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Human TCR Transgenic Bet v 1-Specific Th1 Cells Suppress the Effector Function of Bet v 1-Specific Th2 Cells

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Pollinosis to birch pollen is a common type I allergy in the Northern Hemisphere. Moreover, birch pollen-allergic individuals sensitized to the major birch pollen allergen Bet v 1 frequently develop allergic reactions to stone fruits, hazelnuts, and certain vegetables due to immunological cross-reactivity. The major T cell epitope Bet v 1_{142–153} plays an important role in cross-reactivity between the respiratory allergen Bet v 1 and its homologous food allergens. In this study, we cloned and functionally analyzed a human $\alpha\beta$ TCR specific for the immunodominant epitope Bet v 1_{142–153}. cDNAs encoding TCR α - and β -chains were amplified from a Bet v 1_{142–153}-specific T cell clone, introduced into Jurkat T cells and peripheral blood T lymphocytes of allergic and nonallergic individuals, and evaluated functionally. The resulting TCR transgenic (TCRtg) T cells responded in an allergen-specific and costimulation-dependent manner to APCs either pulsed with Bet v 1_{142–153} peptide or coexpressing invariant chain::Bet v 1_{142–153} fusion proteins. TCRtg T cells responded to Bet v 1-related food and tree pollen allergens that were processed and presented by monocyte-derived dendritic cells. Bet v 1_{142–153}-presenting but not Bet v 1_{4–15}-presenting artificial APCs coexpressing membrane-bound IL-12 polarized allergen-specific TCRtg T cells toward a Th1 phenotype, producing high levels of IFN- γ . Coculture of such Th1-polarized T cells with allergen-specific Th2-differentiated T cells significantly suppressed Th2 effector cytokine production. These data suggest that human allergen-specific TCR can transfer the fine specificity of the original T cell clone to heterologous T cells, which in turn can be instructed to modulate the effector function of the disease initiating/perpetuating allergen-specific Th2-differentiated T cells. *The Journal of Immunology*, 2011, 187: 000–000.

Birch pollen is one of the main causes of pollinosis in the Northern Hemisphere from spring to early summer (1). More than 95% of birch pollen-allergic individuals mount an IgE Ab response against Bet v 1, the major birch pollen allergen (2). T cells from these patients preferentially recognize the immunodominant epitope Bet v 1_{142–153}. This region is located at the highly conserved C terminus of Bet v 1 and shares considerable homology with related food and pollen allergens (3). Recent

studies have demonstrated that various Bet v 1 peptides bind with high affinity to different HLA-DR molecules (4). The structures targeted by both the humoral and cellular immune responses to Bet v 1 are sufficiently characterized at the molecular level to allow the development of knowledge-based therapies for treatment of birch pollen atopic disorders (3, 5).

Individuals allergic to birch pollen frequently develop type I hypersensitivity reactions against certain foods; for example, apple, celery, carrot, or hazelnut (6, 7). Ingestion of these foods causes the oral allergy syndrome, such as an itching, burning, or tingling sensation of the lips, mouth, or pharynx (8, 9). The molecular basis for these symptoms is Bet v 1-specific IgE Abs, which cross-react with tertiary structural determinants on homologous proteins within these foods (10, 11). Moreover, Bet v 1-specific T cells are also efficiently activated by Bet v 1-homologous food allergens (3). But, in marked contrast to IgE-mediated effects, T cell cross-reactivity is not abolished by cooking or by digestion of birch pollen-related food allergens (12) because these processes do not destroy T cell epitopes (13). Thus, ingestion of pollen-related food allergens can lead to aggravation of T cell-mediated immunopathology, such as exacerbation of eczema in patients with atopic dermatitis (14).

In the past, experimental tools have been devised that allow exploration of T cell responses in more detail. For instance, the Ag specificity of T cells can be conveniently transferred to other T cells by $\alpha\beta$ TCR gene transfer (15–17). How might allergy research benefit from recombinant TCRs recognizing major allergens? The answers are severalfold: first, recombinant allergen-specific TCRs should allow the generation of large numbers of specific T cells in a short period of time independent of the allergic status of the donor and the allergy season; second, the availability of well-defined allergen-specific T cells favors the creation of biological model systems in which all three components of the allergen-

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Abbreviations used in this article: aAPC, artificial Ag-presenting cell; Ii, invariant chain; MDDC, monocyte-derived dendritic cell; PB, peripheral blood; TCC, T cell clone; TCRtg, TCR transgenic.

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specific synapse (HLA molecule, allergen peptide, and TCR) are well defined; and third, the recombinant TCR technology should allow engineering of allergen-specific T cells with immunomodulatory capacity (e.g., for adoptive transfer to modulate an existing Th2-dominated allergen-specific response).

Initial steps toward the realization of these objectives have been made possible by the transfer of a human allergen (Art v 1)-specific TCR into human T lymphocytes (18). The resulting TCR transgenic (TCRtg) T cells strictly responded according to the two-signal model described by Bretscher and Cohn (18, 19). In the absence of costimuli, TCRtg T cells receiving solely allergen-specific signal 1 developed a state of unresponsiveness (20). These studies have afforded a rational strategy for anergizing allergen-specific CD4⁺ T lymphocytes.

In the current study, we characterized a Bet v 1-specific TCR that recognizes the immunodominant epitope Bet v 1_{142–153} (3). For this purpose, we isolated cDNA encoding the α - and β -chains of the TCR of a Bet v 1-specific T cell clone (TCC), transferred the TCR chains into Jurkat T cells and peripheral blood (PB) T cells of nonallergic individuals, and studied T cell function upon coculture with APCs presenting allergen plus various combinations of costimulators. Furthermore, we analyzed whether the transferred TCR retained its original fine specificity (i.e., the capacity to cross-react with Bet v 1-related food and pollen allergens). Finally, by combining recently developed APC technologies, we asked whether strictly Th1-polarized TCRtg Bet v 1-specific PB T cells could be conveniently generated and whether such T cells would be able to modulate the effector function of Th2-differentiated cells.

Materials and Methods

Cell lines and primary cells

293 cells and Jurkat TCC 41-19 expressing an IL-2 enhancer/promoter driving luciferase were cultured as described (21). Bet v 1-specific TCCs and immortalized B cells were established from PBMCs of birch pollen-allergic individuals with typical clinical history and positive skin-prick tests to birch pollen extract as described (22). TCR gene usage was determined by RT-PCR with TRAV and TRBV primers (Clontech, Heidelberg, Germany) as described (23). PBMCs from allergic and nonallergic individuals were obtained upon informed consent in accordance with institutional ethics guidelines.

Recombinant allergens

Recombinant allergens (Api g 1.0101; Bet v 1.0101; Dau c 1.0103; Cor a 1.0103; Mal d 1.0108) were purchased from Biomay AG (Vienna, Austria) or produced and purified to homogeneity [Car b 1.0109 (EU283857); Cas s 1.0101 (FJ390843.1); Fag s 1.0101 (FJ390846.1); Que a 1.0301 (EU283863); accession numbers in parentheses were submitted to GenBank] as described elsewhere (24).

Cloning and characterization of $\alpha\beta$ TCRs

mRNA from the Bet v 1_{142–153}-specific HLA-DRB1*07:01-restricted, Th2-like TCC SD334 (3) was amplified with TRAV6.01-forward 5'-CGCGGGAAGCTTGCCACCATGGAGTCATTCCTGGGAGGTGT-3' and TRAC-reverse 5'-CCCGCGGCGCGCTTTAGCTGGACACAGCCGC-3' or TRBV20.01-forward 5'-CGCGGGAAGCTTGCCACCATGCTGCTGCTTCTGCTGCTT-3' and TRBC-reverse 5'-CCCGCGGCGCGCTTTAGAAATCCTTTCTCTTGACCATG-3', digested with NcoI (bold) or HindIII (italics) and NotI (underlined), and gel purified. DNA fragments were ligated into the retroviral expression vector pMMP412 (R.C. Mulligan, Children's Hospital, Boston, MA) and sequenced (VBC Genomics, Vienna, Austria). [Note: HLA nomenclature in this article is given according to the 2010 update and using colons (:) in the allele names to act as delimiters of the separate fields according to Marsh et al. (25).]

Cloning of HLA-DR constructs

mRNA from autologous EBV-immortalized B cells was amplified with HLA-DRB1*07:01-forward 5'-CGCGGGAAGCTTGCCACCATGGTGTGCTGAAGCTCCC-3' and HLA-DRB1*07:01-reverse 5'-CCCGCG-

GCGGCCGCTTTAGCTCAGGAATCCTGTTGGCT-3', digested with HindIII (bold) and NotI (underlined). Gel-purified DNA fragments were ligated into pEAK12 expression vector (EdgeBio, Gaithersburg, MD) and sequenced.

Generation of Bet v 1-containing invariant chain constructs

pcDNA1.1/Amp containing an invariant chain (Ii) with an SfuI-Eco47III cassette instead of CLIP was kindly provided by J. van Bergen (Department of Immunohematology and Blood Transfusion, Leiden, The Netherlands) (26). The following double-stranded oligonucleotides were inserted into SfuI/Eco47III digested pcDNA1.1/Amp-Ii: Bet v 1_{4–15}-upper 5'-CGAAGTACGAGACCGAGACACCAGCGTGATCCCCGC-3', Bet v 1_{4–15}-lower 5'-GCGGGGATCAGCTGGTGGTCTCGGTCTCGTAGTT-3'; Bet v 1_{112–123}-upper 5'-CGTCCATCTGAAGATCTCCAACAAGT-ACCACACCAA-3', Bet v 1_{112–123}-lower 5'-TTGGTGTGGTACTTGT-TGGAGATCTTCAGGATGGA-3'; Bet v 1_{142–153}-upper 5'-CGACCCTGCTGCGCGCCGTGGAGTCTACCTGCTGGC-3', Bet v 1_{142–153}-lower 5'-GCCAGCAGGTAGGACTCCAGCGCGCAGCAGGT-3'. Ii containing CLIP or HA_{309–317} was constructed as described (26). Proper DNA insertion was confirmed by sequence analysis (VBC Genomics).

Transduction of $\alpha\beta$ TCRs into Jurkat and PB T cells

Bet v 1_{142–153}-specific TCR constructs in pMMP412.TRAV6 and pMMP412.TRBV20 or Art v 1_{25–36}-specific TCR constructs pMMP412.TRAV17 and pMMP412.TRBV18 were transduced into Jurkat T cells, bulk or sorted naive CD4⁺CD45RA⁺ PB T cells (Miltenyi, Bergisch Gladbach, Germany; purity of sorted T cells >98%) as described previously (18). In some experiments, a multicistronic strategy was used to express the Bet v 1-specific TCR, linking TRAV6 and TRBV20 via a P2A sequence in an IRES-GFP-containing version of pMMP. TCRtg Jurkat clones were obtained by limiting dilution. (The nucleotide sequences for the Bet v 1_{142–153}-specific TCR α and β chains have been deposited in the GenBank database under accession numbers GQ179994 and GQ179995, respectively; <http://www.ncbi.nlm.nih.gov/genbank/>.)

Immunofluorescence analyses and intracellular cytokine determination

mAbs CD3-FITC (UCHT1), pan TCR $\alpha\beta$ -PE (BMA031), CD4-FITC (VIT4), CD8-PE (VIT8b), and control Ig FITC and PE were obtained from An der Grub (Kaumberg, Austria). TCR β -chain-specific mAbs TRBV12-FITC (56C5) and TRBV20-PE (MPB2D5) were from Immunotech (Marseille, France), and CD45RA (MEM-56) was from Invitrogen (Carlsbad, CA). HLA class II-FITC (1/47) and Ii-FITC (5-329) were kindly provided by O. Majdic (Institute of Immunology, Medical University of Vienna, Vienna, Austria). Staining and flow cytometry was performed as described (18). Intracellular cytokine production was analyzed after pretreatment for 6 h with GolgiStop (1:1500; Becton Dickinson, Palo Alto, CA) using Fix and Perm (An der Grub) and using Abs specific for IFN- γ (25723-PerCP; R&D Systems, Minneapolis, MN), IL-2 (MQ1-17H12-allophycocyanin; eBioscience, San Diego, CA), IL-4 (MP4-25D2-PE; Invitrogen, Camarillo, CA), IL-5 (JES1-39D10-PE; BioLegend, San Diego, CA), IL-10 (JES3-9D7; eBioscience), and IL-13 (JES10-5A2-allophycocyanin; BioLegend).

Generation of artificial APCs

293 cells (3×10^6) were transiently cotransfected with CD80::GPI, CD54::GPI, cathepsin S, HLA-DRA*01:01, and either HLA-DRB1*07:01 or HLA-DRB1*01:01 in combinations as indicated. Alternatively, artificial Ag-presenting cells (aAPCs) were transfected in addition with Ii-allergen fusion constructs Ii::Bet v 1_{4–15}, Ii::Bet v 1_{112–123}, Ii::Bet v 1_{142–153}, or Ii::Art v 1_{25–36} and used 72 h after transfection. For polarization experiments, aAPCs were variably transfected with single-chain IL-12::GPI. IL-12::GPI had been constructed from pORF-hIL-12 (Invivogen, San Diego, CA) with primers IL-12 forward 5'-GCCAAGGAGGGCCACCATGGGTC-3' and IL-12 reverse 5'-CCCGCGCTAGCGGAGCCGCCGCCGCTGCCGCCGCCGCTGCCGCCGCTGCCGCCGCTGAGGAAGCATTCAGATGATCATC-3' followed by digestion with NcoI (bold) and NheI (underlined) and ligation together with a HindIII-NcoI linker into pEAK12_CD80::GPI (21).

Generation of monocyte-derived dendritic cells

Monocyte-derived dendritic cells (MDDCs) were differentiated as described previously (27) and incubated at 2×10^5 /well in 96-well flat-bottom culture plates with allergens (range, 0.01×10^{-6} to 9.4×10^{-6} M) in a total volume of 100 μ l at 37°C overnight. Subsequently, TCRtg Jurkat T cells (1×10^5 /well) were added and cocultured (total volume 200 μ l) for 6 h.

Jurkat IL-2 promoter activity assays

aAPCs expressing the earlier described Ii constructs or aAPCs preincubated with peptides (3×10^{-5} M) Bet v 1₄₋₁₅, Bet v 1₁₁₂₋₁₂₃, Bet v 1₁₄₂₋₁₅₃, Art v 1₂₅₋₃₆, or medium alone (control) for 3 h were generated. Subsequently, Bet v 1₁₄₂₋₁₅₃-specific or Art v 1₂₅₋₃₆-specific TCRtg Jurkat cells (1×10^5) were added to 5×10^4 of the above-described aAPCs and cocultured for 6 h. PMA (10^{-7} M) plus PHA (5 μ g/ml) served as positive and medium alone as negative control. Luciferase activity was assayed as described (21). Stimulation of Jurkat T cells with MDDCs followed an analogous protocol.

T cell proliferation and Th1 polarization

TCRtg T cells (5×10^4 ; starting population bulk PB T cells or naive CD4⁺ CD45RA⁺ T cells) were incubated with 5×10^4 irradiated (60 Gy) aAPCs expressing recombinant immunomodulatory molecules in 96-well flat-bottom plates for 3 d. After 24 to 72 h, cell culture supernatants were harvested and concentrations of IL-2, IL-4, IL-5, IL-10, IL-13, and IFN- γ determined by multiplex analysis (20). Duplicate plates were pulsed after 72 h with methyl-[³H]thymidine (1 μ Ci/well) for 18 h and processed as described (20). Medium alone or anti-CD3/CD28 microbeads (5×10^4) served as controls. Proliferation assays with allergen-specific TCC and Bet

FIGURE 1. Nucleotide and amino acid sequence of the HLA-DRB1*07:01-restricted TCR of the Bet v 1₁₄₂₋₁₅₃-specific TCC SD334. *A*, TCR α -chain sequence. *B*, TCR β -chain sequence. Codon numbering and nomenclature according to the ImMunoGeneTics web resource for T cell receptors (44). The 5'-ends of C-region sequences are shown. CDRs (boxed), P-nucleotides (gray), and amino acids from P-diversification (magenta) and from N-diversification (turquoise) are indicated.

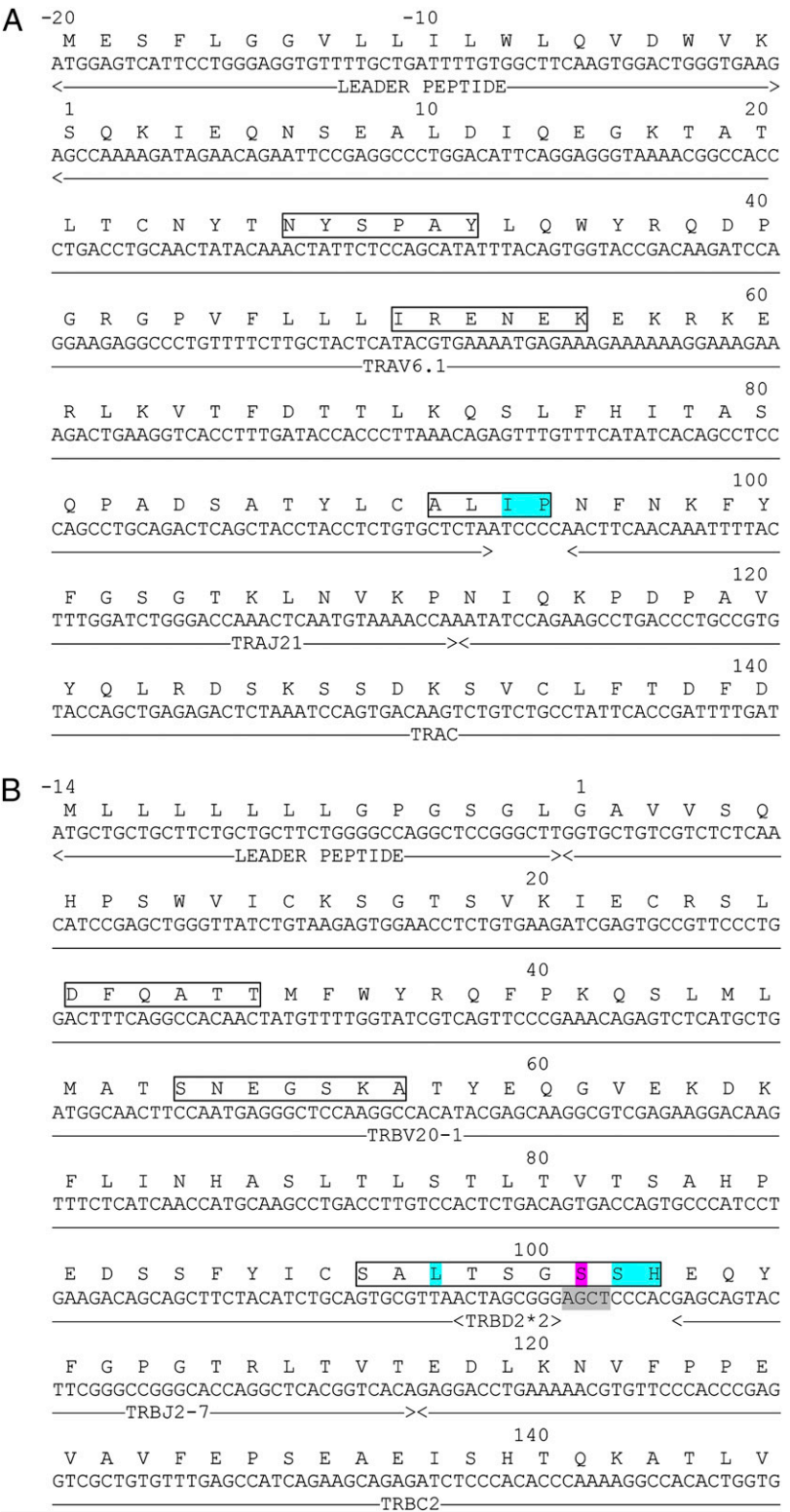
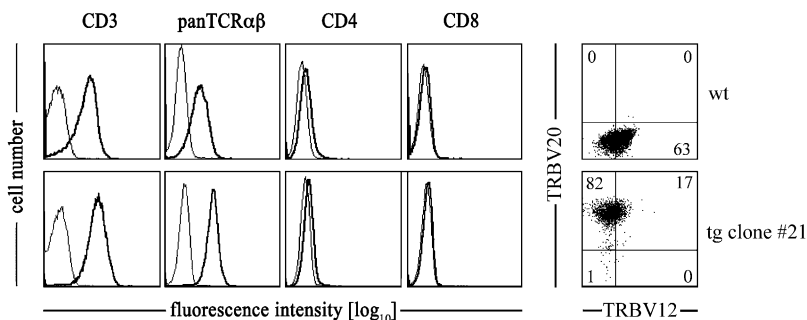


FIGURE 2. Bet v $1_{142-153}$ -specific TCRtg Jurkat T cells. Expression pattern of TCR/CD3, Bet v $1_{142-153}$ -specific transgenic TCR, and coreceptor molecules on wild-type and TCRtg single-cell clone no. 21 are shown. Expression of endogenous TRBV12 and transgenic TRBV20 is shown in the right panel. Numbers indicate percentage values within quadrants. Data are representative of two independently performed experiments.



v 1-related food and pollen allergens were performed as described (3). Th1-polarized cells were generated by coculture with irradiated (60 Gy) allergen-specific aAPCs coexpressing IL-12:GPI for 24 h, harvested, washed extensively, and cytometrically sorted for expression of GFP cotransduced with the recombinant TCR.

Inhibition of effector function of Th2-differentiated T cells

For the generation of TCRtg T cells, CD4⁺CD45RA⁺ PB T cells of allergic and nonallergic individuals were isolated, preactivated for 2 d with CD3/CD28-coated microbeads (ratio 1:2 of beads/cells), followed by transduction with the allergen-specific TCR as described. For Th1 differentiation, T cells were supplemented with IL-2 (100 U/ml; Peprotech) throughout the 9-d culture period. Final Th1 polarization was achieved by coculture of these T cells with irradiated (60 Gy) allergen-specific aAPCs coexpressing IL-12:GPI for 24 h. Th2 differentiation was achieved by supplementing T cells with IL-2 (10 ng/ml; Peprotech), IL-4 (50 ng/ml; Peprotech), anti-IFN- γ mAb (10 μ g/ml; R&D Systems, Minneapolis, MN), and anti-IL-12 mAb (3 μ g/ml; Ustekinumab; Jansen-Cilag, Titusville, NJ) for the 10-d culture period. After harvesting and extensive washing, Th2-differentiated cells (0.5×10^5 /well) and Th1-polarized cells (0.5×10^5 to 1.5×10^5 /well) were either cultured alone or in combination with allergen-specific or allergen-nonspecific aAPCs coexpressing CD80 (5×10^4 /well, irradiated) in 96-well flat-bottom culture dishes in triplicate for up to 72 h. At various times, supernatants were harvested and subjected to multiplex cytokine analyses. After 4 d, cellular proliferation was monitored by pulsing with methyl-[³H]thymidine (1 μ Ci/well) for the last 18 h of cultivation.

Statistical analyses

For multiple group comparisons, a linear mixed-model ANOVA was performed using SPSS software (IBM SPSS, Chicago, IL). For comparison of more than three groups, a Bonferroni correction was applied. Comparison between two groups was performed using the Student *t* test. Statistically significant values are denoted as follows: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

Results

Molecular cloning of the $\alpha\beta$ TCR of a Bet v $1_{142-153}$ -specific, HLA-DRB1*07:01-restricted TCC

A Bet v $1_{142-153}$ -specific CD4⁺ TCC (SD334) was isolated from PB of a birch pollen-allergic individual (HLA-DRB1*07, *15; DRB4*01; DRB5*01; DQB1*02, *06). This clone specifically reacted with the epitope RAVESY located within the immunodominant peptide Bet v $1_{142-153}$ in an HLA-DRB1*07:01-restricted manner (3). cDNA cloning and sequencing revealed that the functional α -chain gene of SD334 is formed by the rearrangement of TRAV6*01 to a TRAJ21*01 segment. The β -chain resulted from a TRBV20-1*01-TRBD2*02-TRBJ2-7*01-TRBC2 rearrangement (Fig. 1). In the α -chain, two non-templated codons resulting from N-diversification are present, whereas the C-terminal TRAV6*01 codon is deleted. In the β -chain CDR3 region, the C-terminal codon of TRBV20-1 has been deleted with P- and N-diversification giving rise to three non-germline-encoded codons at the 3'-end of TRBD2*02.

Retroviral transfer and expression of the Bet v $1_{142-153}$ -specific TCR

cDNAs encoding the Bet v $1_{142-153}$ -specific TCR α - and β -chains were introduced by retroviral transduction into Jurkat T cells harboring a human IL-2 promoter/enhancer controlling luciferase

expression (21). The tgTRBV20⁺ TCR was expressed on >99.8% of the cells of Bet v 1 TCRtg Jurkat clone no. 21 obtained by limiting dilution from bulk cultures of transduced cells (Fig. 2). Both the parental line and clone no. 21 expressed CD3 and TCR $\alpha\beta$, low levels of CD4, and no CD8. Similar results were obtained with other clones (data not shown).

The Bet v $1_{142-153}$ -specific TCR is functionally intact in Jurkat T cells

To assess whether Bet v 1-specific TCRtg Jurkat T cells retain the antigenic specificity of the original TCC, Jurkat clone no. 21 was

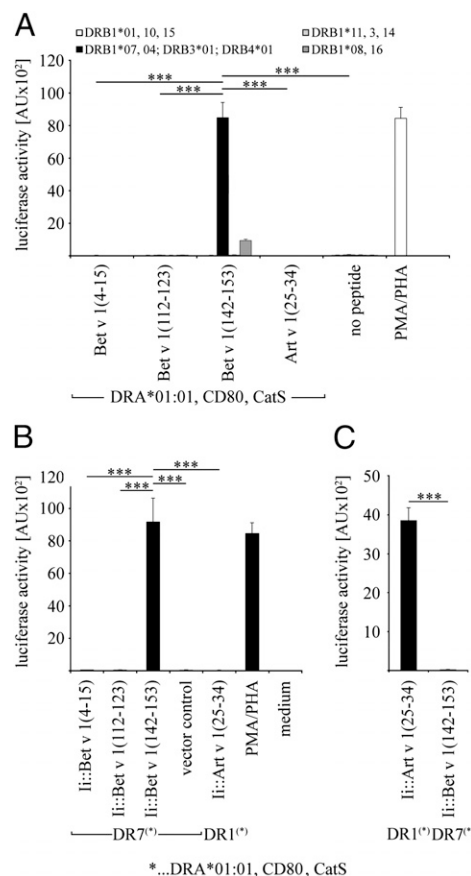


FIGURE 3. Functional activity of the transgenic Bet v $1_{142-153}$ -specific TCR. A, IL-2 promoter activity of Bet v 1 TCRtg Jurkat T cells upon coculture with aAPCs transfected with the indicated pools of HLA class II cDNAs and pulsed with the indicated peptides or medium (no peptide). PMA/PHA (white bar) served as positive control (*n* = 3). Recognition of aAPCs expressing Ii-borne allergenic peptides. B and C, aAPCs expressing the indicated molecules and Ii::fusion proteins or vector control were cocultured with Bet v $1_{142-153}$ -specific (*n* = 6) (B) or Art v 1-specific (*n* = 6) (C) TCRtg Jurkat T cells. Medium or PMA/PHA in the absence of APCs served as controls. Mean values + SEM of triplicates are shown. ****p* < 0.001. AU, arbitrary units; CatS, cathepsin S.

coincubated with aAPCs expressing pools of defined HLA class II molecules in medium alone or after pulsing with saturating concentrations of Bet v 1_{4–15}, Bet v 1_{112–123}, Bet v 1_{142–153} peptides or with Art v 1_{25–36} peptide, the immunodominant epitope of the major mugwort pollen allergen Art v 1, the latter serving as a negative control. Fig. 3*A* illustrates the specific recognition by Jurkat clone no. 21 of Bet v 1_{142–153}-peptide presenting by HLA-DRB1*07:01⁺ aAPCs, resulting in a significant increase in IL-2 promoter activity ($p < 0.001$). Coculture of Jurkat clone no. 21 with Bet v 1- or Art v 1-pulsed aAPCs expressing a collection of non-DR7 HLA class II molecules consistently revealed negative results. Experiments with aAPCs expressing single HLA-DRB1 specificities confirmed the HLA-DRB1*07:01 restriction of Jurkat clone no. 21 (data not shown). Half-maximal stimulation (ED_{50}) was achieved with $0.30 \pm 0.07 \mu\text{M}$ Bet v 1_{142–153} peptide (data not shown). Wild-type Jurkat T cells did not react with Bet v 1_{142–153} but otherwise responded well to the superantigen staphylococcal enterotoxin E (data not shown).

aAPCs harboring an Ii::Bet v 1_{142–153} fusion construct along with expression of HLA-DRA*01:01, HLA-DRB1*07:01, CD80::GPI, CD54::GPI, and cathepsin S also significantly stimulated ($p < 0.001$) Bet v 1_{142–153}-specific TCRtg Jurkat T cells comparable with the positive control PMA/PHA (Fig. 3*B*). In contrast, clone no. 21 was not activated by aAPCs transfected with Ii::Bet v 1_{4–15}, Ii::Bet v 1_{112–123}, or control vector. Furthermore, Art v 1_{25–36} had no stimulatory effect on Bet v 1_{142–153}-specific TCRtg T cells in the context of HLA-DR1, which otherwise significantly ($p < 0.001$) stimulated Art v 1-specific TCRtg Jurkat T cells (Fig. 3*C*). Generally, T cell activation was moderate in the absence (stimulation index: 3.2 ± 0.6 fold background) but became pronounced in the presence (stimulation index: 24.2 ± 3.4 fold background) of costimulation, for example, by CD80 (Supplemental Fig. 1).

Bet v 1_{142–153}-specific TCRtg T cells cross-react with Bet v 1-related food and pollen allergens

In a further step aimed at evaluating the fine specificity of the recombinant TCR, TCRtg Jurkat T cells were incubated with HLA-

DR7⁺ MDDCs pulsed with Bet v 1, Bet v 1-related food (Dau c 1, carrot; Api g 1, celery; Mal d 1, apple) or pollen (Cor a 1, hazelnut; Cab a 1, horn beam; Cas s 1, chestnut; Fag s 1, beech; and Que a 1, oak) allergens. Fig. 4*A* and 4*B* show that, apart from Bet v 1 (ED_{50} $0.22 \pm 0.01 \mu\text{M}$), the food-derived Api g 1 (ED_{50} $0.78 \pm 0.09 \mu\text{M}$) and the pollen-derived Cor a 1 (ED_{50} $0.16 \pm 0.05 \mu\text{M}$) and Car b 1 (ED_{50} $0.38 \pm 0.08 \mu\text{M}$) were recognized by TCRtg T cells. In contrast, Dau c 1 was only poorly stimulatory, and Mal d 1 remained unrecognized. Among the pollen allergens, Cas s 1 was poorly recognized, with responses seen only at the highest concentrations, whereas Que a 1 and Fag s 1 remained unrecognized by the TCRtg T cells. Art v 1 from mugwort used as negative control did not activate TCRtg Jurkat T cells (data not shown). The original TCC SD334 from which the TCR was derived displayed an almost identical reaction pattern to the tested food and pollen allergens when incubated with $0.3 \mu\text{M}$ allergens (Fig. 4*C*, 4*D*). Neither reactivity with Bet v 1 nor cross-reactivity with related allergens was observed when HLA-DR7[−] MDDCs were used as aAPCs (data not shown).

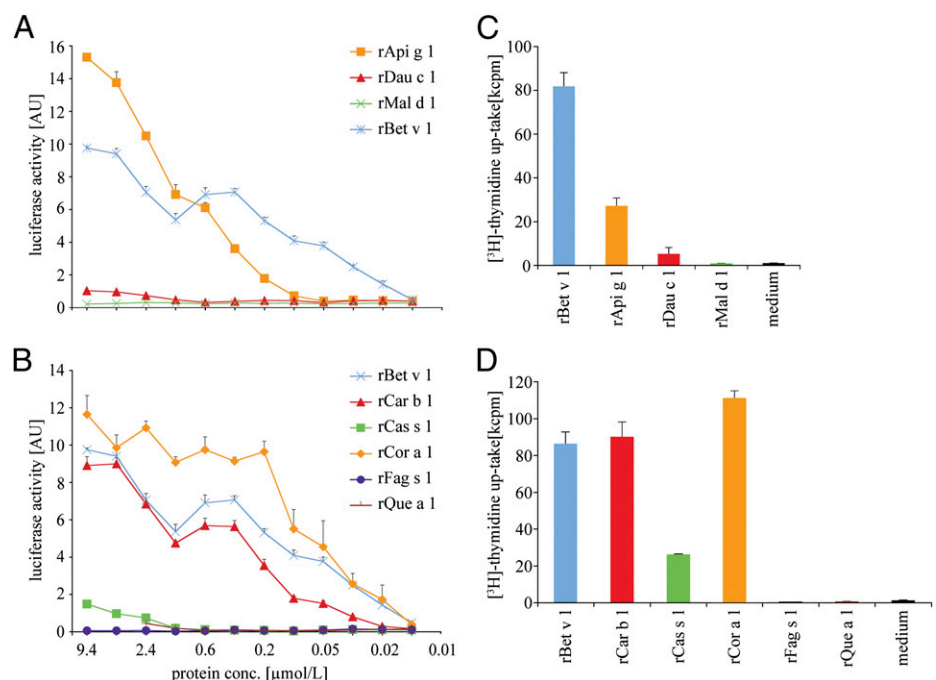
The Bet v 1_{142–153}-specific TCR is functionally active in human PB T cells

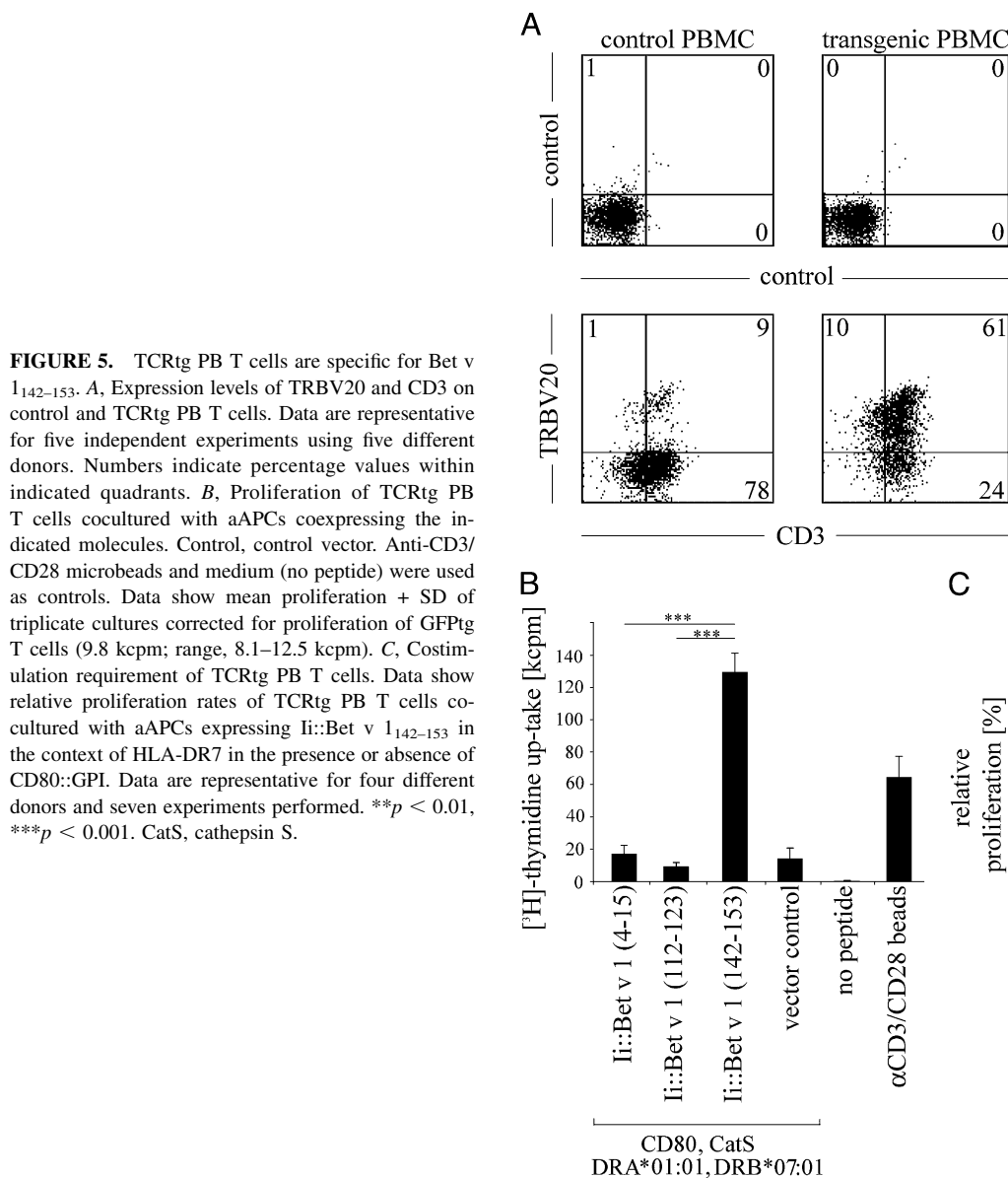
Next, we tested whether it was possible to generate human Bet v 1-specific T lymphocytes from nonallergic individuals. PBLs from five donors (HLA-DR7⁺ or HLA-DR7[−]) were transduced with the Bet v 1_{142–153}-specific TCR, resulting in $63 \pm 2\%$ compared with $7 \pm 2\%$ CD3⁺TRBV20⁺ T cells in TCR versus control transduced T cells, respectively (Fig. 5*A*). TCRtg T cells from all four donors significantly proliferated in an HLA-DR7-restricted manner upon coincubation with aAPCs coexpressing Ii::Bet v 1_{142–153} but not with aAPCs coexpressing Ii::Bet v 1_{4–15} or Ii::Bet v 1_{112–123} (Fig. 5*B*). Moreover, Ii::Bet v 1_{142–153}-induced proliferation was strictly costimulation dependent (Fig. 5*C*).

Th1 polarization of TCRtg PB T lymphocytes by aAPCs coexpressing HLA/allergen plus membrane-bound IL-12

We next asked whether aAPCs could polarize de novo-generated, TCRtg allergen-specific T cells from nonallergic and allergic

FIGURE 4. The Bet v 1_{142–153}-specific TCR cross-reacts with Bet v 1-related food and pollen allergens. *A* and *B*, HLA-DR7⁺ MDDCs preincubated overnight with Bet v 1 and Bet v 1-related (A) food or (B) pollen allergens were cocultured the next day with TCRtg Jurkat cells for 6 h before luciferase activity was determined. Allergens: Bet v 1 (birch), Api g 1 (celery), Dau c 1 (carrot), Mal d 1 (apple), Cor a 1 (hazelnut), Car b 1 (hornbeam), Cas s 1 (chestnut), Fag s 1 (beech), Que a 1 (oak). *C* and *D*, Proliferation of the original TCC from which the TCR was derived in response to (C) food and (D) pollen allergens. Data show mean values $\pm$ SD of triplicate cultures representative of two independently performed experiments. AU, arbitrary units; conc., concentration.





donors toward the Th1 phenotype. For that purpose, we created aAPCs expressing HLA/allergen in the absence or presence of the costimulatory molecule CD80 and/or membrane-bound IL-12 (28). Allergen-specific aAPCs coexpressing CD80 induced significant levels (>40 pg/ml) of IFN- γ , IL-2, and IL-13, whereas no IL-4 (data not shown) and only minute amounts of IL-10 were detectable (Fig. 6 and Supplemental Table I). Significantly, coexpression of membrane-bound IL-12 (p40::p35::GPI fusion protein) (28) in the absence of CD80 strongly increased IFN- γ secretion levels during primary allergen-specific stimulation ($p < 0.05$), which was even more pronounced in the additional presence of CD80 ($p < 0.05$). When compared with CD80 costimulated T cells, IL-12-costimulated allergen-specific T cells (in the absence of CD80) produced significantly less IL-2 and IL-13 (<15 pg/ml; $p < 0.05$ and $p < 0.001$, respectively). In the absence of specific Ag, the levels of CD80-induced or IL-12-induced cytokines were consistently low similar to cytokine levels obtained upon stimulation of T cells with signal 1 alone (Fig. 6 and Supplemental Table I). In contrast, the combination of CD80 and IL-12 led to Ag-independent IFN- γ production. However, the additional presence of an Ag-specific signal 1 increased IFN- γ levels by 23.0 ± 10.4 fold ($p < 0.01$).

Similar results were obtained with an Art v 1-specific TCR introduced into T cells of an HLA-DR1⁺ individual (Supplemental Table II). Comparable Th1-biased polarization was observed, irrespective of whether bulk PB or naive CD4⁺CD45RA⁺ PB T cells were used as the starting population (data not shown).

Next, we assessed cytokine expression levels in allergic and nonallergic individuals ($n = 5$) also on the cellular level by flow cytometry. Fig. 7 shows a clear-cut increase in the percentage of IFN- γ -positive cells in allergic and nonallergic donors upon costimulation with IL-12 compared with that of T cells receiving signal 1 alone (mean $\pm$ SD: 5.3 ± 1.6 fold and 4.6 ± 1.6 fold, respectively). Of note, the IL-12-induced percentage of IFN- γ -producing cells was even higher than upon CD80 costimulation (mean $\pm$ SD: 1.9 ± 1.3 fold and 2.4 ± 0.3 fold, respectively). In contrast, the percentages of IL-2- and IL-13-positive cells remained low compared with CD80-costimulated cells (mean $\pm$ SD of IL-2: $0.4 \pm 0.1\%$ and $0.9 \pm 0.6\%$ versus $5.7 \pm 2.1\%$ and $6.5 \pm 3.1\%$, respectively; mean $\pm$ SD of IL-13: $0.9 \pm 0.6\%$ and $1.3 \pm 0.5\%$ versus $3.6 \pm 0.9\%$ and $5.2 \pm 2.4\%$, respectively). Similar intracellular cytokine pattern was obtained in an HLA-DR1-positive individual and applying the Art v 1-specific TCR (Supplemental Fig. 2).

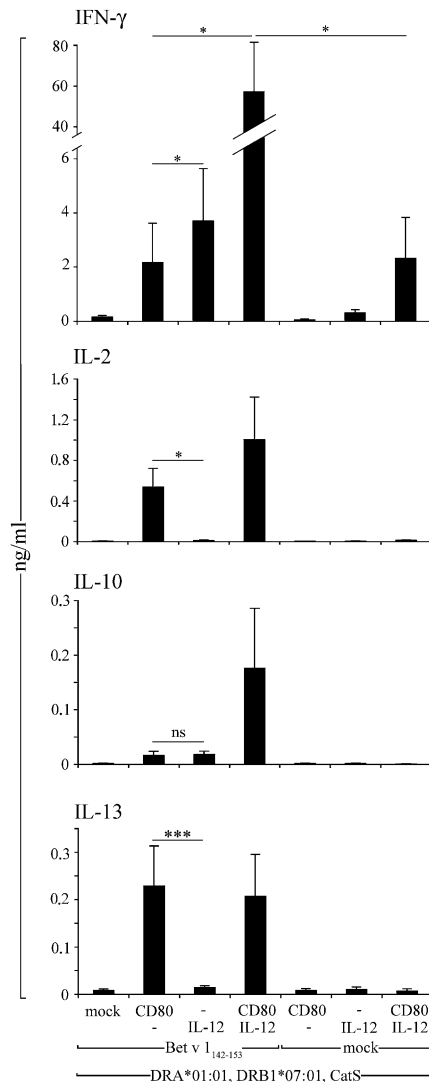


FIGURE 6. Predominant IFN- γ secretion by Bet v $_{142-153}$ -TCRtg PB T cells stimulated with aAPCs expressing Bet v $_{142-153}$ in the context of HLA-DR7 plus membrane-bound IL-12. TCRtg allergen-specific T cells were generated from allergic and nonallergic donors ($n = 6$) and stimulated with irradiated (60 Gy) aAPCs equipped with or without (mock) the indicated costimulatory molecules in the presence (Li::Bet v $_{142-153}$) or absence (mock) of allergen. Data show mean cytokine levels (ng/ml) of supernatants (24 h) of triplicates + SEM. * $p < 0.05$, *** $p < 0.001$. CatS, cathepsin S; ns, not significant.

Allergen-specific Th1-polarized TCRtg T cells inhibit the effector function of allergen-specific Th2-differentiated T cells

To investigate whether IL-12-induced Th1 cells, differentiated in the absence of CD80 costimulation, could inhibit allergen-specific Th2 cells, we introduced the TCR into naive CD4⁺CD45RA⁺ T cells of allergic and nonallergic donors undergoing Th1 (as described earlier) or Th2 polarization. Fig. 8 shows that both cell types when cultured individually secreted typical signature cytokines upon restimulation with Bet v $_{142-153}$ -specific aAPCs coexpressing CD80. However, upon coculture of Th2-polarized cells of allergic (Fig. 8A) or nonallergic (Fig. 8B) individuals with syngeneic Th1-polarized cells at a 1:2 ratio (i.e., 0.5×10^5 Th2 cells to 1×10^5 Th1 cells), Th2-differentiated T cells significantly reduced production of their signature cytokines IL-5 and IL-13 ($p < 0.01$, respectively) and also significantly downregulated IL-2 and IL-10 production ($p < 0.01$ and $p < 0.05$, respectively).

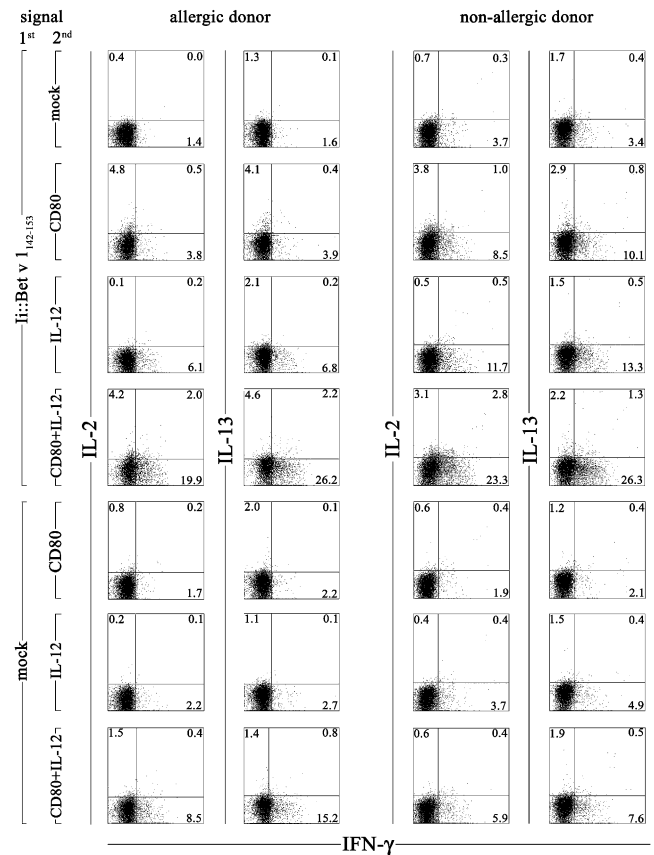


FIGURE 7. Intracellular cytokine levels of allergen-specific TCRtg T cells upon stimulation with different forms of allergen-specific or non-specific APCs. TCRtg allergen-specific T cells were generated from allergic ($n = 2$) and nonallergic ($n = 3$) donors and stimulated with the indicated irradiated (60 Gy) aAPCs in the presence or absence of allergen (1st signal: Li::Bet v $_{142-153}$) and equipped or nonequipped with costimulatory molecules (2nd signal). After 24 h, cocultures were incubated with GolgiStop (Becton Dickinson) for 6 h, followed by intracellular cytokine staining using Fix and Perm (An der Grub) and analysis by flow cytometry. Data show dual parameter dot plots of one representative allergic and nonallergic individual. Markers were set according to the results obtained with nonbinding control Abs. Numbers indicate percentage positive cells in respective quadrants.

vely) (Fig. 8A, 8B). Subset-specific cytokine secretion was strongly dependent on allergen-specific activation by aAPCs coexpressing CD80, as only minute quantities of cytokines (e.g., IFN- $\gamma < 10$ pg/ml) were secreted in the absence of allergen-specific aAPCs (data not shown) or in the presence of aAPCs expressing the non-cognate Bet v $_{14-15}$ peptide and CD80 (Fig. 8, white bars). Furthermore, allergen specificity was also confirmed by determination of intracellular cytokine levels (Fig. 9 and Supplemental Fig. 3).

The observed downregulation of Th2-signature cytokine levels was also apparent when looking at the single-cell level of T cells from allergic individuals using intracellular cytokine staining. In fact, the percentage of IL-4-, IL-5-, and IL-13-positive cells was reduced from 3.3 to 0.7%, 1.0 to 0.3%, and 1.9 to 1.0%, respectively (Fig. 9A). These experiments also revealed that IL-2 consumption does not seem to be the major factor accounting for the observed decrease of IL-2 levels in cocultures, as also the percentage of IL-2-positive cells within the Th2 population was reduced upon coculture from 12.3% in Th2 cultures to 3.1% in Th1/Th2 cultures. Similar results were obtained with allergic and nonallergic donors (data not shown). Data concerning intracellular cytokine levels were confirmed by using an Art v $_{125-36}$ -specific TCR and T cells from

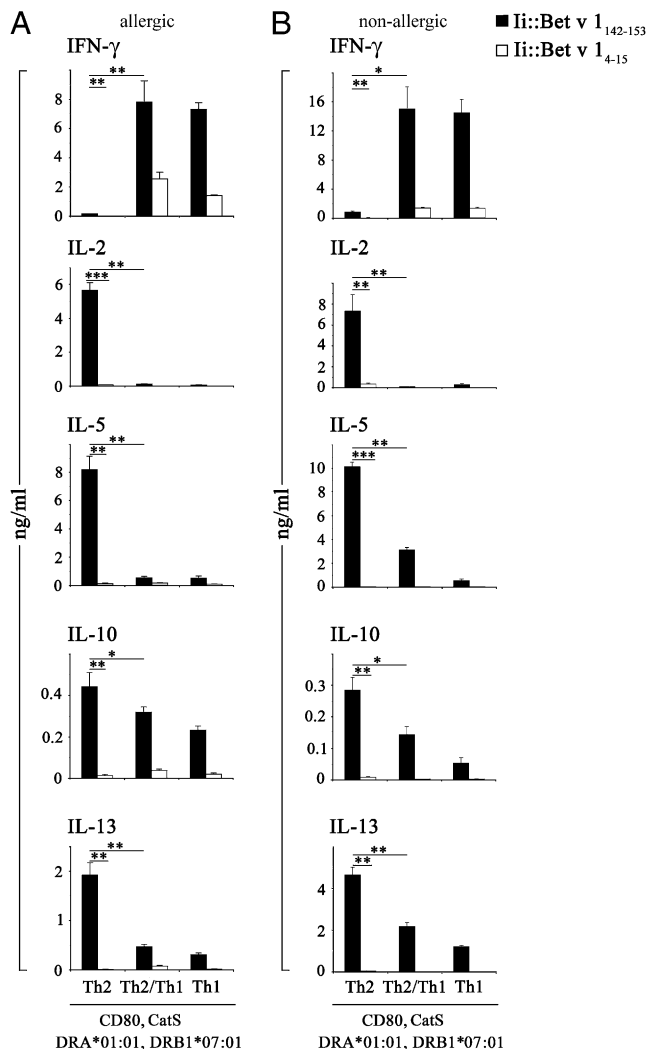


FIGURE 8. Allergen-specific TCRtg Th1-polarized T cells suppress the effector function of allergen-specific Th2-differentiated T cells. **A** and **B**, Naive CD4⁺CD45RA⁺ T cells from (A) allergic and (B) nonallergic individuals were transduced with the Bet v 1_{142–153}-specific TCR and cultured under Th2-differentiating conditions in the presence of IL-2, IL-4, anti-IFN-γ, and anti-IL-12. Th1-polarized T cells were generated by coculture of Bet v 1_{142–153}-specific TCRtg T cells with APC Bet v 1_{142–153} peptide in the context of HLA-DR7 and membrane-bound IL-12. Data show mean + SD of secreted cytokine levels of individual Th cell types or Th1/Th2 cocultures upon incubation with allergen-specific (Ii::Bet v 1_{142–153}, black bars) or allergen-nonspecific (Ii::Bet v 1_{4–15}, white bars) aAPCs coexpressing CD80 for 48 h (IFN-γ, IL-2) or 72 h (IL-5, IL-10, IL-13). Data are representative of four individuals and seven experiments performed. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. CatS, cathepsin S.

an HLA-DR1-positive individual (Supplemental Fig. 3). Cytokine expression was strictly Ag dependent, as no significant numbers of cytokine-positive cells were detectable when aAPCs used for coculture experiments presented the noncognate Bet v 1_{4–15} peptide in the presence of CD80 (Fig. 9B). In summary, these results demonstrate the concept that the effector function of human Th2-differentiated T cells can be efficiently inhibited by allergen-specific TCRtg Th1-polarized T cells generated by priming with allergen-specific aAPCs coexpressing membrane-bound IL-12.

Discussion

In the current study, we have cloned, sequenced, and functionally characterized the TCR α- and β-chains of a CD4⁺ TCC (SD334)

specific for the immunodominant T cell epitope of the major birch pollen allergen Bet v 1. Jurkat T cells as well as PB T cells of nonallergic individuals stably expressing this transgenic TCR after retroviral transduction specifically reacted with Bet v 1_{142–153} peptide-pulsed aAPCs, with aAPCs expressing Ii::Bet v 1_{142–153} fusion proteins, and with MDDC-processed Bet v 1 protein. Reactivity of TCRtg T cells depended on coexpression of costimulatory molecules such as CD80. The fine specificity of the TCC for Bet v 1-related food and pollen allergens was preserved in the TCRtg T cells. Coexpression of membrane-bound IL-12 on allergen-presenting aAPCs induced TCRtg T cells derived from allergic and nonallergic individuals to secrete high levels of IFN-γ, whereas IL-2 and IL-13 levels remained low, compatible with the induction of a Th1-polarized phenotype. Of note, analysis of cytokines at the single-cell level revealed similar results. Such Th1-polarized T cells, upon allergen-specific but not -nonspecific activation, significantly inhibited the effector function of Th2-differentiated allergen-specific T cells derived from naive CD4⁺ CD45⁺ T cells. Similar results were obtained with an HLA-DR1-restricted TCR recognizing the mugwort major pollen allergen, Art v 1_{25–34}, confirming the general validity of our findings.

Birch pollen allergy and its associated oral allergy syndrome significantly reduce the quality of life of affected individuals throughout the year (8). Individuals with accompanying atopic dermatitis may additionally suffer cutaneous exacerbations (e.g., aggravation of atopic eczema) upon ingestion of Bet v 1-related food allergens (13). Significantly, Bet v 1-related food allergy cannot be palliated by conventional specific immunotherapy, leaving this aspect of birch pollen allergy currently intractable (29). To contribute to a better understanding of the Bet v 1-specific T cell response, in this study we devised a novel tool, a molecularly defined and transferable Bet v 1_{142–153}-specific food and pollen cross-reactive TCR, which allows the creation of biological model systems in which the Bet v 1-specific T cell response can be studied in greater detail and which can be subjected to hypothesis-based modulation. In the Bet v 1-specific model system, all three components of the allergen-specific synapse (restriction element, immunodominant peptide, TCR) are well defined at the molecular level allowing the reproducible study and evaluation of new allergen variants/formulations for specific immunotherapy. The system also provides a solid basis for the establishment of “birch pollen-specific TCRtg mice” to study cross-reactivity and late-phase reactions in vivo in the near future. In principle, the recombinant TCR could also be used to evaluate the contribution of allergen-specific IgE in T cell-dominated in vivo model systems, for example, by adoptive transfer of recently developed mAbs with allergen specificity (30).

Upon transfer into heterologous T cells the Bet v 1_{142–153}-specific TCR retained its typical cross-reactivity with the Bet v 1-related food allergen Api g 1 from celery and its weak but significant cross-reactivity with Dau c 1 from carrot. Similarly, cross-reactivity remained strong with the tree pollen allergens Cor a 1 from hazelnut and Car b 1 from hornbeam and weak with Cas s 1 from chestnut (3). Notably, the presence of the immunodominant epitope RAVESY, representing the amino acid residues 145–150 of Bet v 1, was not an absolute requirement for cross-reactivity with related food and pollen allergen. Only one of the cross-reactive allergens (i.e., Car b 1) has complete sequence identity at positions 145–150 with Bet v 1. Although Cor a 1 (hazelnut) and Api g 1 (celery) have one or three amino acid exchanges in the core binding region, respectively, they still functioned as highly cross-reactive allergens. These results together with the reliable identification of anchor residues and the availability of a high-throughput cellular test system should be useful for the design

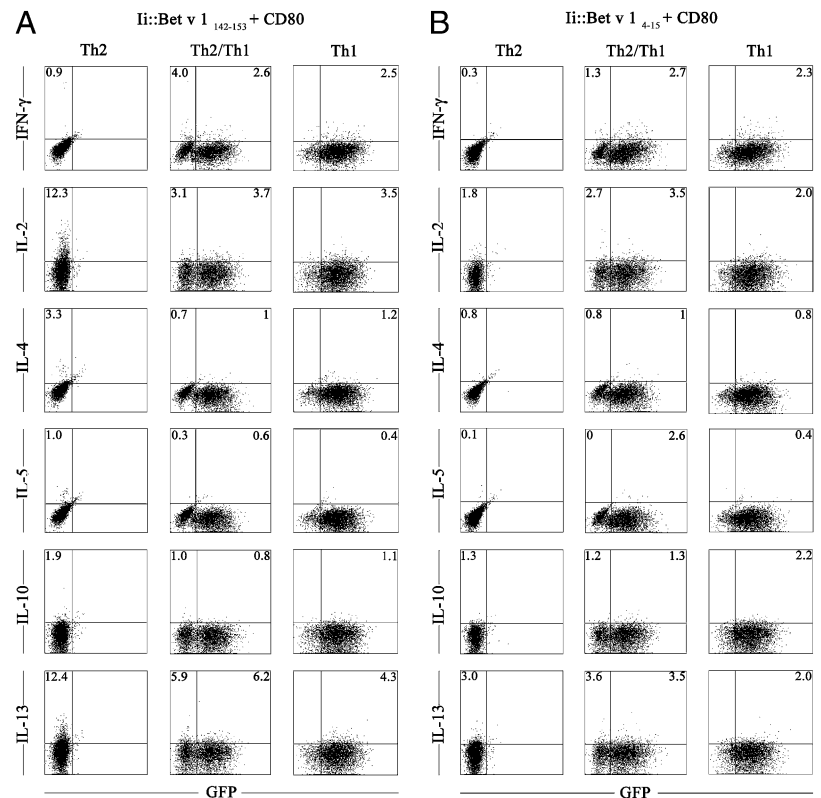


FIGURE 9. Intracellular cytokine levels of polarized allergen-specific T cells upon individual culture and coculture. *A* and *B*, Th1 (GFP⁺) and Th2 (GFP⁻) differentiated T cells from an allergic individual cocultured with (*A*) allergen-specific (Ii::Bet v 1₁₄₂₋₁₅₃) or (*B*) allergen-nonspecific (Ii::Bet v 1₄₋₁₅) aAPCs coexpressing CD80 were analyzed for intracellular cytokine levels individually or upon Th1/Th2 coculture. After 48 h, cultures were incubated with GolgiStop (Becton Dickinson) for 6 h, followed by intracellular cytokine staining using Fix and Perm (An der Grub) and the indicated directly conjugated cytokine-specific mAbs and analyzed by flow cytometry. Data show dual parameter dot plots of one representative experiment of several performed ($n = 5$). Markers were set according to negative controls; numbers indicate percentage positive cells within the GFP⁻ and GFP⁺ cell fractions.

of therapeutically valuable immunomodulatory peptide ligands covering the immunodominant C-terminal epitope of Bet v 1 in the future.

In allergic individuals, the net output of the cognate allergen/APC–T cell interaction is an imbalanced, Th2-dominated immune response favoring IgE production along with recruitment and arming of potent effector cells (31) such as eosinophils and mast cells. It is generally believed that, among other mechanisms, Th1 cells inhibit the de novo generation of Th2 cells by virtue of their secreted signature cytokines. Soluble IL-12 is a potent inducer of Th1 cells (32, 33); however, systemic administration of the cytokine has been shown to induce a plethora of adverse reactions (34, 35). To provide a more general solution for this problem and to restrict the action of cytokines to the site of Ag presentation, we have resorted to membrane attachment of cytokines to aAPCs (28). In this report, we sought to assess whether allergen-specific aAPCs expressing membrane-bound IL-12, in the absence of CD80 coexpression, would induce Th1-polarized Bet v 1-specific T cells. Our results clearly demonstrate that membrane-tethered IL-12p35::p40 fusion proteins in concert with HLA/allergen complexes elicited allergen-specific TCRtg T cells with a strict Th1 phenotype, exemplified by the sole secretion of large amounts of IFN- γ (Fig. 6 and Supplemental Table I). The increased IFN- γ levels were mirrored by elevated numbers of IFN- γ -producing cells as determined by intracellular cytokine measurements at the single-cell level (Fig. 7). Importantly, similar results were obtained irrespective of whether T cells were derived from allergic or nonallergic individuals. Of note, Th1 polarization was not a salient feature of the Bet v 1-specific TCR but was similarly observed with a mugwort, Art v 1₂₅₋₃₄-specific, TCR (Supplemental Table II and Supplemental Fig. 2). Moreover, we have demonstrated that such Th1-polarized T cells are capable of dominantly inhibiting the effector function of Th2-differentiated T cells with the same allergen specificity. Besides the significantly reduced Th2-signature cytokine levels in coculture supernatants, these experiments also revealed distinct

inhibition of cytokine production at the single-cell level (Fig. 9). This indicates that cytokine consumption/deprivation (36) might not play a major role for the observed downregulation of cytokine levels in cocultures. Our findings with TCRtg T cells (i.e., inhibition of cytokine synthesis) are consistent with previous results obtained with Th2-differentiated human and murine TCCs, which were stimulated Ag-specifically in the presence of exogenously added IFN- γ (37, 38). In principle, redirection of Th cell polarization represents a potentially important strategy for treating Th2 cell-dominated diseases, such as type I allergies. However, future studies, which certainly are beyond the scope of this investigation, will have to address the question whether the Th2 cells under investigation have retained their flexibility and were thus diverted from their previous phenotype. Alternatively, suppression with subsequent deletion from cultures could explain the effects observed. The availability of well-characterized Bet v 1-specific TCRs along with adjustable aAPCs and noncellular Ag-presenting platforms (20) offers the possibility to generate allergen-specific, Th1-polarized T lymphocytes ex vivo on a large scale (e.g., for subsequent adoptive transfer into severely allergic individuals in the future).

Before initiation of such studies, however, it remains to be explored whether and how polarized T cells could be applied in vivo to restore the Th1/Th2 balance without harming the host. In fact, certain allergic diseases have a biphasic T cell response in which a systemic allergen-specific Th2 response is followed by a more Th1-dominated organ pathology, as for example in atopic dermatitis (39). Along those lines, also IL-17 has been described to have a dual nature in the pathogenesis of allergic diseases (40). While IL-17 is required for the establishment of asthma in sensitized mice, it suppresses asthma in the effector phase of the disease (41). Consequently, as of today our current study cannot exclude that adoptive transfer of allergen-specific Th1 cells might enhance disease-promoting mechanisms in the recipient. To clarify these important issues experimentally in the near future,

humanized “allergy mice,” expressing the relevant Bet v 1-specific TCR and the human restriction elements described in this article, would offer an attractive model.

As an alternative to the above strategy, the engineering and application of allergen-specific regulatory T cells modified by direct gene transfer appears to represent an attractive option. In a pilot study, we have transduced PB T cells with multicistronic expression cassettes (42) harboring Bet v 1-specific TCR α - and β -chains along with regulatory genes, such as the *FOXP3* gene, as a single translation product. This maneuver endows T cells with Ag specificity, a regulatory cell surface phenotype, and regulatory capabilities. The multicistronic approach reliably prevents inappropriate expression of single-gene products, avoiding the potential generation of undesired T cell specificities. Recently, we have demonstrated that Bet v 1-specific TCR/Foxp3 double-transgenic T cells suppress the effector function of Bet v 1-specific T cells in an activation-dependent manner (43).

In conclusion, the availability of well-characterized allergen-specific TCRs offers a broad range of possibilities for creating hypothesis-based, human-relevant in vitro and in vivo model systems to study the multifaceted aspects of allergic diseases. Biologically relevant pathways amenable to immunomodulation have been experimentally addressed in this report. The findings are of potential clinical importance and should also provide novel insights into the pathophysiology of allergic diseases and their possible cure in the future.

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Disclosures

The authors have no financial conflicts of interest.

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SUPPLEMENTAL DATA FIGURE LEGENDS

FIGURE S1. Activation of Bet v 1₁₄₂₋₁₅₃-specific TCRtg T-cells is co-stimulation dependent. IL-2 promoter activity of **A**, Bet v 1₁₄₂₋₁₅₃-specific TCRtg Jurkat T-cells (n=4) or **B**, Art v 1₂₅₋₃₄-specific TCRtg Jurkat T-cells (n=3) upon co-culture with aAPC transfected with the indicated HLA class II cDNAs and Ii in the absence (grey bars) or presence (black bars) of the co-stimulatory molecule CD80. PMA/PHA (white bars) served as positive medium (no peptide) as negative control. Data show mean values + SD of triplicates and are representative for the number of experiments indicated. AU, arbitrary units. ***, $p < 0.001$.

FIGURE S2. Intracellular cytokine levels of allergen-specific T-cells upon stimulation with different forms of allergen-specific APC. TCRtg allergen-specific T-cells were generated from non-allergic (n=3) and allergic donors (n=2) and stimulated with the indicated irradiated (60 Gy) aAPC equipped with co-stimulatory molecules (2nd signal) in the presence or absence of allergen (1st signal: Bet v 1₁₄₂₋₁₅₃). After 24 hours, co-cultures were incubated with Golgi-Stop (Becton Dickinson) for 6 hours, followed by intracellular staining using Fix and Perm (An der Grub) and the indicated directly conjugated cytokine-specific mAbs and analyzed by flow cytometry. Data show dual parameter dot plots of representative examples of several experiments performed (n=5). Numbers indicate percent positive cells in respective quadrant.

FIGURE S3. Intracellular cytokine levels of polarized allergen-specific T-cells upon co-culture. Th1 (CFSE⁺) and Th2(CFSE⁻) differentiated T-cells from a non-allergic individual, co-cultured with **A**, allergen-specific (Art v 1₂₅₋₃₄) or **B**, allergen-nonspecific (Bet v 1₁₄₂₋₁₅₃) aAPC were analyzed for intracellular cytokine levels individually or upon co-culture for 48 hours. Subsequently, co-cultures were incubated with Golgi-Stop (Becton Dickinson) for 6 hours, followed by intracellular staining using Fix and Perm (An der Grub) and the indicated

directly conjugated cytokine-specific mAbs and analyzed by flow cytometry. Data show dual parameter dot plots of representative examples of several experiments performed (n=5). Numbers indicate the percent positive cell fractions within the CFSE⁻ and CFSE⁺ cell fractions. CFSE, Carboxy-fluorescein succinimidyl ester.

Figure S1

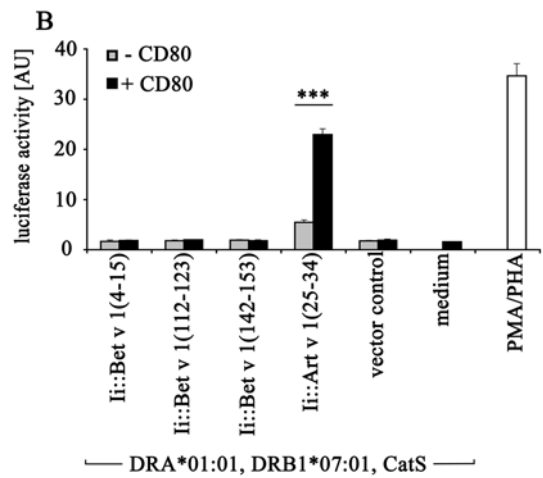
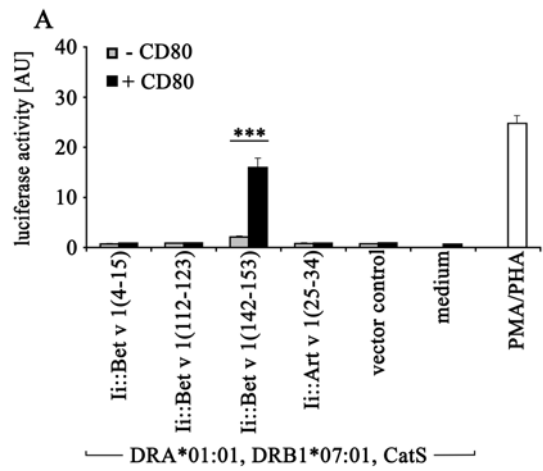


Figure S2

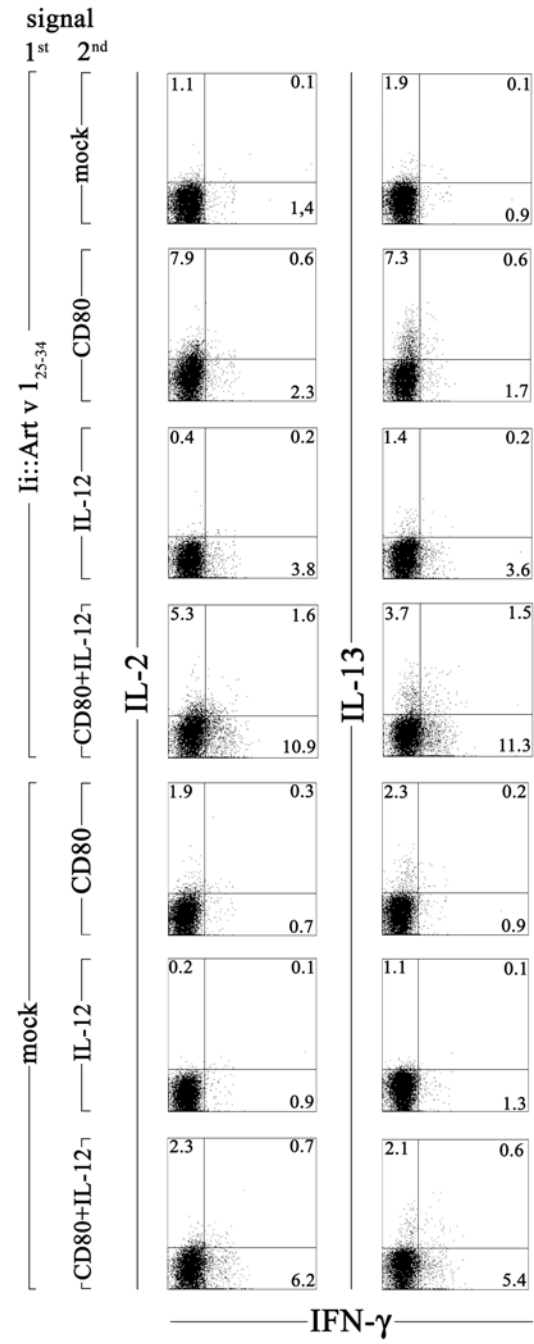


Figure S3

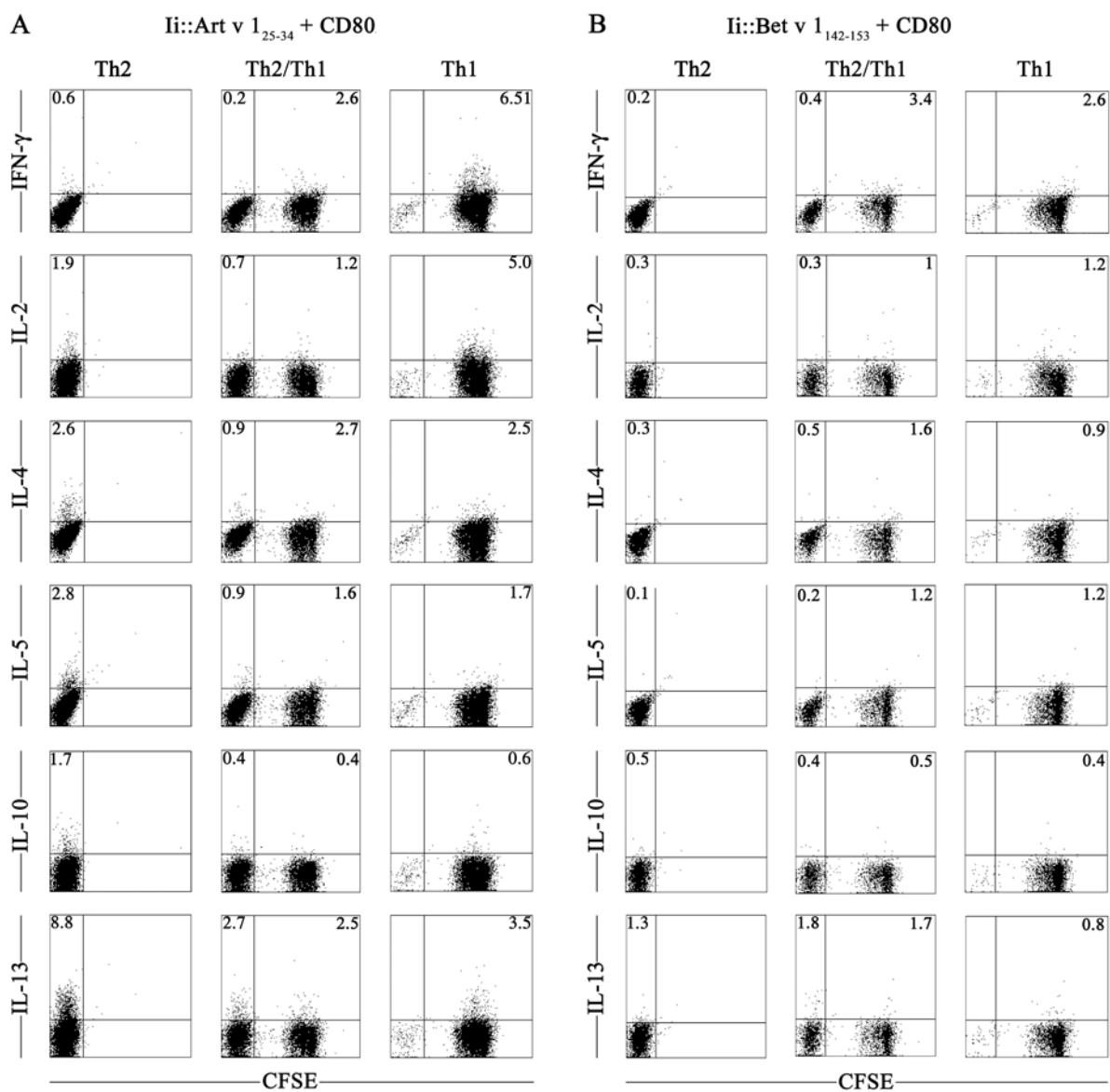


TABLE SI. Predominant IFN- γ secretion by TCRtg PB T-cells stimulated with aAPC expressing Bet v 1₁₄₂₋₁₅₃ in the context of HLA-DR7 plus membrane-bound IL-12

		1 st signal													
		Ii::Bet v 1 ₁₄₂₋₁₅₃								mock					
		mock	CD80		IL-12		2 nd signal		CD80		IL-12		CD80 + IL-12		
			pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	
donor #1	Cytokines	pg/ml	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	
	IFN- γ	36 $\pm$ 10 ^a	149 $\pm$ 10	100 ^b	1165 $\pm$ 384	782	32750 $\pm$ 12070	21980	10 $\pm$ 5	7	115 $\pm$ 34	77	154 $\pm$ 46	103	
	IL-2	11 $\pm$ 4	659 $\pm$ 65	100	22 $\pm$ 6	3	1463 $\pm$ 291	222	10 $\pm$ 9	2	4 $\pm$ 4	1	18 $\pm$ 10	3	
	IL-4	0	0	100	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	
	IL-10	3 $\pm$ 1	13 $\pm$ 2	100	20 $\pm$ 4	154	80 $\pm$ 12	615	0	0	1 $\pm$ 1	8	2 $\pm$ 0	15	
	IL-13	0	47 $\pm$ 2	100	4 $\pm$ 3	9	33 $\pm$ 1	70	0	0	0	0	0	0	
donor #2	IFN- γ	17 $\pm$ 0	133 $\pm$ 18	100 ^b	3367 $\pm$ 1420	2531	77210 $\pm$ 4190	58053	4 $\pm$ 4	3	35 $\pm$ 6	26	73 $\pm$ 21	55	
	IL-2	12 $\pm$ 5	1324 $\pm$ 17	100	37 $\pm$ 6	3	2871 $\pm$ 163	217	7 $\pm$ 0	1	2 $\pm$ 4	0	19 $\pm$ 4	1	
	IL-4	0	0	100	0	n.d.	1 $\pm$ 1	n.d.	0	n.d.	0	n.d.	0	n.d.	
	IL-10	2 $\pm$ 1	15 $\pm$ 1	100	41 $\pm$ 4	273	78 $\pm$ 3	520	0	0	0	0	1 $\pm$ 1	7	
	IL-13	4 $\pm$ 3	73 $\pm$ 7	100	23 $\pm$ 5	32	61 $\pm$ 8	84	0	0	0	0	0	0	
donor #3	IFN- γ	104 $\pm$ 8	601 $\pm$ 92	100 ^b	974 $\pm$ 101	162	4045 $\pm$ 198	673	38 $\pm$ 17	6	139 $\pm$ 15	23	626 $\pm$ 197	104	
	IL-2	9 $\pm$ 1	509 $\pm$ 78	100	9 $\pm$ 1	2	415 $\pm$ 80	82	8 $\pm$ 2	2	17 $\pm$ 4	3	1 $\pm$ 1	0	
	IL-4	0	0	100	0	n.d.	1 $\pm$ 1	n.d.	0	n.d.	0	n.d.	0	n.d.	
	IL-10	0	1 $\pm$ 2	100	5 $\pm$ 4	500	38 $\pm$ 11	3800	0	0	0	0	1 $\pm$ 1	100	
	IL-13	17 $\pm$ 11	391 $\pm$ 44	100	31 $\pm$ 4	8	391 $\pm$ 66	100	11 $\pm$ 3	0	35 $\pm$ 7	9	3 $\pm$ 5	1	
donor #4	IFN- γ	187 $\pm$ 28	975 $\pm$ 122	100 ^b	1328 $\pm$ 151	136	3354 $\pm$ 683	344	129 $\pm$ 28	13	344 $\pm$ 50	35	1002 $\pm$ 63	103	
	IL-2	3 $\pm$ 1	76 $\pm$ 5	100	4 $\pm$ 1	5	76 $\pm$ 14	100	5 $\pm$ 1	7	10 $\pm$ 3	13	1 $\pm$ 1	1	
	IL-4	0	0	100	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	
	IL-10	0	2 $\pm$ 2	100	5 $\pm$ 4	250	49 $\pm$ 9	2450	0	0	0	0	0	0	
	IL-13	5 $\pm$ 6	172 $\pm$ 13	100	13 $\pm$ 2	8	124 $\pm$ 24	72	8 $\pm$ 7	5	9 $\pm$ 8	5	0	0	
allergic donor #1	IFN- γ	460 $\pm$ 54	9287 $\pm$ 2640	100 ^b	13155 $\pm$ 142	136	145991 $\pm$ 21662	1572	1 $\pm$ 2	0	574 $\pm$ 236	6	9657 $\pm$ 1565	104	
	IL-2	4 $\pm$ 0	535 $\pm$ 46	100	2 $\pm$ 1	0	779 $\pm$ 126	146	1 $\pm$ 1	0	0	0	32 $\pm$ 10	6	
	IL-4	0	0	100	0	n.d.	0	n.d.	0	n.d.	0	n.d.	2 $\pm$ 0	n.d.	
	IL-10	1 $\pm$ 1	12 $\pm$ 2	100	8 $\pm$ 1	67	85 $\pm$ 2	708	0	0	1 $\pm$ 1	8	0	0	
	IL-13	0	119 $\pm$ 34	100	1 $\pm$ 1	1	72 $\pm$ 8	61	0	0	0	0	9 $\pm$ 1	8	
allergic donor #2	IFN- γ	96 $\pm$ 16	1874 $\pm$ 125	100 ^b	2232 $\pm$ 242	119	8014 $\pm$ 619	428	140 $\pm$ 20	7	695 $\pm$ 178	37	2441 $\pm$ 643	1302	
	IL-2	3 $\pm$ 1	120 $\pm$ 23	100	3 $\pm$ 1	3	420 $\pm$ 46	350	8 $\pm$ 3	7	2 $\pm$ 0	2	11 $\pm$ 4	9	
	IL-4	0	0	100	0	n.d.	0	n.d.	0	n.d.	0	n.d.	33 $\pm$ 5	n.d.	
	IL-10	3 $\pm$ 6	53 $\pm$ 13	100	30 $\pm$ 4	57	725 $\pm$ 38	1368	8 $\pm$ 7	15	6 $\pm$ 6	11	0	n.d.	
	IL-13	20 $\pm$ 5	570 $\pm$ 100	100	12 $\pm$ 10	2	561 $\pm$ 38	98	28 $\pm$ 3	5	14 $\pm$ 12	2	30 $\pm$ 11	5	

^a Mean cytokine levels of triplicates $\pm$ SD.

^b Percent cytokine levels compared to allergen/HLA plus CD80 stimulated T-cells.

n.d., not determined.

TABLE SII. Predominant IFN- γ secretion by TCRtg PB T-cells stimulated with aAPC expressing Art v 1₂₅₋₃₄ in the context of HLA-DR1 plus membrane-bound IL-12

		1 st signal												
		Ii:Art v 1 ₂₅₋₃₄						mock						
						2 nd signal								
		mock	CD80		IL-12		CD80 + IL-12		CD80		IL-12		CD80 + IL-12	
Cytokines	pg/ml	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	
donor #5	IFN-γ	534±71 ^a	2008±177	100 ^b	3399±640	169	7276±1138	362	170±17	8	966±150	48	3966±757	198
	IL-2	5±1	76±9	100	4±1	5	27±2	36	10±3	13	2±0	3	24±6	32
	IL-4	0	0	100	0	n.d.	0	n.d.	0	n.d.	0	n.d.	11±3	n.d.
	IL-10	7±4	19±0	100	10±10	53	51±7	268	1±2	5	6±6	32	0	n.d.
	IL-13	293±46	1732±98	100	144±19	8	1190±59	69	100±46	6	45±6	3	292±76	17

^a Mean cytokine levels of triplicates $\pm$ SD.

^b Percent cytokine levels compared to allergen/HLA plus CD80 stimulated T-cells.

n.d., not determined.