

Molecular and functional analysis of the antigen receptor of Art v 1-specific helper T lymphocytes

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Background: Ninety-five percent of patients with mugwort allergy are sensitized to Art v 1, the sole major allergen in mugwort (*Artemisia vulgaris*) pollen. Sixty-nine percent of patients recognizing the single immunodominant T-cell epitope Art v 1₂₅₋₃₆ have an HLA-DRB1*01 phenotype.

Objective: We studied cloning and functional expression of a human $\alpha\beta$ T-cell receptor (TCR) specific for Art v 1₂₅₋₃₆.

Methods: TCR chains were RT-PCR amplified from an Art v 1₂₅₋₃₆-specific T-cell clone, retrovirally transferred, and functionally tested in Jurkat T cells or alternatively in peripheral blood T lymphocytes of nonallergic individuals.

Results: The α -chain of the TCR is composed of TRAV17 and TRAJ45 segments, and the β -chain uses TRBV18, TRBD1, and TRBJ2-7. Analyses of 23 other Art v 1-specific T-cell clones did not reveal preferential usage of the TRAV17, TRBV18, or other TCR gene families. Efficient TCR transfer into Jurkat T cells was shown by binding of TCR V β 18-specific mAb and DRB1*0101/Art v 1 tetramers. Transgenic Jurkat T cells specifically recognized syngeneic EBV B cells pulsed with Art v 1₂₅₋₃₆ peptide and artificial antigen-presenting cells expressing invariant chain::Art v 1 fusion proteins. Moreover, transfer of the TCR into peripheral blood lymphocytes generated T cells that were Art v 1 reactive. Activation of transgenic T cells by artificial antigen-presenting cells was strictly dependent on costimulation.

Conclusion: For the first time, a detailed molecular and functional analysis of a human allergen-specific TCR is presented.

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Key words: Type I allergy, mugwort pollinosis, Art v 1, human T-cell receptor, T-cell receptor transgenic T cells, artificial antigen-presenting cell

T lymphocytes play a central role in the pathogenesis of allergic diseases. During sensitization, priming of allergen-specific (CD4⁺) T_H2 cells results in the production of T_H2 cytokines, which are responsible for class switching to the ϵ immunoglobulin heavy chain, allowing IgE production by B cells.¹ Once primed, allergen-specific T cells critically contribute to late-phase reactions and allergic inflammation in target organs (eg, airways and skin).² The clonotypic specificity of T cells is brought about by the T-cell receptor (TCR), which shows dual specificity for the antigen (allergen) and for self (MHC) determinants.³ Signal transduction by the TCR is conducted by means of noncovalently associated invariant CD3 subunits.⁴⁻⁶

The antigen specificity of T cells can be transferred to other T cells by means of TCR $\alpha\beta$ gene transfer.⁷⁻⁹ TCR-transgenic (tg) model systems have contributed greatly to our understanding of the immune response to autoantigens and infectious agents¹⁰⁻¹² and have shed light on fundamental processes in T-cell development.^{5,13,14} Although TCR gene usage by allergen-specific T lymphocytes has been studied to a considerable degree in the past,¹⁵⁻¹⁹ no molecular or functional analyses of cloned and ectopically expressed human allergen-specific TCRs have been reported thus far. Consequently, biologic model systems based on human allergen-specific TCRs are still missing.

An ideal human allergen-specific model system would comprise an immunodominant epitope, which is presented by a well-characterized restriction element expressed by the majority of the individuals affected. These features apply to mugwort pollen allergy: mugwort (*Artemisia vulgaris*) is widely spread throughout the European temperate climate zone, North America, and parts of Asia and is one of the main causes of hay fever in late summer and autumn.²⁰ More than 95% of patients with mugwort allergy are sensitized to Art v 1, the major allergen in mugwort pollen. Analyzing the T-cell response to Art v 1 by using a panel of specific T-cell lines and clones revealed that the presentation of the sole immunodominant epitope (Art v 1₂₅₋₃₆) is highly associated with HLA-DRB1*01.^{21,22} Thus allergy to Art v 1 is characterized by a uniform T-cell response.

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The nucleotide sequences for the Art v 1-specific T-cell receptor α and β chains have been deposited in the GenBank database under accession numbers EU030677 and EU030678, respectively.

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Abbreviations used

aAPC:	Artificial antigen-presenting cell
CLIP:	Class II-associated invariant chain peptide
FITC:	Fluorescein isothiocyanate
HA:	Influenza hemagglutinin
Ii:	Invariant chain
PB:	Peripheral blood
PBL:	Peripheral blood lymphocytes
PE:	Phycoerythrin
SEE:	Staphylococcal enterotoxin E
SI:	Stimulation index
TCR:	T-cell receptor
tg:	Transgenic
WT:	Wild-type

In the present study we addressed the feasibility of engineering allergen-specific T lymphocytes by means of genetic TCR transfer. The molecular and functional characterization of human allergen-specific TCRs as critical tools of defined model systems in which all 3 components of the “allergen-specific synapse” are well defined (ie, restriction element, immunodominant peptide, and TCR) will provide important insights into the pathophysiology of allergic diseases and their possible future cure.

METHODS**Cell lines and primary cells**

For this investigation, 293 cells, 293 OKT3scFv::GPI transfectants, 23 Raji cells, and Jurkat clone 41-19 expressing an IL-2 enhancer/promoter-driving luciferase were cultured as previously described.²³ Art v 1-specific T-cell lines, T-cell clones, and immortalized B cells were established from peripheral blood lymphocytes (PBLs) of individuals with mugwort pollen allergy with a typical clinical history and positive skin prick test responses to mugwort pollen extract, as described.²² The TCR gene usage was determined by means of RT-PCR with TRAV and TRBV primers (Clonetechn, Heidelberg, Germany), as previously described.²²

Cloning and characterization of $\alpha\beta$ TCRs

mRNA from the Art v 1-specific T_H0 cell clone SSR20, which on antigen-specific stimulation produced 22 pg/mL IL-4 and 75 pg/mL IFN- γ , was amplified with TRAV17 forward 5'-CGCGGGAAGCTTGCCACCATGGAAA CTCTCTGGGAGTG-3' and TRAC reverse 5'-CCC GCGGCGGCGGCTT TAGCTGGACCACAGCCGC-3' or TRBV18 forward 5'-CGCGGG AAGCTTGCCACCATGGCCAACTCTGCTATGGAC-3' and TRBC reverse 5'-CCC GCGGCGGCGGCTTTAGAAATCCTTCTCTTGACCATG-3', digested with *Nco*I (bold) and *Not*I (underlined), and gel purified. DNA fragments were ligated into the retroviral expression vector pMMP412 (R.C. Mulligan, Childrens Hospital, Boston, Mass) and DNA sequenced (VBC Genomics, Vienna, Austria).

Cloning of HLA-DR constructs

SSR (EBV-immortalized B cells) mRNA was amplified with HLA-DRA forward 5'-CGCGGGAAGCTTGCCACCATGGCCATAAGTGGAGTCCC-3' and HLA-DRA reverse 5'-CCC GCGGCGGCGGCTTTACAGAGGCC CCTGCG-3' or HLA-DRB1*0101 forward 5'-CGCGGGAAGCTTGCCAC CATGGTGTGTCTGAAGTCCC-3' and HLA-DRB1*0101 reverse 5'-CCC GCGGCGGCGGCTTTAGCTCAGGAATCCTGTTGGCT-3', digested with *Hind*III (bold) and *Not*I (underlined), and gel purified. DNA fragments were ligated into pEAK12 expression vector (EdgeBio, Gaithersburg, Md) and DNA sequenced.

Generation of invariant chain constructs

pcDNA1.1/Amp containing an invariant chain (Ii) with a *Sfi*I-*Eco*47III cassette instead of class II-associated Ii peptide (CLIP) was kindly provided by J. vanBergen (Immunohematology and Blood Transfusion, Leiden, The Netherlands).²⁴ The double-stranded Art v 1₂₅₋₃₄ oligonucleotides Art v 1₂₅₋₃₄ upper 5'-CGAAGTGCATCGAGTGGGAGAAGGCCAGCACCAGGC-3' and Art v 1₂₅₋₃₄ lower 5'-GCCTGGTGCTGGGCTTCTCCCACTCG ATGCACTT-3' were inserted into *Sfi*I/*Eco*47III-digested pcDNA1.1/Amp-Ii. CLIP and HA₃₀₉₋₃₁₇ containing Iis were constructed as previously described.²⁴ Length variants of Art v 1 were constructed by means of PCR with codon optimized Art v 1²⁵ as template amplification with the primers codon optimized Art v 1₁₋₁₀₉ forward 5'-CGCGGGTTCGAAGATGGCCGG CAGCAAGCTG-3' and codon optimized Art v 1₁₋₁₀₉ reverse 5'-GGGCGCGGCGAGCGCTTGGTGGGTGCTGGGGGG-3'; codon optimized Art v 1₁₅₋₅₀ forward 5'-CGCGGGTTCGAAGAGCGGCAAGTGCGA CAACAAG-3' and codon optimized Art v 1₁₅₋₅₀ reverse 5'-GGGCGCG GGCAGCGCTTGGCAGAAGCAGCTCTCCTTGCC-3'; codon optimized Art v 1₂₀₋₄₅ forward 5'-CGCGGGTTCGAAGAACAAGAAGTGCGACAA GAAG-3' and codon optimized Art v 1₂₀₋₄₅ reverse 5'-GGGCGCGGGG AGCGCTTGCTTGCCGGCTCCCGCTT-3'; and codon optimized Art v 1₁₋₅₇ reverse 5'-GGGCGCGGGCAGCGCTTGGCTCTTGCTGCAGTC GAAGTA-3'. After PCR amplification, digestion, and gel purification, the DNA fragments were ligated into the pcDNA1.1/Amp-Ii expression vector, followed by DNA sequencing (VBC Genomics).

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

Amphotropic TCR $\alpha\beta$ -delivering retroviruses were produced by means of transient transfection with standard procedures.²⁶ Briefly, 293 OKT3scFv::GPI cells were transfected with pMD.gagpol (Moloney murine leukemia virus *gag-pol*) pMD-VSV-G (vesicular stomatitis virus protein G; both provided by Dr R. C. Mulligan),²⁷ pMMP412.TRAV17, and pMMP412.TRBV18. Alternatively, pMMP412.GFP was used as a control. Jurkat clone 41-19 or peripheral blood (PB) T cells were transduced with viral supernatant in the presence of 8 μ g/mL polybrene (Sigma, St Louis, Mo). Before transduction, PB T cells were pre-stimulated with anti-CD3/CD28 substituted microbeads (Dynabeads, Dynal Biotech, Oslo, Norway) and optionally with 300 U/mL IL-2 (Peprotech, London, United Kingdom). Jurkat clones were obtained by limiting dilution.

Immunofluorescence analyses

The mAbs CD3-fluorescein isothiocyanate (FITC; UCHT1), pan-TCR $\alpha\beta$ -phycoerythrin (PE; BMA031), CD2-FITC (TS2.18), CD4-FITC (VIT4), CD8-PE (VIT8b), and control immunoglobulin FITC and PE were obtained from ADG (Kaumberg, Austria). TCR V β 8-FITC (56C5) and TCR V β 18-PE (BA62) were from Immunotech (Marseille, France). HLA class II-FITC (1/47) and Ii-FITC (5-329) were provided by O. Majdic (Institute of Immunology, MUV, Vienna, Austria). Surface and intracellular staining, as well as flow cytometric analyses, were performed as previously described.²⁸ For tetramer staining, Jurkat cells (4×10^5) were incubated with 4 μ L of PE-labeled DRB1*0101/Art v 1 peptide-specific tetramer (Beckman Coulter, Marseille, France) containing Art v 1₁₉₋₃₆ (NKKCDKKCIEWEKAQHGA) at 37°C in the dark for 2 hours, washed once, and fixed in 200 μ L of 0.5% paraformaldehyde-PBS at room temperature for 1 hour, followed by immediate flow cytometric analysis.

Jurkat luciferase activity assays

SSR EBV-immortalized B cells (5×10^4 , HLA-DRB1*0101⁺) were pre-incubated with the peptides (3×10^{-5} mol/L) Art v 1₁₉₋₃₀, Art v 1₂₅₋₃₆, Art v 1₉₄₋₁₀₅, influenza HA306-318 or medium alone, respectively, for 3 hours. Subsequently, TCR tg Jurkat cells or wild-type (WT) Jurkat cells (1×10^5) were added and cocultured with presensitized syngeneic EBV cells or peptide alone for 6 hours. Alternatively, artificial antigen-presenting cells (aAPCs; 5×10^4) expressing HLA-DR1, CD54, CD80, and Ii constructs served as stimulators. Staphylococcal enterotoxin E (SEE; 0.3×10^{-6} mol/L; Toxin Technologies, Sarasota, Fla) or PHA (5μ g/mL) plus phorbol 12-myristate

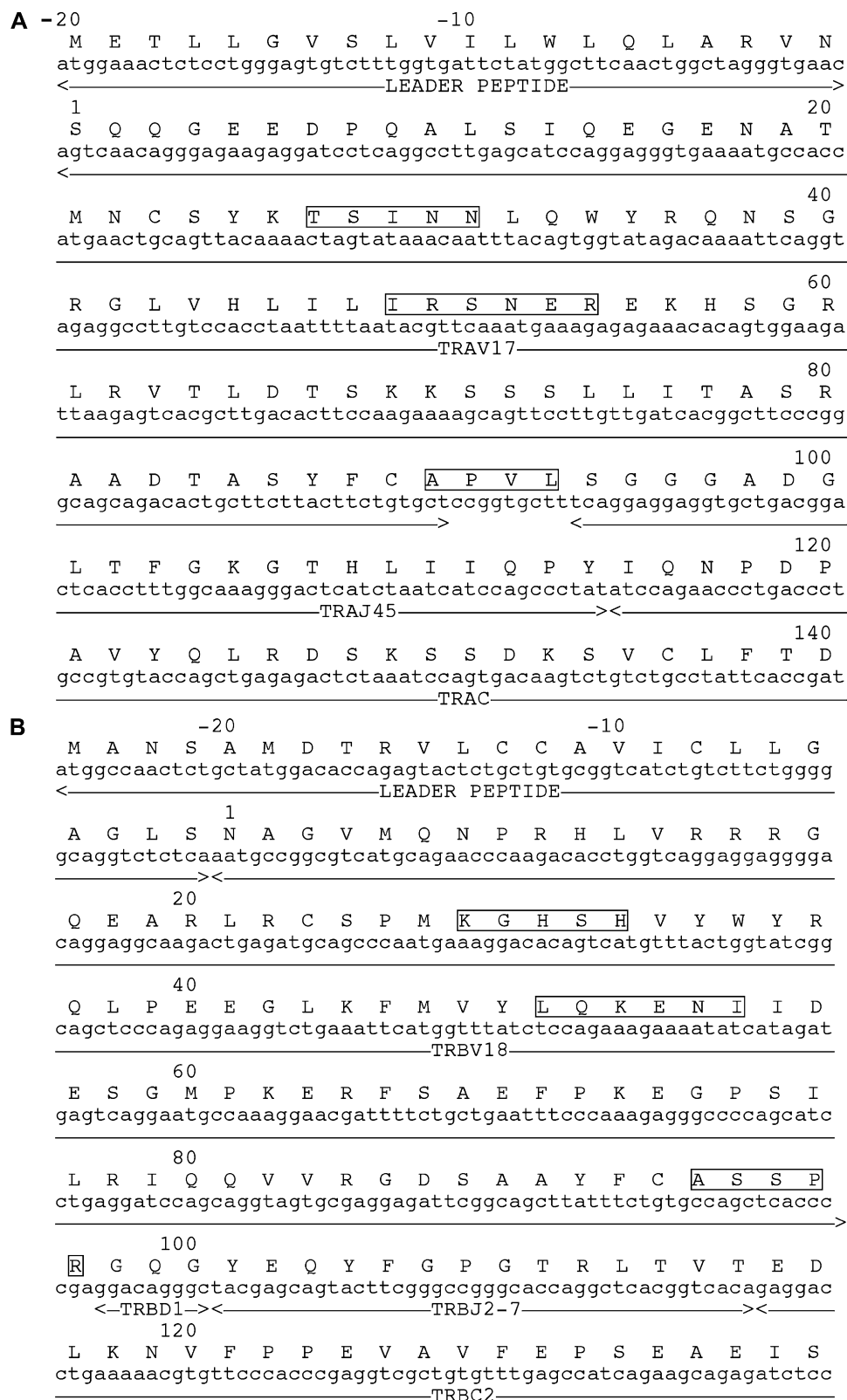


FIG 1. Nucleotide and predicted amino acid sequence of the HLA-DRB1*0101-restricted TCR of the Art v 1-specific T-cell clone SSR20. **A**, TCR α -chain sequence. **B**, TCR β -chain sequence. Codon numbering and nomenclature are according to the international immunogenetics information system.²⁹ Only the 5'-ends of the constant-region sequences are shown. Complementarity-determining regions are boxed.

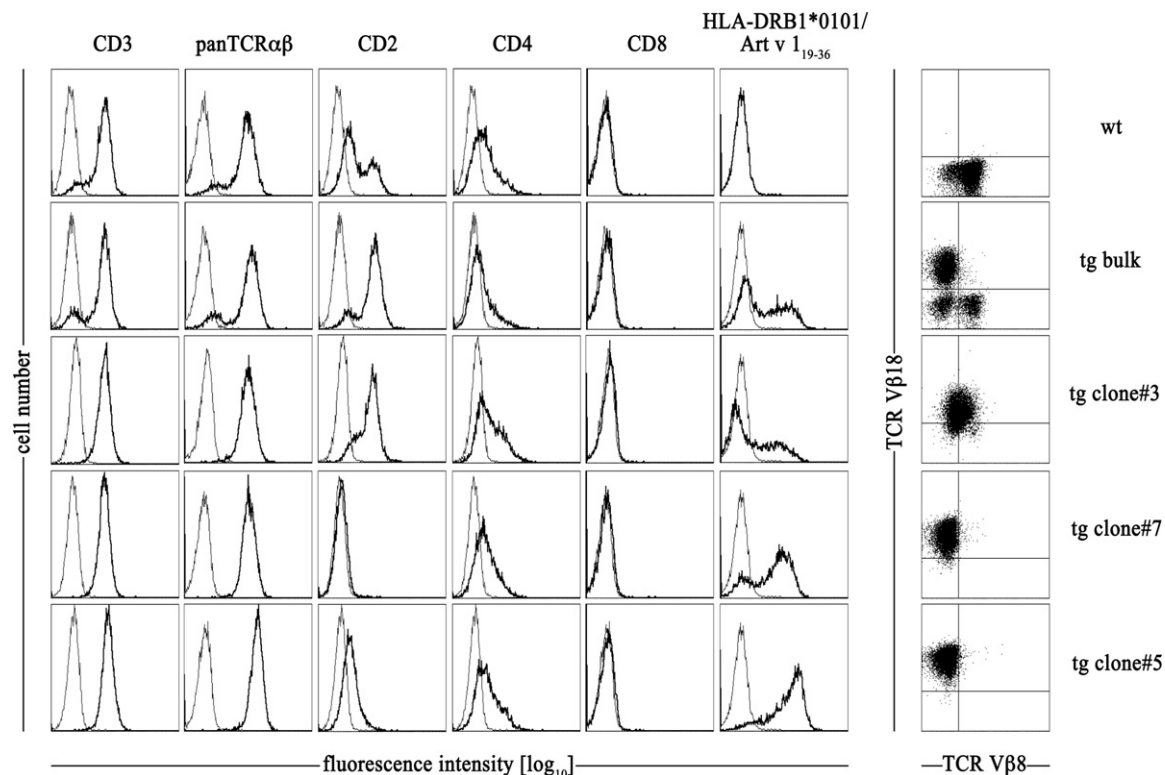


FIG 2. Expression pattern of endogenous TCR, Art v 1-specific tg TCR, and accessory molecules on WT, bulk TCR tg, and TCR tg single-cell clones (nos. 3, 5, and 7) of Jurkat T cells are shown. Binding of specific mAbs or Art v 1-specific tetramers (HLA-DRB1*0101/Art v 1₁₉₋₃₆; **bold lines**) is compared with control antibody binding (*thin lines*). Two-parameter dot-plot analysis of Jurkat cells stained with TCR Vβ8 and TCR Vβ18 mAbs (*right panel*). Data are representative of 2 independently performed experiments.

13-acetate (10^{-7} mol/L) served as positive controls. Luciferase activity was assayed as previously described.²⁸

Generation of aAPCs

In this study, 293 cells (3×10^6) were transiently cotransfected with HLA-DRA*0101, HLA-DRB1*0101, CD54, CD80, cathepsin S,²⁸ and Ii constructs or empty control vector in combinations as indicated and used 72 hours after transfection.

T-cell proliferation assays

TCR tg T cells (5×10^4 per well) were incubated with aAPCs (5×10^4 per well irradiated with 60 Gy) expressing HLA-DRA*0101, HLA-DRB1*0101, Ii::Art v 1₂₅₋₃₄, Ii::HA₃₀₉₋₃₁₇, CD80, and cathepsin S combined, as indicated in 96-well flat-bottom tissue-culture plates for 3 days. Cells were pulsed with [methyl-³H] thymidine (1 μ Ci per well) for another 18 hours and processed as previously described.²³ Medium alone or anti-CD3/CD28 microbeads (5×10^4) served as controls.

RESULTS

Molecular cloning of the $\alpha\beta$ TCR of an Art v 1₂₅₋₃₆-specific, HLA-DRB1*0101-restricted T-cell clone

The Art v 1-specific CD4⁺ T-cell clone (SSR20) was isolated from the blood of a subject with mugwort pollen allergy (HLA-DRB1*0101, *0301; DRB3*0101; DQB1*0201, *0501) shortly after the mugwort pollen season. The clone specifically reacted to stimulation with the immunodominant peptide of Art v 1, Art

v 1₂₅₋₃₆, in an HLA-DRB1*0101-restricted manner.²¹ The TCR α - and β -chains of SSR20 were RT-PCR amplified, ligated into the retroviral expression vector pMMP412, and DNA sequenced. The functional α -chain gene of SSR20 was formed by the rearrangement of a V α gene segment of the TRAV17 gene family to a TRAJ45 segment (Fig 1, nomenclature according to Lefranc et al²⁹). The β -chain of SSR20 is derived from a TRBV18-TRBD1-TRBJ2-7-TRBC2 rearrangement (Fig 1). The junctional or N-diversification in CDR3s is low for both chains. In the α -chain 3 nongermline-encoded codons are present, whereas the 2 C-terminal TRAV17 codons have been deleted. CDR3 β is composed of germline sequences, except for the addition of a single arginine. Assessment of 23 T-cell clones of 10 additional subjects with Art v 1 allergy revealed that 12% of the clones expressed TRAV17 and 9% of the clones expressed TRBV18, indicating that Art v 1₂₅₋₃₆-specific T-cell responses are not confined to TCRs consisting of the TRAV17 and TRBV18 gene families.

Retroviral transfer and expression of the Art v 1-specific TCR

In a first instance expression of the cloned TCR was tested by means of retroviral transduction of Jurkat T cells harboring a human IL-2 promoter/enhancer controlling luciferase expression.²³ Immunofluorescence staining of bulk transductants revealed stable TCR Vβ18 expression in approximately 60% of cells (Fig 2). Expression levels of CD3, pan-TCRαβ, and CD4 were comparable in WT and TCR tg Jurkat cells in bulk

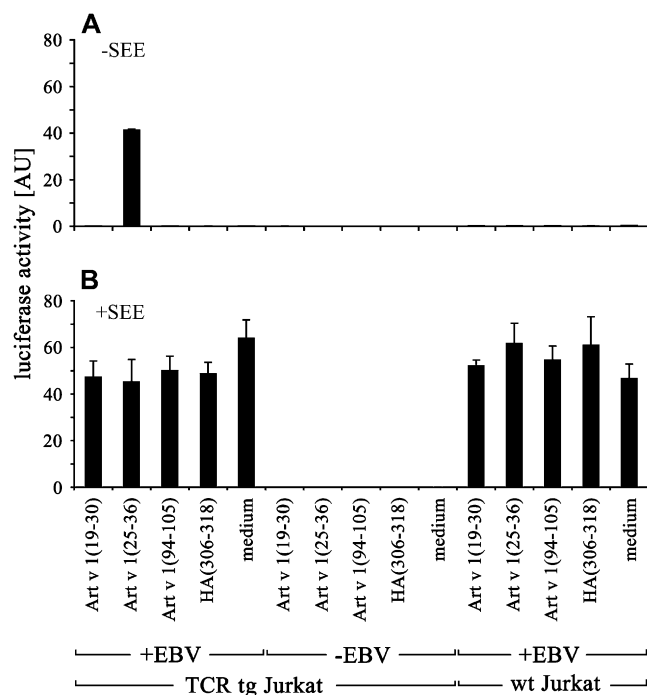


FIG 3. The Art v 1–specific TCR in tg Jurkat T cells is functionally active. **A**, IL-2 promoter activity of Art v 1 TCR tg or WT Jurkat T cells on coculture with or without HLA-DRB1*0101⁺ EBV B cells and the indicated peptides or with medium alone. **B**, Similar to Fig 3, A, but supplemented with SEE. Data show mean values $\pm$ SDs of triplicate cultures representative of several independently performed experiments.

transductants, as well as in single-cell clones, whereas, for example, CD2 expression levels showed considerable variation (Fig 2). A similar variability was observed for V β 8 and V β 18 expression on single-cell clones. Clone 3 showed residual coexpression of the endogenous V β 8 chain, whereas clones 5 and 7 showed mutual exclusive expression of the tg V β 18 chain (Fig 2), which might be caused by different levels of overexpression (long terminal repeat–driven or through copy number) of the tg TCR and competition for the constitutively expressed CD3 complex. HLA-DRB1*0101/Art v 1₁₉₋₃₆–specific tetramers bound to TCR transductants exclusively (Fig 2), indicating that on transfer the tg TCR retained its capability to specifically interact with the restriction element/peptide complex against which it had been selected *in vivo*.

The allergen-specific TCR is functionally intact in TCR tg Jurkat T cells

To determine whether Jurkat T cells transduced with the allergen-specific TCR recognize the HLA-DRB1*0101–restricted immunodominant T-cell epitope Art v 1₂₅₋₃₆^{21,22} also in functional terms, the TCR transductants (subclone 5) were cocultured with syngeneic EBV B cells that had been pulsed with saturating concentrations of Art v 1₂₅₋₃₆ or control peptides from Art v 1 (Art v 1₁₉₋₃₀ or Art v 1₉₄₋₁₀₅) or influenza hemagglutinin (HA₃₀₆₋₃₁₈). Fig 3, A, shows specific recognition of Art v 1₂₅₋₃₆ by the TCR tg Jurkat T cells, as revealed by IL-2 promoter–driven luciferase activity. Similar results were obtained with other subclones or the bulk transductants (not shown). Of note, residual coexpression of endogenous TCR chains, as observed for clone 3, did not influence

subsequent antigen-specific activation (not shown). EBV B cells pulsed with control peptides from Art v 1 (Art v 1₁₉₋₃₀ or Art v 1₉₄₋₁₀₅), the DRB1*0101/0401–restricted peptide from influenza hemagglutinin (HA₃₀₆₋₃₁₈),³⁰ or medium alone were unable to stimulate TCR tg T cells. Cellular activation induced by Art v 1₂₅₋₃₆ was strictly dependent on the presence of antigen-presenting cells because no T-cell stimulation was observed on cocultivation of TCR tg Jurkat T cells with peptides in the absence of EBV B cells (Fig 3, A). The immunodominant Art v 1₂₅₋₃₆ peptide did not activate WT Jurkat T cells, which were perfectly able to mount HLA class II–dependent responses in the presence of SEE (Fig 3, B). Dose-response determinations revealed that TCR tg Jurkat T cells required between 7.6 and 33.4 μ mol/L (12.4–54.8 μ g/mL) peptide for half-maximal stimulation (not shown).

TCR tg Jurkat T cells recognize Art v 1 borne by engineered invariant chain

The HLA CLIP occupies the peptide-binding groove during assembly and transport and becomes exchanged to antigenic peptides in endosomes/lysosomes.²⁴ Does the allergen-specific TCR also recognize Art v 1, which was genetically exchanged against CLIP in Ii expression constructs?

To answer this question, we generated aAPCs by transfecting human 293 cells with HLA-DRA*0101, HLA-DRB1*0101, CD80, CD54, and cathepsin S plus Ii constructs harboring different length variants of a codon-optimized version of the Art v 1 gene.²⁵ Alternatively, 293 cells were transfected with the control Ii constructs Ii::HA309-317 or Ii::CLIP. 293 Cells transfected with the Ii::Art v 1 versions, all harboring the minimal Art v 1₁₂₇₋₃₄ epitope,²¹ were able to stimulate the Art v 1–specific tg Jurkat T cells similar to the positive control PHA/phorbol 12-myristate 13-acetate (Fig 4, A). No stimulation was induced by 293 cells transfected with Ii::HA309-317, Ii::CLIP, or control vector, although these cell types were perfectly able to functionally bind SEE (Fig 4, B). However, no SEE-dependent response was observed in the absence of aAPCs. Fig 4, C, shows that HLA Class II expression was similar on all aAPC types, whereas Ii expression levels provided a more heterogeneous picture (Fig 4, D). Ii::Art v 1₁₋₁₀₉ fusion protein repeatedly showed the weakest expression, which also resulted in a weaker stimulatory capacity of Ii::Art v 1₁₋₁₀₉–transfected aAPCs.

Significantly, T-cell activation was only moderate in the absence of costimulation (stimulation index [SI], 3.2 ± 0.34); however, it became clear-cut when costimulatory molecules, such as CD80, were coexpressed (SI, 44.7 ± 8.7 ; Fig 5). In our system coexpression of CD80, together with the intercellular adhesion molecule 1 (CD54), on aAPCs revealed only moderate additional synergy (SI, 58.1 ± 15.2 ; Fig 5), which might reflect already maximal stimulation by CD80 alone, making a further enhancement by CD54 impossible.

Transfer of allergen-specific TCR into human PBLs

In a next step we tested whether it was possible to generate human Art v 1–specific T lymphocytes from nonallergic individuals. For that purpose, PBLs from 6 donors (HLA-DRB1*0101 positive or negative) were transduced with the Art v 1–specific TCR. Transduction efficiencies ranged from 10% to 30%, as determined by flow cytometry of CD3⁺ TCR V β 18⁺ T cells (Fig 6, A). TCR tg T cells from all donors specifically proliferated on

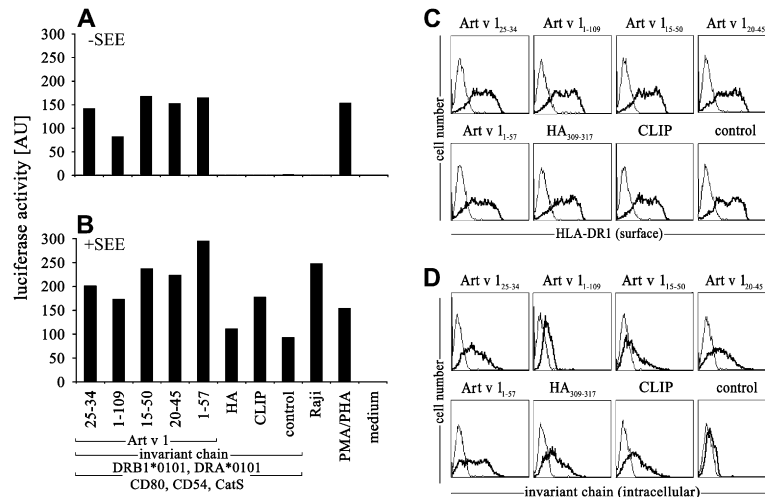


FIG 4. Art v 1-specific TCR tg Jurkat T cells recognize li-encoded allergenic peptides presented by aAPCs. **A**, IL-2 promoter activity of TCR tg Jurkat T cells (bulk) cocultured with 293 cells expressing HLA-DRB1*0101, HLA-DRB1*0101, CD80::GPI, CD54::GPI, cathepsin S, and the indicated forms of li or controls. Coculture with Raji B cells, PHA plus phorbol 12-myristate 13-acetate (*PMA*), or medium served as a control. **B**, Similar to Fig 4, A, but supplemented with SEE. Expression levels of HLA-DR1 (**C**) or li (**D**) in 293 aAPC transfectants (bold lines) superimposed by negative control staining (thin lines) is also shown.

coinocubation with aAPCs expressing li::Art v 1₂₅₋₃₆ but not with aAPCs expressing li::HA₃₀₉₋₃₁₇. Control-transduced T cells (green fluorescent protein) of HLA-DRB1*0101-negative donors showed residual reactivity with HLA-DRB1*0101-expressing aAPCs, possibly reflecting alloreactivity, which was not observed with responder T cells from HLA-DRB1*0101-positive donors. Moreover, stimulation of TCR tg PB T cells was strongly dependent on CD80-mediated costimulation. Within 10 days, up to 1×10^7 Art v 1-specific T cells can be generated from 10 mL of PB of various donors. This cell number can be further increased by a factor of approximately 3 by supplementation of TCR tg T cells with growth factors, such as IL-2. Thus PB T cells from non-allergic donors can be genetically modified to react with a well-defined allergen, such as Art v 1.

DISCUSSION

We cloned, sequenced, and transferred the TCR α - and β -chain genes of a CD4⁺ T-cell clone (SSR20) specific for the immunodominant T-cell epitope of the major mugwort pollen allergen Art v 1 to human T lymphocytes. The human Jurkat T-cell line, as well as PB T cells of nonallergic individuals, showed clear-cut expression of the tg TCR and specifically reacted with Art v 1₂₅₋₃₆ peptide-pulsed autologous EBV B cells, as well as with aAPCs expressing li::Art v 1 fusion proteins. Significantly, stimulation of TCR tg T cells was dependent on coexpression of costimulatory molecules, such as CD80 or CD86 (not shown) and, to a much lesser degree, on CD58 (not shown). To our knowledge, this is the first report describing the successful genetic transfer of a human allergen-specific TCR into human T lymphocytes.

We here attempted to contribute to a better definition of the critical molecular players within the “allergen-specific synapse” formed between antigen-presenting cells and T cells. Whereas immunodominant peptides and corresponding restriction elements of major allergens have been characterized in detail during the past 2 decades,^{31,32} the information on the molecular and functional definition of human allergen-specific TCRs remained scarce. The availability of recombinant, allergen-specific TCRs

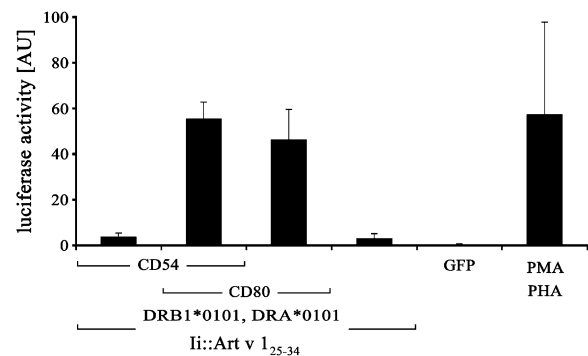


FIG 5. Efficient stimulation of Art v 1-specific Jurkat T cells requires costimulation. IL-2 promoter activity of TCR tg Jurkat T cells incubated with 293 cells cotransfected with DRA*0101, DRB1*0101, li::Art v 1₂₅₋₃₄, CD54, CD80, green fluorescent protein (*GFP*), or control plasmid alone or combined as indicated or phorbol 12-myristate 13-acetate (*PMA*) plus PHA. Data show mean values $\pm$ SDs of triplicate cultures representative of 3 independently performed experiments.

will allow us to set up biologic model systems in the future in which all 3 components of the allergen-specific synapse (ie, the restriction element, the immunodominant peptide, and the corresponding TCR) are defined at a molecular level and are based on a relevant allergic disease. Currently, allergen-specific T cells have to be expanded from the blood of patients with clinically well-defined allergy, a cumbersome task that is only successful shortly after the pollen season. In addition, the overall cell yields are often negligible. The transgenic approach allows the generation of high numbers of allergen-specific T cells in a short period of time, independent of the allergic status of the donor and the allergy season. Future studies will have to show whether and how allergen-specific TCR tg T cells can be polarized to exert defined effector functions. The steadily growing number of modified/recombinant allergens³³ (allergen [peptide] fragments,³⁴ trimers,³⁵ conjugates,³⁶ shuffled allergens,³⁷ or hybrid molecules³⁸) demands test systems able to predict their immunologic behavior. Allergen-

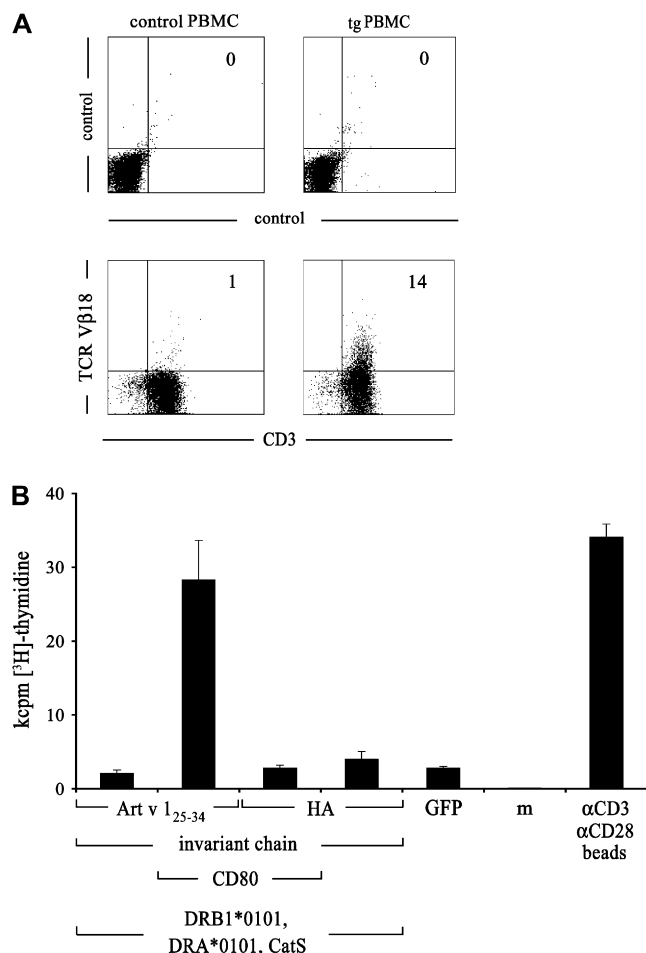


FIG 6. TCR tg PB T cells proliferate on challenge with li::Art v 1₂₅₋₃₆-expressing aAPCs. **A**, Expression levels of TCR Vβ18 and CD3 on control and tg PB T cells. Numbers indicate percentage of CD3⁺ TCR Vβ18⁺ cells. **B**, Proliferation of human PBMCs transduced with Art v 1-specific TCR cocultured with aAPCs coexpressing the indicated molecules or alternatively with microbeads coated with CD3 plus CD28 mAbs or with medium alone. Data show mean values $\pm$ SDs of triplicate cultures corrected by mean values (2.6 kcpm; range, 0–8.2 kcpm) of green fluorescent protein (GFP)-transduced tg T cells. Data are representative of several independently performed experiments.

specific tg T cells might represent such a standardizable tool to, for example, test for retained T-cell reactivity of modified/recombinant allergens.

In addition, the li-based sensitization strategy,²⁴ which we adapted for allergenic peptides in this report, might prove to be useful to define by means of recombinant DNA technology—altered peptide ligands of immunodominant allergens. Conventionally, altered peptide ligands are created by introducing single amino acid changes into peptide ligands of MHC, generating peptides able to influence autoimmune diseases but also allergies.^{39–42} The li-based allergen (peptide) expression system in combination with, for example, error-prone PCR approaches,⁴³ might serve as a novel high-throughput screening approach for such purposes.

Moreover, expressible allergen-specific TCRs might also lay the groundwork for the generation of valuable *in vivo* systems in the future, such as TCR tg “allergy mice” based on a human disease and based on human immune receptors. Allergy mice will represent powerful biologic systems to analyze T cell-dependent

(but also T cell-independent) pathways influencing allergic diseases with the required profoundness in the future.

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Clinical implications: Expressible allergen-specific TCRs might contribute to a better definition of the “allergen-specific synapse” and thus the processes leading to allergic diseases and their cure.

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