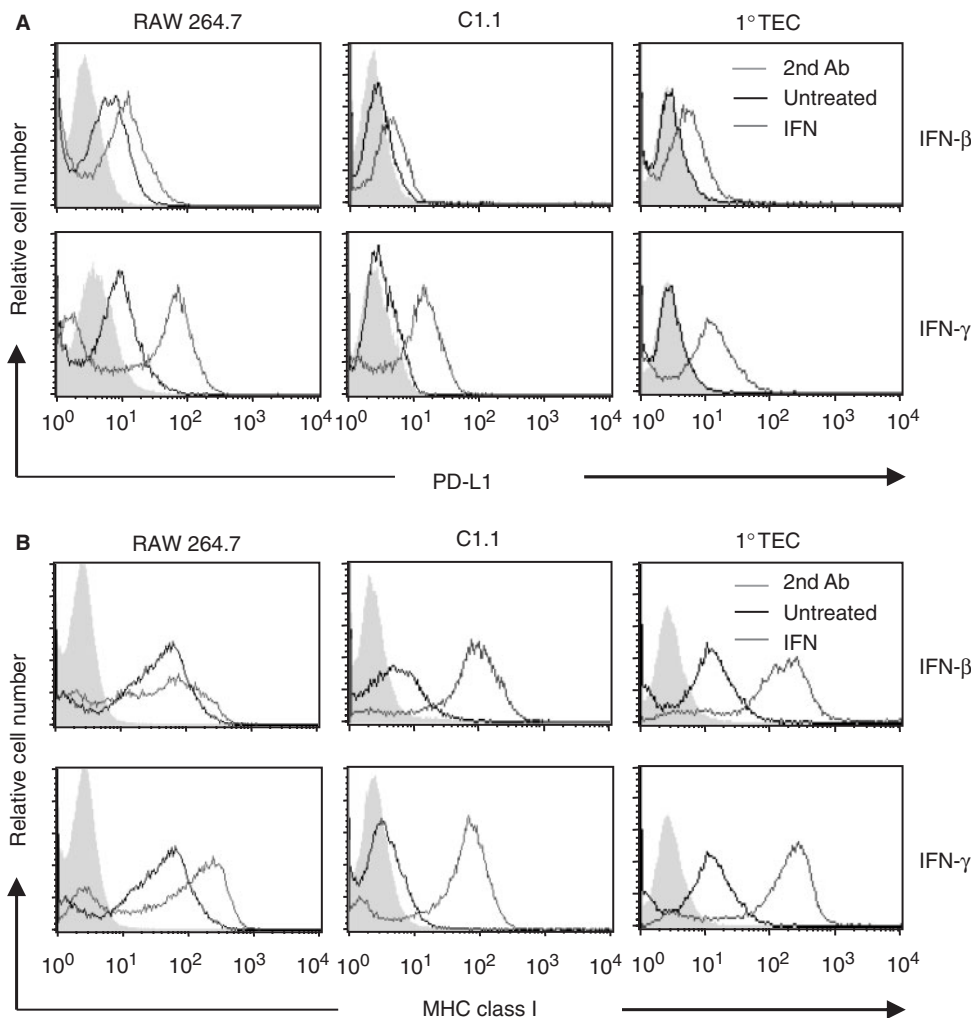

Fig. 2. PD-L1 and MHC class I expression on renal TEC stimulated with IFN-β (200 U/ml) or IFN-γ (100 U/ml) for 48 h. Murine macrophage line RAW264.7, renal TEC line C1.1 and primary TEC cultures generated from C3H mice (1° TEC) were stained with anti-mouse PD-L1 (**A**) or MHC class I (**B**) mAbs for flow cytometric analysis. Results are representative of four independent experiments.

upregulate PD-L1. Cells were then collected, washed and loaded with $OVA_{257-264}$ peptide. Anti-PD-L1 mAb was added to block PD-L1 on TEC before activating the resting OT-1 $CD8^+$ T cells. Blockade of PD-L1 significantly increased OT-1 $CD8^+$ T cell activation, as revealed by higher amounts of IFN-γ, compared to those with TEC without PD-L1 blockade (Figure 6). The reaction was antigen-specific and MHC class I-restricted, since irrelevant β-$Gal_{497-504}$ peptide-pulsed TEC failed to induce OT-1 T cell activation and C1.1 cells (H-$2K^k$) pulsed with $OVA_{257-264}$ peptide did not induce IFN-γ production by OT-1 T cells (data not shown). These results indicated that PD-L1-expressing renal epithelial cells modulated the antigen-specific $CD8^+$ T cell activation.

PD-L1 partially protected TEC from cytolysis by OT-1 CTL

We then examined whether OVA-presenting TEC would be the direct targets of OT-1 $CD8^+$ CTL, and whether epithelial PD-L1 plays a functional role on

CTL responses. For this purpose, $OVA_{257-264}$ peptide-pulsed B6 renal TEC, pretreated with IFN-α, IFN-β and IFN-γ or untreated, were used as target cells to study the cytotoxicity of OT-1 $CD8^+$ T cells. Resting OT-1 $CD8^+$ T cells isolated from naïve OT-1 mice failed to kill $OVA_{257-264}$ peptide-presenting target cells, including renal B6 TEC or EL-4 cells (data not shown). In contrast, *in vitro*-stimulated/ activated OT-1 $CD8^+$ T cells demonstrated a strong cytotoxicity when $OVA_{257-264}$ peptide-presenting B6 TEC or EL-4 cells were used as targets (Figure 7A). Only basal cytolysis of target cells was observed when TEC and EL-4 cells were pulsed with the irrelevant H-$2K^b$ peptide β-$Gal_{497-504}$ (Figure 7A). Importantly, when IFN-β and IFN-γ-stimulated TEC were used as targets in the same CTL assay, significantly lower cytolysis was observed compared with untreated TEC. The lysis of TEC treated with IFN-α did not show significant difference when compared with untreated TEC (Figure 7A). Considering that IFN-β or IFN-γ treatment upregulated high-surface PD-L1 molecule expression, this may be indirect evidence that the surface PD-L1 protects TEC from the cytolysis by CTL. To further clarify this point, anti-PD-L1 anti-bodies were added to block the surface PD-L1 during CTL assay. As shown in Figure 7B, the PD-L1 Ab blockade led to a significant increase of cytolysis of TEC treated with IFN-β or IFN-γ, respectively, while adding anti-PD-L1 mAb to untreated or IFN-α-treated TEC did not alter the cytolytic effect of OT-1 $CD8^+$ T cells. Taken together, these results demonstrate that PD-L1 expressed on renal TEC plays an inhibitory role on CTL responses, reducing T cell activation and protecting TEC from the killing by $CD8^+$ CTL.
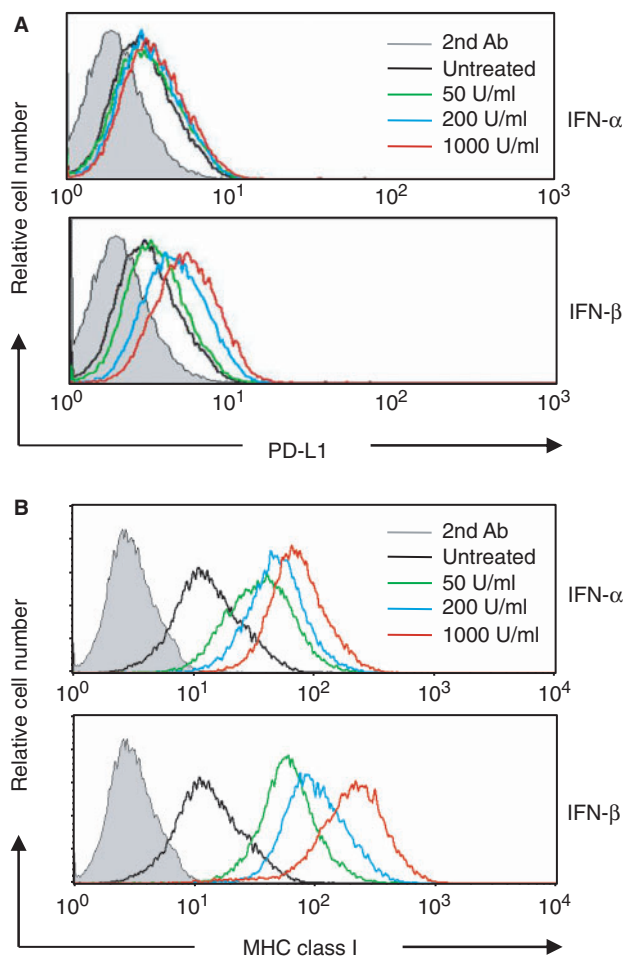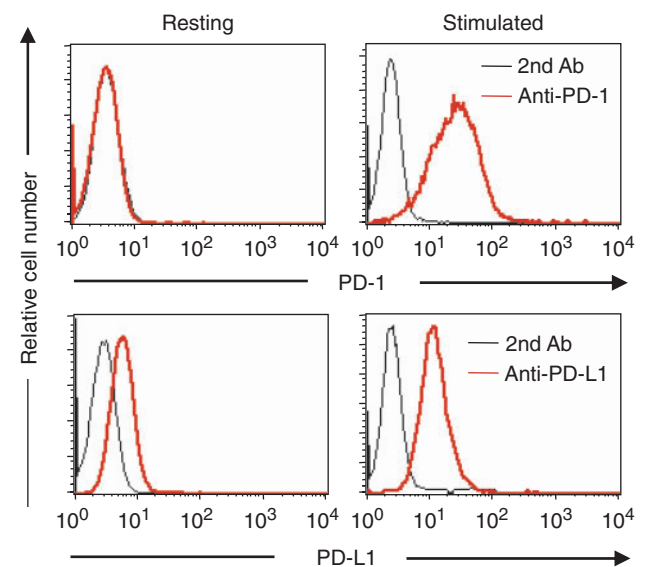

Fig. 3. PD-L1 and MHC class I expression on renal TEC stimulated with type I IFNs. C1.1 cells were stimulated with the indicated concentrations of IFN-α or IFN-β for 48 h, and were stained with anti-mouse PD-L1 (**A**) or MHC class I (**B**) mAbs for flow cytometric analysis. Results are representative of two independent experiments.

Fig. 4. Flow cytometric analysis of PD-1 and PD-L1 expression on OT-1 $CD8^+$ T cells. Resting or $OVA_{257-264}$ peptide-stimulated OT-1 $CD8^+$ T cells were stained with anti-mouse PD-1 or PD-L1 mAbs for flow cytometric analysis. Results are representative of two independent experiments.

Discussion

In this study we demonstrate that the expression of PD-L1 on TEC is strongly and rapidly upregulated by IFN-β and IFN-γ, but not by IFN-α. Furthermore, we show that the PD-L1/PD-1 pathway inhibits antigen-specific CD8$^+$ T cell activation by renal TEC. In addition, the blockade of PD-L1 on TEC leads to an enhanced cytolytic activity of CTL in an MHC class I-restricted manner. PD-L1 on renal TEC, therefore, provides a negative signal for antigen-specific CD8$^+$ T cell responses.

CD8$^+$ T cells are major contributors in cell-mediated immune responses in tubulointerstitial renal diseases and are responsible for the tubular destruction [4,16]. However, the mechanisms that regulate the intrarenal CD8$^+$ T cell activity have not been investigated in detail. Apart from MHC class I complex/TCR inter-actions that are crucial for initiating T cell activation, a variety of co-stimulatory and co-inhibitory pathways are known to influence the cytolytic potential of CD8$^+$ T cells [9,10,17]. Among those co-stimulatory

pathways, B7/CD28 provides the most prominent activation signal for CD8$^+$ T cells. However, as B7.1 and B7.2 are hardly expressed by renal TEC, the relevant co-stimulatory pathways might be different for TEC-driven CD8$^+$ T cell responses. Similarly, the co-inhibitory B7/CTLA-4 interaction might also not operate on TEC for the same lack of expression of B7 molecules. Considering that only limited co-stimulatory or co-inhibitory molecules such as PD-L1, ICOS-L and CD40 are expressed in renal TEC (our unpublished data), thus PD-L1 may be one of the major co-inhibitory molecules regulating renal epithelial CD8$^+$ CTL responses. Our current findings that the epithelial PD-L1/PD-1 pathway has an immunological regulatory function on CD8$^+$ CTL responses are also consistent with other studies, including cancer immunity, pathogen-induced CD8$^+$ T cell responses, autoimmune diseases and organ transplantation [9,10,18].

PD-L1 is aberrantly expressed by various human carcinomas [18–23]. The expression of PD-L1 by tumour cells can impair tumour-specific
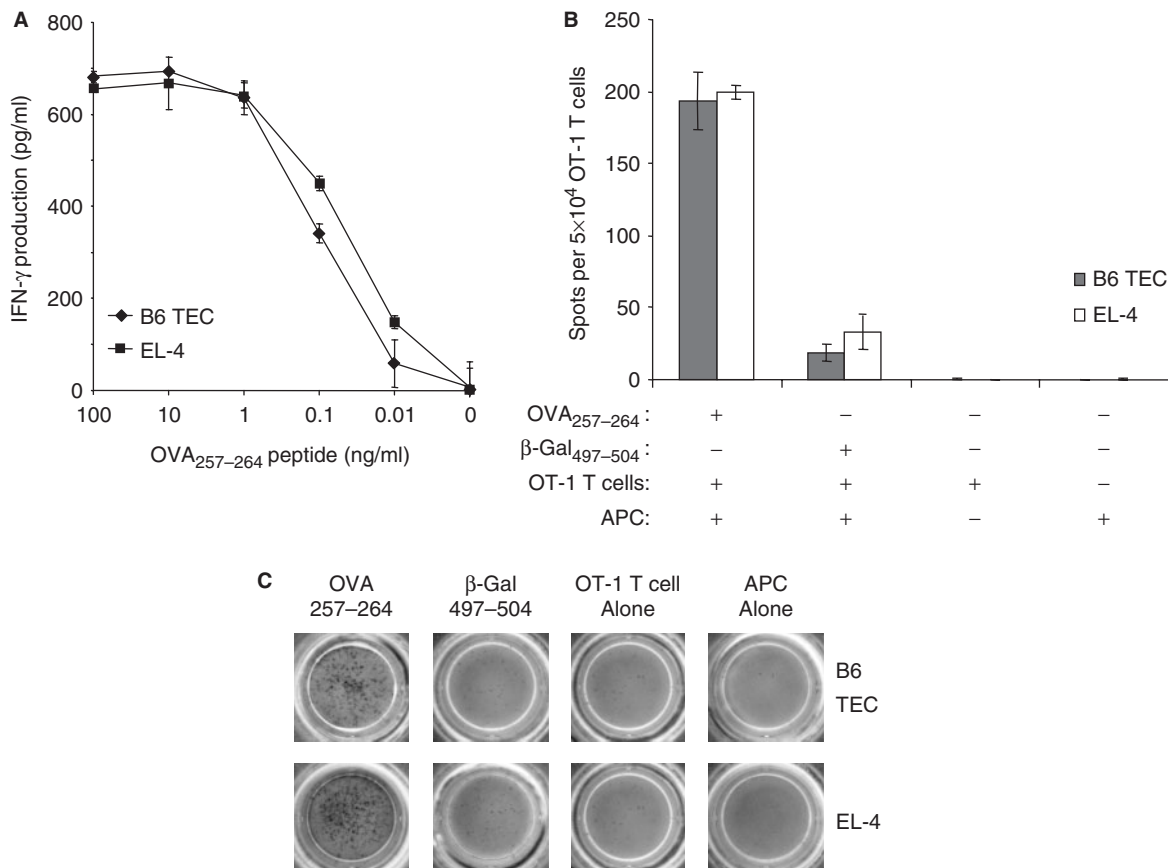

Fig. 5. OVA-presenting renal TEC activate resting OT-1 CD8$^+$ T cells to produce high amounts of IFN-γ. (**A**) Antigen titration assay. Untreated primary B6 TEC or EL-4 cells (1×10^4 per well) pulsed with increasing concentrations of OVA$_{257-264}$ peptide were co-incubated with resting OT-1 CD8$^+$ T cells (5×10^4 per well). After 24 h, OT-1 CD8$^+$ T cell activation was determined with IFN-γ ELISA. Results represent the mean amount of IFN-γ ± SD. (**B** and **C**) IFN-γ ELISPOT assay. Untreated B6 TEC or EL-4 cells (1×10^4 per well) were pulsed with 100 ng/ml of OVA$_{257-264}$ or β-Gal$_{497-504}$ peptide, then were co-incubated with resting OT-1 CD8$^+$ T cells (5×10^4 per well) in pre-coated murine IFN-γ ELISPOT plates. After 18 h, plates were developed, and spots were counted as described in 'Materials and methods'. Wells containing only TEC or OT-1 CD8$^+$ T cells were included as controls. Data represent the mean spots per well ± SD. Representative individual ELISPOT wells with different experimental setting are shown in **C**.

T cell functions, resulting in defective host anti-tumour immunity. For example, high PD-L1 expression in human renal cell carcinomas is correlated with tumour invasiveness and markedly increased the risk of death from cancer [19]. In an animal study, resistance of PD-L1-positive tumour cells to the cytolysis by tumour-specific CTL has been observed, and this could be abrogated by the treatment with anti-PD-L1 or anti-PD-1 antibodies [24].

Interesting results have been obtained regarding the role of PD-L1 in chronic infections. Barber *et al.* [25] demonstrated that the *in vivo* blockade of either PD-L1 or its receptor PD-1 with antibodies enhanced lymphocytic choriomeningitis virus (LCMV)-specific CD8$^+$ T cell responses, and restored the function of those 'exhausted' CD8$^+$ T cells during chronic LCMV infection. It is tempting to speculate that the IFN-stimulated expression of PD-L1 on TEC could protect intratubular viruses such as Epstein–Barr or the BK polyoma virus, promoting, therefore, their cytopathic effects [26,27].
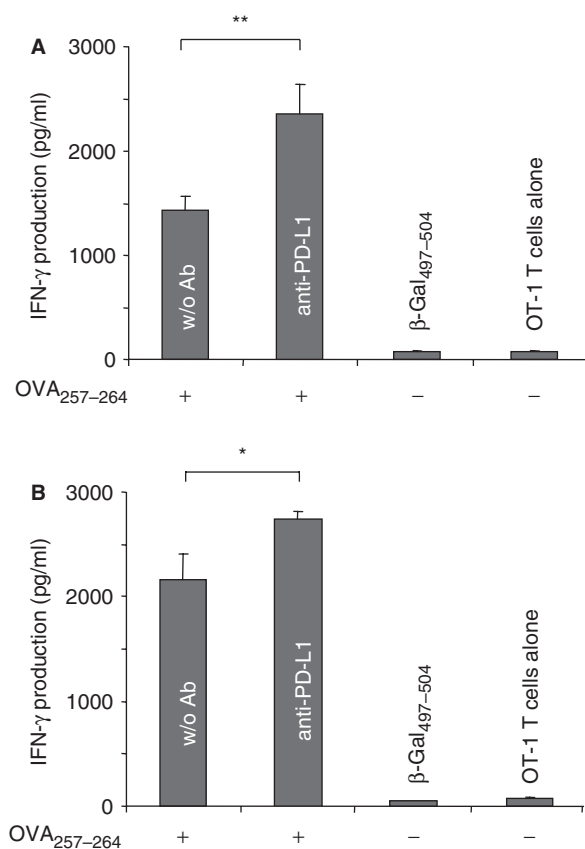

Fig. 6. Blockade of PD-L1 on renal TEC increased OVA peptide-specific OT-1 CD8$^+$ T cell activation. IFN-β (**A**) or IFN-γ (**B**) stimulated, OVA$_{257-264}$ or β-Gal$_{497-504}$ peptide-pulsed (100 ng/ml) renal B6 TEC (2×10^4 per well) were co-incubated with resting OT-1 CD8$^+$ T cells (1×10^5 per well) in the presence or absence of the PD-L1 blocking mAbs (final concentration 10 μg/ml) for 24 h. OT-1 CD8$^+$ T cell activation was determined by IFN-γ production from the collected supernatants with IFN-γ ELISA. Data represent the mean amount of IFN-γ ± SD. One out of the four independent experiments is shown. *$P \leq 0.05$; **$P \leq 0.001$, when compared with TEC without Ab blockade (Student's *t*-test).

PD-L1 is also an important control molecule in autoimmunity and transplant rejection. Mice that are deficient in PD-L1 and/or PD-1 have a strong tendency to develop autoimmune diseases [28,29]. In disease models such as autoimmune diabetes and experimental autoimmune encephalomyelitis (EAE), upregulated PD-L1 expression has been observed [30–33]. Enhanced expression of PD-L1 has also been reported in allograft transplants of kidney, heart, skin and islets of pancreas, indicating that PD-L1 plays a role in the downregulation of the rejection process [2,34–36]. Finally, PD-L1 is involved in feto-maternal tolerance mechanisms [37]. Together, these data suggest that the PD-L1/PD-1 pathway plays an important role in peripheral and parenchymal immune tolerance.

During inflammation, the tubulointerstitium and/or TEC are exposed to many pro-inflammatory cytokines that are produced by infiltrating immune cells. We have previously reported that IFN-γ treatment led to the strong increase of PD-L1 expression in TEC [2]. An additional important finding in this study is that the type I interferon IFN-β is able to induce strong PD-L1 expression in TEC and that the IFN-β-induced PD-L1 on TEC reduced specific CD8$^+$ CTL responses. IFN-β is the principal immune modulation agent to treat patients with multiple sclerosis (MS) [38–40]. Schwarting *et al.* [41] reported that IFN-β treatment prolonged survival of MRL-*Fas^{lpr}* mice with experimental systemic lupus erythematosus (SLE) and reduced kidney pathology. The molecular mechanisms of IFN-β treatment on autoimmune diseases still remain unclear, but one hypothesis might be that the administration of IFN-β is able to induce an upregulation of PD-L1 on peripheral professional APC and on non-lymphoid tissues, thereby downregulating T cell proliferation and the infiltration of leukocytes. Indeed, Schreiner *et al.* [40] showed that the therapeutic application of IFN-β leads to a significant increase of PD-L1 level in MS patients, suggesting that PD-L1 might be responsible, at least in part, for the protective effect in this therapy. More detailed studies are required to test this hypothesis

IFN-γ is a pivotal pro-inflammatory cytokine in immune-mediated renal diseases [42]. However, its immunoregulatory effect *in vivo* is still unclear so far. Some studies showed that IFN-γ is essential for the disease development [43,44]; whereas others reported that endogenous IFN-γ plays a protective role in renal organ-specific autoimmunity [45]. Although IFN-γ treatment showed immunoregulatory effect to downregulate specific CTL responses under our *in vitro* experimental conditions, its *in vivo* role of organ-specific immunomodulation still needs to be elucidated.

The results obtained from the current study were based on an *in vitro* experimental system that used primary TEC cultures and antigen-specific CD8$^+$ CTL from OT-1 transgenic mice to study the co-stimulatory function of epithelial PD-L1. The *in vivo* role of intrarenal PD-L1 on modulating
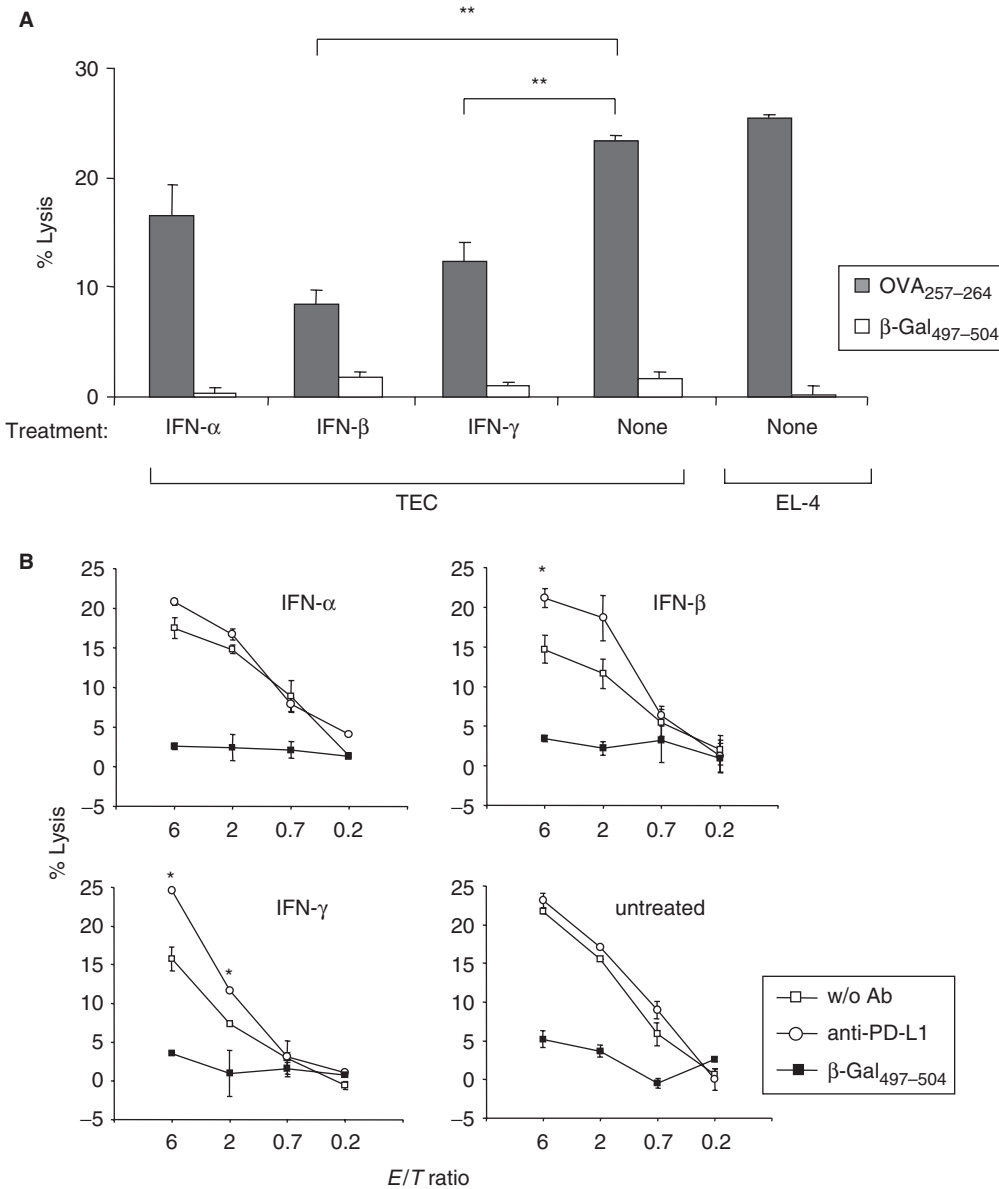
Fig. 7. High PD-L1-expressing TEC were partially protected from cytolysis by activated OT-1 CD8^{+} CTL. (**A**) IFN-α, IFN-β or IFN-γ treated or untreated renal B6 TEC or EL-4 cells pulsed with 10 ng/ml of OVA$_{257-264}$ or β-Gal$_{497-504}$ peptide were co-incubated with activated OT-1 CD8^{+} CTL as an E/T ratio 10:1 (1×10^5 of CTL to 1×10^4 of TEC per well) for CTL assay. After 4 h of incubation, specific cytolysis of targets was measured with the CytoTox 96[®] non-radioactive cytotoxicity assay and calculated as described in 'Materials and methods'. *$P \le 0.05$; **$P \le 0.001$, when compared with untreated TEC (Student's t-test). (**B**) CTL assay was performed by using IFN-α, IFN-β or IFN-γ pre-treated or untreated, OVA$_{257-264}$ peptide-pulsed B6 TEC as targets as described in (**A**), in the presence or absence of anti-mouse PD-L1 mAb (10 μg/ml). TEC pulsed with β-Gal$_{497-504}$ peptide was used as control. Data represent the mean % lysis ± SD. One out of three independent experiments is shown. *$P \le 0.05$; **$P \le 0.001$, when compared with TEC without Ab blockade (Student's t-test).

T cell responses requires further verification. Nevertheless, considering the evidence that the *in vivo* PD-L1 expression is markedly enhanced in kidneys with tubulitis in renal allograft rejection [2] and in lupus nephritis [46], and our previous findings that the renal epithelial PD-1/PD-L1 pathway inhibits antigen-specific CD4^{+} T helper cell activation *in vitro* [2], our data indicated that the renal epithelial PD-L1/PD-1 pathway may inhibit both CD4^{+} and CD8^{+} T cell responses and protect the renal epithelial cells from the attack of CTL.

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