Therapeutic effects of soluble human leukocyte antigen G2 isoform in lupus-prone MRL/lpr mice

Short title: Immunosuppressive effects of HLA-G2 in SLE

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Abstract (<200 words)

Human leukocyte antigen (HLA)-G, a non-classical HLA class I molecule, has one of the splicing isoforms, HLA-G2, which lacks one domain (α2) and forms a non-covalent homodimer. HLA-G2 is expressed on placental cells, regulatory T cells, tumor cells, and virus-infected cells, and is involved in immunosuppression. The major isoform of HLA-G, HLA-G1, binds to leukocyte immunoglobulin (Ig)-like receptor (LILR) B1 and LILRB2, on the contrary, HLA-G2 binds to only LILRB2. We previously reported that HLA-G2 bound LILRB2 more strongly than HLA-G1 and also to paired Ig-like receptor (PIR)-B, a mouse homolog of LILRBs. Furthermore, HLA-G2 showed immunosuppressive effects in both collagen-induced arthritis (CIA) and atopic dermatitis-like model mice. In this study, we examine *in vivo* effects of HLA-G2 in systemic lupus erythematosus (SLE) model mice. HLA-G2 showed the suppression of the typical SLE symptoms such as serum anti-dsDNA antibody level and urinary albumin index. Furthermore, HLA-G2 tended to downregulate B-lymphocyte stimulator (BLyS) production. This is the first observation of the immunosuppressive effects of HLA-G2 isoform in SLE model mice, suggesting that HLA-G2 could be a useful therapeutic agent for SLE.

Key words; HLA-G2, LILRB2, PIR-B, APCs, SLE

Abbreviations: Human leukocyte antigen (HLA), Beta-2-microglobulin (β2m), Leukocyte immunoglobulin-like receptor (LILR), Dendritic cell (DC), Paired immunoglobulin-like receptor (PIR), Antigen presenting cell (APC), Collagen-induced arthritis (CIA), Systemic lupus erythematosus (SLE), B lymphocyte stimulator (BLyS), B cell activating factor belonging to the tumor necrosis factor family (BAFF), size exclusion chromatography (SEC)

1. Introduction

Human leukocyte antigen-G (HLA-G) is a low polymorphic non-classical HLA class I molecule. HLA-G is expressed as variable forms such as alternative splicing isoforms (HLA-G1 to -G7) and their disulfide and/or non-disulfide-linked dimer and multimers [1-3]. The structure of HLA-G1 isoform is very similar to those of classical HLA class I molecules consists of heavy chain, beta-2-microglobulin (β2m) and a peptide, and its structure, function and expression have been studied well. The finding of the individuals who possess the homozygous *HLA-G*0105N* null allele lacking functional HLA-G1 isoform suggested that HLA-G2 isoform could substitute the HLA-G1 function because they could express α2 domain-deleted HLA-G2 [4]. HLA-G1 specifically binds to inhibitory immune receptors, leukocyte immunoglobulin (Ig)-like receptor B1 (LILRB1) and B2 (LILRB2) [5]. LILRB1 is constitutively expressed on both myeloid and lymphoid lineage cells and suppresses the broad innate and acquired immune responses. LILRB2 expression is more restricted on myeloid lineage cells, monocytes, macrophages and dendritic cells (DCs). Interestingly, β2m-free HLA-G molecules such as HLA-G2 and β2m-free HLA-G1 selectively bound to LILRB2, but not to LILRB1. This suggests that HLA-G1 and HLA-G2 can transmit the inhibitory signals on the different immune cells. The mouse homolog of LILRBs, paired Ig-like receptor B (PIR-B) is expressed by

antigen presenting cells (APCs) including myeloid-lineage immune cells and B cells and has the potential to bind with HLA-G1 and HLA-G2 molecules [6, 7]. Thus, PIR-B has been thought to be a mouse ortholog of LILRB2 on its distribution and function. HLA-G1 also showed therapeutic effects in atopic dermatitis-like model mice suggesting that HLA-G can inhibit Th2/Th17-mediated immune responses as well [8]. Furthermore, we previously reported the *in vivo* immunosuppressive effects of HLA-G1 and HLA-G2 using collagen-induced arthritis (CIA) mice [6, 7]. Surprisingly, both HLA-G1 and -G2 maintained a suppressive effect for over one month by a subcutaneous single administration in the left footpad [6, 7].

Systemic lupus erythematosus (SLE) is a clinically heterogeneous chronic autoimmune inflammatory disease characterized by multisystem organ involvement, polyclonal B cell activation, high-level of autoantibody production against a wide range of nuclear, cytoplasmic and cell surface autoantigens, immune complex deposition, and vasculitis [9]. In contrast to rheumatoid arthritis, biological drugs targeting effector molecules are still limited in SLE. Belimumab is the first drug approved for the treatment of SLE and blocks the B lymphocyte stimulator (BLyS, also known as B cell activating factor belonging to the tumor necrosis factor family (BAFF)), which is upregulated in the patients and correlates with disease activity [10]. However, novel therapeutic drugs for

SLE have been expected for long-term satisfaction of patients.

In the present study, we hypothesized that the immune suppressive function of HLA-G2, which can target specific suppression of APCs, might be effective to heterogeneous immune disorders such as SLE. We verified the *in vivo* effects of HLA-G2 using MRL/lpr mice by administrating its soluble form. HLA-G2 inhibits the typical SLE symptoms, serum anti-double strand DNA (dsDNA) antibody level and urinary albumin index. HLA-G2 also showed tendency to suppress BLyS production in mice. Taken together, HLA-G2 could be suggested as a new therapeutic application for SLE.

2. Materials and methods

2.1. Preparation of recombinant HLA-G2 protein

The ectodomain of HLA-G2 (α1-α3 domains, Gly1-Trp182) which has five nonsynonymous substitutions for sufficient expression was expressed as inclusion bodies in ClearColi BL21(DE3) competent cells (Lucigen, Middletown, WI, USA) as previously described [7]. The HLA-G2 inclusion bodies were refolded by a dilution method for 3 days at 4˚C and purified by size exclusion chromatography (SEC) using a HiLoad 26/60 Superdex 75 pg column (GE Healthcare, Chicago, IL, USA) equilibrium with 20 mM Tris-HCl pH 8.0, 100 mM NaCl (Figure 1A black arrow). The purity of the HLA-G2 protein was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis under reduced or non-reduced condition using 15% acrylamide gel by Coomassie Brilliant Blue staining (Figure 1B). Since HLA-G2 has an exposed free Cysteine residue, Cys42, which can naturally form disulfide-bonded further multimers [11], the HLA-G2 homodimer partially formed disulfide-linked multimers. These disulfide-linked HLA-G2 multimers maintained the receptor binding activity comparable to HLA-G2 homodimer by surface plasmon resonance analysis (data not shown). HLA-G2 molecule has flexible structure unlike HLA-G1 [11], however, we could confirm the purified HLA-G2 protein could be stable for two weeks by SDS-PAGE under non-

reducing condition (data not shown). Thus, we prepared the HLA-G2 recombinant protein every two weeks and used major peak fraction (Figure 1A black arrow) for administration. For injection to mice, the purified HLA-G2 were replaced the buffer with autoclaved phosphate-buffered saline (PBS) by dialysis.

2.2. Mice

Seven weeks old female MRL/MpJ-*lpr/lpr* mice were purchased from Japan SLC (Shizuoka, Japan) and bred under specific-pathogen-free conditions. All experiments were performed in accordance with the guidelines of the Committee of Ethics on Animal Experiments in Hokkaido University.

2.3. Injection and sampling

After eight days of housing, the mice were divided into two groups (HLA-G2 and control PBS group, n = 8) and injected 15 μg of HLA-G2 in PBS or 200 μL of PBS as a negative control by intraperitoneal administration (Day 0). The mice were injected in a same way and their body weight were measured for twice a week until Day 95. Plasma and urine were collected from each mouse every 2 or 3 weeks (Day -8, 22, 41, 62, 76, 90 ,105, 118).

2.4. ELISA analysis

The anti-dsDNA titer and the BLyS concentrations in plasma were measured using LBIS Mouse Anti-dsDNA ELISA Kit (FUJIFILM Wako Shibayagi, Gunma, Japan) and Mouse BAFF/BLyS/TNFSF13B Quantikine® ELISA Kit (R & D Systems, Inc., Minneapolis, MN, USA), respectively, according to the instruction manuals. The albumin and creatinine concentrations in urine were measured using LBIS Mouse Albumin ELISA Kit (FUJIFILM Wako Shibayagi, Gunma, Japan) and Urinary Creatinine ELISA Kit (TRANS GENIC INC, Fukuoka, Japan), respectively, according to the instruction manuals.

2.5. Statistical analysis

Statistically significant differences were calculated using Mann-Whitney *U*-test. $p < 0.05$ were considered as statistically significant difference.

3. Results

3.1. Suppression of SLE symptoms by HLA-G2

To examine the effect of the HLA-G2 in SLE, we used the MRL/MpJ-*lpr/lpr* mice which possess a mutation in the *lpr* gene that encodes Fas and shows systemic autoimmunity symptoms by producing various autoantibodies. The autoantibodies form immune complexes with autoantigens, and their deposition on tissues throughout the body cause various symptoms such as glomerulonephritis or arthritis as observed in SLE [12, 13].

To consider the dose and the frequency of administration, we first administered HLA-G2 (750 μg/kg (9 ~ 13 μg/mouse)), which was prepared by refolding method described in Materials and Methods (Figure 1), once two weeks by intraperitoneal administration. No clear difference was observed between the HLA-G2 and PBS treated groups (data not shown). Therefore, in the present study, we determined to administer HLA-G2 (15 μg/mouse) or PBS (200 μL) twice a week intraperitoneally for 95 days (Figure 2A). Furthermore, we examined whether the effects last after stop the administration until 118th day. To evaluate the effect of HLA-G2 protein on the SLE model mice, we collected mice plasma and urine every 2 or 3 weeks (Figure 2A).

The typical symptoms of MRL/lpr mice, anti-dsDNA antibodies in the plasma and urinary protein [12, 13], were assessed and compared between HLA-G2 and PBS groups.

The anti-dsDNA antibody level was gradually increased in the PBS group until 90th day, and then, some mice showed spontaneous recovery (Figure 3A, Supplementary Table 1). In the HLA-G2 group, the antibody titer slightly increased in the same manner as the PBS group until Day 41, but it did not show further increase during HLA-G2 treatment, and this suppression was maintained even after the end of administration (Figure 3A, Supplementary Table 1). On Day 90, the anti-dsDNA antibody titer in HLA-G2 group was significantly lower than that of PBS group (Figure 3A).

The urinary albumin index, an indicator of the progression of glomerulonephritis, begun to increase from Day 62 in PBS group (Figure 3B, Supplementary Table 1). However, the index was suppressed during the treatment in HLA-G2 group, while the difference did not reach statistical significance due to the variation in each group (Figure 3B). Once HLA-G2 administration stopped, the urinary albumin index was elevated, and the average of each group was similar (Figure 3B, Supplementary Table 1). Importantly, the HLA-G2 did not cause negative effect on mice, as reflected by a lack of weight loss compared with PBS group (Figure 2B).

3.2. Suppression of BLyS production by HLA-G2 in SLE mice

HLA-G1 and HLA-G2 show different receptor preference in human as described above,

however, they bind to the same receptor, PIR-B, which is an inhibitory receptor expressed on antigen presenting cells in mice. HLA-G1 and HLA-G2 suppressed the arthritis in CIA mice [6, 7]. Furthermore, HLA-G1 isoform could suppress the production of effector molecules such as IL-17 and IL13 and showed therapeutic effect in atopic dermatitis-like mice [8]. Thus, we examined whether HLA-G2 administration could inhibit the overexpression of a significant effector molecule, BLyS, which is observed in both MRL/lpr mice and the SLE patients. The BLyS concentration in the plasma on Day 90, the end of the HLA-G2 administered day and Day 118, after 28 days of final administration was shown in Figure 4 and Supplementary Table 1. While there was no statistically significant difference between HLA-G2 group and PBS group (P value = 0.17 by Mann-Whitney U-test), HLA-G2 showed tendency to downregulate the BLyS production. These results suggested that HLA-G2 may contribute to the therapeutic phenotype *in vivo* by modulation of the excess effector molecules including BLyS.

Discussion

In the present study, we showed that HLA-G2 recombinant protein have potential to suppress the clinically heterogeneous disease, SLE. Recently, kinds of biologics targeting cytokine molecules, immune checkpoint receptors and their ligands showed therapeutic success especially in rheumatoid arthritis and cancers [14, 15]. On the other hand, the treatment of SLE can induce remission and its disease heterogenicity, which force patients with SLE to still have unsatisfactory chronic symptoms. HLA-G molecules are known as immunosuppressive molecules and mainly function at the maternal-fetal interface during pregnancy. Furthermore, regulatory T cells, tumor cells and virus-infected cells also express HLA-G molecules to inhibit surrounding immune cell activations. We have examined *in vivo* therapeutic effects of HLA-G1 and HLA-G2 molecules [6-8]. Both HLA-G1 and HLA-G2 could significantly suppress the inflammation in CIA mice, and especially, HLA-G2 was effective with lower effective dose than HLA-G1 isoform in CIA mice [6, 7]. In mice, these immunosuppressive functions were suggested to be transmitted through PIR-B expressed on APCs, whose distribution is similar to human LILIRB2. In regard to the strength of receptor binding, the apparent dissociation constant (K_d) of the HLA-G2-LILRB2 interaction was 1.7 nM with avidity effects due to homodimerization and further multimerization through disulfide-bond by Cys42 [11]. This association is

much stronger than those of the binding of both HLA-G1-LILRB2 and HLA-G1 dimer toward LILRB2 [5, 16, 17]. Thus, among the variable HLA-G molecular types, we recently focused on non-disulfide-linked homodimer of α2 domain-deleted HLA-G2 for therapeutic application.

The distribution of these HLA-G2 receptors suggests that HLA-G2 modulates the APC function *in vivo*. Recently, Belimumab targeting autoreactive B cells by inhibition of BLyS, which is elevated in the sera of SLE patients, was approved for the treatment of patients with active refractory SLE. HLA-G2 showed the potential to suppress BLyS production in the plasma, suggesting that the binding of HLA-G2 to PIR-B suppressed the BLyS production via B cells or APCs in mice. This is consistent with the previous results demonstrating the PIR-B stimulation by HLA-G1 or HLA-G2 suppressed broad immune effector functions targeting Th1, Th2 and Th17 responses [6-8]. In human, HLA-G2–LILRB2 signaling in APCs might be located at the upstream of BLyS production by B cells due to loss of LILRB2 expression in B cells. The stimulation of LILRB2 by HLA-G1 isoform induces the development of tolerogenic DCs in LILRB2 transgenic mice and human monocyte-derived DCs [18]. Ristich et al. also reported that HLA-G1-modified tolerogenic DCs induced anergic and immunosuppressive effector T cells [18]. The DCs differentiated to tolerogenic APCs in HLA-G2-treated MRL/lpr mice would affect on

autoreactive B cells through the modulation of effector T cell function *in vivo*. Therefore, it was expected that the induction of tolerogenic APCs by HLA-G molecules could promote the suppression of excess immune responses regardless of cell type.

The HLA-G2 can be useful as therapeutic reagent, but, the role and the protein expression level of HLA-G2 in healthy and the patients with immune diseases are still unclear because any HLA-G2-specific antibody has not been available so far. To clarify the amount of HLA-G2 molecule in the sera for the comparison between healthy individuals and the patients is necessary to develop HLA-G2 as a novel therapeutic agent. HLA-G2 exists stably as a homodimer [11] but can also naturally form further multimer through Cys42 by natural oxidation. HLA-G2 multimer possible to possess much higher affinity and avidity to LILRB2 on the cell surface. Furthermore, the administration to "male" MRL/lpr mice resulted in no symptoms in both HLA-G2 and PBS groups (anti-dsDNA antibodies, urinary protein and BLyS concentration; data not shown) and no side effect such as inflammation or body weight loss was observed. This supports that injected HLA-G2 transiently suppress through APC functions when the immune response is overactivated. However, to examine the long-term effect *in vivo* including tumorigenesis and viral infections is necessary. To elucidate the immunosuppressive effects of HLA-G2 in SLE, it will be important in future to evaluate the functional importance and

quantitative effects of HLA-G2 in human.

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References

[1] Carosella ED, Rouas-Freiss N, Tronik-Le Roux D, Moreau P, LeMaoult J: HLA-G: An Immune Checkpoint Molecule. Adv Immunol 2015;127:33.

[2] Kamishikiryo J, Maenaka K: HLA-G molecule. Curr Pharm Des 2009;15:3318.

[3] Kuroki K, Maenaka K: Immune modulation of HLA-G dimer in maternal-fetal interface. Eur J Immunol 2007;37:1727.

[4] Le Discorde M, Le Danff C, Moreau P, Rouas-Freiss N, Carosella ED: HLA-G*0105N null allele encodes functional HLA-G isoforms. Biol Reprod 2005;73:280.

[5] Shiroishi M, Tsumoto K, Amano K, Shirakihara Y, Colonna M, Braud VMet al. : Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. Proc Natl Acad Sci U S A 2003;100:8856.

[6] Kuroki K, Hirose K, Okabe Y, Fukunaga Y, Takahashi A, Shiroishi Met al. : The long-term immunosuppressive effects of disulfide-linked HLA-G dimer in mice with collagen-induced arthritis. Hum Immunol 2013;74:433.

[7] Takahashi A, Kuroki K, Okabe Y, Kasai Y, Matsumoto N, Yamada Cet

al. : The immunosuppressive effect of domain-deleted dimer of HLA-G2 isoform in collagen-induced arthritis mice. Hum Immunol 2016;77:754.

[8] Maeda N, Yamada C, Takahashi A, Kuroki K, Maenaka K: Therapeutic application of human leukocyte antigen-G1 improves atopic dermatitis-like skin lesions in mice. Int Immunopharmacol 2017;50:202.

[9] Kotzin BL: Systemic lupus erythematosus. Cell 1996;85:303.

[10] Lopez P, Rodriguez-Carrio J, Caminal-Montero L, Mozo L, Suarez A: A pathogenic IFNalpha, BLyS and IL-17 axis in Systemic Lupus Erythematosus patients. Sci Rep 2016;6:20651.

[11] Kuroki K, Mio K, Takahashi A, Matsubara H, Kasai Y, Manaka Set al. : Cutting Edge: Class II-like Structural Features and Strong Receptor Binding of the Nonclassical HLA-G2 Isoform Homodimer. J Immunol 2017;198:3399.

[12] Reilly CM, Gilkeson GS: Use of genetic knockouts to modulate disease expression in a murine model of lupus, MRL/lpr mice. Immunol Res 2002;25:143.

[13] Richard ML, Gilkeson G: Mouse models of lupus: what they tell us and what they don't. Lupus Sci Med 2018;5:e000199.

[14] Sarzi-Puttini P, Ceribelli A, Marotto D, Batticciotto A, Atzeni F:

Systemic rheumatic diseases: From biological agents to small molecules. Autoimmun Rev 2019;18:583.

[15] Sun L, Chen L, Li H: Checkpoint-modulating immunotherapies in tumor treatment: Targets, drugs, and mechanisms. Int Immunopharmacol 2019;67:160.

[16] Shiroishi M, Kuroki K, Ose T, Rasubala L, Shiratori I, Arase Het al. : Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. J Biol Chem 2006;281:10439.

[17] Shiroishi M, Kuroki K, Rasubala L, Tsumoto K, Kumagai I, Kurimoto Eet al. : Structural basis for recognition of the nonclassical MHC molecule HLA-G by the leukocyte Ig-like receptor B2 (LILRB2/LIR2/ILT4/CD85d). Proc Natl Acad Sci U S A 2006;103:16412.

[18] Ristich V, Liang S, Zhang W, Wu J, Horuzsko A: Tolerization of dendritic cells by HLA-G. Eur J Immunol 2005;35:1133.

Figure legends

Figure 1. Purification of HLA-G2 and PEG-G2 protein.

(A) The refolded HLA-G2 was purified by SEC using Hiload 26/60 Superdex 75 pg column and collected the peak fractions indicated by the black arrow. (B) Collected HLA-G2 was checked the purity by SDS-PAGE analysis under non-reduced condition.

Figure 2. Administration of HLA-G2 into MRL/lpr mice.

(A) The schedule of intraperitoneal administrations (Day 0-95) and sampling date (black arrows, Day -8, 22, 41, 62, 76, 90, 105, 118). (B) Changes in body weight of MRL/MpJ-lpr/lpr mice administered with HLA-G2 (black) and PBS (gray). Each bar indicates mean ± Standard deviation (SD).

Figure 3. Anti-dsDNA antibody titer in plasma.

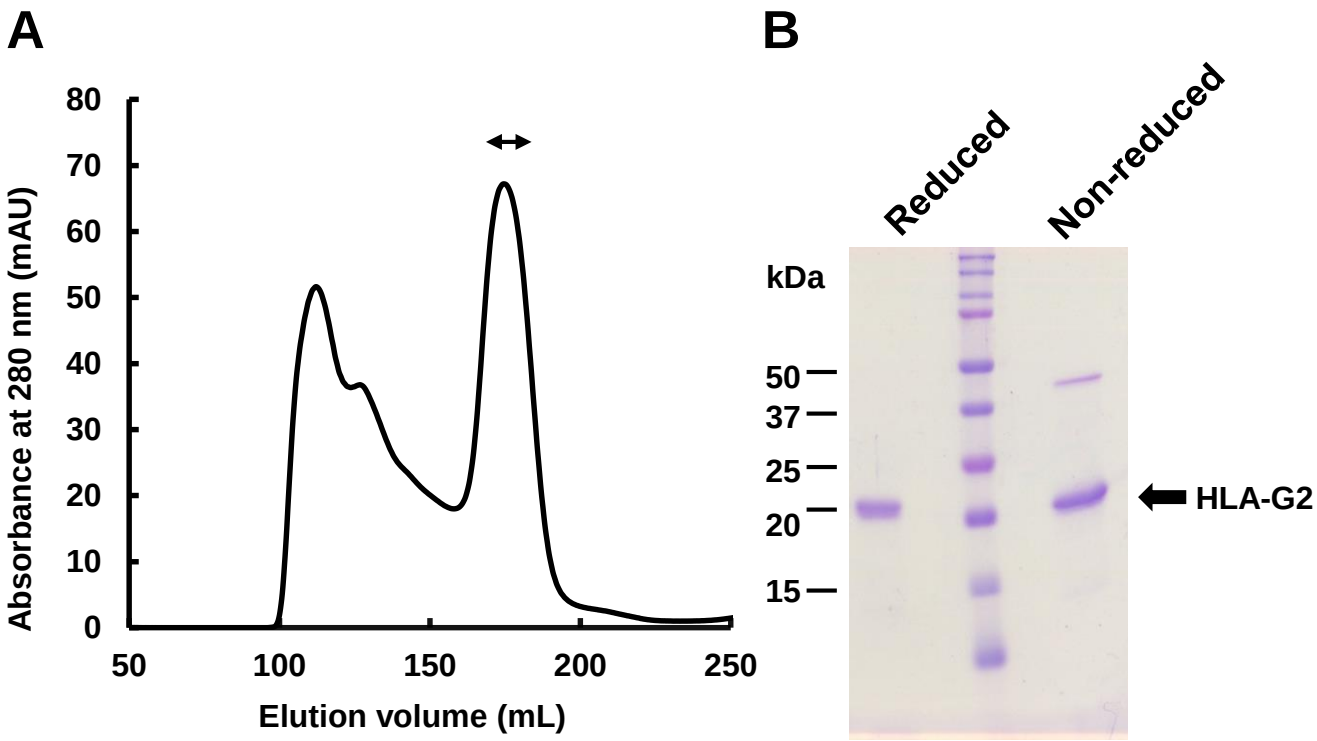
(A) Anti-dsDNA antibody titer in plasma of mice administered with HLA-G2 or PBS. (B) Urinary albumin index of mice administered with HLA-G2 or PBS.

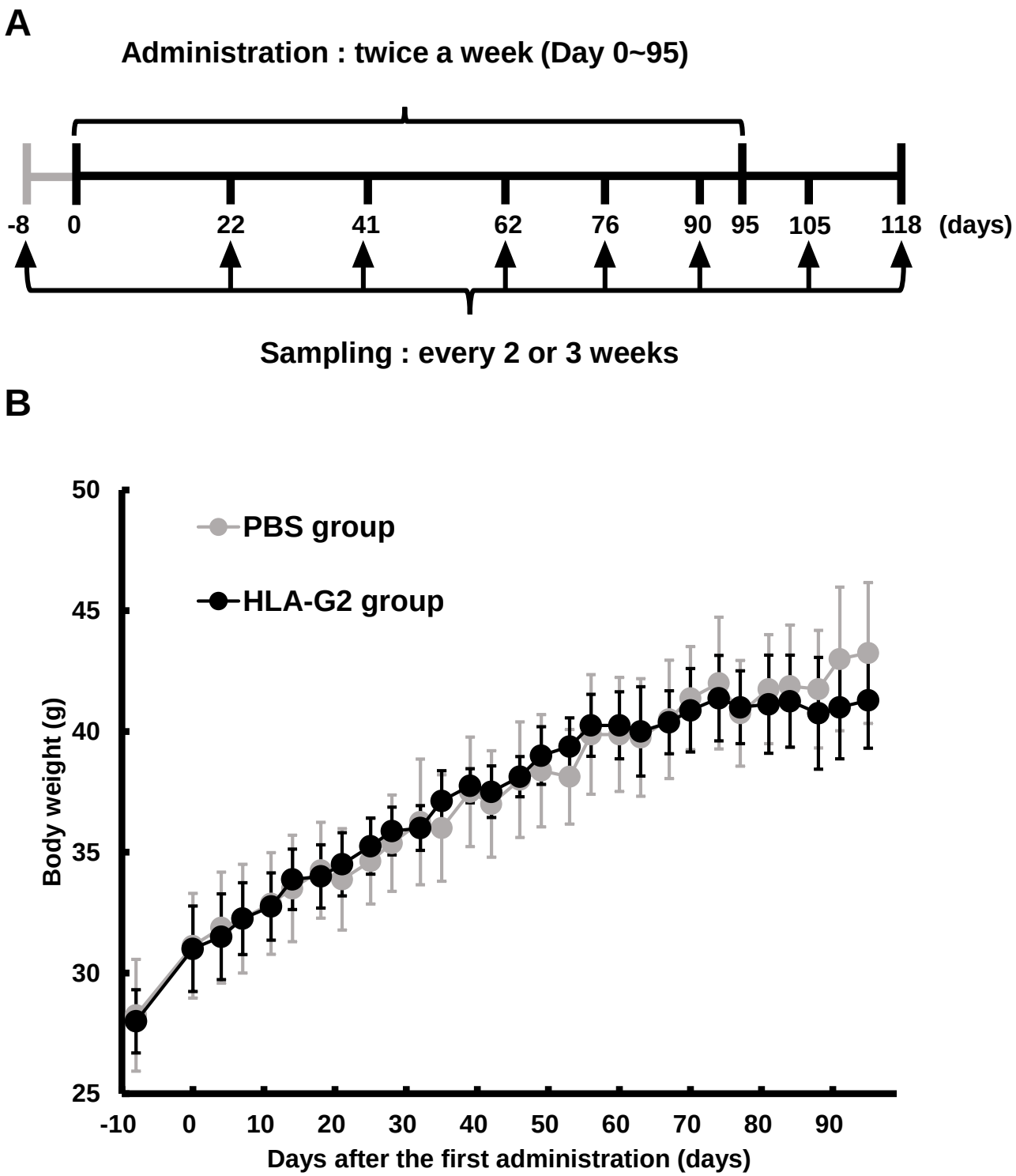
Administration period was indicated by black line underneath of days. Each bar indicates mean + SD. Dot blots for value of each mouse were also shown. Statistically significant differences were shown as p value (** $p < 0.01$).

Figure 4. BLyS concentration in plasma.

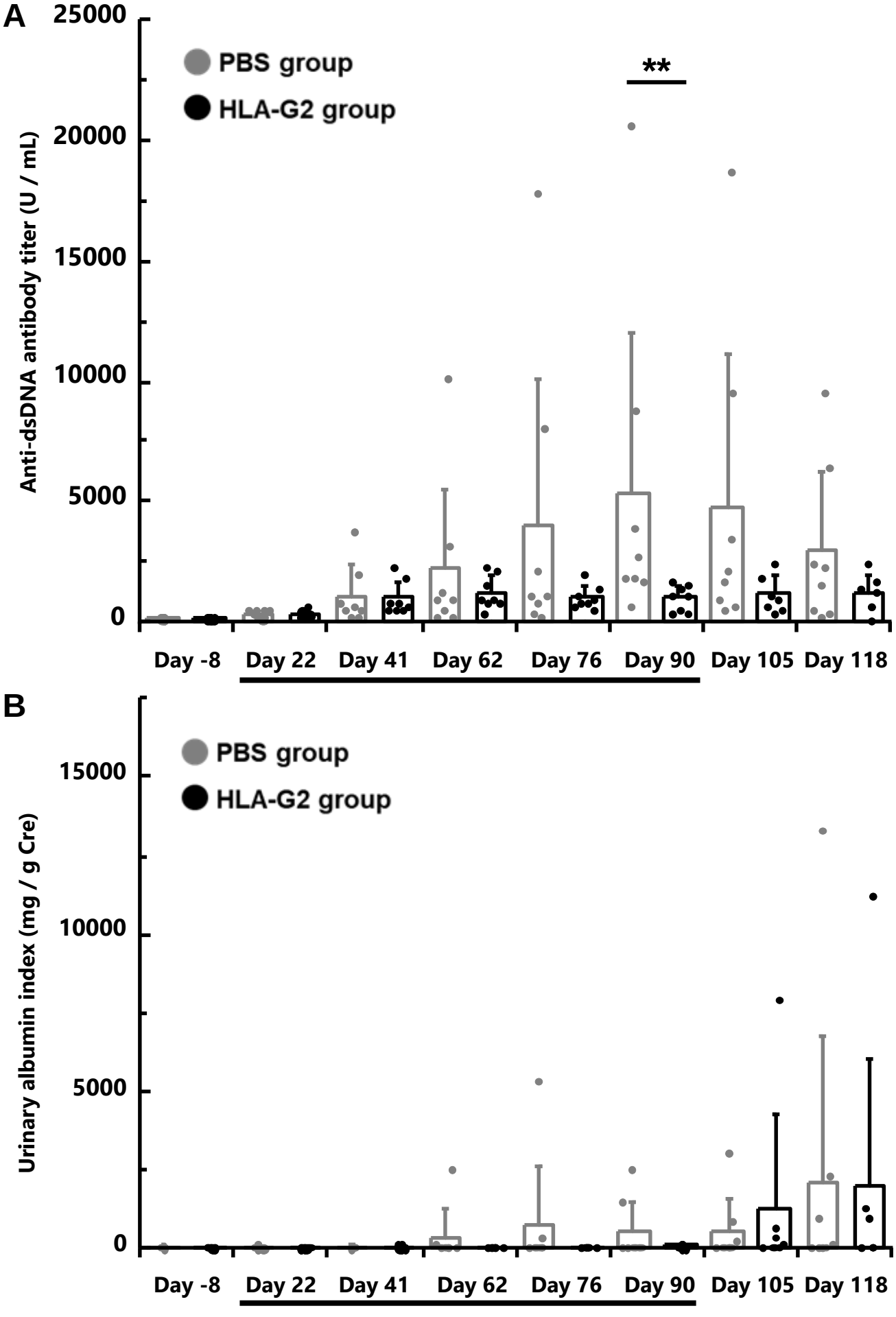
BLyS concentration in plasma of mice administered with HLA-G2 or PBS on Day 90 and Day 118. Administration period was indicated by black line underneath of days. Each bar indicates mean + SD. Dot blots for value of each mouse were also shown.

Watanabe et al., Figure 1

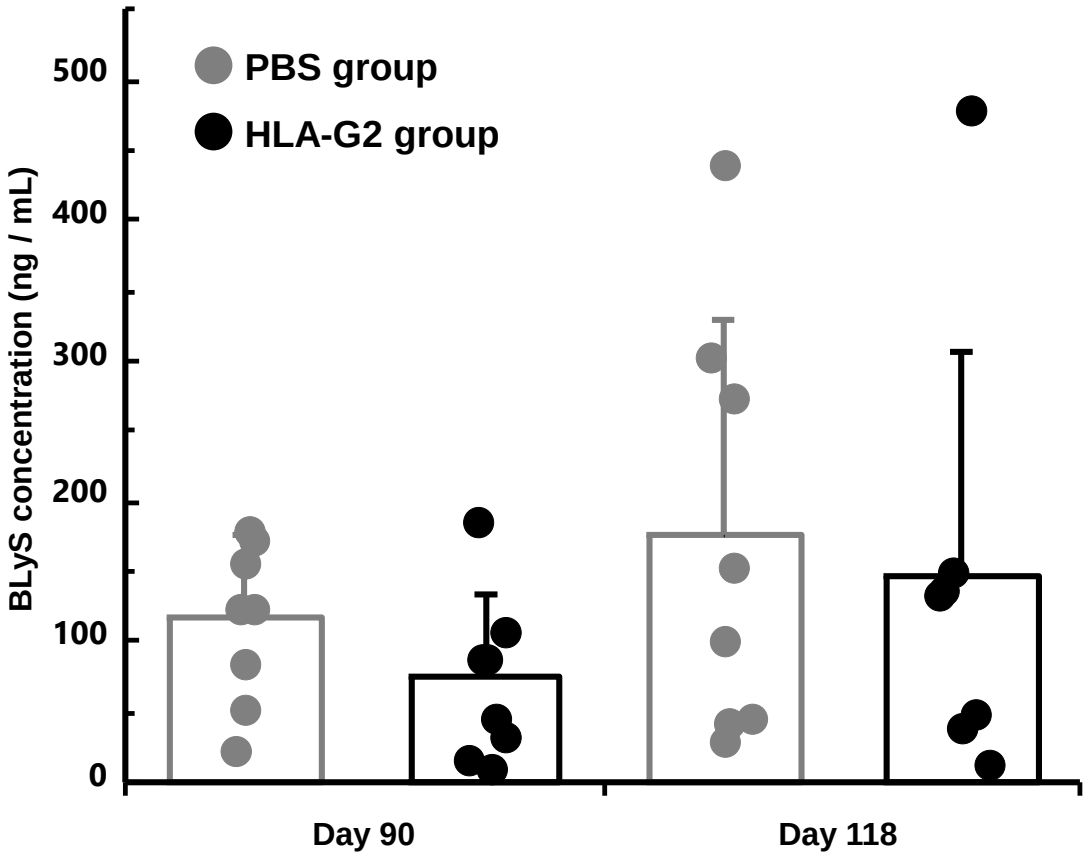



A
Administration : twice a week (Day 0~95)
-8
0
22
41
62
76
90
95
105
118
(days)
Sampling : every 2 or 3 weeks
B
PBS group
HLA-G2 group
Body weight (g)
50
45
40
35
30
25
-10
0
10
20
30
40
50
60
70
80
90
Days after the first administration (days)

Watanabe et al., Figure 3



Watanabe et al., Figure 4



Supplementary Table 1.

	Anti-dsDNA antibody titer (U / ml)		Urinary albumin index (mg / g Cre)		BLyS concentration (ng / ml)	
	PBS group	HLA-G2 group	PBS group	HLA-G2 group	PBS group	HLA-G2 group
Day -8	123.5 ± 70.6	117.8 ± 78.4	34.3 ± 18.9	23.4 ± 13.8	N.D.	N.D.
Day 22	337.8 ± 176.5	350.7 ± 162.7	36.7 ± 63.5*	18.4 ± 12.8	N.D.	N.D.
Day 41	1078.8 ± 1249.1	981.4 ± 650.0	29.8 ± 26.3 #	48.7 ± 55.6 #	N.D.	N.D.
Day 62	2154.2 ± 3352.1	1198.0 ± 722.7	346.9 ± 894.5	16.5 ± 10.4	N.D.	N.D.
Day 76	3958.3 ± 6188.0	996.3 ± 505.0	732.9 ± 1854.7	17.6 ± 9.4	N.D.	N.D.
Day 90	5260.5 ± 6695.0	989.7 ± 549.2	500.4 ± 943.6	66.6 ± 57.9	118.8 ± 57.4	76.4 ± 58.1
Day 105	4693.8 ± 6384.1	1116.0 ± 761.0*	529.8 ± 1045.0	1293.7 ± 2936.7*	N.D.	N.D.
Day 118	4660.2 ± 7927.9	1169.3 ± 766.2*	2101.7 ± 4624.1	1933.8 ± 4030.8*	177.4 ± 150.4	147.1 ± 157.9*

(n = 8, *: n = 7, #: n = 5)

Appendix A. Supplementary data

Supplementary Table 1.

Anti-dsDNA antibody titer, Urinary albumin index and BLyS concentration values. Data are shown as mean ± SD.