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Unexpected T cell regulatory activity of anti-histone H1 autoantibody: Its mode of action in regulatory T cell-dependent and -independent manners

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ABSTRACT

Induction of anti-nuclear antibodies against DNA or histones is a hallmark of autoimmune disorders, but their actual contribution to disease predisposition remains to be clarified. We have previously reported that autoantibodies against histone H1 work as a critical graft survival factor in a rat model of tolerogeneic liver transplantation. Here we show that an immunosuppressive anti-histone H1 monoclonal antibody (anti-H1 mAb) acts directly on T cells to inhibit their activation in response to T cell receptor (TCR) ligation. Intriguingly, the T cell activation inhibitory activity of anti-H1 mAb under suboptimal dosages required regulatory T (Treg) cells, while high dose stimulation with anti-H1 mAb triggered a Treg cell-independent, direct negative regulation of T cell activation upon TCR cross-linking. In the Treg cell-dependent mode of immunosuppressive action, anti-H1 mAb did not induce the expansion of $CD4^{+}$ $Foxp3^{+}$ Treg cells, but rather potentiated their regulatory capacity. These results reveal a previously unappreciated T cell regulatory role of anti-H1 autoantibody, whose overproduction is generally thought to be pathogenic in the autoimmune settings.

1. Introduction

Development of antinuclear autoantibodies is a cardinal feature of autoimmune diseases such as systemic lupus erythematosus (SLE) and Sjögren's syndrome, and those who are suffering from lupus disorders show high antibody titers against nuclear components including DNA, histones, and non-histone ribonucleoproteins (*e.g.* Sm, SS-A/Ro, and SS-B/La antigens) [1–3]. Although it has been widely accepted that antinuclear autoantibody titer is highly correlated with disease severities [4], their actual involvement in the lupus predisposition is still controversial. Several lines of evidence has suggested a causative role of anti-DNA autoantibodies in the development of glomerulonephritis [5–7], while others have reported that multiple antinuclear autoantibodies against DNA and/or histones are involved in the immune complex formation at the glomerular basement membranes [8]. Liang et al. [9] have shown that anti-DNA but not other anti-nucleosome autoantibodies from lupus mice-prone mice are pathogenic *in vivo*. In the clinical settings, Li et al. [10] have shown by using glomerular proteome arrays that anti-DNA autoantibody response is associated with lupus disease activity while anti-histone autoreactivity does not.

We have previously identified autoreactive antibodies against linker histone H1 as a critical immunosuppressive factor induced in a rat model of tolerogeneic liver transplantation [11], in which orthotopically transplanted DA rat liver is accepted in PVG rat without any immunosuppressive treatments [12–14]. In this model, anti-histone H1 (anti-H1) antibody response is transiently induced 2–3 weeks after liver transplantation, and potently suppresses alloimmune response as well as prolongs allograft survival [11]. We also have shown that pre-vaccination with histone H1 or its peptide mimotope recognized by an anti-H1 antibody induces T cell unresponsiveness and enhancement of graft survival [15,16]. These results suggest anti-H1 autoantibodies and/or H1-specific immune response negatively regulates alloimmune response to establish tolerogeneic status, but detailed molecular and cellular basis of this regulatory action remains to be investigated.

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To elucidate immunosuppressive mechanism of anti-H1 autoantibodies, we have generated an anti-H1 monoclonal antibody (mAb) that potently inhibits allogeneic mixed lymphocyte reaction [17]. We show here that the anti-H1 mAb acts directly on T cells to negatively regulate their activation through Treg cell-dependent and -independent mechanisms. Our data provide a hitherto-unknown mode of T cell regulatory action fulfilled by anti-histone autoantibodies, which are generally thought to be associated with autoimmune manifestations.

2. Materials and methods

2.1. Reagents and mice

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO), Katayama Chemical (Osaka, Japan) or Nakalai Tesque (Kyoto, Japan) unless specifically indicated. C57BL/6 (B6) and BALB/c mice (female, 5 week old) were obtained from Charles River Laboratories (Yokohama, Japan). These mice were maintained under specific pathogen-free conditions, and fed CRF-1 diet (Oriental Yeast, Tokyo, Japan) and sterile distilled water *ad libitum*. All animal experiments were carried out using protocols reviewed and approved by the Committee on Animal Experimentation of Hiroshima University.

2.2. Hybridoma cell culture

A hybridoma cell line 16G9 secreting anti-H1 mAb (16G9 mAb) was generated as described [17]. The 16G9 hybridoma was cultured in Iscove's Modified Dulbecco's Medium (Invitrogen,Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Biowest, Nuaille, France) and Penicillin–Streptomycin (Invitrogen) at 37 °C in 5% CO_2/95% air. For serum-free culture, 16G9 cells (1×10^5 cells/ml) were inoculated in CD Hybridoma Medium (Invitrogen) added with GlutaMax-I (Invitrogen) and Penicillin–Streptomycin, and were cultured for seven days.

2.3. Purification of anti-H1 mAb

Serum-free culture supernatant of 16G9 hybridoma was applied to ammonium sulfate precipitation (50% saturation), and precipitate was dissolved in 20 mM sodium phosphate buffer (NaPB, pH 7.4) followed by dialysis at 4 °C. The 16G9 mAb was purified using H1-immobilized HiTrap NHS-activated HP column (1 ml column volume, GE Healthcare Life Sciences, Uppsala, Sweden), on which 1 mg of calf thymus histone H1 (Roche Diagnostics, Mannhein, Germany) was coupled following the manufacturer's protocols. After application of the sample onto the column, 16G9 mAb was eluted with 0.1 M glycine–HCl (pH 2.7) followed by neutralization with 1 M Tris–HCl (pH 9.0). The purified mAb was desalting with PD-10 Column (GE Healthcare Life Sciences), was lyophilized and stored at −80 °C until use.

2.4. T cell activation and mixed lymphocyte reaction (MLR)

Splenic B6 T cells were purified using Mouse Pan T cell Isolation Kit II (Miltenyi Biotec, Bergisch Glakbach, Germany), and cultured in RPMI-1640 medium (Sigma–Aldrich) supplemented with 10% FBS, 50 mM 2-mercaptoethanol, and Penicillin–Streptomycin. For T cell activation, B6 T cells (1×10^5 cells) were stimulated with immobilized anti-mouse CD3ε (1 μg/ml, eBioscience, San Diego, CA) with or without anti-mouse CD28 (0.5 μg/ml, eBioscience) for 48 h. For pharmacological activation, T cells were stimulated with phorbol 12-myristate 13-acetate (PMA, 40 ng/ml) and ionomycin (1 μM) for 48 h. T cell proliferation was analyzed using BrdU Labeling and Detection Kit III (Roche Diagnostics) with a Wallac 1420 ARVOsx Multilabel Counter (Perkin–Elmer, Downers Grove, IL). For murine allogeneic MLR, splenic $CD11c^+$ dendritic cells (DC, from BALB/c mice) were prepared using Mouse CD11c MicroBeads (Miltenyi Biotec), and treated with 25 μg/ml of mitomycin C (MMC in 10% FBS/RPMI-1640) at 37 °C for 30 min. Then the stimulator DC (5×10^3 cells) were mixed with responder B6 T cells (5×10^4 cells), and cultured for 72 h. Allogeneic T cell response was detected by above BrdU incorporation-based analytical procedures.

2.5. IL-2 analysis

B6 total T cells (1×10^5 cells) were stimulated with anti-CD3 upon co-stimulation with 16G9 mAb or isotype IgM (10 μg/ml each) for 24 or 48 h, and secretion of IL-2 was measured by sandwich enzyme-linked immunosorbent assay using reagents and instructions from BD Biosciences. For IL-2 supplementation assay, recombinant IL-2 (BD Biosciences) was added to above T cell cultures.

2.6. Cell death analysis

T cell viability was determined by trypan blue dye exclusion assay. Apoptotic T cells were stained with fluorescein isothiocyanate (FITC)-conjugated annexin V (Invitrogen), and analyzed on a FACSCalibur flow cytometer (BD Biosciences).

2.7. Depletion of Treg cells and in vitro suppression assay of Treg cells

To deplete $CD25^+$ Treg cells, B6 T cells (1×10^7 cells) were serially labeled with anti-mouse CD25-phycoerythrin (PE) MicroBeads and anti-PE MicroBeads (Miltenyi Biotec), and were applied onto a LD column (Miltenyi Biotec) to deplete $CD25^+$ Treg cells. To analyze the function of Treg cells upon co-stimulation with anti-H1 mAb, B6 T cells (1×10^5 cells) were stimulated with or without immobilized anti-CD3 upon co-stimulation with 16G9 mAb or isotype IgM (2 μg/ml each) for 24 h. Then Treg cells were purified from the stimulated T cells using Mouse $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (Miltenyi Biotec). Resulting Treg cells ($0.8–2.5 \times 10^4$ cells, final T:Treg cell ratio of 1:0.3–1:1) were mixed with B6 T cells (2.5×10^4 cells), and cultured upon stimulation with anti-CD3 for 72 h (summarized in Fig. 3C). To test the direct effect of 16G9 mAb on Treg cells, freshly isolated $CD4^+CD25^+$ Treg cells were used instead of total T cells. Suppressor activities of those Treg cell samples were analyzed using BrdU Labeling and Detection Kit III (Roche Diagnostics) with a Wallac 1420 ARVOsx Multilabel Counter (Perkin-Elmer).

2.8. Fluorescence-activated cell sorting (FACS) analysis

To test the effect of anti-H1 mAb on the expansion of $CD4^+$-$Foxp3^+$ Treg cells, B6 T cells were activated with anti-CD3 upon co-stimulation with 16G9 or isotype IgM mAbs (2 μg/ml each) for 24 or 48 h. The stimulated T cells were collected and dissolved in 0.5% FBS/0.02% NaN_3/PBS, and incubated with anti-mouse CD16/32 (clone #93, eBioscience). Then the T cells were stained with FITC-conjugated anti-mouse CD4 (BD Biosciences), followed by labeling with PE-conjugated anti-Foxp3 using Anti-Mouse/Rat Foxp3 Staining Set (eBioscience). $CD4^+Foxp3^+$ Treg cell numbers were analyzed by a FACSCalibur flow cytometer (BD Biosciences). To analyze the reactivity of anti-H1 mAb on the T cell surface, 16G9 mAb and isotype control IgM were biotinylated using EZ-Link Sulfo-NHS-SS-Biotin following manufacturer's instruction. Total B6 T cells (pre-incubated with anti-CD16/32) were stained with these biotinylated mAbs, followed by labeling with PE-conjugated

streptavidin (BD Biosciences). For competition assay, biotinylated 16G9 mAb was pre-incubated with 10 μg/ml of histone H1 or ovalbumin (OVA, Sigma–Aldrich) at 4 °C overnight. The T cell surface reactivity of 16G9 mAb was analyzed on the FACSCalibur apparatus.

2.9. Statistical analysis

Statistical analysis was performed by unpaired Student's *t*-test, and *P* value less than 0.05 was evaluated as the difference being statistically significant.

3. Results and discussion

3.1. An immunosuppressive anti-H1 mAb negatively regulates T cell activation in Treg cell-dependent and -independent manners

We have previously identified anti-H1 autoantibodies as a critical graft survival factor induced in a rat model of tolerogeneic liver transplantation [11]. To elucidate mechanisms underlying their immunosuppressive action, we have generated an anti-H1 mAb (16G9 mAb) that potently inhibits allogeneic MLR of rat splenocytes [17]. To start testing its regulatory effect on T cells, we first confirmed that 16G9 mAb actually suppressed MLR in which purified C57BL/6 (B6) T cells were stimulated with mitomycin C (MMC)-treated $CD11c^+$ DC from BALB/c mice (data not shown). Furthermore, we found that 16G9 mAb also inhibited proliferation of purified B6 T cells upon stimulation with immobilized anti-CD3 mAb in a dose-dependent manner (Fig. 1A). The T cell proliferation inhibitory effect of 16G9 mAb was also evident against B6 T cells stimulated with serial concentrations of anti-CD3 mAb (Fig. 1B). These results indicate that this immunosuppressive anti-H1 mAb can act directly on purified T cells to inhibit their activation in response to TCR ligation.

We next tested whether Treg cells were essential for this T cell activation inhibitory effect of 16G9 mAb. To this end, we magnetically depleted a Treg cell population from B6 T cells by using anti-CD25 mAb-immobilized microbeads, and tested immunosuppressive effect of 16G9 mAb against those Treg cell-depleted T cells upon TCR cross-linking by anti-CD3 mAb. FACS analysis confirmed that 97% of $CD4^+CD25^+$ or 93% of $CD4^+Foxp3^+$ Treg cell population was successfully eliminated from our B6 T cell preparations (data not shown). Intriguingly, we found that the T cell activation inhibitory effects of 16G9 mAb under suboptimal doses (2.5 μg/ml and 5 μg/ml) were abolished upon depletion of Treg cells (shown in Fig. 2A). By contrast, immunosuppressive activity of 16G9 mAb with a higher mAb dose (10 μg/ml) was almost intact even in the absence of Treg cells; the T cell proliferation was completely suppressed as comparable to those of unstimulated baseline levels (shown by a black bar in Fig. 2A). We next tested whether Treg cell population was also requited for the immunosuppressive action of suboptimal dose of 16G9 mAb against murine allogeneic MLR in which Treg cell-depleted B6 T cells were stimulated with MMC-treated BALB/c DC. Also in this system, MLR inhibitory activity of 16G9 mAb with suboptimal doses (1.25–5 μg/ml), but not with a higher dosage (10 μg/ml), was abrogated when Treg cells were depleted from responder B6 T cells (Fig. 2B). We observed that co-stimulation with 2 5 μg/ml and 5 μg/ml of 16G9 mAb still significantly inhibited MLR as compared with those stimulated with isotype control IgM, but impaired T cell regulatory capacity upon Treg cell depletion was evident when compared with those seen in the Treg cell-sufficient total T cells (Fig. 2B). We assume that these suboptimal mAb dosages could induce an intermediate immunosuppressive effect in which both Treg cell-dependent and -independent modes of MLR inhibitory activities were co-elicited.

These facts collectively suggest that our immunosuppressive anti-H1 mAb negatively regulates T cell activation through Treg cell-dependent and -independent mechanisms depending on its antibody concentrations.

3.2. Suboptimal dose of immunosuppressive anti-H1 mAb potentiates T cell regulatory capacity of Treg cells

We next assessed how above Treg-cell dependent mode of immunosuppressive action was fulfilled by anti-H1 16G9 mAb.

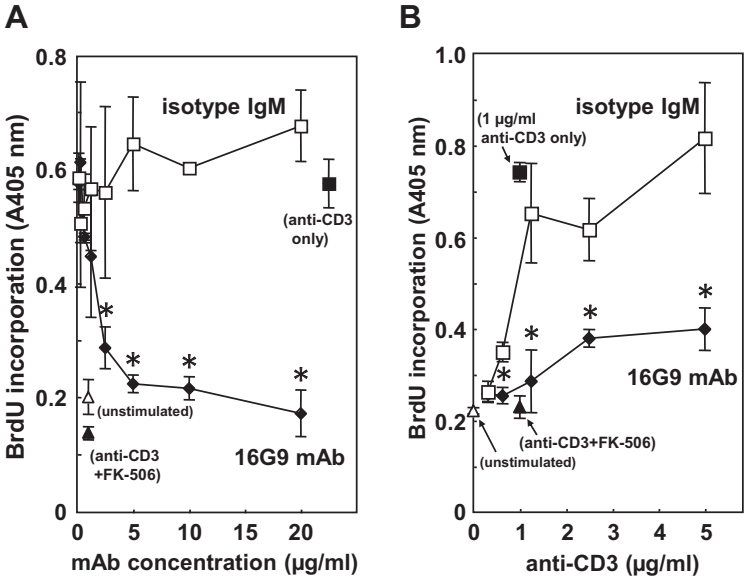

Fig. 1. An immunosuppressive anti-H1 mAb (16G9 mAb) directly acts on T cells to suppress their activation in response to TCR ligation. (A) 16G9 mAb suppresses anti-CD3-mediated T cell activation in a dose-dependent manner. Purified B6 total T cells were stimulated with immobilized anti-CD3 (1 μg/ml) upon supplementation with 16G9 mAb (closed diamonds) or isotype control IgM mAb (open squares). T cell proliferations of unstimulated cultures (open triangles) and those stimulated only with anti-CD3 (closed squares) are indicated as positive and negative controls, respectively. Effect of FK-506 (10 ng/ml) on this T cell activation system is also shown as a positive control of T cell immunosuppression. $^*P < 0.05$ vs. isotype control IgM. (B) 16G9 mAb shows potent T cell activation inhibitory activity under a serial concentration of anti-CD3.$^*P < 0.05$ vs. isotype IgM.

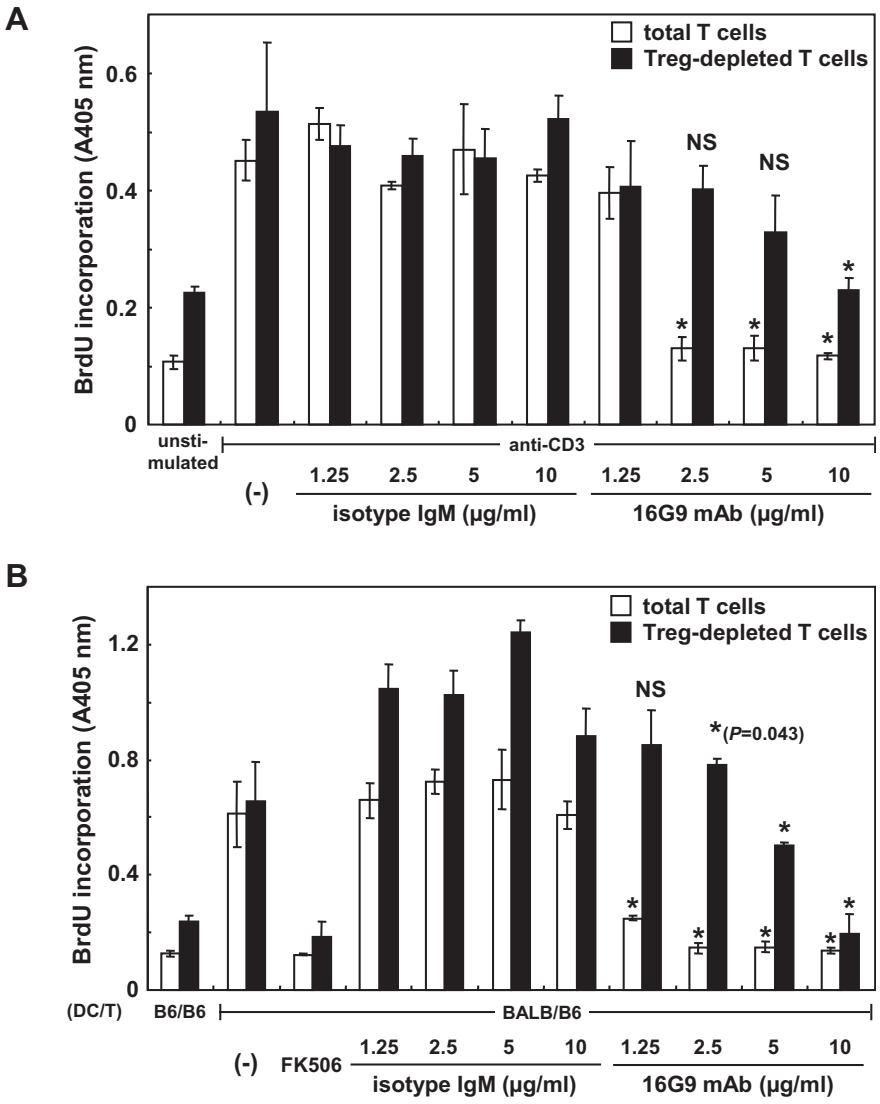

Fig. 2. An immunosuppressive 16G9 mAb negatively regulates anti-CD3-mediated T cell activation in Treg cell-dependent and -independent manners. (A) T cell activation inhibitory activity of 16G9 mAb under suboptimal dosages (2.5 μg/ml and 5 μg/ml) but not with a high dose (10 μg/ml) is abrogated upon depletion of $CD25^+$ Treg cells. $^*P < 0.05$ vs. isotype IgM; NS, not significant vs. isotype IgM. (B) Depletion of $CD25^+$ Treg cell population impairs MLR inhibitory action fulfilled by suboptimal doses of 16G9 mAb (1.25–5 μg/ml) but not by a high dose (10 μg/ml). B6 total T cells or Treg cell-depleted T cells were stimulated with MMC-treated allogeneic $CD11c^+$ DC from BALB/c mice. $^*P < 0.05$ vs. isotype IgM; NS, not significant vs. isotype IgM. Note that abrogation of MLR inhibitory action upon Treg cell depletion is unequivocally observed upon supplementation with 2.5 μg/ml and 5 μg/ml of 16G9 mAb, although their responder T cell responses are still significantly higher than those treated with isotype control IgM.

First, we tested whether co-stimulation with suboptimal dose of 16G9 mAb triggered expansion of Treg cell population. FACS analysis indicated that 24 to 48 h co-stimulation with a suboptimal dose (2 μg/ml) of 16G9 mAb did not affect the cell number of $CD4^+$-$Foxp3^+$ Treg cells (Fig. 3A and B), suggesting that the Treg cell-dependent T cell regulatory action of 16G9 mAb was not attributable to the expansion of Treg cells. We next tested the possibility that suboptimal dose of 16G9 mAb induced functional up-modulation of Treg cells. To address this issue, we co-stimulated total B6 T cells with 2 μg/ml of 16G9 mAb in together with TCR cross-linking for 24 h, and then purified $CD4^+CD25^+$ Treg cells *via* magnetic cell sorting. Thereafter, the T cell regulatory activity of those 16G9 mAb-co-stimulated Treg cells was tested against freshly isolated B6 T cells upon stimulation with immobilized anti-CD3 (procedures summarized in Fig. 3C). We found that immunosuppressive activity of 16G9 mAb-co-stimulated Treg cells was significantly enhanced as compared with those stimulated with isotype-matched control IgM mAb (left panel of Fig. 3D). This functional up-modulation of Treg cells was not seen in the absence of anti-CD3 stimulation (right panel of Fig. 3D), suggesting that TCR signal was also critical for the co-stimulatory effect of 16G9 mAb to positively regulate Treg cells. We also found that 16G9 mAb failed to enhance immunosuppressive activity of Treg cells when we performed this co-stimulation experiment using purified $CD4^+CD25^+$ Treg cells instead of total T cells (Fig. 3E). This suggests that non-Treg conventional T cell population is needed for the 16G9 mAb-driven enhancement of immunosuppressive potency of Treg cells. Taken together, these results indicate that the Treg cell-dependent T cell regulatory capacity of 16G9 mAb accompanies functional up-modulation of Treg cells.

3.3. High dose of anti-H1 mAb negatively regulates TCR signaling to fulfill T cell regulatory capacity

We next analyzed the mode of action by which a high dose (10 μg/ml) of 16G9 mAb negatively regulates T cell activation in a Treg cell-independent fashion. In addition to its immunosuppressive effect on anti-CD3-activated T cells (shown in Fig. 1A and B),

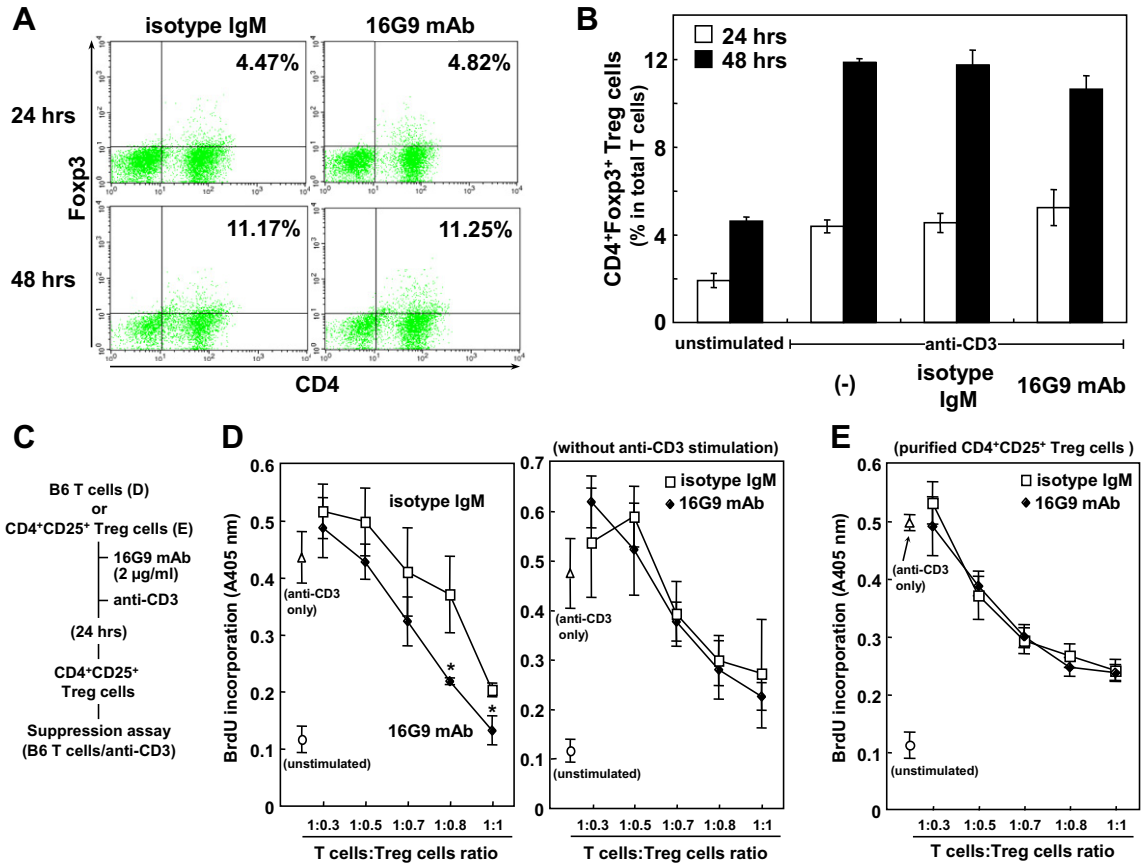

Fig. 3. Suboptimal dose of 16G9 mAb does not induce the expansion of Treg cells, but rather potentiates their regulatory capacity. (A and B) Co-stimulation with sub-optimal dose of 16G9 mAb does not affect the Treg cell numbers. Purified B6 T cells were stimulated with 2 μg/ml of 16G9 mAb or isotype control IgM upon TCR cross-linking for 24 or 48 h. Then the cell numbers of $CD4^+Foxp3^+$ Treg cell population were analyzed by FACS. (C) Schematic procedures to assess the effect of suboptimal dose of 16G9 mAb on the immunosuppressive function of Treg cells. B6 total T cells (for D) or purified $CD4^+CD25^+$ Treg cells (for E) were co-stimulated with 2 μg/ml of 16G9 mAb upon TCR ligation for 24 h, and immunosuppressive activity of resultant Treg cells was analyzed for their inhibitory capacity against anti-CD3-mediated T cell activation of freshly isolated B6 T cells. (D) Left; Co-stimulation of T cells with suboptimal dose of 16G9 mAb up-modulates T cell regulatory capacity of $CD4^+CD25^+$ Treg cells. $^*P < 0.05$ vs. isotype IgM. Right; TCR signal is essential for the functional up-modulation of Treg cells fulfilled by 16G9 mAb. (E) Co-stimulation with suboptimal dose of 16G9 mAb fails to positively regulate immunosuppressive action of purified $CD4^+CD25^+$ Treg cells.

the stimulation with 16G9 mAb suppressed T cell proliferation mediated by immobilized anti-CD3 and anti-CD28 mAbs (Fig. 4A). The high dose of 16G9 mAb also inhibited pharmacological T cell activation upon stimulation with PMA/ionomycin that bypassed TCR ligation (Fig. 4A), implicating that this mAb did not suppress TCR-membrane proximal signaling events, but rather negatively regulated downstream TCR signaling pathway to fulfill T cell regulatory activity. Indeed, we also found that the co-stimulation with high dose 16G9 mAb significantly inhibited IL-2 secretion from T cells (Fig. 4B), and that adding back of exogenous IL-2 cancelled its immunosuppressive activity (Fig. 4C), further supporting that the co-stimulation with high dose of 16G9 mAb negatively regulates TCR signaling to inhibit T cell activation. This T cell activation inhibitory effect of 16G9 mAb was not caused by the induction of T cell apoptosis or cell death, as demonstrated by FACS analysis of annexin V staining (Fig. 4D) and trypan blue dye exclusion assay (data not shown).

Finally, we confirmed by FACS analysis that 16G9 mAb actually reacted with B6 T cells (upper panel of Fig. 4E). Exogenous addition of histone H1, but not irrelevant antigen ovalbumin (OVA), specifically abrogated the cell surface binding of 16G9 mAb (lower panels of Fig. 4E), suggesting the existence of histone H1-like cross-reactive antigens on the T cell surface. Since we found that non-Treg conventional T cell population was also required for the 16G9 mAb-driven functional up-modulation of Treg cells (Fig. 3E), conventional T cells rather than Treg cells *per se* could be a primary target of the anti-H1 mAb to execute its T cell regulatory activity in both Treg cell-dependent and -independent manners. Identification of responsible cell surface antigens from conventional T cells is now underway to delineate molecular mechanisms underlying the T cell regulatory action of anti-H1 mAb. Another unsolved issue is how suboptimal dose of anti-H1 mAb up-modulates Treg cell function through the help of conventional T cells and TCR signaling (Fig. 3D and E). Our preliminary observation indicates that co-stimulation with sub-optimal doses of 16G9 mAb (2.5–5 μg/ml), but not with a high mAb dosage (10 μg/ml), induces IL-10 secretion from total T cells upon TCR ligation (unpublished data), although actual involvement of the induced IL-10 in the functional enhancement of Treg cells has yet to be clarified.

In summary, here we show that an immunosuppressive anti-histone H1 autoantibody negatively regulates T cell activation through Treg cell-dependent and -independent mechanisms. Our results provide a hitherto-unknown mode of T cell regulatory action of anti-histone autoantibodies, which are generally recognized as proinflammatory markers and/or mediators of autoimmune disorders. Interestingly, evidences similar to our results have recently been accumulated that autoantibodies and/or natural antibodies show immunosuppressive and disease-protective capacities in several autoimmune-prone mice such as NZB/W F1 mice [18], MRL/*lpr*

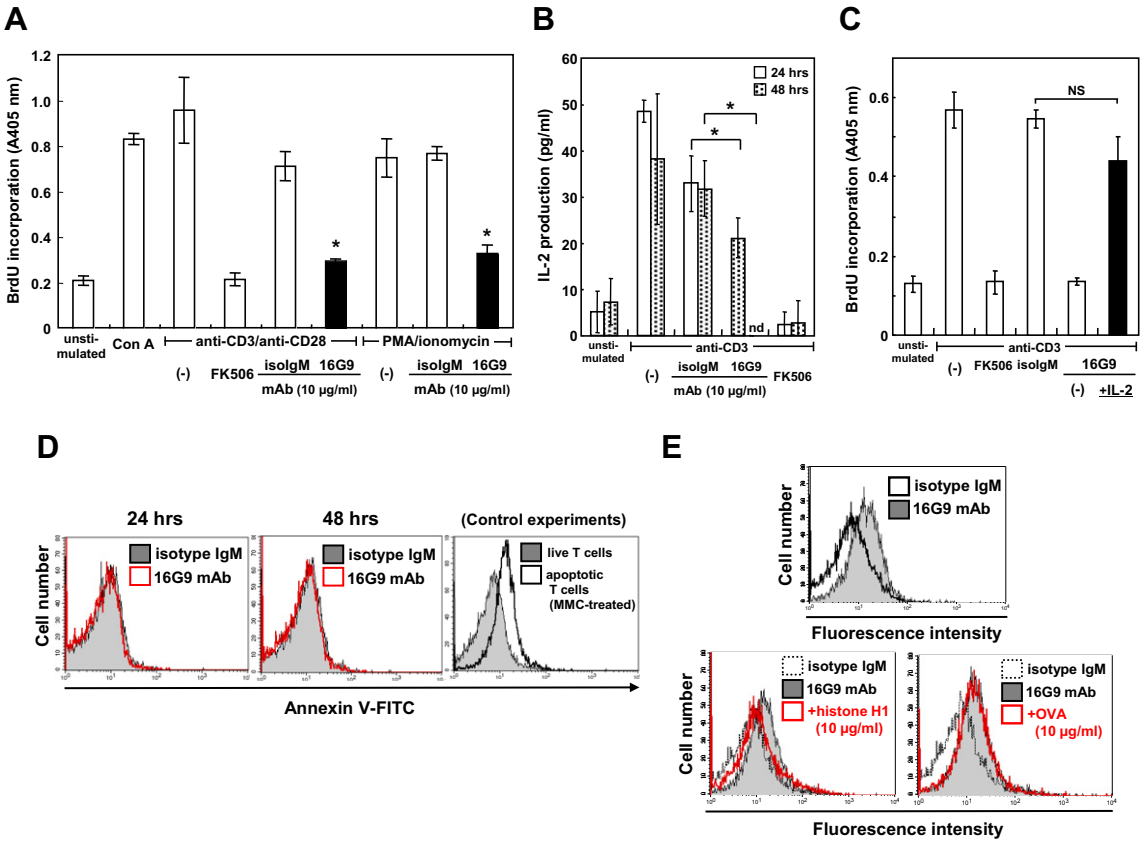

Fig. 4. (A) Co-stimulation of B6 T cells by high dose of 16G9 mAb (10 μg/ml, for 48 h) inhibits T cell activation upon stimulation with anti-CD3/anti-CD28 as well as with PMA/ionomycin that bypasses TCR ligation. $^{*}P < 0.05$ vs. isotype IgM (isoIgM). (B) The high dose co-stimulation with 16G9 mAb suppresses IL-2 secretion from T cells upon TCR cross-linking. $^{*}P < 0.05$ vs. isoIgM; nd, not detectable (<3.9 pg/ml). (C) Exogenous addition of IL-2 abrogates immunosuppressive activity of 16G9 mAb. B6 T cells treating with anti-CD3 were co-stimulated with 10 μg/ml of 16G9 mAb in the presence of IL-2 (10 ng/ml) for 48 h. NS, not significant vs. isoIgM. (D) The high dose co-stimulation with 16G9 mAb does not induce T cell apoptosis in response to TCR ligation. B6 T cells were activated with anti-CD3 upon stimulation with 10 μg/ml of 16G9 mAb for 24 or 48 h, and apoptotic cell death was assessed by FACS analysis of annexin V staining. Results of MMC-treated apoptotic T cells (24 h) and freshly isolated T cells were shown as positive and negative controls, respectively. (E) Upper; Specific reactivity of 16G9 mAb on the cell surface of B6 T cells. Lower; T cell surface reactivity of 16G9 mAb was competitively inhibited by exogenous addition of histone H1 (left) but not by OVA (right).

mice [19], and TCRα-deficient mice spontaneously developing chronic colitis [20]. These facts together with our data raise a possibility that those 'regulatory antibodies (Abreg)' could represent a new class of tolerogeneic factor to negatively regulate harmful T cell response in partly collaboration with Treg cells.

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