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博 士 （医 学） 学 位 論 文

Human cartilage glycoprotein-39 (HC gp-39) is highly expressed on Foxp3^{+} Treg cells, and inhibits the proliferation and cytokine production of antigen-specific Th1 and Th17 cells in GPI-induced arthritis model.

(Human cartilage glycoprotein-39 (HC gp-39)は GPI 誘導関節炎モデルにおいて Foxp3^{+} Treg 細胞に高発現し、抗原特異的 Th1 および Th17 の増殖および サイトカイン産生を抑制する)

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CONTENTS

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the multiple joints. In patients with RA, several reports indicate the presence of clonally expanded blood and synovial $CD4^+$ T cells. Recently, biologic agents targeting T cell co-stimulation have clear therapeutic benefits on patients with RA, suggesting $CD4^+$ T cells play a crucial role in the pathogenesis. However, their precise function to RA is poorly defined. In this study, we focused on specific molecule in antigen-specific $CD4^+$ T cells and explored the mechanisms in GPI-induced arthritis (GIA) model.

We evaluated highly expressed molecules of $CD4^+$ T cells from splenocytes of GIA mice by GeneChip analysis, and we focused on human cartilage glycoprotein-39 (HC gp-39). HC gp-39 is a well-known autoantigen in rheumatoid arthritis (RA). However, the exact localization, fluctuation, and function of HC gp-39 in RA are unknown. Therefore, using a GIA model, we investigated these aspects of HC gp-39 in arthritis.

The production of HC gp-39 was highly detected on the early phase of GIA (day 7) and the HC gp-39 mRNA was significantly increased on splenic $CD4^+$ T cells on day 7 but not on $CD11b^+$ cells. Moreover, to identify the characterization of HC gp-39^+CD4^+ T cells, we assessed the analysis of Th subsets. As a result, HC gp-39 was dominantly expressed in $CD4^+CD25^+Foxp3^+$ Treg cells, but not in Th1 and Th17 cells. Furthermore, to investigate the effect of HC gp-39 to $CD4^+$ T cells, we assessed T cell proliferation assay and cytokine production from $CD4^+$ T cells using recombinant HC gp-39. We found that GPI-specific T cell proliferation, and IFNγ or IL-17 production were clearly suppressed by addition of recombinant HC gp-39. Antigen-specific expression of HC gp-39 in splenic $CD4^+CD25^+$ $Foxp3^+$ Treg cells occurs in the induction phase of GIA, and addition of recombinant HC gp-39 suppresses antigen-specific T cell proliferation and cytokine production, suggesting that HC gp-39 in $CD4^+$ T cells might play a regulatory role in arthritis.

Chapter. 1 Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of multiple joints and variable disease outcomes. Biologic agents targeting proinflammatory cytokines, such as TNFα and IL-6, as well as T cell costimulation have clear therapeutic benefits on patients with RA [1], although its etiology is poorly understood.

Glucose-6-phosphate isomerase (GPI) is an autoantigen that was identified in KxB/N mice [2]; it also induces arthritis in DBA/1 mice after a single immunization (GPI-induced arthritis: GIA) [3, 4]. In this model, the development of arthritis depends on T cells, B cells, and inflammatory cytokines, and the therapeutic effectiveness of biologics closely resembles that for human RA [4, 5]. Notably, the GPI-reactive T cells of this model are skewed to Th1 and Th17 cells, and T cell targeting therapies such as anti-CD4, CTLA-4 Ig, anti-ICOS, and anti-IL-17antibodies are clearly effective in treating GIA [3-6], whereas IFNγ receptor deficient mice were less susceptible to GIA [7], highlighting the critical role of antigen-specific T cells. Thus, unique expression of

an unknown molecule in autoreactive T cells might mirror a corresponding unique expression in RA.

Human cartilage glycoprotein-39 (HC gp-39; also termed BRP-39 or chitinase 3-like 1) is a well- known biomarker of several inflammatory autoimmune diseases:, elevated serum levels of HC gp-39 are seen in patients with RA, systemic lupus erythematosus (SLE), multiple sclerosis (MS) and asthma [8-10]. Indeed, elevated serum levels of HC gp-39 in RA are positively correlated with inflammatory cytokines and arthritis biomarkers such as IL-6 and CRP [11]. Also, HC gp-39 has been the focus of studies investigating it as a candidate autoantigen in RA [12-14], owing to its different expression in cartilage and synovial tissues, and to a possible link with the HLA-DR4 shared epitope [12, 13]. HC gp-39 protein is expressed in neutrophils, macrophages, chondrocytes, and synoviocytes [15]. Lee et al reported that in a HC gp-39 deficient murine model of asthma, disease state was improved as a result of diminished antigen-induced Th2 cell responses, suggesting an essential role in antigen sensitization by Th2 cells [16, 17]. However, the expression of HC gp-39 and its function in T cells in arthritis remain unclear.

In this report, we show that HC gp-39 is upregulated in antigen-specific $CD4^+CD25^+$ $Foxp3^+$ Treg cells in the early phase of arthritis, and that addition of HC gp-39 suppresses the proliferation and cytokine production of antigen-specific $CD4^+$ T cells, suggesting a crucial role for HC gp-39 in the regulation of T cells in arthritis.

Chapter 2. Materials and Methods

2-1. Mice

DBA/1 male mice and C57BL/6 male mice were obtained from Charles River Laboratories (Yokohama, Japan). All mice used in these studies were aged between 6 and 8 weeks and maintained under specific pathogen-free conditions. All experiments were approved by and conducted in accordance with the guidelines of the Ethics Review Committee for Animal Experimentation of the University of Tsukuba.

2-2. The mouse model of arthritis

Recombinant human GPI was prepared as described previously (4). The mice were immunized by intradermal injection of 300 μg recombinant human GPI-GST in emulsified Freund's complete adjuvant (CFA) (Difco, Detroit, MI, USA). Control mice were immunized with 100 μg GST in CFA. The arthritic mice were assessed visually, and changes in each paw were scored on a scale 0 to 3-; a score of 0 indicated no evidence of inflammation-; of 1, subtle inflammation or localized

edema-; of 2, easily identified swelling but localized to the dorsal or ventral surface of the paws-; and of 3, swelling of all aspects of the paws. The maximum possible score per mouse was 12.

2-3. GeneChip Analysis

Splenic $CD4^+$ T cells were collected from three DBA/1 mice (arthritis susceptible) and three C57BL/6 mice (arthritis resistant, as a control) after immunization of GPI on day7. Total RNA was extracted from these $CD4^+$ T cells using ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan), and then 15 μg of RNA was used for cDNA synthesis by reverse transcription followed by the synthesis of biotinylated cRNA through *in vitro* transcription. After cRNA fragmentation, hybridization with mouse 430A2.0 GeneChip (Affymetrix, Santa Clara, CA, USA) with probes for 43,000 mouse gene ESTs (expressed sequence tags) was performed in accordance with the protocol provided by the manufacturer. Analysis was performed by gene expression software (GEO- GSE66912).

2-4. ELISA

Sera were obtained from DBA/1 mice after GPI immunization (days 0, 7, 14, 28), or culture supernatants in which the splenocytes were stimulated with anti-CD3/28 and GPI for 48 and 24 hours, respectively, were collected. The HC gp-39 protein in the sera and culture supernatants was analyzed using the HC gp-39 ELISA kit (R&D Systems, Minneapolis, MN).

2-5. Quantitative PCR analysis

After GPI immunization (days 0, 7, 14, 28), the spleens, lymph nodes and joints were isolated, cut into small pieces, and passed through cell strainers (BD Biosciences, Erembodegem, Belgium) to obtain single-cell suspensions. $CD4^+$, $CD11b^+$ and CD19 cells were isolated from the splenocytes by means of magnetic-activated cell sorting (MACS) (miltenyi Biotech, Bergisch Gladback, Germany). The remaining cells were washed with phosphate-buffered saline (PBS). Total RNA was extracted with Isogen reagents (Nippon Gene Inc, Tokyo, Japan)

according to the manufacturer's. cDNA was obtained by reverse transcription with a commercially available kit (PrimeScript RT Master Mix; Takara). For quantitative PCR, we used a TaqMan Assay-on-Demand gene expression product (Applied Biosystems, Foster City, CA, USA). The expression levels of HC gp-39 and GAPDH (assay ID Mm00901477_m1 and Mm99999915_g1, respectively; Applied Biosystems) were normalized relative to the expression of GAPDH. Analysis was performed with an ABI Prism 7500 apparatus (Applied Biosystems) under the following conditions: inactivation of possible contaminating amplicons with AmpErase UNG (Applied Biosystems) for 2 minutes at 50°C initial denaturation for 10 minutes at 95°C, followed by 45 thermal cycles of 15 seconds at 95°C and of 60 seconds at 60°C.

2-6. Western blot analysis

Splenocytes were obtained from DBA/1 mice after GPI immunization (days 0, 7, 14, 28). Cells were lyzed overnight in ice-cold lysis buffer (50mM Tris-HCl pH7.4, 5mM $MgCl_2$, 2mM PMSF, 0.5% NP-40). The lysates were centrifuged at

15,000 rpm for 30 minutes at 4°C,and the supernatants collected. The lysates were subjected to electrophoresis on SDS-polyacrylamide gel (7.5%-15%). After transfer, the membranes were blocked for 60 minutes with Block Ace solution and then incubated with rat anti-HC gp-39 or rabbit anti-α actin diluted in Can Get Signal solution 1 (Toyobo Corporation, Osaka, Japan). After 60 minutes, the membranes were washed with PBS in 0.05% Tween-20 and incubated with mouse anti-rat HRP and goat anti-rabbit HRP diluted in Can Get Signal solution 2. The proteins were visualized by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech NJ USA) according to the manufacturer's instructions. Analysis was performed with an ImageQuant LAS 4000 apparatus (Fujifilm).

2-7. Fluorescent activated cell-sorting (FACS) analysis

Splenocytes obtained from day 7 DBA/1 mice after GPI immunization were restimulated with GPI or control for 24 hours in vitro. After that, these cells were stained with specific antibodies to CD4 (biolegend), HC gp-39 (Bioss), IFNγ (biolegend), IL-17 (biolegend) and Foxp3 (ebioscience). Intracellular staining was

used BD Cytofix/Cytoperm Plus Fixtation/Permeabilization kit (BD biosciences) and Treg staining kit. Flow cytometry was conducted using a FACSCalibur flow cytometer (BD Biosciences), and data were analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

2-8. Cell proliferation assay

Naïve $CD4^+$ T cells (2 x 10^6 cells) isolated from splenocytes or GPI-reactive $CD4^+$ T cells from lymph node on day 7 after GPI immunization were stained with 1.25 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) for 7 minutes. The stained cells were stimulated with 1 μg/ml anti-CD3/28 antibodies or 5.0μg/ml GPI in the presence of antigen presenting cells, respectively. Then, 10 μg/ml recombinant mouse HC gp-39 (R&D) (rHC gp-39) or as a control ovalbumin (OVA) was added in the presence of 1 x 10^6 cells/ml in 96-well round-bottom plates for 72 hours.

2-9. Co-culture

The mice were killed on day 7 after GPI immunization, and $CD4^+$ T cells and $CD11c^+$ cells were obtained from the lymph nodes and spleens, respectively, by means of MACS. The $CD4^+$ T cells and $CD11c^+$ cells were then co-cultured with 5 μg/ml GPI in the absence or presence of rHC gp-39, and as a control protein, ovalubumin (OVA) at a ratio of 5:1 for 72 hours. The supernatants were collected, and IFNγ and IL-17, measured using ELISA (R&D).

2-10. Histologic examinations

The hind paws and ankles were obtained from GIA mice (day 14). These samples were fixed in 10% formalin and stained with goat anti-HC gp-39 antibody (Santa Cruz Biothecnology, Santa Cruz CA) and hematoxylin.

2-11. Statistical analysis

All data were expressed as means ± SEMs. Differences between groups were examined for statistical significance using the *t* test. Probability values less than 0.05 were considered to indicate statistical significance.

Chapter 3. Results

3-1. HC gp-39 was identified as a highly expression molecules in arthritic CD4$^+$T cells by GeneChip analysis.

CD4$^+$ T cells are indispensable for both the induction and the effector phase of GIA [4]. To identify the highly expressed molecules of CD4$^+$ T cells in GIA, we assessed the GeneChip analysis. The expression of mRNA profile was examined in splenic CD4$^+$ T cells from DBA/1 mice (arthritis susceptible) and C57BL/6 mice (arthritis resistant) after immunization of GPI on day 7 (induction phase). Twenty genes were upregulated on the CD4$^+$ T cells from DBA/1 mice on GeneChip (GEO-GSE66912, Table.1). Among them, we focused on the molecules contribute to immune system such as HC gp-39.

3-2. HC gp-39 was highly detected in serum and CD4$^+$ T cell from GIA mice

To determine the correlation between HC gp-39 and GIA, the time-course production and expression of the protein was analyzed. For the control, mice were

190 immunized with a tagged protein (GST). Serum HC gp-39 levels were clearly elevated on day 7 (onset of arthritis), and then gradually increased, peaking on day 14 (peak of arthritis), where after they subsided to the basal level by day 28 (attenuate of arthritis) (Fig. 1a). We also assessed the fluctuated expression of HC gp-39 at the splenocytes by quantitative PCR and Western blotting. The expression of HC gp-39 was highly 195 detected in the splenocytes of GIA mice on day 7 (Fig. 1b). Next, the splenocytes from GIA mice were separated into $CD4^+$ T cells, $CD11b^+$ cells and $CD19^+$ cells. Then, fluctuation of HC gp-39 mRNA was analyzed in the individual cell populations. Of these, $CD4^+$ T cells had clear antigen-specific upregulation of HC gp-39 mRNA on days 7 and 14, which was confirmed by quantitative PCR. On the other hand, the 200 $CD11b^+$ cells showed higher HC gp-39 expression on days 7 and 14, but a comparable amount of expression was noted in the control- GST- immunized mice. $CD19^+$ cells demonstrated only weak upregulation of HC gp-39 on day 14 (Fig. 1c). These findings suggest that arthritis-specific overproduction of HC gp-39 is dominated by $CD4^+$ T cells.

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3-3. HC gp-39 was highly expressed in $CD4^+CD25^+$ T cells, but not Th1 and Th17 cells in GIA

To confirm GPI-specific overproduction of HC gp-39 in splenocytes, we cultured splenocytes from GPI immunized mice with GPI or control stimulation and the production of HC gp-39 was analyzed. The production of HC gp-39 in the supernatants was induced by GPI stimulation, but not by control stimulation (Fig. 2a). This findings was also confirmed by FACS staining of HC gp-39 expression in $CD4^+$ T cells (Fig. 2b). As the GIA model is clearly skewed to Th1 and Th17 cells in arthritic conditions [3-5], to analyze HC gp-39 overexpression in Th cell subsets such as Th1 and Th17 cells, we performed intracellular FACS staining. We did not detect clear overexpression in either the Th1 or the Th17 cells as compared with the control stimulation cells (Fig. 2c). To further reveal HC gp-39 positive T cell populations, we also screened $CD25^+$ cells, because it was reported that depletion of $CD25^+$ cells results in non-limiting arthritis in a model of GIA [18]. HC gp-39 positive cell population was detected in $CD4^+CD25^+$ T cells when compared with the control stimulation cells.

3-4. HC gp-39 was expressed in $CD4^+CD25^+Foxp3^+$ Treg cells and produced as a soluble protein

Next, to confirm the HC gp-39 protein association between serum and $CD4^+CD25^+$ T cells, we examined the soluble HC gp-39 proteins from GPI-reactive $CD4^+CD25^+$ T cells using ELISA system. We purified $CD4^+CD25^+$ T cells from GIA from lymph node on day 7, then co-cultured with antigen-presenting cells (APC) with GPI for 96 hours. The production of HC gp-39 from GPI-reactive $CD4^+CD25^+$ T cells was clearly overproduced. Moreover, to abolish the possibility that the HC gp-39 was produced by APC, we also cultured APC only, but the production of HC gp-39 from APC was not detected (Fig. 2e). We also checked the expression of HC gp-39 in Treg cells ($CD4^+CD25^+Foxp3^+$ T cells) and effector T cells ($CD4^+CD25^-$ T cells). As a result, HC gp-39 was highly detected on Treg cells compared with effector T cells (Fig. 2f). Taken together, these findings suggest that HC gp-39 is dominantly expressed in GPI-specific $CD4^+CD25^+$ $Foxp3^+$Treg cells, but not in Th1 and Th17 cells in GIA.

3-5. Antigen-specific T cell proliferation and cytokine production are suppressed by addition of recombinant HC gp-39

We also speculated that overproduction of HC gp-39 can enhance or suppress T cell proliferation. The addition of recombinant HC gp-39 did not suppress the proliferation of $CD4^+$ T cells induced by CD3 and CD28 stimulation, which was confirmed by CFSE labeling (Fig. 3a). Moreover, if we used antigen-specific culture conditions, T cell proliferation was suppressed by addition of HC gp-39 (Fig. 3b). Finally, antigen-specific overproduction of both IFNγ and IL-17 was clearly suppressed by HC gp-39 (Fig. 3c). These findings suggest that HC gp-39 itself may suppress antigen-specific T cells, especially Th1 and Th17 cells, in the early phase of arthritis.

3-6. Localization of HC gp-39 protein in cells infiltrated into the arthritic synovium

HC gp-39 is basically a cartilage-dominant protein. To check the association between joint specific fluctuation of HC gp-39 and arthritis, we checked the time

course of HC gp-39 expression in joints. HC gp-39 was clearly overexpressed on day 14 and was accompanied by joint swelling (Fig. 4a). Next, immunohistochemical analysis was conducted to determine the distribution of HC gp-39 in the arthritic joints. HC gp-39 was clearly detected in the cells infiltrating the arthritic synovium, which included neutrophils and monocytes (Fig. 4b). For detailed investigation of the expression of HC gp-39 in these cells, the arthritic joints were harvested and the acquired cells were analyzed by FACS. HC gp-39 was expressed mainly in the CD4 negative cells, suggesting different localization and upregulation of this molecule in splenocytes within joints (Fig. 4c).

Chapter. 4 Discussion

In this study, we clearly detected unexpected expression of HC gp-39 in antigen-specific T cells of a GIA model, particularly in $CD4^+CD25^+Foxp3^+$ Treg cells.

HC gp-39 is also known as YKL-40, BRP-39, and chitinase 3-like 1 [19]. It is a secretory protein that is upregulated in a wide variety of inflammatory disorders [7-11, 15, 19]. It is also expressed by a variety of cells, including macrophages, neutrophils, synoviocytes, and chondrocytes [19, 20]. Its functional roles such as augmention of Th2 immunity, regulation of apoptosis, and stimulation of alternative macrophage activation have recently been elucidated [16, 17].

In RA, increased levels of HC gp-39 have been demonstrated in plasma [8] and synovial fluid, and it has also been plotted as a multi- biomarker [21]. As a major constituent of human cartilage, HC gp-39 is considered a candidate autoantigen in RA [12-14]. Intranasal administration of HC gp-39 in collagen type II (CII)-induced arthritis after immunization (days 20, 25, 30) clearly suppressed arthritis and was associated with decreased levels of anti-CII antibody, whereas administration of CII over the

same time course was ineffective. In addition, HC gp-39 administration before immunization with CII was found to be ineffective [22]. Joosten et al. speculated that HC gp-39 might trigger a regulatory mechanism by cross- tolerance. However, we expected that one of the explanations for this suppressive mechanism after CII immunization is the blocking of antigen-specific T cell proliferation in the arthritic condition, which was confirmed by the findings depicted in Figure 3B.

In terms of the T cell subtype of HC gp-39, Lee et al. confirmed that BRP-39 (HC gp-39) plays a critical role in the pathogenesis of Th2-driven asthma [16, 20], however, we could not find any antigen Th2- type cells in our arthritis model. We also checked antigen-specific Th1 and Th17 cells, but those cells were mostly HC gp-39 negative (Fig. 4c).

Frey et al. confirmed that depletion of $CD25^+$ cells (before immunization) in GIA results in sustained severe arthritis [18]. In this study, we demonstrated that HC gp-39 is expressed on $CD4^+CD25^+Foxp3^+$ Treg cells. Moreover, it is possible that the HC gp-39 from $CD4^+CD25^+$ T cells and in serum is the same molecule (Fig.2e). Thus, HC gp-39 produced by Treg cells suppress the production of IFNγ and IL-17 via

295 inhibition of proliferation on antigen-specific Th1 and Th17, which may in turn control self-limiting GIA in vivo.

Among the peripheral blood mononuclear cells of RA patients, the cellular source of HC gp-39 was identified as $CD16^+$ monocytes [23]. In our experiments, the $CD11b^+$ cells were indeed the major source of HC gp-39 in splenocytes, although the
300 control immunization mice also expressed comparable amounts of HC gp-39 (Fig. 1c). We demonstrated that antigen-specific T cells themselves express HC gp-39 in the early phase of arthritis. Intranasal administration of HC gp-39 was also conducted in human RA, but in phase II, the treatment lacked efficacy when compared with the placebo [24]. We need to further characterize whether human antigen-specific T cells
305 express HC gp-39 in the induction phase of RA.

Chapter. 5 Acknowledgment

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Chapter. 7 Figure lengends

Figure 1.

Systemic upregulation of HC gp-39 is dominant in CD4$^+$ T cells in the early phase of GIA

(A) Serum HC gp-39 from DBA/1 mice immunized with GPI (■; n = 5) or control (GST-protein) (□; n = 5) was measured by ELISA. (B) The expression of HC gp-39 mRNA (upper panel) in splenocytes was analyzed by quantitative PCR after GPI immuniazation (■; n = 5) or control immunization (□; n = 5). The expression of HC gp-39 (lower panel) in splenocytes was detected by Western blot analysis. (C) The expression of HC gp-39 mRNA in CD4$^+$ T cells, CD11b$^+$ cells and CD19$^+$ cells was analyzed by quantitative PCR after GPI immunization(■; n = 5) or control immunaization (□; n = 5). **p <0.01, t- test.

Figure 2.

HC gp-39 in CD4$^+$ T cells was increased by GPI stimulation, particularly in the CD25$^+$ population

(A) Splenocytes from GPI immunized DBA/1 mice on day 7 were restimulated with GPI or control for 24 hours in vitro, and production of HC gp-39 in the culture supernatants was measured by ELISA. (B) Splenocytes obtained on day 7 were restimulated with GPI or control for 24 hours in vitro, and FACS of HC gp-39 in CD4$^+$ T cells, (C) IFNγ or IL-17 cells, and (D) CD25$^+$ cells was performed, (to gate the CD4$^+$ T cells). (E) CD4$^+$CD25$^+$ T cells were sorted from from lymph node on day7, and co-cultured APC with GPI for 96 hours. Then, the HC gp-39 from CD4$^+$CD25$^+$T cells in the culture supernatants was measured by ELISA. (F) In the same way as Fig 2C, the expression of HC gp-39 on Treg cells was analyzed by FACS. *$p < 0.05$, **$p < 0.01$, by *t*-test.

Figure 3.

Recombinant HC gp-39 suppressed the proliferation and cytokine production of GPI-specific CD4$^+$ T cells. (A) CD4$^+$ T cells from naïve DBA/1 mice were stained

with carboxyfluorescein diacetate succinimidyl ester (CFSE-DA) and stimulated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) antibodies in the absence or presence of recombinant HC gp-39 (rHC gp-39) for 72 hours in vitro. (B, C) Lymph nodes were collected from GPI- immunized DBA/1 mice on day 7, and cells were stained with CFSE-DA cultured with stimulation media, GPI, or control in the absence or presence of rHC gp-39 or OVA for 72 hours in vitro. Then, the proliferation of GPI-specific $CD4^+$T cells was analyzed by FACS, and the production of IFNγ and IL-17 in the supernatants, analyzed by ELISA. $^*p < 0.05$, *t*-test.

Figure 4.

Fluctuation and localization of HC gp-39 in arthritic joints

(A) Expression of HC gp-39 mRNA in joints were analyzed by quantitative PCR after GPI immunization (■; $n = 5$) or control immunization (□; $n = 5$). (B) Localization of HC gp-39 in the joints on day 14 was detected by immunofluorescence. (C) Cells isolated from the joints of GIA mice on day 14 were analyzed by FACS. $^*p < 0.05$, *t*-test.

Table

Table 1.

The identification of the strong expression molecule in $CD4^+$T cells with GeneChip analysis

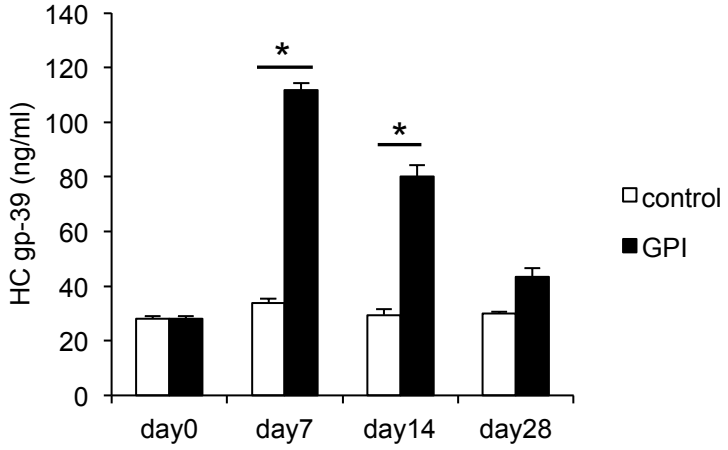
Symbol	NCBI accession no.	Description	Fold change
Stab2	NM_138673.1	stabilin 2	4
Scg5	AK019337.1	secretogranin V	4
Fpr2	NM_008039.1	formyl peptide receptor 2	3.8
Ighg1	AF466769.1	immunoglobulin heavy constant gamma 1 (G1m marker)	3.6
Stfa3	NM_025288.1	stefin A3	3.3
Hp	NM_017370.1	haptoglobin	3.2
Spon1	BC020531.1	spondin 1a	3.2
Cap1	BC005446.1	CAP, adenylate cyclase-associated protein 1	3.1
Chi3l1	BC005611.1	chitinase 3-like 1 (cartilageglycoprotein-39)	3
Ccr1	BC011092.1	chemokine (C-C motif) receptor 1	2.9
Pla2g7	AK005158.1	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	2.9
Serpinb1a	AF426024.1	serine (or cysteine) peptidase inhibitor, clade B, member 1a	2.7
Cd177	BC027283.1	CD177 antigen	2.7
Slfn4	AF099975.1	schlafen 4	2.7
Anxa1	NM_010730.1	annexin A1	2.7
Mlycd	NM_019966.1	malonyl-CoA decarboxylase	2.7
Hebp1	AF117613.1	heme binding protein 1	2.6
Olfm1	D78264.1	olfactomedin 1	2.6
Supt16	AW536705	suppressor of Ty 16	2.6
Ifitm1	BC027285.1	interferon induced transmembrane protein 1	2.5

465

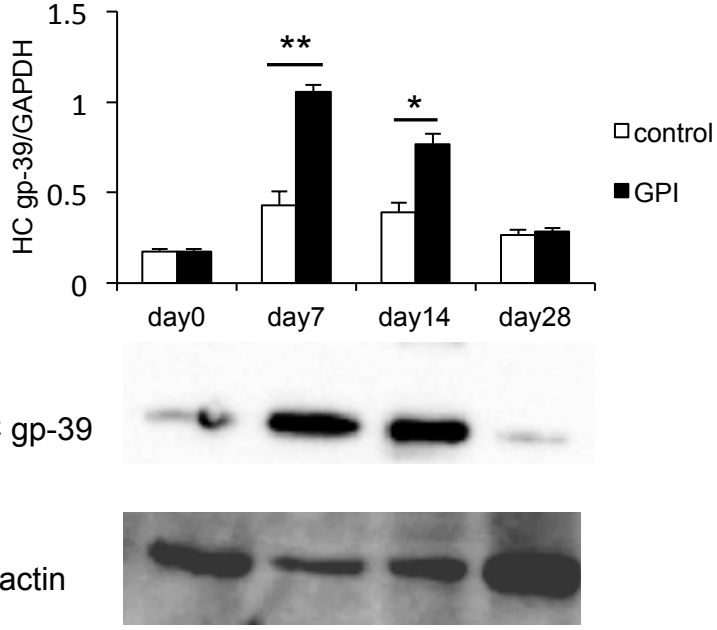
The mRNA expression profile of splenic $CD4^+$ T cells was examined by microarray in DBA/1 mice (arthritis susceptible) and B6 mice (arthritis resistant) after immunization of GPI and then, the highly expression molecules which is GIA specific was selected (20 molecules).

Figure.1

a


HC gp-39 (ng/ml)
140
120
100
80
60
40
20
0
*
*
day0
day7
day14
day28
control
GPI

b


HC gp-39/GAPDH
1.5
1
0.5
0
**
*
day0
day7
day14
day28
control
GPI
HC gp-39
actin

c

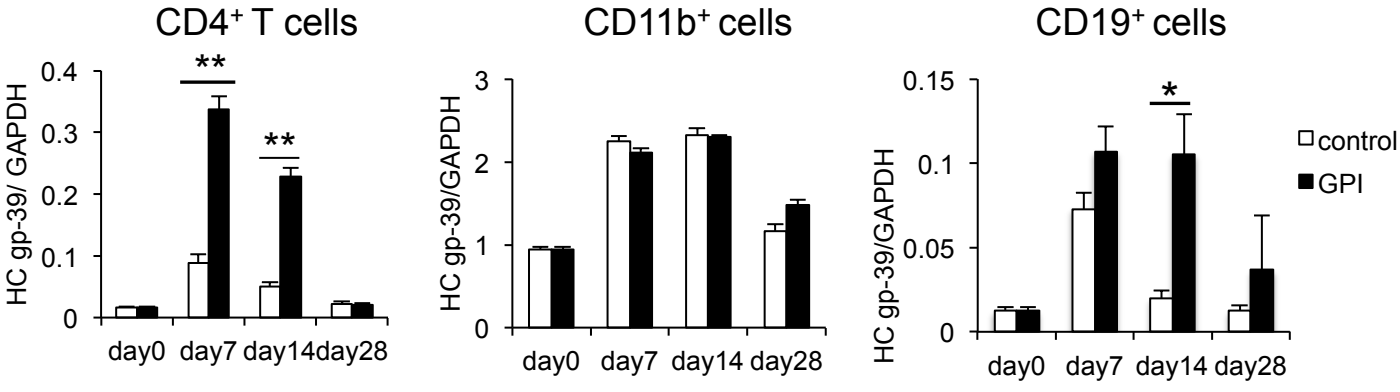
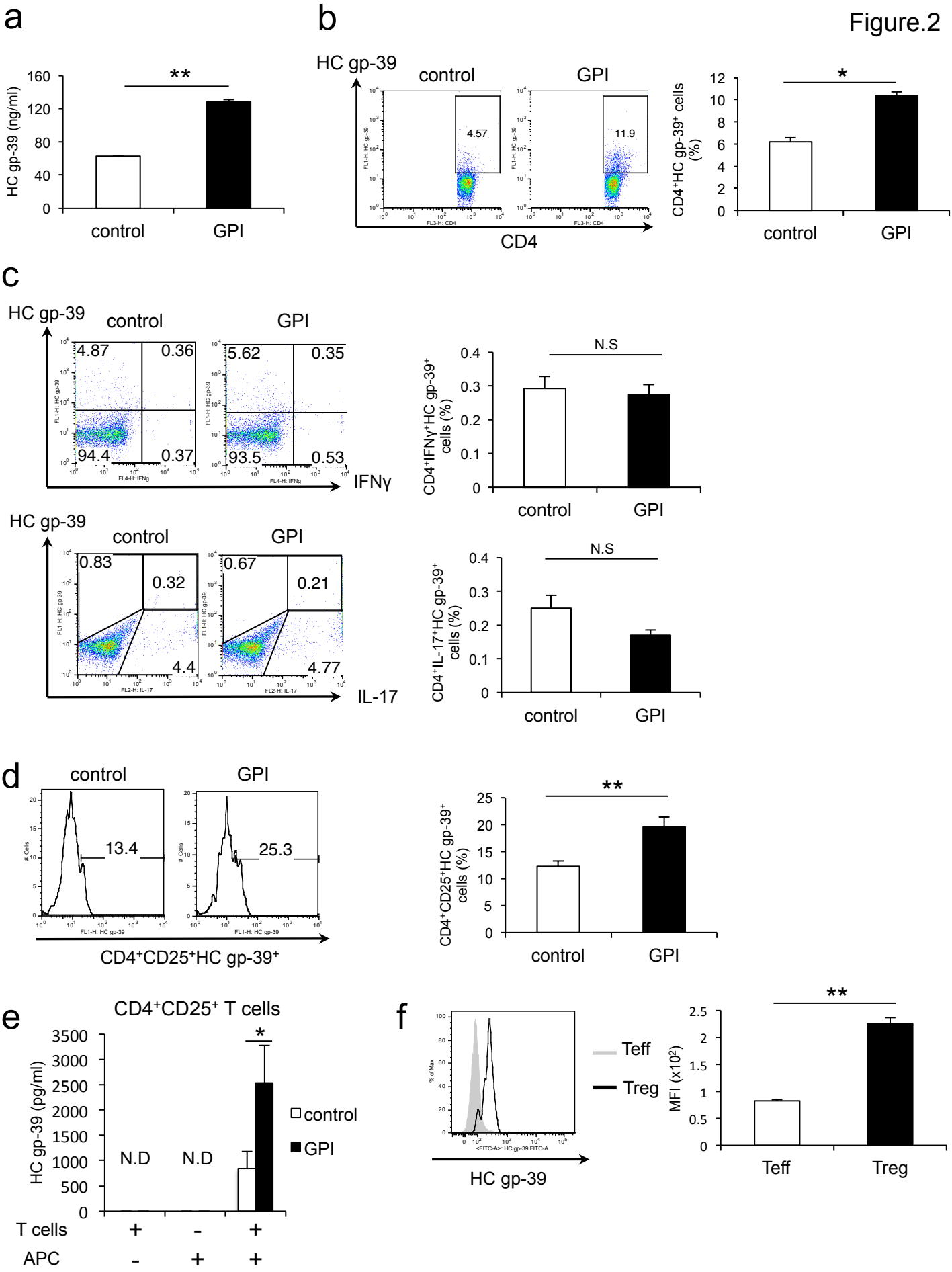
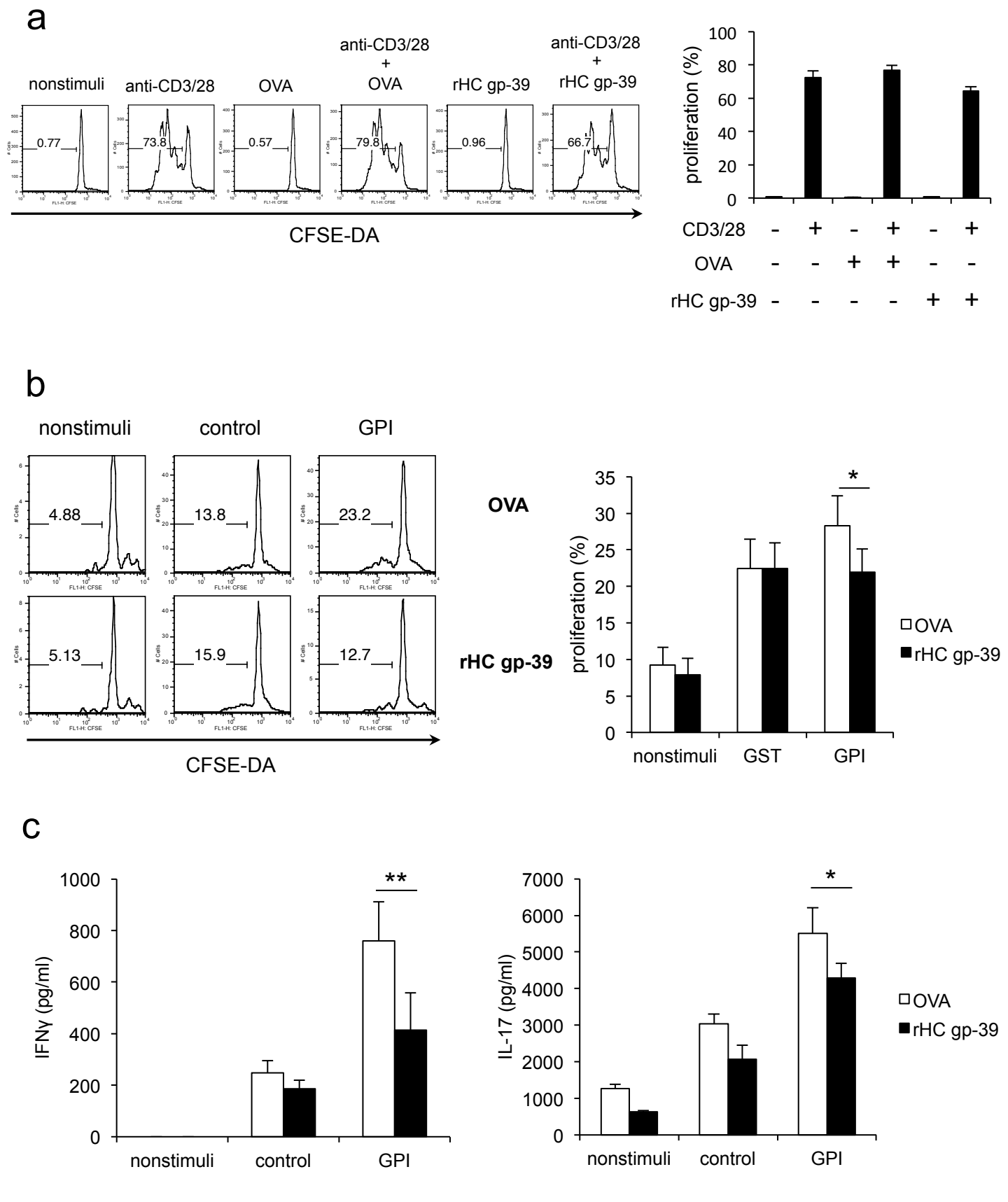
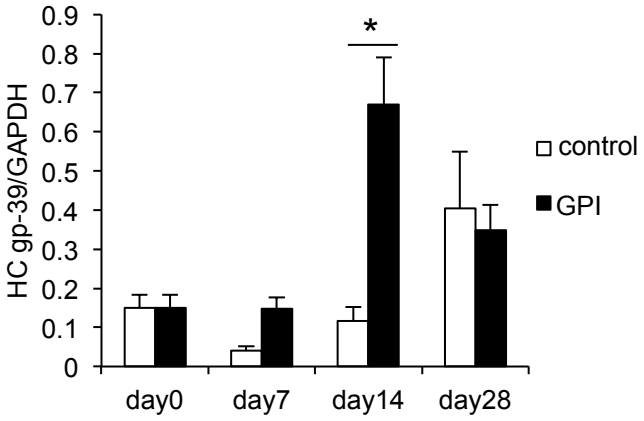

CD4+ T cells
HC gp-39/ GAPDH
0.4
0.3
0.2
0.1
0
**
**
day0
day7
day14
day28
CD11b+ cells
HC gp-39/GAPDH
3
2
1
0
day0
day7
day14
day28
CD19+ cells
HC gp-39/GAPDH
0.15
0.1
0.05
0
*
day0
day7
day14
day28
control
GPI

Figure.2

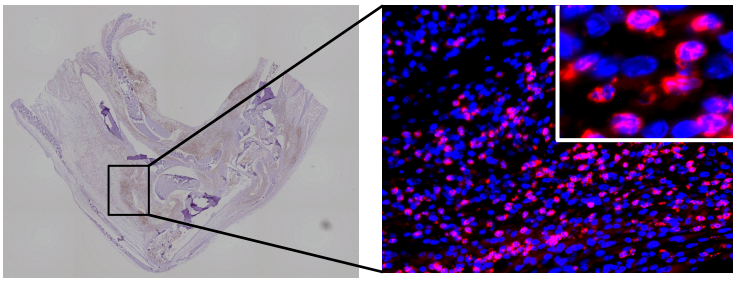

a
HC gp-39 (ng/ml)
160
120
80
40
0
**
control
GPI
b
HC gp-39
control
GPI
4.57
11.9
FL1-H: HC gp-39
FL3-H: CD4
CD4
CD4+HC gp-39+ cells (%)
12
10
8
6
4
2
0
*
control
GPI
c
HC gp-39
control
GPI
4.87
0.36
94.4
0.37
5.62
0.35
93.5
0.53
FL4-H: IFNg
IFNγ
CD4+IFNγ+HC gp-39+ cells (%)
0.4
0.3
0.2
0.1
0
N.S
control
GPI
HC gp-39
control
GPI
0.83
0.32
4.4
0.67
0.21
4.77
FL2-H: IL-17
IL-17
CD4+IL-17+HC gp-39+ cells (%)
0.4
0.3
0.2
0.1
0
N.S
control
GPI
d
control
GPI
13.4
25.3
Cells
FL1-H: HC gp-39
CD4+CD25+HC gp-39+
CD4+CD25+HC gp-39+ cells (%)
25
20
15
10
5
0
**
control
GPI
e
CD4+CD25+ T cells
HC gp-39 (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
*
N.D
N.D
control
GPI
T cells + - +
APC - + +
f
% of Max
<FITC-A>: HC gp-39 FITC-A
HC gp-39
Teff
Treg
MFI (x10²)
2.5
2
1.5
1
0.5
0
**
Teff
Treg


a
nonstimuli
anti-CD3/28
OVA
anti-CD3/28 + OVA
rHC gp-39
anti-CD3/28 + rHC gp-39
0.77
73.8
0.57
79.8
0.96
66.7
CFSE-DA
proliferation (%)
100
80
60
40
20
0
CD3/28 - + - + - +
OVA - - + + - -
rHC gp-39 - - - - + +
b
nonstimuli
control
GPI
4.88
13.8
23.2
5.13
15.9
12.7
OVA
rHC gp-39
CFSE-DA
proliferation (%)
35
30
25
20
15
10
5
0
*
nonstimuli
GST
GPI
OVA
rHC gp-39
c
IFNγ (pg/ml)
1000
800
600
400
200
0
**
nonstimuli
control
GPI
IL-17 (pg/ml)
7000
6000
5000
4000
3000
2000
1000
0
*
nonstimuli
control
GPI
OVA
rHC gp-39

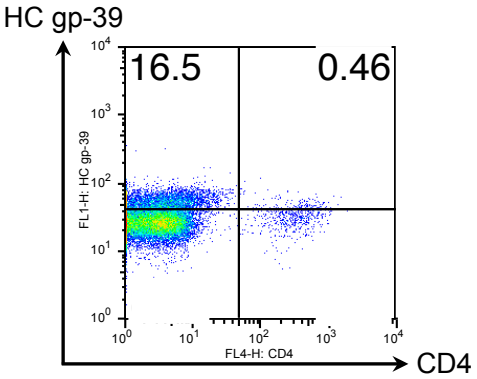
a


HC gp-39/GAPDH
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
day0
day7
day14
day28
control
GPI

b



c


HC gp-39
16.5
0.46
FL1-H: HC gp-39
FL4-H: CD4
CD4