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CD155 (PVR/Nect15) Mediates a Costimulatory Signal in CD4⁺ T Cells and Regulates Allergic Inflammation

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Although Th1 and Th2 cells are known to be involved in allergic inflammatory diseases, the molecular mechanisms underlying their differentiation are incompletely understood. In this study, we identified CD155 as a costimulatory molecule on CD4⁺ T cells. Importantly, CD155-mediated signaling induced Th1 development in both humans and mice, as evidenced by production of IFN- γ and upregulation of *Tbx21* transcription; these effects were independent of IL-12 but dependent on NF- κ B-induced autocrine IFN- γ that triggered positive feedback via STAT1 activation. Mice genetically deficient in CD155 or treated with anti-CD155 Ab exhibited attenuated Th1-type contact hypersensitivity. Thus, CD155 plays an important regulatory role in helper T cell differentiation and allergic diseases. *The Journal of Immunology*, 2015, 194: 000–000.

Allergic diseases are caused by hypersensitivity reaction of immune responses and are commonly classified according to the type of immune responses. The Th2 immune response is involved in IgE production and mediates type I allergic responses (so-called immediate hypersensitivity), which are due to mast cell activation induced by the crosslinking of Fc ϵ RI with an IgE immune complex (1, 2). In contrast, the Th1 immune response leads to delayed-type hypersensitivity through the production of proinflammatory cytokines from Th1 and CD8⁺ T cells. Imbalance in the numbers of Th1 and Th2 cells is responsible for many allergic inflammatory disorders and correction of the imbalance may ameliorate allergic inflammation. For example, the administration of recombinant IFN- γ or IL-12, which induce the polarization of CD4⁺ naive Th cells into Th1 cells, attenuates allergic airway inflammation (3, 4).

Allergic contact dermatitis (ACD) is one of the most common skin diseases, affecting 15–20% of the general population worldwide. Contact hypersensitivity (CHS) is the most frequently used murine model of human ACD (5). The pathogenesis of CHS is based on delayed-type hypersensitivity, which is mediated mainly by Th1 immune responses. The CHS reaction is divided into the

sensitization and elicitation phases. Ag-specific Th1 cell clones differentiate from naive CD4⁺ Th cells during the sensitization phase; later on, re-exposure to Ag activates Th1 and CD8⁺ T cells, thus inducing cytokine-mediated inflammation during the elicitation phase. Recent evidence has demonstrated that regulatory T cells, Th17 cells, and dermal dendritic cells (DC) also play important roles in the pathogenesis of CHS (5). Moreover, CHS reactions are attenuated in mice deficient in CD28, OX40L, and LFA-1 (6–8), suggesting that these costimulatory molecules are involved in the development of CHS.

Th1 cells produce IFN- γ , IL-2, and TNF- α and are involved in cellular immune responses against intracellular pathogens. The differentiation of Th1 cells from naive CD4⁺ T cells is driven mainly by IL-12 produced by APCs. IL-12-mediated signaling in CD4⁺ T cells activates STAT4, which induces IFN- γ production (9). IFN- γ promotes the phosphorylation of STAT1 by binding to the receptor for IFN- γ that is expressed on CD4⁺ T cells, resulting in upregulation of T-bet, a master regulator of Th1 development. Thus, Th1 development is reinforced by autocrine IFN- γ via the STAT1 and T-bet signaling pathways in a positive-feedback manner (10, 11). However, although IL-12-deficient mice show severely impaired Th1 responses, they still carry low levels of Th1 cells, suggesting that these cells might alternatively be developed through an IL-12-independent mechanism (12).

CD155, initially identified as a receptor for poliovirus in humans (13), is a member of poliovirus receptor-related (PRR) family of adhesion molecules, which consists of CD111 (nectin-1/PRR-1), CD112 (nectin-2/PRR-2), nectin-3 (PRR-3), and nectin-4 (PRR-4). The CD155 transcript is ubiquitously expressed in most organs in both humans and mice (13–15) and on various hematopoietic cells in mice, including T cells, macrophages, and DC (16). CD155 binds to another PRR family member, nectin-3, as well as the matrix protein vitronectin, thereby mediating cell–cell or cell–matrix adhesion, respectively, and cell migration (17). Additionally, CD155 is a ligand for the Ig-like receptors DNAM-1 (CD226), Tactile (CD96), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) on T cells and NK cells (18–22). Mice deficient in CD155 show diminished humoral immune responses and thymic retention of mature CD8⁺ cells (16, 23–25). Although several studies have demonstrated various functions of CD155, particularly on nonhematopoietic cells, the role of CD155 in lymphocyte function remains undefined.

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Abbreviations used in this article: ACD, allergic contact dermatitis; alum, aluminum hydroxide; CHS, contact hypersensitivity; cIg, control Ig; DC, dendritic cell; DNCB, 1-chloro-2,4-dinitrobenzene; IFN- γ R α , IFN- γ receptor α -chain; PRR, poliovirus receptor-related; Tg, transgenic; WT, wild-type.

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In this study, we investigated the role of CD155 in lymphocyte function and immune responses. We show that CD155-mediated signaling plays an important role in the pathogenesis of contact hypersensitivity.

Materials and Methods

Mice

C57BL/6 and BALB/c mice were purchased from Clea Japan (Tokyo, Japan). *Ifng*^{-/-} mice and *Rag1*^{-/-} mice were purchased from The Jackson Laboratory (Bar Harbor, ME). CD155-deficient (*Pvr*^{-/-}) mice were generated as described (16) and were crossed with OT-II transgenic (Tg) mice (26). DNAM-1-deficient (*Cd226*^{-/-}) mice generated as described (27) were crossed with *Rag1*^{-/-} mice. All mice were housed and bred under specific pathogen-free conditions at the Laboratory Animal Resource Center (University of Tsukuba, Ibaraki, Japan). All animal experiments were performed according to the guidelines of the Animal Ethics Committee of the Laboratory Animal Resource Center, University of Tsukuba.

Human samples

Human peripheral blood was obtained from healthy volunteers after obtaining informed consent. Human cord blood was purchased from the RIKEN BioResource Center (Ibaraki, Japan). All experiments with samples of human blood were approved by the Ethics Review Board of the University of Tsukuba.

Abs and reagents

Anti-mouse CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD28 (37.51), CD62L (MEL-14), CD44 (IM7), B220 (RA3-6B2), CD49b (DX5), CD11c (HL3), CD11b (M1/70), Ly6G (1A8), Siglec-F (E50-2440), IFN- γ receptor α -chain (IFN- γ R α ; GR20), IFN- γ (XMG-1.2), IL-4 (11B11), IL-17A (TC11-18H10.1), and IL-12 (C17.8) mAbs; anti-human CD3 (UCHT1), CD4 (RPA-T4), CD8 (RPA-T8), CD19 (HIB19), CD56 (B159), CD28 (CD28.2), CD45RA (HI100), CD45RO (UCHL1), and CD44 (G44-26) mAbs; isotype-matched control Abs; and secondary Abs and fluorescein-conjugated streptavidin were purchased from BD Biosciences (San Jose, CA). Anti-human CD14 (TUK4) mAb was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Anti-mouse IFN- γ R α (2E2) was purchased by BioLegend (San Diego, CA). Anti-mouse T-bet (4B10) mAbs were purchased from eBioscience (San Diego, CA). Anti-mouse p-STAT1 (42H3) mAb and STAT1, IkB α polyclonal Abs, and anti- α -tubulin mAb were purchased from Cell Signaling Technology (Danvers, MA, USA), and anti-mouse NF- κ B polyclonal Abs and anti-lamin B polyclonal Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-mouse β -actin (AC15) mAb was purchased from Sigma-Aldrich (St. Louis, MO). For ELISA, anti-mouse IFN- γ (AN-18), IL-2 (JES6-1A2), and IgE (R35-72) mAbs were used as capture Abs, and biotinylated anti-mouse IFN- γ (R4-6A2), IL-2 (JES6-5H4), and IgE (R35-118) mAbs were used as detection Abs; these mAbs all were purchased from BD Biosciences. Streptavidin-conjugated HRP was purchased from GE Healthcare Biosciences (Little Chalfont, U.K.). Anti-mouse CD155 mAb (TX56, rat IgG2a), which blocks the interaction between CD155 and its ligand DNAM-1 (28), anti-mouse DNAM-1 mAb (TX42, rIgG2a), and anti-human CD155 mAb (TX24) were generated in our laboratory (27, 29). Recombinant mouse and human IL-2 were purchased from BD Biosciences. NF- κ B inhibitor JSH-23 was purchased from Merck Millipore (Darmstadt, Germany).

Flow cytometry

Flow cytometric analysis and cell sorting were performed by using FACSCalibur, LSRFortessa, and FACSARIA flow cytometers (BD Biosciences). FlowJo software (Tree Star, Ashland, OR) was used for data analysis.

Isolation of CD4⁺ T cells

CD4⁺ T cells were isolated from the spleens of wild-type (WT) mice by using anti-CD4 MACS technology (Miltenyi Biotec) according to the manufacturer's instructions. Naive CD62L^{hi}CD44^{low}CD4⁺ T cells and CD45RA^{hi}CD45RO⁺CD4⁺ T cells were sorted from mouse spleens or human cord blood, respectively, by using FACSARIA (BD Biosciences).

Stimulation of CD4⁺ T cells

To examine tyrosine phosphorylation of CD155, CD4⁺ T cells were stimulated with anti-CD155 mAb or control Ig (cIg; 10 μ g/ml) and then

cross-linked with anti-rat IgG secondary Ab (10 μ g/ml). For proliferation assays, CD4⁺ T cells were stimulated for 48 h with plate-bound anti-CD3 mAb and either plate-bound rat IgG2a isotype control or anti-CD155 mAb (20 μ g/ml). CFSE-labeled CD4⁺ T cells were stimulated for 48 h with plate-bound anti-CD3 mAb and either plate-bound rat IgG2a isotype control or anti-CD155 mAb (20 μ g/ml). CFSE dilution was measured by flow cytometry. For analysis of IkB α , CD4⁺ T cells were stimulated with plate-bound anti-CD3 and anti-CD155 mAbs. In the case of Erk and NF- κ B, CD4⁺ T cells were stimulated with soluble anti-CD3 and anti-CD155 mAbs, followed by cross-linking with secondary Abs. Mouse and human CD4⁺ naive T cells were stimulated for 6 d with plate-bound anti-CD3 mAb (1 μ g/ml) and anti-CD155 mAb (20 μ g/ml) in the presence of IL-2 (20 ng/ml). For coculture assays, splenocytes were treated with 50 μ g mitomycin C. Naive CD4⁺ T cells (10^5) and the splenocytes (2×10^6) were cocultured for 6 d with OVA₃₂₃₋₃₃₉ peptide (1 μ M) in the presence or absence of neutralizing anti-IL-12 mAb or anti-IFN- γ mAb (40 μ g/ml).

Analysis of intracellular cytokines

Cells (10^5 – 10^6 /ml) were stimulated with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 2 h, after which brefeldin A (10 μ g/ml; Sigma-Aldrich) was added and cells were cultured for another 2 h. Cells then were harvested, resuspended, stained for intracellular cytokines (Fix and Perm Kit, Invitrogen) according to the manufacturer's instructions, and analyzed by flow cytometry.

ELISA

The concentration of mouse IFN- γ and IL-2 in culture supernatants and IgE, IgG1, and IgG2a in sera were measured by ELISA using capture and detection mAbs against mouse IFN- γ , IL-2, and IgE. Mouse IL-4 and human IFN- γ and IL-4 concentrations were measured by using ELISA kits from BD Biosciences, and mouse and human IL-17A levels were measured by using ELISA kits from R&D Systems; all assays were performed according to the manufacturers' instructions.

Biochemistry

After respective stimulations as described in *Materials and Methods*, cells were lysed by using a buffer containing 1% Nonidet P-40, protease inhibitors, and phosphatase inhibitors, as described (30). Total cell lysates were immunoprecipitated by using control Ab or anti-CD155 mAb. Proteins were separated by SDS-PAGE under nonreducing conditions, immunoblotted by using anti-phosphotyrosine (4G10) (0.3 μ g/ml) Ab, and reblotted with anti-CD155 mAb (2 μ g/ml). For analysis, IkB α and NF- κ B-stimulated cells were lysed by using 1% Nonidet P-40 lysis buffer as described above, or by a cytoplasmic and nuclear extraction kit (Invent, Eden Prairie, MN), respectively, and immunoblotted with appropriate Abs, according to the manufacturer's instructions.

Quantitative RT-PCR

Total RNA was isolated from cell pellets by using Isogen reagent (Nippon Gene, Tokyo, Japan). For reverse transcription, we used 2 μ g total RNA and a final volume of 20 μ l with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). Real-time PCR analysis of *Tbx21*, *Gata3*, *Rorc*, *Foxp3*, and *Ifng* was performed by using an ABI 7500 sequence detector (Applied Biosystems), the Power SYBR Green PCR master mix (Applied Biosystems), and appropriate primers. Primer sequences of the target genes are: *Tbx21*, forward, 5'-AGC AAG GAC GGC GAA TGT T-3', reverse, 5'-GGG TGG ACA TAT AAG CGG TTC-3'; *Gata3*, forward, 5'-TTA TCA AGC CCA AGC GAA GG-3', reverse, 5'-CAT TAG CGT TCC TCC TCC AGA G-3'; *Rorc*, forward, 5'-GGA GGA CAG GGA GCC AAG TT-3', reverse, 5'-CCG TAG TGC ATC CCA GAT GAC T-3'; *Foxp3*, forward, 5'-CCC ATC CCC AGG AGT CTT G-3', reverse, 5'-ACC ATG ACT AGG GGC ACT GTA-3'; *Ifng*, forward, 5'-ACA GCA AGG CGA AAA AGG ATG-3', reverse, 5'-TGG TGG ACC ACT CGG ATG A-3'; *Actb*, forward, 5'-ACT GTC GAG TCG CGT CCA-3', reverse, 5'-GCA GCG ATA TCG TCA TCC AT-3'. The β -actin level was measured as an internal control to normalize data. The thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The mRNA level was determined relative to that in mouse spleen. All values were determined in triplicate.

Induction of CHS

Aliquots (200 μ l) of 5% 1-chloro-2,4-dinitrobenzene (DNCB; Sigma-Aldrich) in ethanol were applied to the shaved abdomens of mice. Five days later, mice were challenged by the application of 1% DNCB (40 μ l

total) to the inner and outer sides of the left ear and of ethanol only to the right ear. Response was assessed by using a digital thickness gauge (G-1A; Ozaki, Tokyo, Japan) to measure ear thickness. Net ear swelling was calculated as the difference between the thickness of the right and left ears of each mouse. For the transfer model, either WT or *Pvr*^{-/-} CD4⁺ T cells (10⁷) and WT CD8⁺ T cells (10⁶) were injected i.v. into *Rag1*^{-/-} or *Rag1*^{-/-} *Cd226*^{-/-} mice. One week after transfer, CHS was induced as described above. Five days after sensitization, draining lymph nodes were harvested, and cytokine production was analyzed by flow cytometry. For treatment of Ab, mice were injected i.v. with anti-CD155 mAb (TX56) or control mAb (MP Biomedicals, Solon, OH) at 2 h prior to sensitization (anti-CD155 dose, 1.0 mg) or challenge (0.5 mg).

Induction of eosinophilic airway inflammation

WT and *Pvr*^{-/-} BALB/c mice were injected i.p. with OVA (100 µg) plus aluminum hydroxide (alum) on days 0 and 7. On days 14, 15, and 16, these mice were challenged intranasally with OVA (10 µg in PBS) or PBS alone. On day 18, the numbers of total cells and eosinophils in bronchoalveolar lavage fluid were determined by using flow cytometry. Serum IgE, IgG1, and IgG2a levels on days 0 and 14 were measured by using ELISA.

Histology

Mouse ears and lungs were fixed with formalin and then stained with H&E for histopathologic examination by light microscopy (×200 objective).

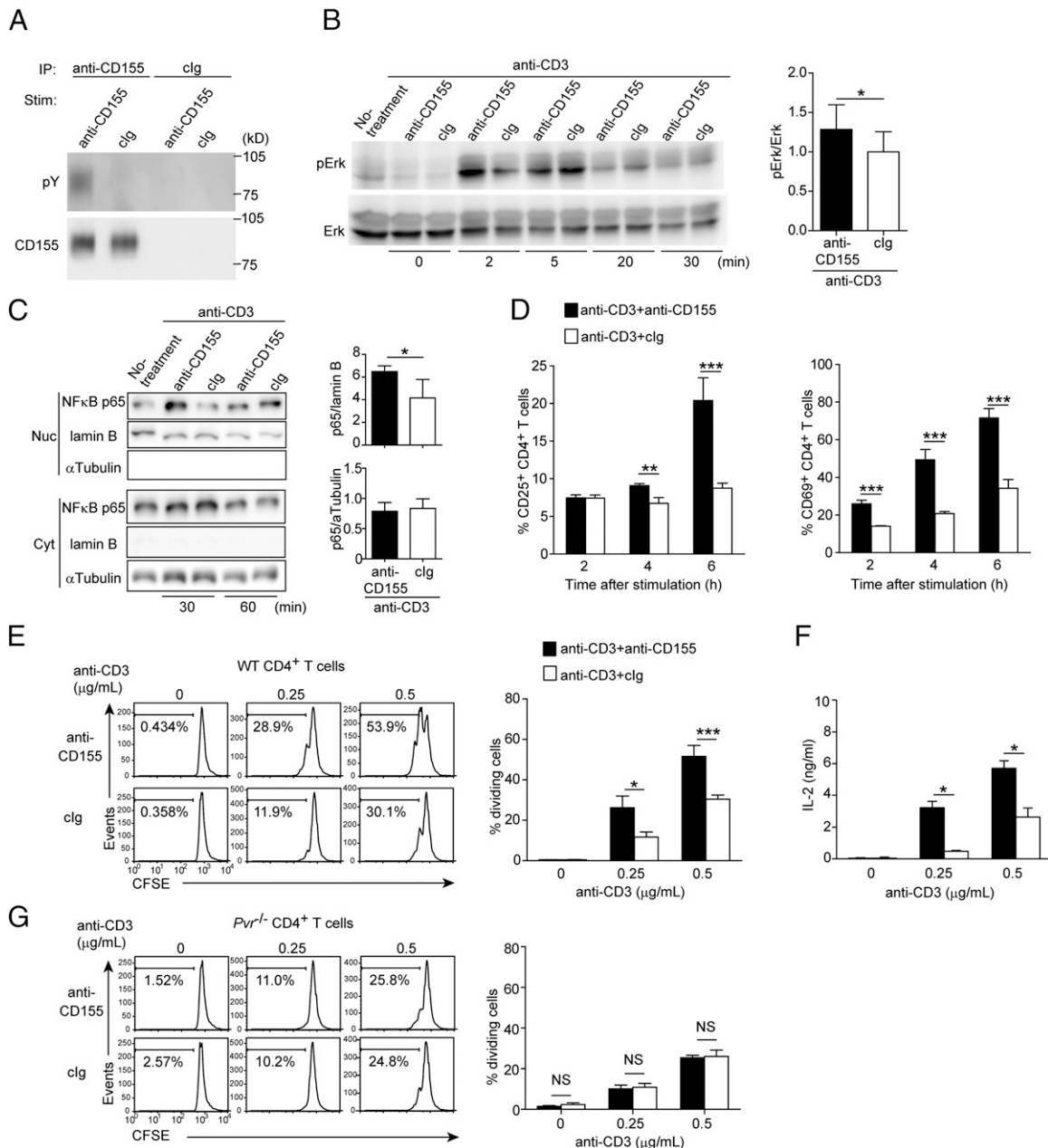


FIGURE 1. CD155 mediates a costimulatory signal in CD4⁺ T cells. **(A)** Immunoblot analysis of phosphorylated CD155 (blotted with anti-phosphotyrosine [pY]) and CD155 (blotted with anti-CD155 mAb [CD155]) in mouse CD4⁺ T cells that were stimulated with anti-CD155 mAb or isotype control (cIg) and immunoprecipitated (IP) with anti-CD155 mAb or cIg. **(B and C)** Immunoblot analysis of phosphorylated Erk and Erk (B) or p65 NF-κB of nuclear (Nuc) and cytoplasmic (Cyt) extracts (C) of mouse CD4⁺ T cells at indicated time points after stimulation with anti-CD3 plus either anti-CD155 mAb or cIg. Bar graphs show the relative amount of phosphorylated Erk to total Erk at 2 min (B) or of NF-κB p65 at 30 min (C). **(D–G)** Frequencies of CD25⁺ and CD69⁺ CD4⁺ T cells analyzed by flow cytometry (D), proliferation assay by CFSE dilution (E), and IL-2 production according to ELISA (F) after stimulation of WT CD4⁺ T cells with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg. **(G)** Proliferation assay by CFSE dilution after stimulation of CD155-deficient (*Pvr*^{-/-}) CD4⁺ T cells with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg. The mean ± 1 SD is shown. Data are representative of three or four (B–F) or two (A and G) independent experiments. **p* < 0.05, ***p* < 0.01, ****p* < 0.005.

Statistical analysis

Statistical analyses were performed by using the unpaired or paired Student *t* test (GraphPad Prism 5, GraphPad Software, La Jolla, CA). A *p* value <0.05 was considered statistically significant.

Results

CD155 mediates a costimulatory signal in CD4⁺ T cells

To analyze the function of CD155 in immune responses, we first investigated the expression profile of CD155 on spleen cells. CD155 was broadly expressed on both myeloid and lymphoid lineage cells. Of note, CD155 was expressed most strongly on CD4⁺ T cells (Supplemental Fig. 1A). Stimulation of CD4⁺ T cells with plate-bound anti-CD3 plus anti-CD28 mAbs further augmented the expression of CD155 (Supplemental Fig. 1B). Therefore, we focused our present study on the role of CD155 in immune responses by CD4⁺ T cells. CD4⁺ T cells purified from the spleen were stimulated with anti-CD155 mAb. Immunoblot analysis demonstrated that CD155 became tyrosine phosphorylated after stimulation (Fig. 1A), suggesting that CD155 is a signal-transducing molecule in CD4⁺ T cells. We next examined whether CD155 mediates a costimulatory signal in CD4⁺ T cells. Erk is phosphorylated after stimulation of the TCR with a cognate Ag or anti-CD3 mAb (31). Stimulation of CD4⁺ T cells with anti-CD155 together with anti-CD3 mAb augmented Erk phosphorylation as early as 2 min after stimulation, compared with stimulation with control mAb and anti-CD3 mAb (Fig. 1B). Furthermore, stimulation of CD4⁺ T cells with anti-CD155 mAb plus anti-CD3 mAb markedly increased activation of NF- κ B, as determined by translocation of p65 NF- κ B from the cytoplasm into the nuclei

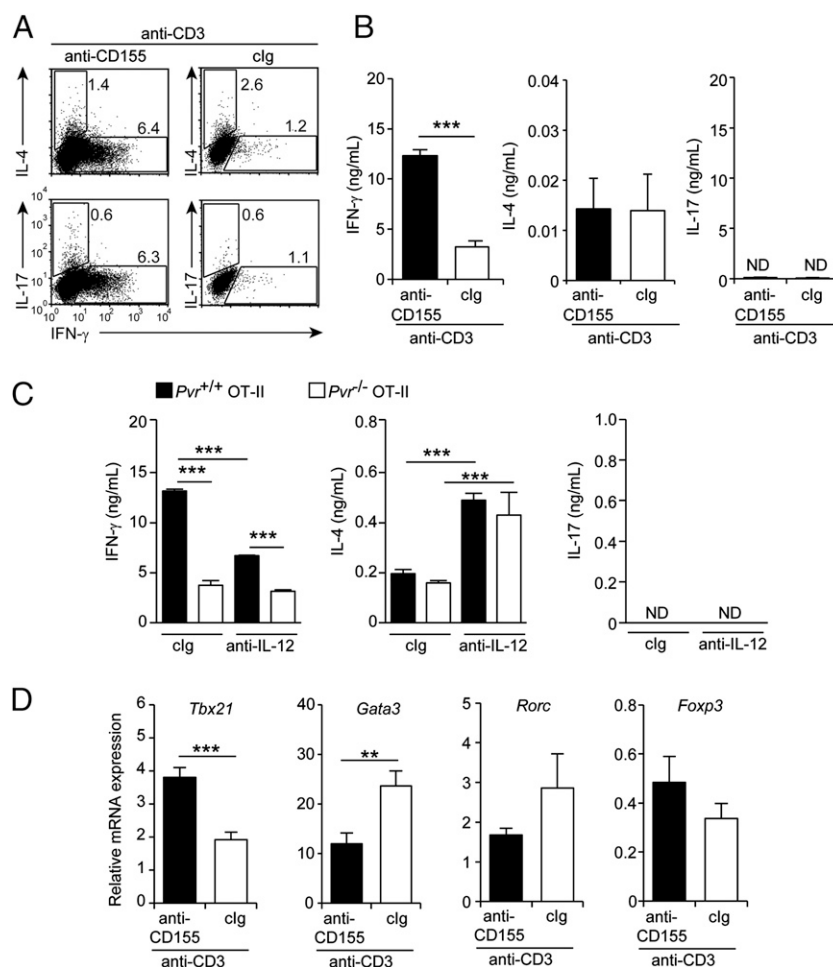
(Fig. 1C), increased number of activated CD4⁺ T cells (Fig. 1D), augmented proliferation of CD4⁺ T cells (Fig. 1E), and increased IL-2 production by CD4⁺ T cells (Fig. 1F), compared with stimulation with control mAb plus anti-CD3 mAb. In contrast, stimulation with anti-CD155 mAb plus anti-CD3 mAb did not augment the proliferation of CD155-deficient (*Pvr*^{-/-}) CD4⁺ T cells (Fig. 1G). Taken together, these results suggest that CD155 is a costimulatory molecule in CD4⁺ T cells.

CD155 mediates IL-12-independent Th1 development from naive CD4⁺ T cells

We next investigated whether CD155-mediated signal is involved in development of Th cell subsets. Naive CD4⁺ T cells, prepared by sorting CD3⁺CD4⁺CD62L^{hi}CD44^{dull} cells from the spleen by using flow cytometry, were stimulated for 6 d with anti-CD3 mAb and either plate-bound anti-CD155 or control mAb in the presence of IL-2 and analyzed for cytokine production. Stimulation with anti-CD155 and anti-CD3 mAbs significantly increased the production of IFN- γ but not IL-4 or IL-17 by CD4⁺ T cells, compared with stimulation with control mAb and anti-CD3 mAb (Fig. 2A, 2B). These results suggest that CD155 is involved in the development of Th1 cells. Because the *in vitro* Th1 differentiation assay was free from IL-12, these results suggest that CD155 is involved in IL-12-independent Th1 development from naive CD4⁺ T cells.

To examine the costimulatory function of CD155 under more physiological conditions, we cocultured OVA-specific naive CD4⁺ T cells derived from *Pvr*^{+/+} or *Pvr*^{-/-} OT-II Tg mice with WT splenocytes loaded with OVA peptide for 6 d. *Pvr*^{+/+} CD4⁺ T cells produced significantly more IFN- γ than did *Pvr*^{-/-} CD4⁺ T cells,

FIGURE 2. CD155 mediates IL-12-independent Th1 development. (**A** and **B**) Cytokine analyses of IFN- γ , IL-4, and IL-17 in the cytoplasm (**A**) or culture supernatant (**B**) after stimulation of CD4⁺ naive T cells from C57BL/6 mice with plate-bound anti-CD3 plus anti-CD155 mAbs or clg in the presence of IL-2 for 6 d, followed by restimulation with PMA plus ionomycin for 4 h (**A**) or with anti-CD3 mAb for 48 h (**B**). Numbers indicate the percentages of the population in each region (**A**). (**C**) ELISA of cytokines in culture supernatants of CD4⁺ naive T cells obtained from *Pvr*^{+/+} or *Pvr*^{-/-} OT-II Tg mice after coculture for 6 d with WT splenocytes loaded with OVA peptide in the presence or absence of anti-IL-12-neutralizing mAb, followed by washing and restimulation with anti-CD3 mAb for 48 h. (**D**) Quantitative RT-PCR of gene expression in CD4⁺ T cells after stimulation with plate-bound anti-CD3 plus anti-CD155 mAbs or clg for 3 d. The mean $\pm$ 1 SD is shown. Data are representative of three (**A** and **B**) or two (**C** and **D**) independent experiments. ***p* < 0.01, ****p* < 0.005.



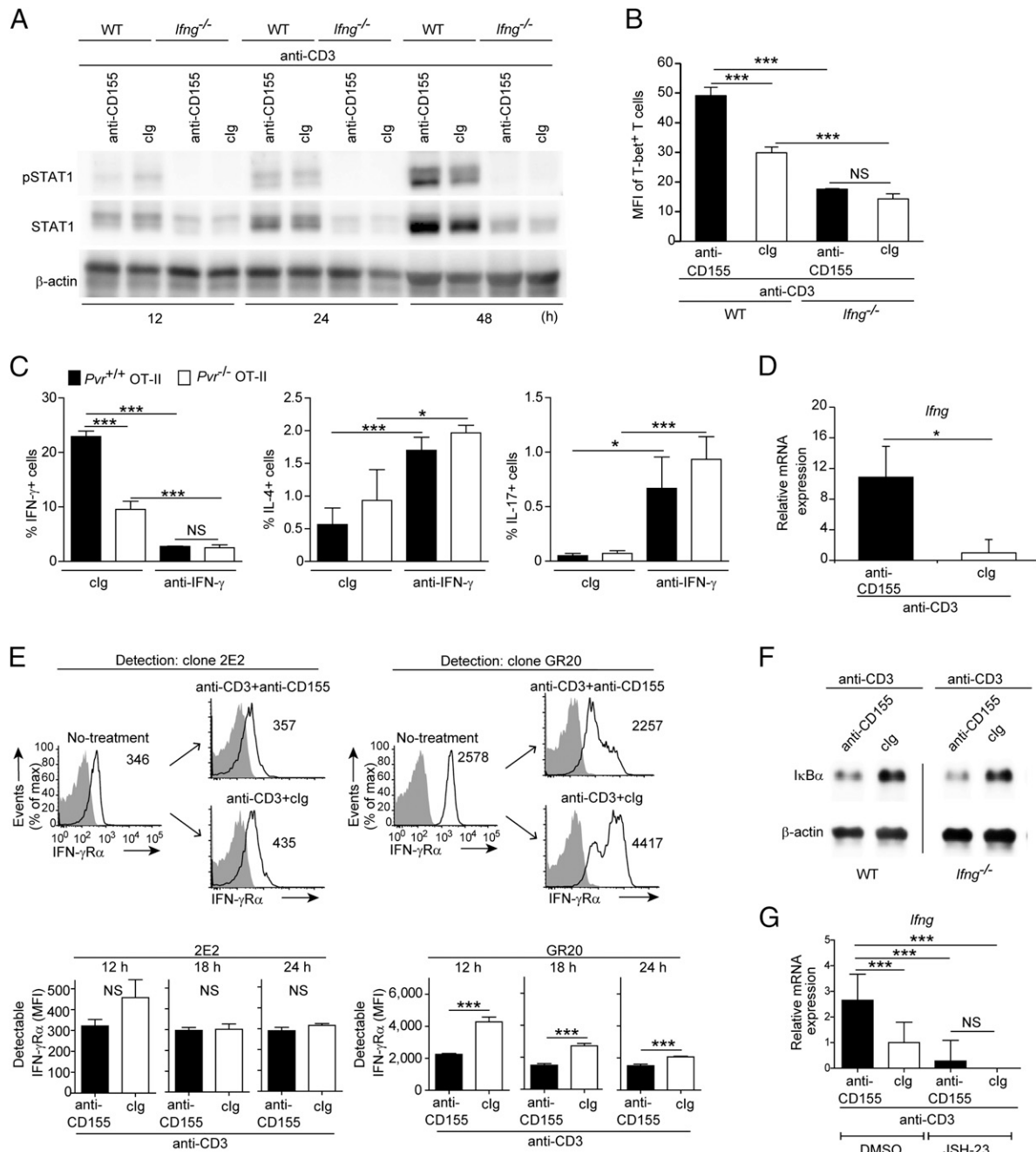


FIGURE 3. CD155 mediates IFN- γ -dependent Th1 development. **(A)** Immunoblot analysis of p-STAT1 and STAT1 of WT or *Ifng*^{-/-} CD4⁺ T cells simulated for indicated times with plate-bound anti-CD3 plus either anti-CD155 mAb or cIg. **(B)** Flow cytometric analysis of T-bet of WT or *Ifng*^{-/-} CD4⁺ T cells simulated for 48 h with plate-bound anti-CD3 plus either anti-CD155 mAb or cIg. MFI, mean fluorescence intensity. **(C)** Cytokine analyses of IFN- γ , IL-4, and IL-17 in the cytoplasm after stimulation of CD4⁺ naive T cells obtained from *Pvr*^{+/+} or *Pvr*^{-/-} OT-II Tg mice after coculture for 6 d with WT splenocytes loaded with OVA peptide in the presence or absence of anti-IFN- γ -neutralizing mAb, followed by washing and restimulation with PMA plus ionomycin for 4 h. **(D)** Quantitative RT-PCR of *Ifng* gene expression in CD4⁺ naive T cells after stimulation with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg for 10 h. **(E)** Flow cytometric analysis of IFN- γ R α detected by clone 2E2 or GR20 (open histogram) in CD4⁺ naive T cells after stimulation with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg for 12 h. Shaded histograms indicate staining with isotype control. Numbers in histograms indicate MFI. Bar graphs show the average MFI. **(F)** Immunoblot analysis of IkB α in WT or *Ifng*^{-/-} CD4⁺ T cells at 15 min after stimulation with plate-bound anti-CD3 plus either anti-CD155 mAb or cIg. The lanes were run on the same gel but were noncontiguous. **(G)** Quantitative RT-PCR of *Ifng* gene expression in CD4⁺ naive T cells after stimulation with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg in the presence of 50 μ M JSH-23 or DMSO for 10 h. The mean $\pm$ 1 SD is shown. Data are representative of two independent experiments. * p < 0.05, *** p < 0.005.

indicating that CD155 plays an important role in Th1 development when naive CD4⁺ T cells are stimulated by using a cognate Ag and APCs that express DNAM-1, a natural ligand for CD155 (Fig. 2C, Supplemental Fig. 1C). In contrast, IL-4 and IL-17 production was comparable between *Pvr*^{+/+} and *Pvr*^{-/-} CD4⁺ T cells. The addition of a neutralizing anti-IL-12 mAb into the

assay still permitted significantly greater IFN- γ production from *Pvr*^{+/+} CD4⁺ T cells than from *Pvr*^{-/-} CD4⁺ T cells (Fig. 2C), indicating that CD155 does not necessarily require IL-12 to induce Th1 development. Alternatively, the generation of Th1 cells from naive CD4⁺ T cells after stimulation with anti-CD3 and anti-CD155 was comparable to that after stimulation with anti-CD3

plus control Ab in the presence of exogenous IL-12 and anti-IL-4 (Th1 condition) (Supplemental Fig. 2A). Moreover, the coculture of *Pvr*^{+/+} naive CD4⁺ T cells and APCs in the presence of a cognate Ag generated Th1 cells at a comparable level to that of *Pvr*^{-/-} CD4⁺ T cells and APCs under the Th1 condition (Supplemental Fig. 2B), indicating that CD155 is not necessarily required for Th1 differentiation from naive CD4⁺ T cells when in the presence of adequate exogenous IL-12.

The transcription of *Tbx21*, which encodes T-bet, was upregulated in CD4⁺ T cells after stimulation with anti-CD155 and anti-CD3 mAbs (Fig. 2D). In contrast, these cells showed downregulation of *Gata3*, a master regulator of Th2 development (Fig. 2D). There was no difference in expression of *Rorc*, which encodes retinoic acid-related orphan receptor γ t, and *Foxp3*, master regulators of Th17 and regulatory T cells, respectively, between CD4⁺ T cells stimulated with anti-CD155 mAb plus anti-CD3 mAb and those stimulated with control mAb and anti-CD3 mAb (Fig. 2D). Taken together, CD155-mediated signal is involved in IL-12-independent Th1 development.

CD155 mediates IFN- γ -dependent Th1 development from naive CD4⁺ T cells

We next investigated the molecular mechanism of Th1 development induced by CD155-mediated signaling. Because STAT1 activation upregulates T-bet expression and Th1 development (10), we examined whether CD155 mediates STAT1 phosphorylation. Although stimulation of CD4⁺ T cells with anti-CD3 mAb plus control mAb moderately induced STAT1 phosphorylation, costimulation with plate-bound anti-CD155 together with anti-CD3 mAbs markedly increased both the expression and phosphorylation of STAT1 (Fig. 3A), suggesting that CD155-mediated Th1 development is dependent on the STAT1 pathway. Because IFN- γ also activates STAT1, we investigated whether CD155-mediated

activation of STAT1 required IFN- γ . STAT1 expression and phosphorylation were negligible in *Ifng*^{-/-} CD4⁺ T cells after stimulation with anti-CD3, regardless of whether anti-CD155 mAb was included (Fig. 3A), indicating that the CD155-associated induction of STAT1 expression and phosphorylation is dependent on IFN- γ . Moreover, CD155-mediated upregulation of T-bet expression was undetectable in *Ifng*^{-/-} CD4⁺ T cells (Fig. 3B). A neutralizing anti-IFN- γ mAb decreased IFN- γ production from both *Pvr*^{+/+} and *Pvr*^{-/-} OT-II CD4⁺ T cells at the same level after coculture with WT splenocytes loaded with OVA peptide (Fig. 3C). These results suggest that CD155-mediated STAT1 phosphorylation and subsequent Th1 development were dependent on IFN- γ .

We therefore investigated whether CD4⁺ T cells produced IFN- γ before STAT1 phosphorylation after stimulation with anti-CD155 mAb. We found that *Ifng* transcript was significantly upregulated in naive CD4⁺ T cells just 10 h after stimulation with plate-bound anti-CD155 mAb and anti-CD3 mAb compared with stimulation with control mAb and anti-CD3 mAb (Fig. 3D). We also found that the expression of IFN- γ R α on CD4⁺ T cells was comparable regardless of stimulation with anti-CD155 mAb, as analyzed by using an anti-IFN- γ R α mAb 2E2 (Fig. 3E). However, when we used GR20 anti-IFN- γ R α mAb, which, unlike 2E2 mAb, competes with IFN- γ for binding to IFN- γ R α , GR20 mAb binding was significantly suppressed on CD4⁺ T cells after stimulation with anti-CD155 mAb and anti-CD3 mAb, compared with stimulation with control mAb and anti-CD3 mAb (Fig. 3E), suggesting that autocrine IFN- γ bound to IFN- γ R α on CD4⁺ T cells stimulated with anti-CD155 mAb and anti-CD3 mAb significantly more than on CD4⁺ T cells stimulated with control mAb and anti-CD3 mAb before STAT1 phosphorylation. Taken together, these results indicated that CD155-mediated signal induced STAT1-independent production of IFN- γ , triggering the positive feed-

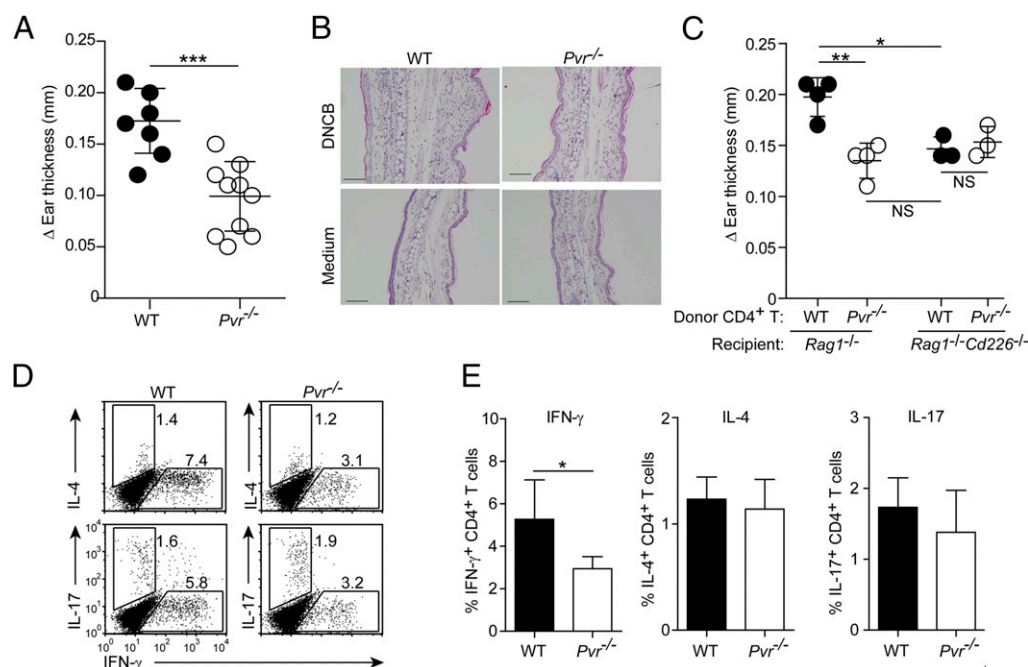


FIGURE 4. CHS is attenuated in CD155-deficient mice. (**A** and **B**) Net swelling of the left ear at 24 h after challenge of WT ($n = 7$) or *Pvr*^{-/-} ($n = 10$) mice, which had been immunized with DNCB onto the abdomen on day 0 and challenged with DNCB onto the left ear or with medium alone onto the right ear on day 5. (**B**) H&E-stained mouse ear tissues at 24 h after challenge. Scale bars, 100 μ m. (**C**) Net ear swelling on day 6 in *Rag1*^{-/-} ($n = 4$ per group) or *Rag1*^{-/-} *Cd226*^{-/-} ($n = 3$ per group) mice, into which CD4⁺ T cells from WT or *Pvr*^{-/-} mice had been transferred i.v. together with WT CD8⁺ T cells 1 wk before immunization with DNCB, followed by challenge on day 5 with DNCB (left ear) or medium alone (right ear). (**D** and **E**) Intracellular analysis of cytokines in CD4⁺ T cells obtained from axillary lymph nodes of WT ($n = 3$) or *Pvr*^{-/-} ($n = 3$) mice on day 5 after immunization with DNCB. Numbers indicate the percentage of cells in each region. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

back of IFN- γ -dependent STAT1 phosphorylation and Th1 development. It remains undetermined how CD155 upregulates STAT1-independent IFN- γ expression. However, we found that stimulation with anti-CD155 and anti-CD3 mAbs induced degradation of I κ B α significantly more than that with control mAb and anti-CD3 mAbs even in the absence as well as in the presence of IFN- γ (Fig. 3F). Moreover, the expression of *Ifng* gene was significantly decreased in the presence of NF- κ B inhibitor (JSH-23) regardless of the stimulation of CD155 and CD3 (Fig. 3G). Taken together, these results suggest that CD155 triggers *Ifng* gene transcription via NF- κ B.

CHS is attenuated in CD155-deficient mice

Th1 cells play an important role in CHS (32). Because CD155-mediated costimulatory signaling is involved in Th1 development, we investigated whether CD155 deficiency affects the development of CHS. WT and *Pvr*^{-/-} mice were sensitized by using epicutaneous application of DNCB and then were challenged with DNCB on the left ear and medium alone on the right ear. The net swelling in the left ear, as determined by the difference in the thicknesses of the left and right ears, of both WT and *Pvr*^{-/-} mice was increased at 24 h after antigenic challenge. However, swelling was significantly greater in WT than *Pvr*^{-/-} mice (Fig. 4A). Supporting these results, histological analysis demonstrated that the ear swelling at 24 h after application of DNCB was considerably greater in WT mice than in *Pvr*^{-/-} mice (Fig. 4B).

Because CD155 is expressed on various immune cells, we next examined whether CD155 on CD4⁺ T cells alone was responsible for the difference in the development of CHS between WT and *Pvr*^{-/-} mice. WT or *Pvr*^{-/-} CD4⁺ T cells were transferred with WT CD8⁺ T cells into *Rag1*^{-/-} mice. One week after transfer, mice were sensitized and then challenged with DNCB as de-

scribed above to induce CHS. Ear swelling was significantly greater in the *Rag1*^{-/-} mice that received WT CD4⁺ T cells than in those that received *Pvr*^{-/-} CD4⁺ T cells (Fig. 4C). These results suggest that CD155 on CD4⁺ T cells is solely responsible for the development of CHS. Because DNAM-1, encoded by *Cd226* gene, was expressed on DC (Supplemental Fig. 1C), DNAM-1 on DC might be involved in the development of CD155-mediated CHS as a ligand for CD155. To test this hypothesis, WT or *Pvr*^{-/-} CD4⁺ T cells were transferred with WT CD8⁺ T cells into *Rag1*^{-/-} *Cd226*^{-/-} mice and then CHS was induced in these mice. Ear swelling was comparable between *Rag1*^{-/-} *Cd226*^{-/-} mice that received WT CD4⁺ T cells and those mice that received *Pvr*^{-/-} CD4⁺ T cells (Fig. 4C). These results suggest that DNAM-1 on DC plays an important role in the development of CHS as a ligand for CD155.

To confirm the role of CD155 in Th1 development during CHS, the draining lymph nodes were harvested and cytokine production was analyzed 5 d after DNCB sensitization. Flow cytometric analyses showed that the generation of IFN- γ -producing CD4⁺ T cells was impaired in *Pvr*^{-/-} mice compared with WT mice (Fig. 4D, 4E). However, the generation of IL-4- and IL-17-producing CD4⁺ T cells was comparable between WT and *Pvr*^{-/-} mice. These results indicate that CD155 is involved in the priming phase of Th1 responses after sensitization with DNCB.

CD155 deficiency enhances allergic airway inflammation

We further investigated whether CD155 deficiency reciprocally augments Th2 responses in vivo. Because CD4⁺ T cells derived from BALB/c mice similarly showed Th1 development after stimulation with anti-CD155 mAb together with anti-CD3 mAb (Fig. 5A), we examined allergic airway inflammation in WT or *Pvr*^{-/-} BALB/c mice after immunization i.p. with OVA plus alum

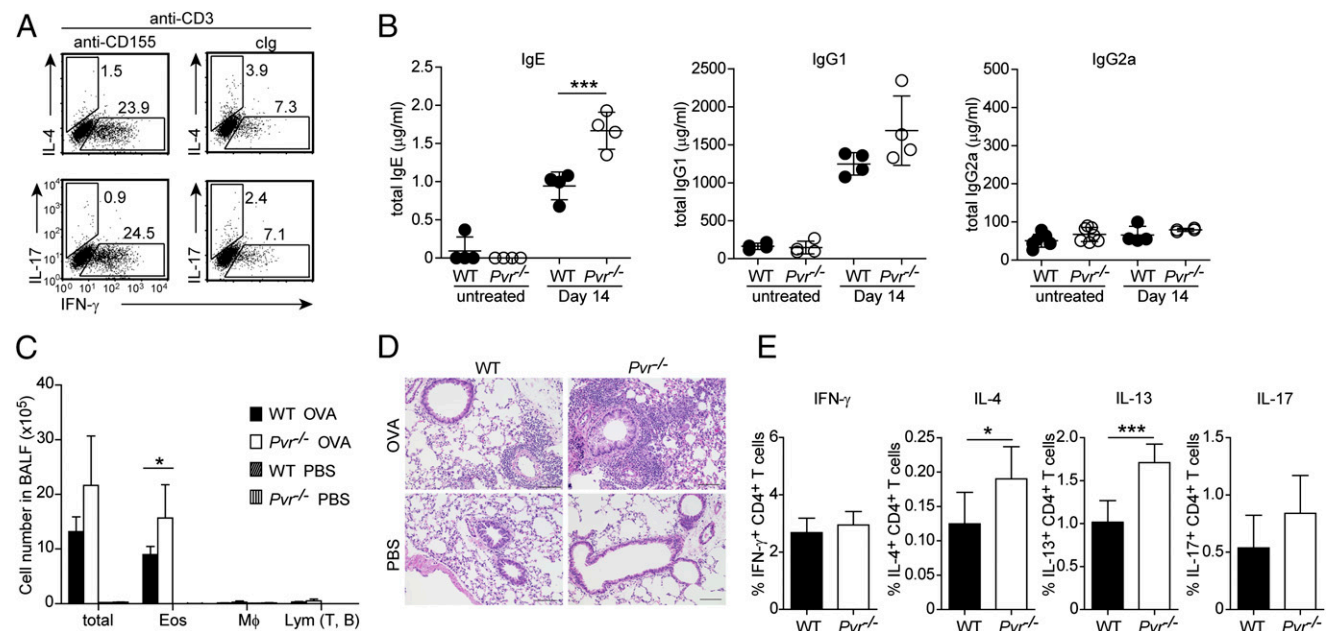


FIGURE 5. CD155 deficiency enhances allergic airway inflammation. **(A)** Cytokine analyses of IFN- γ , IL-4, and IL-17 in the cytoplasm after stimulation of CD4⁺ naive T cells from BALB/c mice with plate-bound anti-CD3 plus anti-CD155 mAbs or clg in the presence of IL-2 for 6 d, followed by restimulation with PMA plus ionomycin for 4 h. Numbers indicate the percentages of the population in each region. **(B)** ELISA of serum IgE, IgG1, and IgG2a in WT or *Pvr*^{-/-} ($n = 4-7$) mice immunized i.p. with OVA plus alum on days 0 and 7. **(C and D)** Flow cytometric analysis of total cells, eosinophils (CD11b⁺Siglec-F⁺), macrophages (CD11b⁺F4/80⁺), and lymphocytes (T cells, CD3⁺B220⁻; B cells, CD3⁺B220⁺) in bronchoalveolar lavage fluid (BALF) (C) and histological analyses of H&E-stained sections of lung tissue (D) from WT ($n = 6$) or *Pvr*^{-/-} ($n = 6$) mice immunized i.p. with OVA plus alum on days 0 and 7, challenged intranasally with OVA or PBS on days 14, 15, and 16, and analyzed on day 18. Scale bars, 100 μ m. **(E)** Intracellular analysis of cytokines in CD4⁺ T cells obtained from spleen of WT ($n = 5$) or *Pvr*^{-/-} ($n = 6$) BALB/c mice on day 11 after immunization i.p. with OVA plus alum on days 0 and 7, followed by restimulation with PMA plus ionomycin for 4 h. The mean $\pm$ 1 SD is shown. Data are representative of two independent experiments. * $p < 0.05$, *** $p < 0.005$.

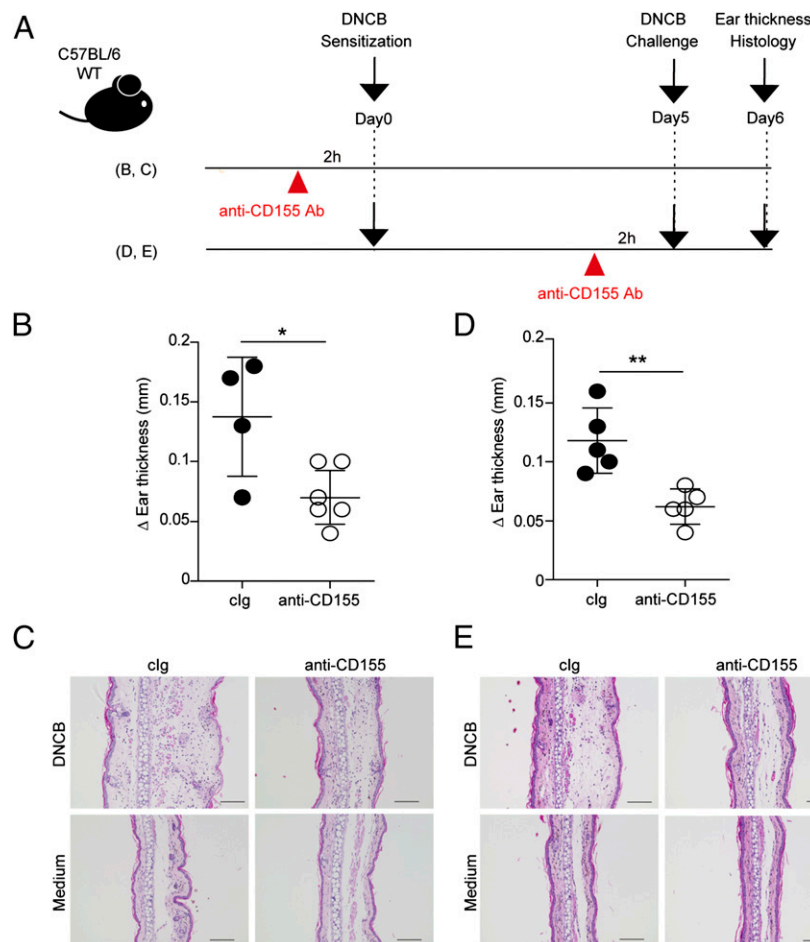


FIGURE 6. Treatment with anti-CD155 mAb suppresses CHS responses. **(A–E)** Schedule of treatment with anti-CD155 mAb to prevent CHS development **(A)**: mice were injected i.v. with anti-CD155 mAb or cIg 2 h before either sensitization **(B and C)** or challenge **(D and E)** with anti-CD155 mAb ($n = 6$ or 5) or cIg ($n = 4$ or 5). Net ear swelling and H&E-stained sections of ear tissue from mice treated with anti-CD155 mAb or cIg before either sensitization **(B and C)** or challenge **(D and E)**. The mean $\pm$ 1 SD is shown. Data are representative of two independent experiments. Scale bars, 100 μ m. * $p < 0.05$, ** $p < 0.01$.

and then challenge intranasally with OVA. *Pvr*^{-/-} mice showed significantly higher concentration of serum IgE than did WT mice on day 14 after the immunization. However, there were no differences in IgG1 and IgG2a levels between WT and *Pvr*^{-/-} mice after the immunization (Fig. 5B). *Pvr*^{-/-} mice exhibited higher numbers of eosinophils in bronchoalveolar lavage fluids (Fig. 5C) and infiltrated inflammatory cells in the lung (Fig. 5D), compared with WT mice, after challenge of OVA. Moreover, the frequency of IL-4- and IL-13-producing cells among total CD4⁺ T cells in the spleen from *Pvr*^{-/-} mice was significantly increased, compared with that from WT mice (Fig. 5E). Taken together, these results suggest that CD155-mediated signal stimulates Th1 pathway and reciprocally suppresses Th2 development both in vitro and in vivo.

Treatment with anti-CD155 mAb suppresses CHS responses

To investigate whether CD155 is a potential molecular target for preventive and therapeutic applications, we treated mice with a neutralizing anti-CD155 mAb, which blocks the interaction between CD155 and DNAM-1 (28), at 2 h before sensitization or challenge with DNCB (Fig. 6A). Suppression of ear swelling was greater in mice that received anti-CD155 mAb before sensitization than in mice that received control mAb (Fig. 6B, 6C). Similarly, administration of anti-CD155 mAb before challenge significantly suppressed CHS responses (Fig. 6D, 6E), suggesting that CD155 may be a candidate target for therapeutic approaches for ACD.

CD155 is involved in Th1 development in humans

We next examined the expression of CD155 on human PBMCs. Whereas CD155 was expressed abundantly on monocytes, it was

scarcely detectable on lymphoid cells, including CD4⁺ and CD8⁺ T cells and NK cells (Supplemental Fig. 3A). However, activation of CD4⁺ T cells through stimulation with anti-CD3 plus anti-CD28 mAbs markedly upregulated the expression of CD155 (Supplemental Fig. 3B). To investigate whether CD155 is involved in Th1 development in human, we purified CD4⁺CD45RA^{hi}CD45RO⁻ naive T cells from the cord blood, stimulated them with anti-CD3 mAb and either anti-CD155 or control Ig, and analyzed subsequent cytokine production. The concentration of IFN- γ , but not IL-4 or IL-17, in the culture supernatants of naive CD4⁺ T cells that had been stimulated with anti-CD155 plus anti-CD3 was significantly higher than that in supernatants from cells

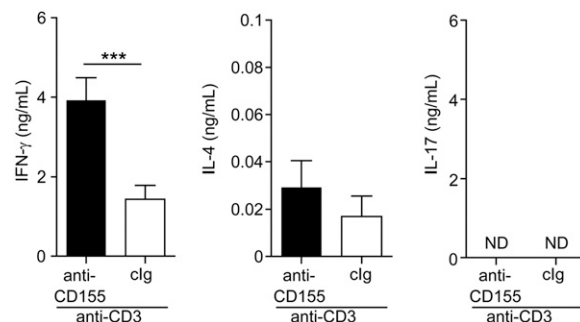


FIGURE 7. CD155 is involved in Th1 development in humans. Cytokine analyses of IFN- γ , IL-4, and IL-17 in the culture supernatant after stimulation of human CD4⁺ naive T cells with plate-bound anti-CD3 plus anti-CD155 mAbs or cIg for 6 d, followed by restimulation with PMA plus ionomycin for or 48 h. The mean $\pm$ 1 SD is shown. Data are representative of three experiments. *** $p < 0.005$.

stimulated with control Ig plus anti-CD3 (Fig. 7). These results indicate that CD155 on CD4⁺ T cells plays an important role in the development of Th1 cells in humans as in mice.

Discussion

CD155 initially was identified as a receptor for poliovirus that was expressed on human epithelial cells (13). Although mice are not infected with poliovirus, we previously identified the mouse counterpart of CD155 (33). CD155 is expressed on various hematopoietic and nonhematopoietic cells in both humans and mice. However, the physiological function of CD155, particularly in hematopoietic cells, is incompletely understood. We previously demonstrated that DNAM-1, which is a counterreceptor for CD155 and is expressed also on CD4⁺ T cells, mediates an activating signal for Th1 development in naive CD4⁺ T cells in human (30). In agreement with this context, Lozano et al. (34) suggested that DNAM-1 on CD4⁺ naive T cells mediates an activating signal for Th1/Th17 differentiation as a result of ligation with CD155. In sharp contrast, the present study demonstrated that CD155 itself mediates a costimulatory signal in CD4⁺ T cells that promotes Th1 development. These results were further supported by the *in vivo* study, which showed that whereas CHS was significantly greater in *Rag1*^{-/-} mice that received WT CD4⁺ T cells than in those that received *Pvr*^{-/-} CD4⁺ T cells, it was comparable between *Rag1*^{-/-} *Cd226*^{-/-} mice that received WT CD4⁺ T cells and those mice that received *Pvr*^{-/-} CD4⁺ T cells. These results suggest that CD155-mediated signal in CD4⁺ T cells triggered by the *trans* interaction with DNAM-1 on APC, rather than DNAM-1-mediated signal in CD4⁺ T cells triggered by the *cis* interaction with CD155 on CD4⁺ T cells, was involved in the development of CHS.

Th1 development is induced through both IL-12-dependent and -independent mechanisms (12). In the present study, we showed that CD155-mediated Th1 development does not require IL-12. Previous reports demonstrated that the costimulatory molecule LFA-1 is involved in Th1 development in an IL-12-independent manner (30, 35), in which Erk phosphorylation is sustained for >30 min. The sustained phosphorylation of Erk downregulates GATA-3 but upregulates T-bet, resulting in Th1 development from naive CD4⁺ T cells (30, 35). CD27, a member of the TNF receptor family, mediates another costimulatory signal that induces Th1 differentiation in an IL-12-independent manner (36, 37), although the molecular mechanisms underlying this effect remain undetermined as yet. Although we showed that the CD155-mediated costimulatory signal also enhances Erk phosphorylation, Erk was dephosphorylated in several minutes in our experiments (Fig. 1B). Therefore, CD155 is involved in Th1 development by a mechanism distinct from LFA-1. Instead, we showed that CD155 induced Th1 development in an autocrine IFN- γ -dependent manner. CD155 also induced NF- κ B activation, which was independent of IFN- γ . Evidence demonstrated that p50 and p60 NF- κ B subunits bind to the IFN- γ promoter and upregulate IFN- γ expression (38). Therefore, CD155 signaling induces IFN- γ secretion via NF- κ B, leading to autocrine IFN- γ -dependent STAT1 activation and the subsequent upregulation of T-bet expression (Supplemental Fig. 4). Previous reports also demonstrated that CD155 recruits the Src homology 2 domain-containing protein tyrosine phosphatase 2 (SHP-2), which activates MAPK (39, 40). How CD155 activates NF- κ B is unknown currently but SHP-2 might also be involved in NF- κ B activation in CD4⁺ T cells. A recent study has demonstrated that the CD155 promoter region contains an NF- κ B binding site (41), suggesting a positive feedback loop in which CD155 and NF- κ B stimulate each other.

The costimulatory signal mediated by CD155 induced Th1 development from naive CD4⁺ T cells in both humans and mice and exacerbated Th1-mediated CHS and ameliorated Th2-mediated airway inflammation in mice. We showed that treatment with anti-CD155 mAb dampened CHS. Treatment with anti-LFA-1 mAb has been reported to similarly inhibit the development of CHS (42). However, although LFA-1 also mediates a costimulatory signal that leads to Th1 development from naive CD4⁺ T cells, mice deficient in LFA-1 or treatment of mice with anti-LFA-1 mAb show impaired migration of T cells to draining lymph nodes (43), leading to overall T cell dysfunction. In contrast, we observed that cell migration to draining lymph nodes was not impaired in CD155-deficient mice, suggesting that CD155 is not involved in T cell migration into lymph nodes. Notably, treatment with anti-CD155 mAb was effective when injected after as well as before sensitization with DNCB Ag. These characteristics of anti-CD155 treatment are advantageous for its clinical use in the prevention of ACD, because most patients have been already sensitized to a specific allergen.

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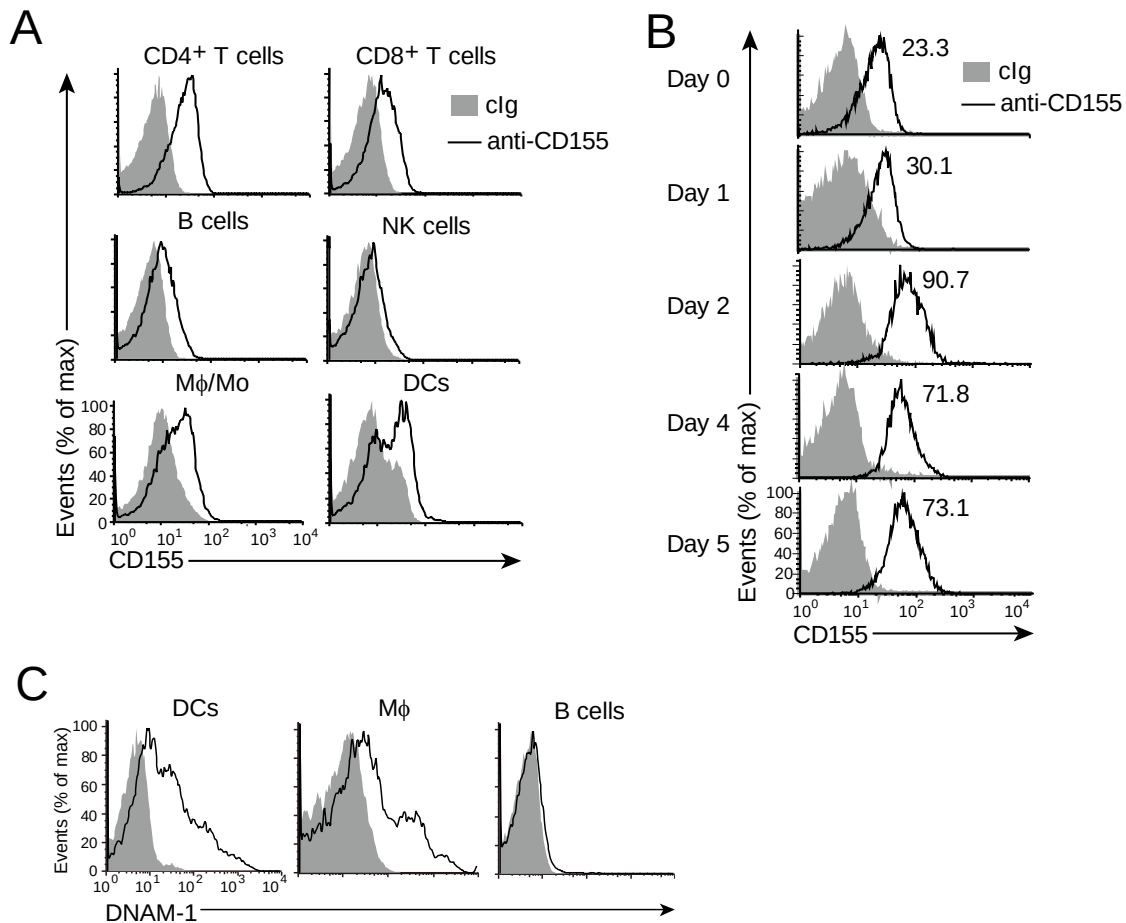
Disclosures

The authors have no financial conflicts of interest.

References

- Wambre, E., E. A. James, and W. W. Kwok. 2012. Characterization of CD4⁺ T cell subsets in allergy. *Curr. Opin. Immunol.* 24: 700–706.
- Galli, S. J., and M. Tsai. 2012. IgE and mast cells in allergic disease. *Nat. Med.* 18: 693–704.
- Holgate, S. T., and R. Polosa. 2008. Treatment strategies for allergy and asthma. *Nat. Rev. Immunol.* 8: 218–230.
- Gavett, S. H., D. J. O'Hearn, X. Li, S.-K. Huang, F. D. Finkelman, and M. Wills-Karp. 1995. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J. Exp. Med.* 182: 1527–1536.
- Honda, T., G. Egawa, S. Grabbe, and K. Kabashima. 2013. Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. *J. Invest. Dermatol.* 133: 303–315.
- Kondo, S., F. Kooshesh, B. Wang, H. Fujisawa, and D. N. Sauder. 1996. Contribution of the CD28 molecule to allergic and irritant-induced skin reactions in CD28^{-/-} mice. *J. Immunol.* 157: 4822–4829.
- Chen, A. I., A. J. McAdam, J. E. Buhlmann, S. Scott, M. L. Lupper, Jr., E. A. Greenfield, P. R. Baum, W. C. Fanslow, D. M. Calderhead, G. J. Freeman, and A. H. Sharpe. 1999. OX40-ligand has a critical costimulatory role in dendritic cell:T cell interactions. *Immunity* 11: 689–698.
- Schmits, R., T. M. Kündig, D. M. Baker, G. Shumaker, J. J. Simard, G. Duncan, A. Wakeham, A. Shahinian, A. van der Heiden, M. F. Bachmann, et al. 1996. LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. *J. Exp. Med.* 183: 1415–1426.
- Murphy, K. M., and S. L. Reiner. 2002. The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2: 933–944.
- Afkarian, M., J. R. Sedy, J. Yang, N. G. Jacobson, N. Cereb, S. Y. Yang, T. L. Murphy, and K. M. Murphy. 2002. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4⁺ T cells. *Nat. Immunol.* 3: 549–557.
- Robinson, D. S., and A. O'Garra. 2002. Further checkpoints in Th1 development. *Immunity* 16: 755–758.
- Magram, J., S. E. Connaughton, R. R. Warriar, D. M. Carvajal, C. Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, and M. K. Gately. 1996. IL-12-deficient mice are defective in IFN γ production and type 1 cytokine responses. *Immunity* 4: 471–481.
- Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello. 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* 56: 855–865.
- Koike, S., H. Horie, I. Ise, A. Okitsu, M. Yoshida, N. Iizuka, K. Takeuchi, T. Takegami, and A. Nomoto. 1990. The poliovirus receptor protein is produced both as membrane-bound and secreted forms. *EMBO J.* 9: 3217–3224.
- Lim, Y.-P., L. C. Fowler, D. C. Hixson, T. Webbe, and N. L. Thompson. 1996. TuAg.1 is the liver isoform of the rat colon tumor-associated antigen pE4 and a member of the immunoglobulin-like supergene family. *Cancer Res.* 56: 3934–3940.

16. Maier, M. K., S. Seth, N. Czeloth, Q. Qiu, I. Ravens, E. Kremmer, M. Ebel, W. Müller, O. Pabst, R. Förster, and G. Bernhardt. 2007. The adhesion receptor CD155 determines the magnitude of humoral immune responses against orally ingested antigens. *Eur. J. Immunol.* 37: 2214–2225.
17. Takai, Y., J. Miyoshi, W. Ikeda, and H. Ogita. 2008. Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat. Rev. Mol. Cell Biol.* 9: 603–615.
18. Shibuya, A., D. Campbell, C. Hannum, H. Yssel, K. Franz-Bacon, T. McClanahan, T. Kitamura, J. Nicholl, G. R. Sutherland, L. L. Lanier, and J. H. Phillips. 1996. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity* 4: 573–581.
19. Bottino, C., R. Castriconi, D. Pende, P. Rivera, M. Nanni, B. Carnemolla, C. Cantoni, J. Grassi, S. Marcenaro, N. Reymond, et al. 2003. Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J. Exp. Med.* 198: 557–567.
20. Tahara-Hanaoka, S., K. Shibuya, Y. Onoda, H. Zhang, S. Yamazaki, A. Miyamoto, S. Honda, L. L. Lanier, and A. Shibuya. 2004. Functional characterization of DNAM-1 (CD226) interaction with its ligands PVR (CD155) and nectin-2 (PRR-2/CD112). *Int. Immunol.* 16: 533–538.
21. Wang, P. L., S. O'Farrell, C. Clayberger, and A. M. Krensky. 1992. Identification and molecular cloning of tactile. A novel human T cell activation antigen that is a member of the Ig gene superfamily. *J. Immunol.* 148: 2600–2608.
22. Yu, X., K. Harden, L. C. Gonzalez, M. Francesco, E. Chiang, B. Irving, I. Tom, S. Ivelja, C. J. Refino, H. Clark, et al. 2009. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat. Immunol.* 10: 48–57.
23. Seth, S., I. Ravens, E. Kremmer, M. K. Maier, U. Hadis, S. Hardtke, R. Förster, and G. Bernhardt. 2009. Abundance of follicular helper T cells in Peyer's patches is modulated by CD155. *Eur. J. Immunol.* 39: 3160–3170.
24. Boles, K. S., W. Vermi, F. Facchetti, A. Fuchs, T. J. Wilson, T. G. Diacovo, M. Cella, and M. Colonna. 2009. A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur. J. Immunol.* 39: 695–703.
25. Qiu, Q., I. Ravens, S. Seth, A. Rathinasamy, M. K. Maier, A. Davalos-Misnitz, R. Forster, and G. Bernhardt. 2010. CD155 is involved in negative selection and is required to retain terminally maturing CD8 T cells in thymus. *J. Immunol.* 184: 1681–1689.
26. Barnaden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based α - and β -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76: 34–40.
27. Iguchi-Manaka, A., H. Kai, Y. Yamashita, K. Shibata, S. Tahara-Hanaoka, S. Honda, T. Yasui, H. Kikutani, K. Shibuya, and A. Shibuya. 2008. Accelerated tumor growth in mice deficient in DNAM-1 receptor. *J. Exp. Med.* 205: 2959–2964.
28. Nabekura, T., K. Shibuya, E. Takenaka, H. Kai, K. Shibata, Y. Yamashita, K. Harada, S. Tahara-Hanaoka, S. Honda, and A. Shibuya. 2010. Critical role of DNAX accessory molecule-1 (DNAM-1) in the development of acute graft-versus-host disease in mice. *Proc. Natl. Acad. Sci. USA* 107: 18593–18598.
29. Tahara-Hanaoka, S., K. Shibuya, H. Kai, A. Miyamoto, Y. Morikawa, N. Ohkuchi, S. Honda, and A. Shibuya. 2006. Tumor rejection by the poliovirus receptor family ligands of the DNAM-1 (CD226) receptor. *Blood* 107: 1491–1496.
30. Shibuya, K., J. Shirakawa, T. Kameyama, S. Honda, S. Tahara-Hanaoka, A. Miyamoto, M. Onodera, T. Sumida, H. Nakauchi, H. Miyoshi, and A. Shibuya. 2003. CD226 (DNAM-1) is involved in lymphocyte function-associated antigen 1 costimulatory signal for naive T cell differentiation and proliferation. *J. Exp. Med.* 198: 1829–1839.
31. Koretzky, G. A., and P. S. Myung. 2001. Positive and negative regulation of T-cell activation by adaptor proteins. *Nat. Rev. Immunol.* 1: 95–107.
32. Wang, B., H. Fujisawa, L. Zhuang, I. Freed, B. G. Howell, S. Shahid, G. M. Shivji, T. W. Mak, and D. N. Sauder. 2000. CD4⁺ Th1 and CD8⁺ type 1 cytotoxic T cells both play a crucial role in the full development of contact hypersensitivity. *J. Immunol.* 165: 6783–6790.
33. Tahara-Hanaoka, S., A. Miyamoto, A. Hara, S. Honda, K. Shibuya, and A. Shibuya. 2005. Identification and characterization of murine DNAM-1 (CD226) and its poliovirus receptor family ligands. *Biochem. Biophys. Res. Commun.* 329: 996–1000.
34. Lozano, E., N. Joller, Y. Cao, V. K. Kuchroo, and D. A. Hafler. 2013. The CD226/CD155 interaction regulates the proinflammatory (Th1/Th17)/anti-inflammatory (Th2) balance in humans. *J. Immunol.* 191: 3673–3680.
35. Wang, Y., K. Shibuya, Y. Yamashita, J. Shirakawa, K. Shibata, H. Kai, T. Yokosuka, T. Saito, S. Honda, S. Tahara-Hanaoka, and A. Shibuya. 2008. LFA-1 decreases the antigen dose for T cell activation in vivo. *Int. Immunol.* 20: 1119–1127.
36. Hendriks, J., L. A. Gravestein, K. Tesselaar, R. A. van Lier, T. N. Schumacher, and J. Borst. 2000. CD27 is required for generation and long-term maintenance of T cell immunity. *Nat. Immunol.* 1: 433–440.
37. Soares, H., H. Waechter, N. Glaichenhaus, E. Mougneau, H. Yagita, O. Mizenina, D. Dudziak, M. C. Nussenzweig, and R. M. Steinman. 2007. A subset of dendritic cells induces CD4⁺ T cells to produce IFN- γ by an IL-12-independent but CD70-dependent mechanism in vivo. *J. Exp. Med.* 204: 1095–1106.
38. Sica, A., L. Dorman, V. Viggiano, M. Cippitelli, P. Ghosh, N. Rice, and H. A. Young. 1997. Interaction of NF- κ B and NFAT with the interferon- γ promoter. *J. Biol. Chem.* 272: 30412–30420.
39. Neel, B. G., H. Gu, and L. Pao. 2003. The “Shp”ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends Biochem. Sci.* 28: 284–293.
40. Oda, T., S. Ohka, and A. Nomoto. 2004. Ligand stimulation of CD155 α inhibits cell adhesion and enhances cell migration in fibroblasts. *Biochem. Biophys. Res. Commun.* 319: 1253–1264.
41. Kamran, N., Y. Takai, J. Miyoshi, S. K. Biswas, J. S. Wong, and S. Gasser. 2013. Toll-like receptor ligands induce expression of the costimulatory molecule CD155 on antigen-presenting cells. *PLoS ONE* 8: e54406.
42. Murayama, M., H. Yasuda, Y. Nishimura, and M. Asahi. 1997. Suppression of mouse contact hypersensitivity after treatment with antibodies to leukocyte function-associated antigen-1 and intracellular adhesion molecules-1. *Arch. Dermatol. Res.* 289: 98–103.
43. Berlin-Rufenach, C., F. Otto, M. Mathies, J. Westermann, M. J. Owen, A. Hamann, and N. Hogg. 1999. Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J. Exp. Med.* 189: 1467–1478.

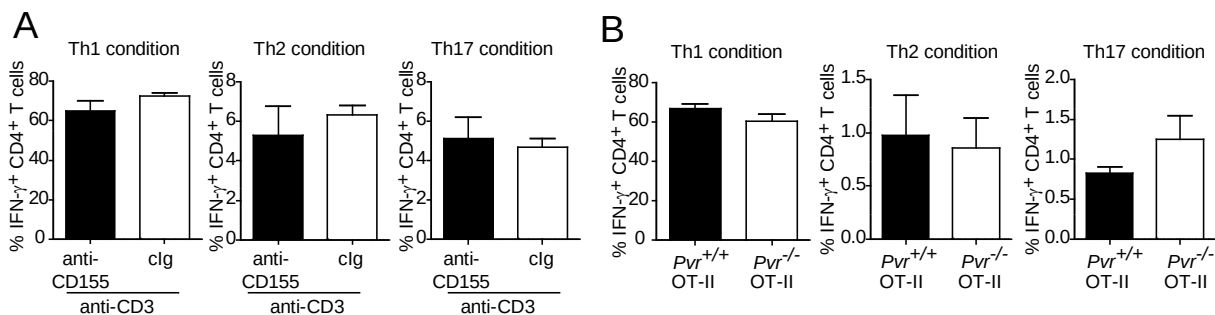


Supplemental Figure 1. CD155 is expressed most strongly on CD4⁺ T cells and DNAM-1, a ligand for CD155, is expressed on APCs.

(A and B) Flow cytometric analysis of mouse CD4⁺ T cells, CD8⁺ T cells, NK1.1⁺ CD3⁻ NK cells, B220⁺ B cells, CD11b⁺ Ly6G⁻ macrophages/monocytes (Mφ/Mo), and CD11c⁺ dendritic cells (DCs) in the spleen stained with biotinylated anti-CD155 mAb (open histogram) or isotype control (clg; shaded histogram), followed by allophycocyanin (APC)-conjugated streptavidin (A). CD4⁺ T cells were stimulated with anti-CD3 (1 μ g/ml) plus anti-CD28 mAbs (1 μ g/ml), and CD155 expression was analyzed. Numbers in histograms indicate mean fluorescence intensities (B).

(C) Flow cytometric analysis of the expression of DNAM-1, (open histogram) on dendritic cells (CD11c⁺ cells), macrophages (Mφ) (CD11b⁺ Ly6G⁻ cells), and B cells (B220⁺ cells) from the spleen of mice. Shaded histograms indicate staining with isotype control.

Data are representative of two independent experiments.

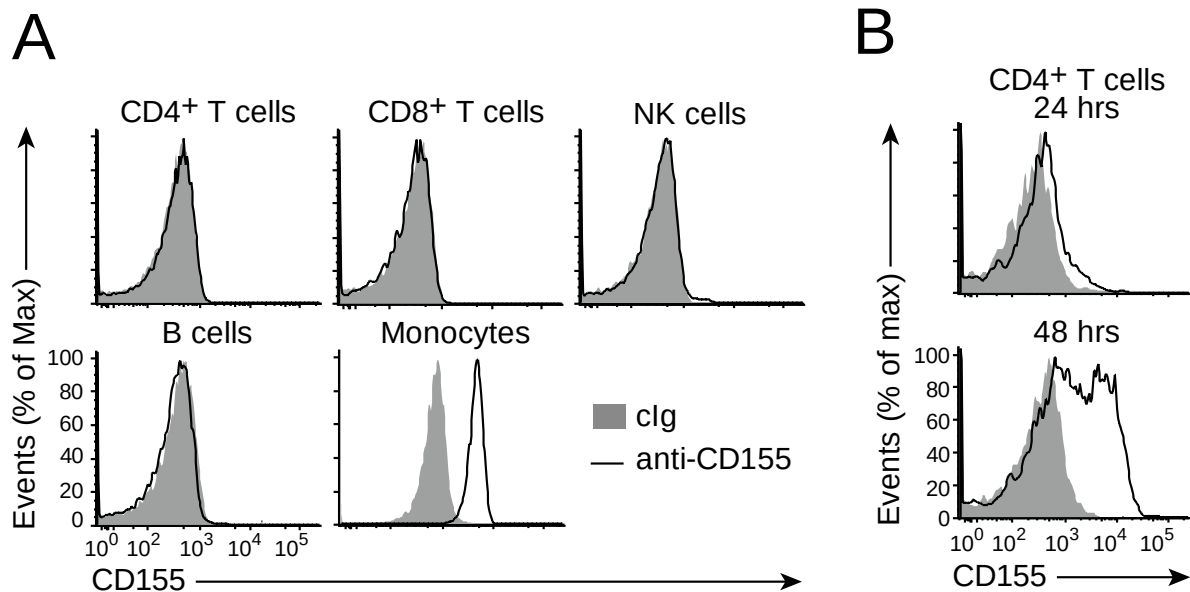


Supplemental Figure 2. CD155 is not necessarily required for Th1 differentiation when in the presence of adequate exogenous IL-12.

(A) Cytokine analyses of IFN- γ in the cytoplasm after stimulation of CD4⁺ naïve T cells from C57BL/6 mice with plate-bound anti-CD3 plus anti-CD155 mAbs or clg in the presence of IL-2 for 6 d, followed by restimulation with phorbol myristate acetate (PMA) plus ionomycin for 4 h. Th1 condition; 20 ng/ml IL-12 plus 2.5 μ g/ml anti-IL-4, Th2 condition; 50 ng/ml IL-4 plus 10 μ g/ml anti-IFN- γ , Th17 condition; 10 ng/ml IL-6 plus 5 ng/ml TGF- β plus 10 μ g/ml anti-IFN- γ plus 2.5 μ g/ml anti-IL-4. Statistical data are indicated.

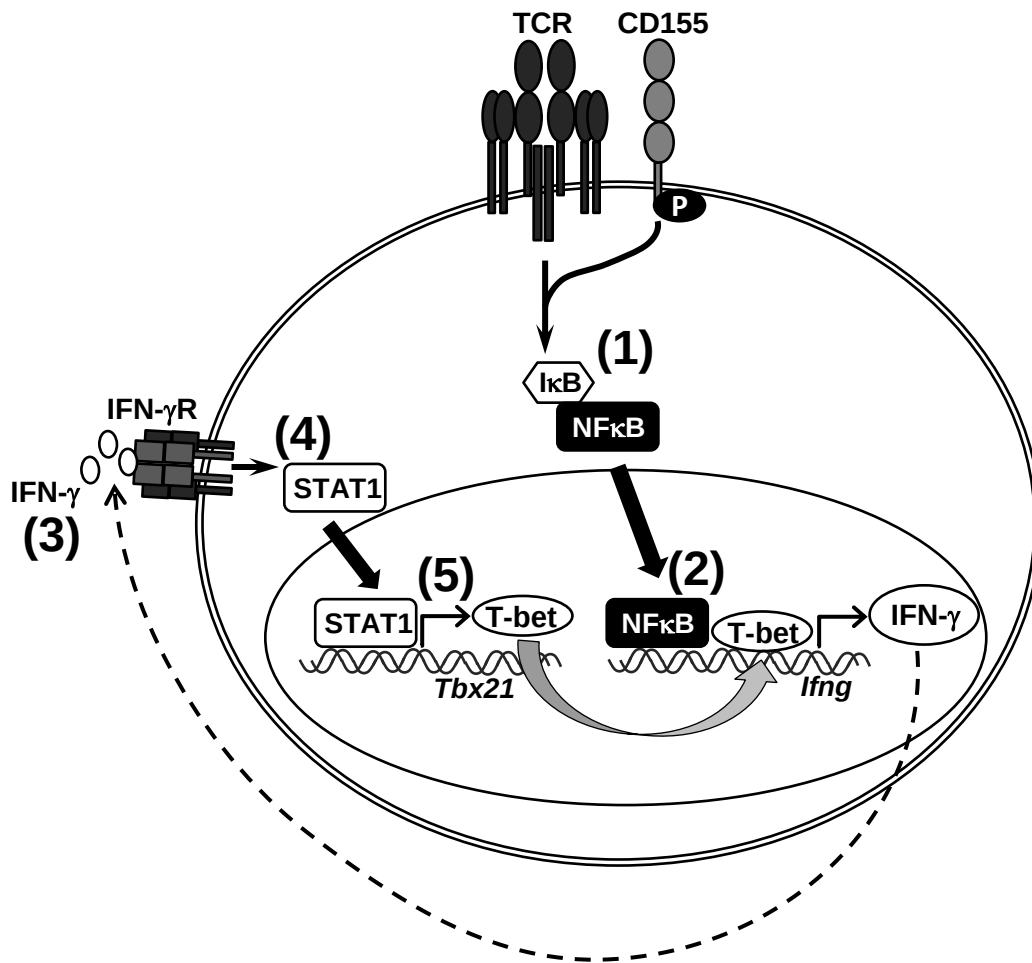
(B) Cytokine analyses of IFN- γ in the cytoplasm after stimulation of CD4⁺ naïve T cells from *Pvr*^{+/+} or *Pvr*^{-/-} OT-II Tg mice after coculture for 6 d with WT splenocytes loaded with OVA peptide, followed by restimulation as in (A).

The mean $\pm$ 1SD is shown. Data are representative of two independent experiments.



Supplemental Figure 3. Expression of CD155 on human peripheral blood.

(A and B) Flow cytometric analysis of human CD4⁺ T cells, CD8⁺ T cells, CD56⁺ CD3⁻ NK cells, CD19⁺ B cells, and CD14⁺ monocytes in the peripheral blood that were stained with PE-conjugated anti-CD155 mAb (open histogram) or isotype control (shaded histogram) (A). Peripheral blood mononuclear cells were stimulated with anti-CD3 (1 μ g/ml) plus anti-CD28 (1 μ g/ml) mAbs for 24 or 48 h in the presence of IL-2 (10 ng/ml), after which CD155 expression was analyzed (B). Data are representative of three experiments.



Supplemental Figure 4. CD155 triggers *Ifng* gene transcription via NFκB.

CD155 cooperates the TCR signaling and promotes NFκB activation (1), which then induces *Ifng* transcription (2). IFN-γ binds to IFN-γ receptor (3) and induces STAT1 activation (4), followed by transcription of *Tbx21* (5), which encodes T-bet, the master regulator of Th1 development.