Hydronephrosis associated with antiurothelial and antinuclear autoantibodies in BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice

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Because most autoimmune diseases are polygenic, analysis of the synergistic involvement of various immune regulators is essential for a complete understanding of the molecular pathology of these diseases. We report the regulation of autoimmune diseases by epistatic effects of two immunoinhibitory receptors, low affinity type IIb Fc receptor for IgG (FcγRIIB) and programmed cell death 1 (PD-1). Approximately one third of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice developed autoimmune hydronephrosis, which is not observed in either BALB/c-*Fcgr2b*$^{-/-}$ or BALB/c-*Pdcd1*$^{-/-}$ mice. Hydronephrotic mice produced autoantibodies (autoAbs) against urothelial antigens, including uroplakin IIIa, and these antibodies were deposited on the urothelial cells of the urinary bladder. In addition, ~15% of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice produced antinuclear autoAbs. In contrast, the frequency of the autoimmune cardiomyopathy and the production of anti–parietal cell autoAb, which were observed in BALB/c-*Pdcd1*$^{-/-}$ mice, were not affected by the additional FcγRIIB deficiency. These observations suggest cross talk between two immunoinhibitory receptors, FcγRIIB and PD-1, on the regulation of autoimmune diseases.

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Most human autoimmune diseases are polygenic, and multiple genetic alterations are involved in the initiation and progression of these diseases. These genetic alterations are supposed to impair some of the critical steps for immunological tolerance, including negative selection of autoreactive T cells in the thymus, anergy/deletion/suppression of autoreactive T cells in the periphery, and deletion and/or inactivation of autoreactive B cells. Linkage analyses in animal models of autoimmune diseases have provided informative results on the polygenic regulation of autoimmune diseases. Many autoimmune-susceptible loci have been identified in each animal model, and the immunological function of each locus has been vigorously analyzed by generating various congenic animals (1–3). However, the mutual relationship of these loci has not been analyzed well because of the many possible combinations and rather limited number of congenic mice that can be established only by repeated backcrossing. Moreover, the low resolution of the linkage analyses on polygenic diseases prevented identification of a single gene from each susceptibility locus. Collectively, our knowledge about the polygenic regulation of autoimmune diseases is very limited.

Programmed cell death 1 (PD-1), an immunoreceptor belonging to the CD28/CTLA-4 family, provides negative co-stimulation to antigen stimulation by recruiting src homology 2 domain–containing tyrosine phosphatase 2, a protein tyrosine phosphatase (4, 5). PD-1 deficiency has been shown to accelerate autoimmune predisposition and to induce autoimmune diseases. PD-1 knockout (*Pdcd1*$^{-/-}$) mice develop lupus-like glomerulonephritis and arthritis on the C57BL/6 background and autoimmune dilated cardiomyopathy (DCM) on the BALB/c background (6–8). Recently, we have reported that PD-1 deficiency accelerates autoimmune diabetes in NOD (nonobese diabetic) mice (9). The development of different forms of autoimmune diseases on different genetic backgrounds of mice indicates that autoimmune phenotypes of *Pdcd1*$^{-/-}$ mice are influenced

by other genetic factors. Conversely, PD-1 deficiency may exaggerate autoimmune predisposition of mice with various genetic alterations.

Fc receptors, which provide either stimulatory or inhibitory signals upon capturing the Fc portion of immunoglobulins, link the humoral and cellular immune systems (10–12). FcγRIA, FcγRIIA, FcγRIIIA, and FcγRIV provide stimulatory signals, whereas low affinity type IIb Fc receptor for IgG (FcγRIIB) provides an inhibitory signal. Because B cells express only FcγRIIB among these Fc receptors, B cells are destined to dampen their activity upon encountering cognate immune complex. FcγRIIB-mediated negative feedback has been shown to be required for the maintenance of tolerance, as *Fcgr2b*$^{-/-}$ mice have been reported to be highly susceptible to experimental autoimmune diseases upon immunization with autoantigens and spontaneously develop systemic lupus erythematosus (SLE)–like syndrome on the C57BL/6 background (10, 11, 13). Again, this spontaneous autoimmunity is strain specific, and BALB/c-*Fcgr2b*$^{-/-}$ mice do not show any autoimmune phenotypes, suggesting a compensatory role of the other inhibitory mechanisms in the regulation of autoimmune diseases in BALB/c-*Fcgr2b*$^{-/-}$ mice (13). The distal region of mouse chromosome 1, which contains the FcγRIIB gene, has been reported to associate with autoimmune symptoms in several strains of mice, including NZB and BXSB. These mice have been shown to express a reduced level of FcγRIIB on activated or germinal center B cells, and a targeted restoration of FcγRIIB expression on B cells efficiently rescued these mice from autoimmune symptoms (14).

Although many immunoregulatory molecules have been identified, including FcγRIIB and PD-1, the mutual relationship of these molecules in the establishment of autoimmune diseases is still ill defined. In this paper, we analyzed the interaction of two immunoinhibitory receptors (FcγRIIB and PD-1) in the regulation of autoimmune diseases by generating their double knockout mice on the BALB/c background. Additional disruption of the FcγRIIB gene did not affect the autoimmune DCM and the production of anti–parietal cell autoantibodies (autoAbs) observed in BALB/c-*Pdcd1*$^{-/-}$ mice. However, 35.3% of the DCM-free BALB/c-*Fcgr2b*$^{-/-}$ *Pdcd1*$^{-/-}$ mice developed hydronephrosis associated with the production of antiurothelial antibodies (Abs), which had not been observed either in BALB/c-*Pdcd1*$^{-/-}$ or BALB/c-*Fcgr2b*$^{-/-}$ mice. Production of antinuclear Abs was also observed only in the double knockout mice of FcγRIIB and PD-1. These results demonstrate cross talk between FcγRIIB and PD-1 in the regulation of autoimmune diseases.

RESULTS AND DISCUSSION

Spontaneous development of hydronephrosis by BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice

We expected the cross between deficiencies of two negative immunoreceptors, FcγRIIB and PD-1, would enhance DCM, and we monitored for the incidence of DCM in BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice. Contrary to our expectations, the incidence of DCM was not different between BALB/c-*Fcgr2b*$^{+/-}$*Pdcd1*$^{-/-}$ mice and BALB/c-*Fcgr2b*$^{-/-}$ *Pdcd1*$^{-/-}$ mice (24.3 vs. 23.3% in 24 wk, respectively). However, 35.3% (18/51) of the DCM-free BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice began to lose weight from 15 wk of age and gradually became moribund. Autopsy examinations revealed that both kidneys were enlarged about twofold in diameter and both renal pelves were translucent, indicating that enlargement of kidneys was caused by obstruction of the urinary flow (Fig. 1 A). The renal cortex was observed only marginally at the edge. The ureter was enlarged at the ureteropelvic junction, but the middle to lower part of the ureter appeared normal. The urinary bladder also looked nor-

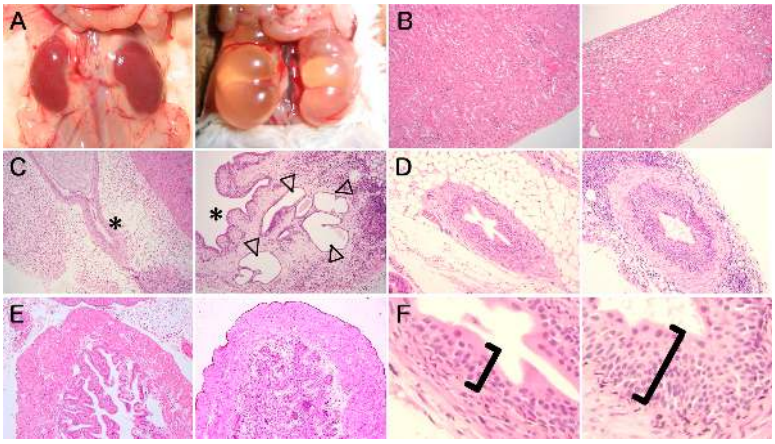

Figure 1. Spontaneous hydronephrosis in BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice. (A) Macroscopic images of a hydronephrotic kidney (right) and a normal kidney control (left) are shown. (B–F) Representative hematoxylin and eosin (H&E) staining histologies of kidney (B), ureteropelvic junction (C), ureter (D), and urinary bladder (E) are shown for hydronephrotic (right) and healthy control (left) mice. (F) High-power fields of ureter. Urothelial cells of hydronephrotic mice (right) are apparently unchanged from those of healthy control mice (left), except for the two- to threefold increase in the number of cells (brackets). Asterisks and arrowheads in C indicate orifice of pelvis and ureteral lumen, respectively. Magnifications: (B and C) 100; (D) 200; (E) 50; (F) 400.

mal; however, urine was hardly detectable in the bladder. From these macroscopic observations, the mice were judged to have developed hydronephrosis.

Microscopic analysis of these hydronephrotic mice revealed severe inflammation along the urinary tract (Fig.1, B–F). The inflammation was most severe at the ureteropelvic junction, and the urinary tract was serpiginous, as evidenced by the appearance of multiple lumens in a section (Fig. 1 C, right, arrowheads). We concluded, therefore, that the urinary tract was most likely blocked at the proximal section of the ureter. The remaining cortex of the kidney appeared normal except for a reduction in the total mass and the moderate enlargement of the tubular architecture (Fig. 1 B). The middle to lower part of the ureter and the urinary bladder appeared normal except for scattered inflammation (Fig. 1 E). There was also severe inflammation at the junction between the ureter and urinary bladder. Inflammation was observed mainly outside the muscular layer, forming distinct zones of T cells and B cells and suggesting the presence of a chronic inflammation (Fig. 1, C and D; and not depicted). The epithelial layer appeared normal except that the cellularity was increased (Fig. 1, D and F). Interestingly, 35.7% (10/28) of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{+/-}$ mice also developed hydronephrosis, suggesting that the FcγRIIB deficiency is critical to the autoimmune response against the urinary tract. Consistently, there was almost no pathological difference in the hydronephrosis of BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{+/-}$ mice and BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice (unpublished data).

Some human SLE patients have been reported to develop bilateral hydronephrosis with concomitant inflammation along the urinary tract (15). Interstitial cystitis is also one of the main complications in SLE and Sjogren's syndrome (16, 17). BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice may thus serve as a good animal model of such complications. IL-9–transgenic mice, known to cause T cell lymphoma, have also been recently shown to develop hydronephrosis, which is dependent on IL-4 and/or IL-13 (18, 19). Although the development of hydronephrosis does not seem to correlate with T cell lymphoma in IL-9–transgenic mice, the role of this cytokine in autoimmunity has not been well analyzed. Compared with the bilateral onset of hydronephrosis in BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice, hydronephrosis in IL-9–transgenic mice is reported to occur unilaterally as well as bilaterally. Therefore, the pathological mechanisms of hydronephrosis seem to be different between IL-9–transgenic and BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice.

Antiurothelial autoAb production

We suspected that the cause of the hydronephrosis observed in the double knockout mice might be the result of autoAb production because deficiency of either FcγRIIB or PD-1 is known to facilitate the production of autoAbs (7, 13). We therefore examined the production of autoAbs against the urinary tract by staining normal mouse tissue sections with sera from hydronephrotic mice. As shown in Fig. 2 (A–D), the epithelial layer of the urinary tract was stained with sera from hydronephrotic mice. This staining was specific to the urothelium in the renal pelvis, ureter, and urinary bladder. All mice with antiurothelial Abs ($n = 28$) were affected with hydronephrosis, whereas 0 out of 33 antiurothelial Ab–negative BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice developed hydronephrosis. We detected IgG1, IgG2a, and IgG2b, but not IgG3, subclasses of antiurothelial Abs (unpublished data). We next examined the deposition of autoAbs on the urothelium of the hydronephrotic mice. All hydronephrotic mice examined were positive for the IgG deposition ($n = 5$). Compared with the serum staining (Fig. 2 D), IgG deposition was most evident on the apical surface of the urothelium (Fig. 2 E).

Recognition of uroplakin IIIa (UPKIIIa) by sera from hydronephrotic mice

We next performed Western blotting to examine the antigen specificity of the antiurothelial Abs. We extracted protein from the urothelial sheet of the urinary bladder and probed with sera from hydronephrotic mice. As shown in Fig. 2 F, all of the sera from hydronephrotic mice recognized a band

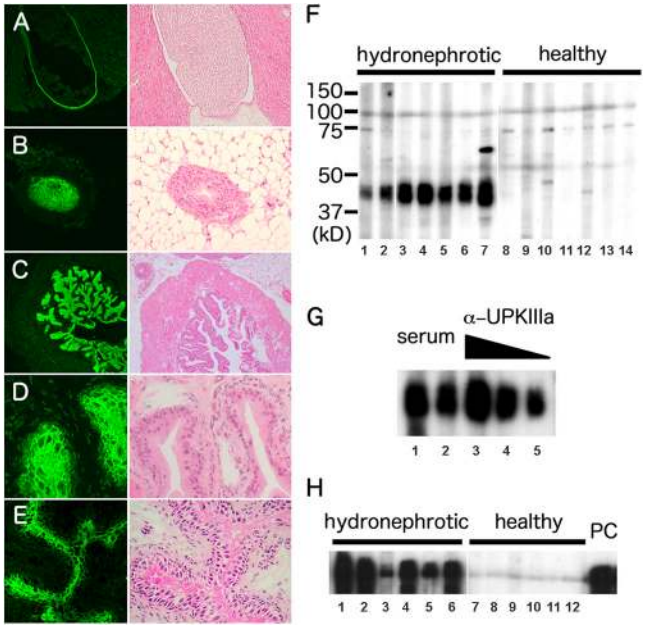

Figure 2. AutoAbs against urothelial cells. (A–D) Sera from hydronephrotic mice recognized urothelial cells of renal pelvis (A), ureter (B), and urinary bladder (C and D). Green signals (left) represent staining by serum IgG. H&E staining of corresponding organs is also shown (right). (E) IgG deposition was strongly observed on the apical surface of the urothelium. (F) All of the sera from hydronephrotic (lanes 1–7) but not from healthy control (lanes 8–14) mice recognized a 45-kD urothelial cell–specific antigen. (G) Sera from two hydronephrotic mice (lanes 1 and 2) and anti-UPKIIIa Ab (lanes 3–5; ×2, ×5, and ×10 dilutions, respectively) recognized similar bands on urothelial extract. (H) Sera from hydronephrotic (lanes 1–6) but not from healthy control (lanes 7–12) mice recognized the recombinant UPKIIIa protein. PC, positive control of anti-UPKIIIa Ab.

around 45 kD, whereas none of the sera from healthy mice with the same genetic background recognized this band. Protein extracts from other organs including the muscular layer of the urinary bladder were negative under the same conditions (unpublished data). UPKIIIa was suspected as a candidate antigen based on its molecular weight and organ specificity (20). We probed the same extract with a commercially available Ab against UPKIIIa. As shown in Fig. 2 G, the anti-UPKIIIa Ab recognized a band with a molecular weight similar to that recognized by sera from hydronephrotic mice. To confirm the identity of the antigen recognized by the sera from hydronephrotic mice, we cloned the cDNA for mouse UPKIIIa by RT-PCR and produced the UPKIIIa protein in *Escherichia coli*. Sera from hydronephrotic but not from healthy control mice recognized UPKIIIa, as shown in Fig. 2 H. Therefore, UPKIIIa was confirmed to be one of the major autoantigens that are targeted by BALB/c-*Fcgr2b*$^{-/-}$ *Pdcd1*$^{-/-}$ hydronephrotic mice.

Transitional cells in the urinary tract contain a large amount of membrane vesicles called urothelial plaques (20). Upon urine load, urinary plaques are released into the plasma membrane, resulting in the increase of the apical surface area of the transitional cells, which allows the subsequent extension of the cell body. UPKIIIa is one of the components of these urinary plaques and plays an essential role in the retention of urinary plaques in the cytoplasm. Mice deficient in UPKIIIa have been reported to have a reduced number of urinary plaques, resulting in impairment of bladder extension with the eventual development of hydronephrosis (20). Although both BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice and *Upk3a*$^{-/-}$ mice eventually develop hydronephrosis, their pathogenic mechanisms are likely to be different. *Upk3a*$^{-/-}$ mice have enlarged ureteral orifices, which results in the backward flow of urine from the urinary bladder to the ureter and the subsequent development of hydronephrosis coupled with hydroureter at the distal portion of the ureter (20). In contrast, hydronephrosis in BALB/c-*Fcgr2b*$^{-/-}$ *Pdcd1*$^{-/-}$ mice seems to be dependent on inflammatory occlusion, accompanied by hydroureter at the proximal part of the ureter. In addition, the deposition of anti-UPKIIIa autoAbs at the urothelium of BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice suggests the involvement of the autoAbs in the pathogenesis of hydronephrosis in BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice.

Production of antinuclear Abs but not anti–parietal cell Abs is regulated synergistically by FcγRIIB and PD-1

In addition to the antiurothelial Abs, some of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice produced anti–parietal cell Abs and antinuclear Abs (Fig. 3, A and B). Most of the mice with anti–parietal cell Abs were affected with severe gastritis (Fig. 3, C and D). Because BALB/c-*Pdcd1*$^{-/-}$ mice also produced anti–parietal cell Abs in a frequency comparable with BALB/c-*Fcgr2b*$^{+/-}$*Pdcd1*$^{-/-}$ and BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice (70, 60, and 80%, respectively; Fig. 3 E), the production of anti–parietal cell Abs seems to be controlled primarily by PD-1 deficiency and not by FcγRIIB deficiency. Anti–parietal cell Abs were not detected in sera from C57BL/6-*Pdcd1*$^{-/-}$ mice (unpublished data).

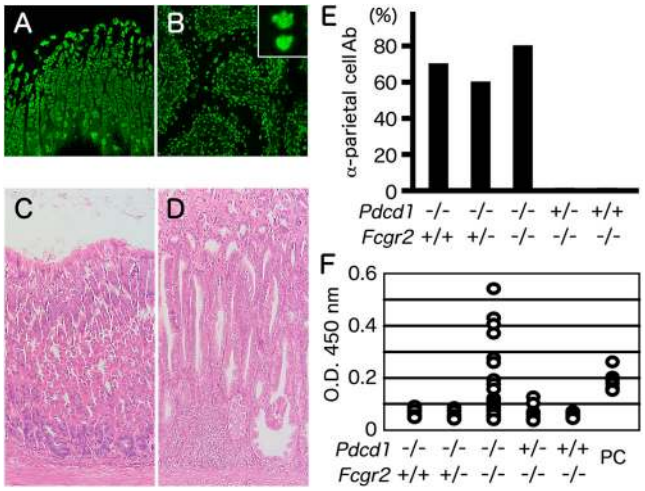

Figure 3. Production of antinuclear Ab and anti–parietal cell Ab by BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice. (A and B) Anti–parietal cell Ab (A) and antinuclear Ab (B) were detected by immunohistochemistry. The inset shows a higher magnification. (C and D) Representative histology of healthy (C) and inflamed stomach (D) by H&E staining. Magnifications, 200. (E) Frequencies of anti–parietal cell Ab–positive mice are shown for indicated genotype. $n = 20$, 10, 40, 23, and 15 mice from left to right. (F) Antinuclear Ab titer was examined for mice with indicated genotypes. PC, positive control of 10-wk-old MRL-lpr-MpJ mice.

We then analyzed the production of antinuclear Abs by ELISA in mice with various genetic backgrounds. Approximately 15% of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice were positive for antinuclear Abs, whereas neither BALB/c-*Fcgr2b*$^{-/-}$ nor BALB/c-*Pdcd1*$^{-/-}$ mice were positive (Fig. 3 F). Similar to the situation observed for antiurothelial Abs, a small fraction of the BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{+/-}$ mice produced a small amount of antinuclear Abs, suggesting that the FcγRIIB deficiency is critical to the autoimmune response against nuclear as well as urothelial antigens.

Synergistic effects of FcγRIIB and PD-1 deficiency on autoimmune phenotypes

We found the spontaneous appearance of novel autoimmune phenotypes in mice deficient in two immunoinhibitory receptors, FcγRIIB and PD-1. As summarized in Table I, hydronephrosis and antinuclear Ab production are not observed in mice that are sufficient in either of the molecules, indicating that these phenotypes require the combined effect of both deficiencies. In contrast, cardiomyopathy and anti–parietal cell Ab production are observed in BALB/c-*Pdcd1*$^{-/-}$ mice and not accelerated drastically by simultaneous deletion of FcγRIIB. BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice may thus serve as a good animal model of bigenic and oligogenic autoimmune diseases. PD-1 has been shown to inhibit antigen receptor signaling in both B cells and T cells, the insuffi-

Table I. Summary of autoimmune phenotypes of BALB/c-*Fcgr2b*$^{-/-}$*Pdcd1*$^{-/-}$ mice

Phenotypes	Genotypes	BALB/c					C57BL/6	
	Pdcd1	−/−	−/−	−/−	+/−	+/+	−/−	+/+
	Fcgr2b	+/+	+/−	−/−	−/−	−/−	+/+	−/−
Cardiomyopathy		+	+	+	−	−	−	−
Anti–parietal cell Ab		+	+	+	−	−	−	ND
Hydronephrosis		−	−	+	+	−	−	−
Antinuclear Ab		−	−	+	±	−	−[a]	+[b]

Spontaneous development of cardiomyopathy and hydronephrosis and production of anti–parietal cell Ab and antinuclear Ab are summarized for mice with the indicated genotypes on either a BALB/c or C57BL/6 background. ND, not done.
[a]Data obtained from reference 6.
[b]Data obtained from reference 13.

ciency of which results in the impairment of both central and peripheral tolerance by facilitating the β selection of T cells in thymus, augmenting the activation and proliferation of T cells and B cells, enhancing the cytotoxic activity of $CD8^+$ T cells, or impairing the anergy induction (4, 5, 21–23). On the other hand, FcγRIIB deficiency has been shown to break peripheral tolerance of B cells by allowing the proliferation of autoAb-secreting B cells and their differentiation to plasma cells (10, 11, 24).

Autoimmune susceptibility of specific organs differs among different strains of mice. For example, NOD mice and NZB mice spontaneously develop type I diabetes and hemolytic anemia, respectively, whereas SJL but not C57BL/6 mice are susceptible to experimental encephalomyelitis. Although MHC molecules essentially determine the organ-specific susceptibility of autoimmune diseases, genetic interactions between specific immune regulators can modify autoimmune susceptibility of organs. An overt autoimmune disease may arise when the threshold to an organ-specific susceptibility is lowered through these genetic interactions. Our present findings indicate that autoreactive lymphocytes against cardiac and gastric antigens, which may be selected in the BALB/c background, can be activated exclusively by PD-1 deficiency, whereas activation of those against urothelial and nuclear antigens are predominantly dependent on FcγRIIB deficiency because the latter phenotypes required homozygous deletion of *Fcgr2b* but not *Pdcd1* (Table I). PD-1 deficiency/insufficiency may be involved additively by augmenting the inflammatory response against these antigens as shown for type I diabetes on NOD mice (9). Spontaneous production of antinuclear Abs by C57BL/6-*Fcgr2b*$^{-/-}$ but not C57BL/c-*Pdcd1*$^{-/-}$ mice and IgG class switching of anti-DNA Ab–producing transgenic B cells in C57BL/6-*Fcgr2b*$^{-/-}$ mice support the idea that FcγRIIB deficiency is critical for immunological tolerance against nuclear antigens (6, 13, 24). FcγRIIB deficiency can also augment humoral immunity in general by accelerating plasma cell differentiation (9, 24).

Recently, single nucleotide polymorphisms (SNPs) for both human PD-1 and FcγRIIB genes have been analyzed in various autoimmune diseases (10, 25). To date, >10 SNPs have been reported both in human PD-1 and FcγRIIB genes. A nonsynonymous SNP in the transmembrane region of FcγRIIB has been reported to associate with SLE in Japanese populations, and the 2B.4 promoter haplotype of the FcγRIIB gene has been reported to associate with SLE in Caucasians (10). Two of the SNPs in the human PD-1 gene were reported to associate with the incidence of SLE, rheumatoid arthritis, and type I diabetes (25–27). Prokunina et al. reported that the A allele of the PD1.3 (PD1.3A), one of the SNPs in the third intron of the human PD-1 gene, associates with the incidence of SLE in Swedish, European-American, and Mexican, but not African-American, populations (25). However, the opposite results have also been reported in Spanish populations; i.e., the PD1.3A allele is rather less frequent among SLE patients in Spain, with statistical significance (28). The effect of these SNPs in the PD-1 gene ($P = 0.006$) seems to vary depending on the genetic background, which is reminiscent of the situation in the mouse. Therefore, it is essential to analyze various immunoregulatory SNPs in combination to completely understand the genetic pathology of autoimmune diseases. However, because disease-promoting SNPs are generally less frequent and the number of possible combinations is enormous, multivariable studies of SNPs are still rather impractical.

Our present analysis clearly demonstrates that FcγRIIB and PD-1 cooperatively regulate autoimmune phenotypes in the mouse, suggesting that some of the human autoimmune diseases may also be regulated by the combination of dysfunction of human FcγRIIB and PD-1 genes. Therefore, it may be beneficial to analyze the combinatorial effect of known SNPs on human FcγRIIB and PD-1 genes in human autoimmune diseases.

MATERIALS AND METHODS

Animals. BALB/c and MRL-lpr-MpJ mice were purchased from Japan SLC. BALB-*Pdcd1*$^{-/-}$ mice and BALB/c-*Fcgr2b*$^{-/-}$ mice were described previously (7, 13). All mouse protocols were approved by the Institute of Laboratory Animals at the Kyoto University Graduate School of Medicine. All animals were maintained under specific pathogen-free conditions.

Western blotting. Urinary bladders were collected from wild-type BALB/c mice and separated into urothelial sheet and muscular layer under the microscope. Crude extracts were prepared with a Polytron homogenizer in lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1% NP-40, and protease inhibitor cocktail [complete; Roche]), separated by SDS-PAGE, and transferred onto Hybond-P filter (GE Healthcare). Filters were incubated with ×200 diluted mouse sera or mouse mAb against UPKIIIa (PROGEN) for 2 h at room temperature and visualized by horseradish peroxidase–labeled anti–mouse IgG Ab (Kirkegaard and Perry Laboratories) with enhanced chemiluminescence system (GE Healthcare).

Immunohistochemistry. Organs were collected from wild-type BALB/c mice and snap frozen in OCT compound (Sakura Finetechnical). Cryosections were fixed with CytoFix (BD Biosciences) and stained with ×100 diluted sera from animals, as indicated in the figures, followed by FITC-

labeled anti–mouse IgG Ab (Southern Biotechnology Associates, Inc.). IgG deposition was analyzed by staining organs from hydronephrotic mice with FITC-labeled anti–mouse IgG Ab. Signals were observed with axiovision (Bio-Rad Laboratories).

Molecular cloning and expression of mouse UPKIIIa protein. Mouse UPKIIIa cDNA was cloned by RT-PCR and confirmed by sequencing. Mouse UPKIIIa cDNA was subcloned into pGEX-6P-1 (GE Healthcare) vector, and GST fusion protein of mouse UPKIIIa was expressed in *E. coli* according to the manufacturer's instructions. GST-UPKIIIa fusion protein was recovered from inclusion body and the purity was >90% as judged by coomassie staining of the SDS-PAGE gel (unpublished data). Recognition of GST-UPKIIIa fusion protein by sera was examined by Western blotting as described in that section.

ELISA. Antinuclear Ab was measured using an antinuclear Ab detection kit (Dade Behring) according to the manufacturer's instructions.

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