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Inhibition of T cell activation through down-regulation of TCR-CD3 expression mediated by an anti-CD90 Ab

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ABSTRACT

We are trying to develop new Abs that can manipulate CD4 T cell responses and are usable as immunosuppressive agents. To this end, we performed functional screening, in which we examined the effect of an Ab on the proliferation of mouse CD4 T cells upon activation. The Ab, LP5, inhibited the activation of CD4 T cells stimulated with an anti-CD3 Ab or peptide antigen. The Ab alone had no stimulatory effect on CD4 T cells. Biochemical experiments demonstrated that LP5 recognized the Thy-1 (CD90) molecule. Interestingly, the treatment of CD4 T cells with LP5 in vitro induced a temporary down-regulation of CD3 expression at the cell surface. TCR molecules were also affected. Other anti-CD90 Abs not inhibitory to CD4 T cell activation failed to induce a reduction in CD3. Experiments in vitro revealed that the down-regulation caused by LP5 is due to an accelerated endocytosis of cell surface CD3. In addition, it was shown that CD3 down-regulation before or in the early stages of T cell activation is critical for the induction of hyporesponsiveness. Experiments in vivo showed that pre-treatment of CD4 T cells with LP5 inhibited the rejection of semi-allogeneic bone marrow transplants. Based on these observations, we propose that CD3 down-regulation without any stimulatory activity against T cells could be one approach to inhibiting T cell activation, and CD90 would be an appropriate target.

1. Introduction

$CD4^+Foxp3^+$ regulatory T cells (Treg cells) have suppressive functions and play important roles in the maintenance of immune homeostasis [1,2]. It has been demonstrated that the manipulation of Treg cells in terms of number and function is useful in the prevention of autoimmune, inflammatory, and graft-versus-host diseases. To increase the number of Treg cells, purified Treg cells have been cultured with IL-2, dendritic cells (DCs) as APCs, and so on [3,4]. In some cases, to increase the suppressive function of Treg cells, it was required to pre-stimulate Treg cells with allogeneic cells before the in vivo transfer of Treg cells since freshly isolated Treg cells did not induce tolerance to bone marrow allografts [5–8]. It was also reported that very large numbers of non-specifically expanded Treg cells were required to inhibit bone marrow allograft rejection [9–11]. In other reports, Treg cells induced from non-Treg cells, so-called iTreg cells, were utilized in manipulating immune responses in vivo [12], although it remained unsolved how long iTreg cells are stable in their suppressive activity. Thus, although Treg cells are an attractive cell population for regulating and manipulating immune responses, there are many complicated procedures involved in manipulating their numbers and functions.

As one approach to manipulating immune responses, we have tried to establish monoclonal Abs exhibiting Treg cell-like suppressive activities. Treg cells can suppress the activation of CD4 T cells stimulated with anti-CD3 Ab and/or Concanavalin A along with APC, allogeneic cells, and anti-CD3/anti-CD28 Ab-coated beads [3,13,14]. However, in the presence of anti-GITR Ab, IL-4, or supernatant (SN) from DCs stimulated with LPS, Treg cells did not exhibit suppressive effects [15–17]. Based on these observations, we tried to establish mAbs that suppress the activation of CD4 T cells in cultures in which Treg cells can exhibit suppressive activity, and at the same time, mAbs that cannot inhibit the activation of CD4 T cells in cultures in which Treg cells cannot exert suppressive activity. Through the functional screenings of hybridomas, we have established some mAbs.

In the present study, we have examined one of those mAbs, LP5, that had almost Treg cell-like properties under the conditions examined. Biochemical experiments revealed that LP5 recognizes Thy-1 (CD90), a small GPI-anchored glycoprotein [18]. In the mouse, CD90 is present on a variety of cell types including thymocytes and peripheral T cells. More recently, CD90 has been used as a marker for lipid rafts in murine T cells [19]. Both stimulatory and negative regulatory roles for CD90 during T cell activation were suggested from studies using CD90-deficient mice or anti-CD90

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Abs [20–25]. In the process of analyzing the functions of LP5, we found that the Ab induces a temporary down-regulation of CD3/TCR expression. It has been shown that TCR/CD3 complexes in T cells are internalized and recycled constitutively [26]. Furthermore, once T cells are activated by an antigen, down-regulation of TCR/CD3 expression at the cell surface is induced [27]. In contrast with this naturally occurring decrease, in this study, we demonstrated that the active down-regulation of CD3 is inducible by an anti-CD90 Ab, and the suppressive effect of LP5 against T cell-activation is dependent on this decrease.

2. Materials and methods

2.1. Animals

BALB/c (H-2^d), CB17.scid, and CBF1 (H-2^{dxb}) mice and Wistar rats were purchased from Japan SLC Inc. (Shizuoka, Japan) or Clea Japan (Tokyo, Japan). DO11.10 mice, OVA peptide (323–339)-specific, MHC class II-restricted, TCR transgenic mice, and DO11.10-RAG2$^{-/-}$ mice, with an RAG2$^{-/-}$ background, were obtained from Taconic Farms (Germantown, NY). All mice were maintained in a specific pathogen-free animal facility and treated in accordance with institutional guidelines for animal care.

2.2. Tumors

Meth A, EL-4, and RLm1 [BALB/c-derived radiation leukaemia, a gift from E. Nakayama (Okayama University, Japan)] [28] were used for the preparation of cell lysates.

2.3. Cell preparation

Splenic cells were incubated at 5×10^7/ml for 45 min at 37 °C with the culture SN of hybridoma cells secreting anti-CD25 Ab (7D4), and rabbit complement diluted to a final concentration of 1:10 (Cedarlane Lab., Canada). The treatment was repeated twice, and the resulting CD25$^+$ cell-depleted cells were then incubated with magnetic beads conjugated with anti-CD4 (GK1.5) Ab (Miltenyi Biotec, Bergisch Gladbach, Germany), washed, and passed through a magnetic column to purify CD4$^+$CD25$^-$ T cells (positive selection). The cells that passed through the magnetic column as non-binding cells were treated with mitomycin C, washed, and used as splenic APCs. In some experiments, CD4 T cells were purified negatively with the use of a CD4$^+$ T cell isolation kit (Miltenyi Biotec) (negative selection).

2.4. Cell culture

CD4 T cells (1×10^4/well) were stimulated with 5% SN of anti-CD3 Ab (145-2C11), which induces maximum proliferation, in the presence of splenic APCs (3×10^4/well), in 96-well round-bottomed plates in DMEM containing 10% heat-inactivated FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine, 10 mM Hepes, 1 mM sodium pyruvate, and 50 μM 2-ME. In some experiments, instead of anti-CD3 Ab SN plus APC, anti-CD3 Ab- and anti-CD28 Ab-conjugated beads (1×10^4/well, Dynal Biotech ASA, Oslo, Norway) were used to stimulate CD4 T cells. The proliferation of T cells (triplicate cultures) was assessed by measuring the incorporation of [^{3}H]TdR (37kBq/well) for the final 4 h of a 3-day culture.

2.5. Abs and reagents

The following Abs were used: anti-CD90 Abs (53-2.1, FF-10 and 30-H12), anti-CD3 (145-2C11 or M-20), anti-CD4 (RM4-5), anti-CD8 (53-6.7), anti-CD45 (30-F11), anti-TCRβ (H57-597), anti-H-2K^b (AF6-88.5), anti-H-2D^d (34-2-12), anti-mouse IgM and anti-rat IgG, all purchased from BD PharMingen, Santa Cruz

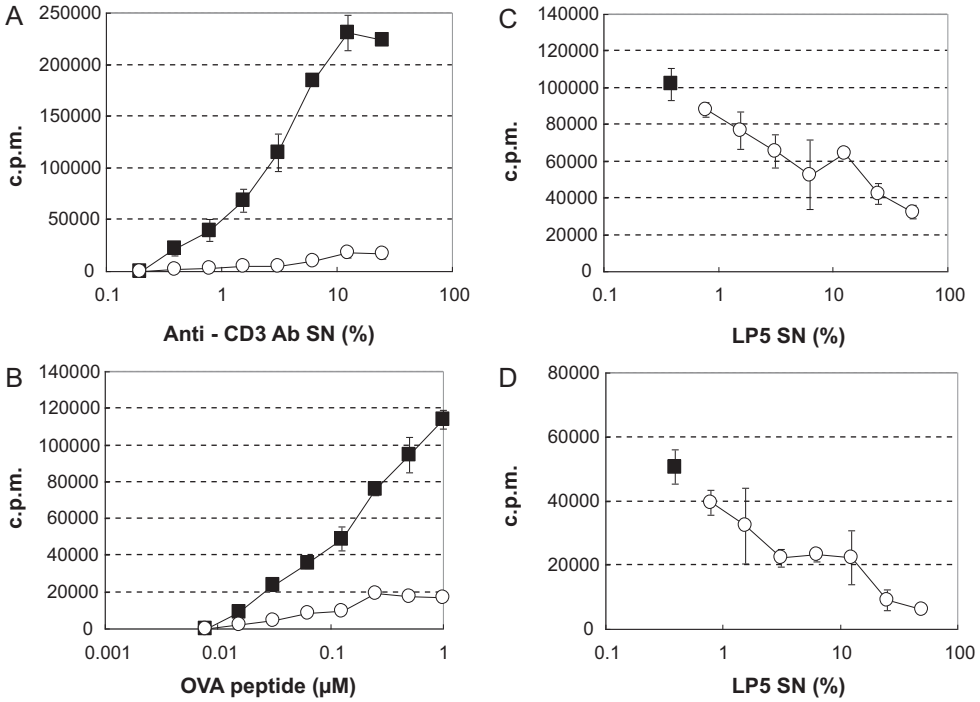

Fig. 1. The Ab LP5 can inhibit the activation of CD4 T cells. (A, B) CD4$^+$CD25$^-$ T cells from DO11.10 mice were cultured with APCs plus the titrated amount of anti-CD3 Ab (A) or $OVA_{323-339}$ peptide (B) in the presence (open symbol) or absence (closed) of LP5 (25% SN). Three days later, cell proliferation was measured. (C and D) CD4$^+$CD25$^-$ T cells from BALB/c mice were cultured with anti-CD3 Ab (5% SN) plus APCs (C), or anti-CD3 Ab/anti-CD28 Ab-coated beads (D) in the presence (open) or absence (closed) of the titrated amount of LP5 Ab. Data are representative of more than five independent experiments.