

IgE Production After Antigen-Specific and Cognate Activation of HLA-DPw4-Restricted T-Cell Clones, by 78% of Randomly Selected B-Cell Donors

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ABSTRACT: The frequency of expression of the MHC class II antigen, HLA-DPw4, in the caucasoid population is approximately 78%, and is unmatched by phenotypic frequencies of other HLA class II molecules. Here we describe three human Der-P1-specific T-cell clones (TCC), restricted by the HLA-DPw4-variant HLA-DPB1*0401, of which two TCC also responded to antigen, presented on HLA-DPB1*0402. Thus, randomly selected caucasoid donors present a 78% chance for a correct match with these HLA-DPw4-restricted TCC. This allows comparative *in vitro* antigen presentation studies with various antigen presenting cells (APC) from different (healthy or diseased) donors without the variable influence of responding T cells. It was subsequently dem-

onstrated that the TCC can be used to study antigen-induced IgE production in randomly selected primary B cells. Cognate HLA-DPw4-restricted antigen presentation caused enhanced immunoglobulin production of IgE, IgG1, IgA and IgM, of which only IgE induction was reversed by addition of anti-IL-4 antibodies. *Human Immunology* 61, 789–798 (2000). © American Society for Histocompatibility and Immunogenetics, 2000. Published by Elsevier Science Inc.

KEYWORDS: B-lymphocyte; T-lymphocyte; histocompatibility antigens class II; immunoglobulin class switching; antigen presentation

ABBREVIATIONS

TCC	T-cell clone
Th	T helper
MHC	major histocompatibility complex
APC	antigen presenting cell
EBV B cell	Epstein-Barr virus transformed B cell

TCR	T-cell receptor
BCR	B-cell receptor
DPT	house dust mite extract (<i>Dermatophagoides pteronissinus</i>)
Der-P1	major allergen of house dust mite
LST	lymphocyte stimulation test

INTRODUCTION

Activation, class switching, and differentiation of primary B cells *in vivo* is dependent on cognate interactions

between the major histocompatibility complex (MHC) class II molecules on the B cell and the correct antigen-specific T-cell receptor (TCR) expressed on T-helper (Th) cells [1]. The TCR does not only “see” the peptide in the groove of the class II molecule but, at the same time, recognizes the isoform of the class II molecule. Nonspecific class II molecules cannot present the antigen to a Th cell, but may induce a nonspecific “mixed lymphocyte reaction,” which *in vivo* is responsible for graft rejection (in the case of organ transplantation) or graft-vs.-host disease (in the case of bone marrow transplantation). The incompatibility between human donors has forced immunolo-

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gists to use inbred mouse strains to study *in vitro* MHC class II restricted cognate cell-cell interactions.

Alternatively some studies have used "allo" recognition between Th cells and the APC from incompatible donors to investigate, for example, the *in vitro* IgE class-switch induction in primary human B cells [2]. These studies indicate that, like in the mouse, human IgE production depends on contact between the MHC class II antigen and the TCR of the T cells. This activates the T cell and causes upregulation of CD40-ligand [3] and cytokine production (*e.g.* IL-4) that subsequently induce differentiation in those B cells that underwent B cell receptor (BCR) triggering [4, 5]. However, other studies have shown that the minimum requirement for IgE synthesis in highly purified B cells is the triggering of CD40 in the presence of IL-4 [6]. Subsequently, the role of BCR triggering, antigen processing, and presentation in a cognate fashion that directly influence the upregulation of important coreceptors such as CD40-ligand, CTLA4, CD40, CD80, and CD86 are left for debate.

The α - and β -chains of HLA-DR, -DQ, and -DP molecules are encoded by the MHC genes A and B and the study of their polymorphic variants is important for both transplantation tissue matching and HLA-disease associations. Immunologic studies in humans or with human tissues are, however, greatly hampered by the MHC diversity leading to incompatibility between donors. Unlike inbred mouse strains, TCC derived from one donor cannot easily be exchanged. Although the HLA-DPB1 gene has been shown to be as polymorphic as the DRB1 and DQB1 ones [7, 8], primed lymphocyte typing has distinguished only six different DP molecules with various predominances in different populations. In the caucasoid population DPw4 is the most frequent among the DPB1 alleles, giving rise to a phenotypic frequency of 78.2% for HLA-DPw4-specificity [9].

HLA-DP molecules have been shown to serve as a restriction element for antigen-specific T cells [10, 11]. Therefore, we hypothesized that TCC, with a restriction for HLA-DPB1*0401, the HLA-DPw4-variant that is expressed in 64% of the caucasoid population, could be used to study antigen-specific interactions with APC derived from randomly selected donors, without the need of laborious pre-selection of HLA-matched donors. TCC with specificity for Der-P1 [12] were tested for compatibility with a panel of Epstein-Barr virus transformed (EBV) B cell lines, covering all restriction possibilities present in the autologous, HLA-DPB1*0401-expressing donor. We were able to select three TCC with the required restriction, expressing different TCR as determined by β -chain sequence analysis [13], which were subsequently studied for their ability to antigen-specifically induce immunoglobulin production in DPw4-compatible B cell donors.

METHODS

Reagents

The culture media used were RPMI-1640 (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal calf serum (FCS) and Iscove's modified Dulbecco's medium (IMDM; GIBCO BRL) containing 10% FCS (HyClone, Logan, UT, USA), 50 μ g/ml soybean lipids (Nattermann, Cologne, Germany) and 90 μ g/ml human transferrin (Behring, Marburg, Germany)/30% FeCl₃ (Sigma, St. Louis, MO, USA). Both media contained 100 IU/ml penicillin, 100 μ g/ml streptomycin and 2 mM L-glutamine (all Gibco BRL). Recombinant human IL-2, IL-4, and GM-CSF were obtained from Novartis Research Institute (Vienna, Austria). Anti-IL-4, clone 1-41-1 was a kind gift of Dr. Franco Di Padova (Novartis Research Institute, Basel, Switzerland) and anti-HLA-DP antibodies clone B7/21 were obtained from Leinco Technologies, Inc. (Ballwin, MO, USA). Tissue culture plates and culture flasks were obtained from Costar (Cambridge, MA, USA) and MaxiSorp plates (Nunc, Denmark) were used for ELISAs. Freeze-dried Dermatophagoides pteronyssinus (DPT) antigen was purchased from ARTU biologicals (Lelystad, The Netherlands).

Isolation and Phenotyping of TCC

Human allergen-specific TCC were isolated from skin biopsies on positive atopy patch test reactions of an atopic dermatitis patient. Isolation, phenotyping and cytokine profiling of the TCC have been reported [12, 14]. The variability between TCR was determined by V β gene usage as described [13]. Briefly, total RNA was isolated, using RNA-SR (Biogenesis, Bournemouth, UK). By reverse transcription with a common TCR-C β primer (C β 3; 5' CTCCTTCCCATTACCCACCAGCT CAGCTC 3'), cDNA from the TCR β -chain was obtained and purified using the Magic DNA Clean-Up System (Promega, Madison, WI, USA). In a semiquantitative PCR, V β gene expression was determined [15], using V β subfamily-specific primer sequences as described by Hu *et al.* [15] for V β 1-20 and Genevée *et al.* [16] for V β 21-24 and the C β 3 primer as used in cDNA synthesis. Sequencing of the PCR products was performed by Dye Deoxy Terminator cycle sequencing (Applied Biosystems Inc., Foster City, CA, USA) with the C β primer, followed by sequence analysis with a Biosystems 373A automated DNA sequencer (Applied Biosystems Inc.).

Isolation of Antigen Presenting Cells

Primary B cells were obtained from tonsils of patients undergoing routine tonsillectomy. The tonsils were finely minced and the resulting cell suspension was en-

riched for B cells using T/non-T rosetting with neuraminidase-treated Sheep red blood cells (SRBC). CD3⁺ and CD14⁺ cells were subsequently depleted by the MACS magnetic separation system (Milteny Biotec, Germany). FACScan (Becton Dickinson, San Jose, CA, USA) analysis generally revealed > 96% CD19 positivity in the remaining B cell fraction.

EBV B cells were generated by depletion of T cells from PBMC using neuraminidase-treated SRBC followed by transformation of the remainder cells with EBV-containing supernatant as described [12].

Primary monocytes were obtained after elutriation [17]. Briefly, monocytes were separated from buffy coats of single blood units by centrifugation through a 0.8 mM sodium citrate-containing Lympho-paque gradient. Cells were resuspended in PBS 0.27 mM EDTA, 1% BSA. Elutriation was performed at 10°C using a JE-6 rotor in a J-6M centrifuge (Beckman Instruments, CA, USA), stepwise reducing the centrifugal force and increasing the medium flow.

HLA Typing

HLA-DR antigens were typed using specific alloantisera by two-color fluorescence assay [18]. HLA-DQA1 and HLA-DQB1 antigens were determined by PCR amplification with sequence-specific primers (PCR-SSP) [19]. High resolution HLA-DPB1 typing of the EBV B cell panel BTL1-BTL9 was performed using sequence based typing by fluorescent sequencing of the amplified polymorphic exon 2 as described [20]. HLA-DPB1 typing in compatible donors was performed using the INNO-LiPA multiprobe, strip-based detection system, based on the reverse hybridization principle (Innogenetics, Gent, Belgium).

Antigen-specific T-Cell Proliferation

APC were incubated overnight in RPMI with plateau-level amounts of antigen, 250 µg/ml DPT for B cells and 50 µg/ml for monocytes and MoDC. After irradiation (4000 rad) they were mixed in triplicate in 96 well U-bottom plates with 4×10^4 T cells that were freshly thawed or in a resting state approximately 14 d after restimulation. Antigens were never left in culture during the lymphocyte stimulation test and the APC:T-cell ratio was 1:1. Cultures were incubated for 5 d in a humidified incubator with 5% CO₂ at 37°C, the last 16 h in the presence of 1 µCi [³H]-thymidine (Amersham, UK). In the 5-d assay, 50 U/ml of IL-2 were added [12, 14]. Plates were harvested on Packard Unifilter microplates (Whatman Group, Canberra) and radioactivity was measured with a Packard TopCount Microplate scintillation counter. Data are presented as mean CPM ± SD of triplicate values.

Induction of IgE Synthesis

After overnight incubation in IMDM of tonsillar B cells with DPT (250 µg/ml), cells were washed and divided over a 96-well U-bottom plate with ten replicates in a concentration of 4×10^4 cells per well. Subsequently, 4×10^4 T cells were added together with 50 U/ml of IL-2. Cultures were incubated for 8 d in a humidified incubator with 5% CO₂ at 37°C. Supernatants were collected, pooled to triplicates, and immunoglobulin content was detected by capture ELISA. Data are presented as mean (ng/ml) ± SD of triplicate values.

Detection of Immunoglobulin Production

The ELISA for IgE, with a sensitivity of 80 pg/ml, was established with monoclonal mouse anti-human-IgE antibodies LE27 and biotinylated BSW17, kindly provided by Dr. Boda Stadler (Inselspital, Bern, Switzerland) and streptavidin-peroxidase (Gibco BRL). Standard curves were prepared with IgE standard serum (Behring).

The ELISA for IgG1, with a sensitivity of 6 ng/ml, was established with mouse anti-human IgG1 (The Binding Site, Birmingham, UK) and rabbit F(ab)₂ anti-human IgG (H+L)-HRP (Southern Biotechnology, Birmingham, AL, USA).

The ELISA for IgA, with a sensitivity of 1.4 ng/ml, was established with rabbit anti-human IgA (DAKO, Glostrup, Denmark) and alkaline phosphatase conjugated goat anti-human IgA (Biosource, Camarillo, CA, USA).

The ELISA for IgM, with a sensitivity of 4.1 ng/ml, was established with mouse anti-human IgM (Nordic, Tilburg, The Netherlands) and alkaline phosphatase conjugated goat anti-human IgM (Biosource). A human immunoglobulin calibrator (The Binding Site) was used for standard curves of IgG1, IgA, and IgM.

The substrate for both IgE and IgG1 ELISAs was a phosphate-citrate buffer containing sodium perborate and tetramethylbenzidine dihydrochloride (both from Sigma, St. Louis, MO, USA); for IgM and IgA, an alkaline phosphatase substrate kit was used (Biorad, Richmond, VA, USA). Plates were read at 450 nm for IgE and IgG1 and 405 nm for IgM and IgA with the SPECTRAMax ELISA reader (Molecular Devices, Sunnyvale, CA, USA) and data were analysed with SOFTmax PRO software.

RESULTS

Identification of Der-P1-specific TCC with HLA-DPB1*0401-restricted TCR

TCC were originally isolated from clinically non-involved patch tested skin biopsies [12] of an atopic dermatitis patient expressing the HLA-DPB1*0401 allele. To investigate which HLA class II molecule on the

TABLE 1 HLA phenotype in a panel of nine EBV B cell lines that were used to determine the TCR restriction in Der-P1-specific TCC

Donor	HLA-DR ^a	HLA-DQA ^b	HLA-DPB ^c
CFB4:2 ^d	2	0102	0101
	3	0501	0401
BTL1	2	0102	0401
	2	0102	0401
BTL2	2	0102	0202
	3	0501	0401
BTL3	2	0102	02012
	5	0501	0601
BTL4	3	0501	02012
	5	0501	0401
BTL5	5	0501	02012
	5	0501	0401
BTL6	2	0103	1301
	w6	0103	2701
BTL7	3	0103	0101
	w6	0501	1401
BTL8	w6	0102	0101
	w6	0102	0401
BTL9	1	0102	0301
	4	?	0601

^a Determined by serology.^b Determined by PCR-SSP method.^c Determined by sequencing.^d The HLA-phenotype of the autologous control donor.

The cell lines were selected by their differential expression of MHC class II molecules, present in the autologous EBV B cell line CFB4:2. Shared specificities with the original donor are presented in bold.

autologous APC was recognized by the TCR of our clones, several Der-P1-specific TCC were tested in an antigen-specific lymphocyte stimulation test (LST) using a panel of heterologous APC donors. For this purpose nine EBV B cell lines with partial overlap in HLA-DR, -DQ, and -DP molecules as shown in Table 1 were selected. T-cell proliferation in response to antigen presented by each EBV B cell line implicated restriction

similarities between autologous and heterologous APC. Previously described TCC were randomly selected for this study. The clones CFTS4:2.20, CFTS4:2.22, CFTS4:2.15, CFTS4:2.24, and CFTS4:3.1 proliferated upon antigen challenge with all HLA-DPB1*0401-expressing B cell lines (Figure 1). CFTS4:7.6 was restricted by HLA-DR2 and for the clones CFTS4:2.80 and CFTS4:2.6, HLA-DR3 positive APC-induced proliferation. HLA-DP restriction was confirmed by antibodies against HLA-DP, which inhibited HLA-DP restricted proliferation dose dependently without inhibiting proliferation in HLA-DR-restricted TCC or phytohemagglutinin-stimulated clones (data not shown).

Next, the TCR sequences of all HLA-DPB1*0401-restricted clones were investigated. First, as described [13], the TCR-V β gene usage was determined using the RT-PCR method followed by sequence analysis of the monoclonal V β -C β PCR products containing the VDJ region sites in the CDR3 region. The N-D β -N (N = randomly inserted nucleotides on both sides of the D β gene) sequences obtained from the above mentioned clones are depicted in Table 2. Finally, three genetically different HLA-DPB1*0401-restricted TCC were identified from one donor, distinguished by randomly inserted nucleotide sequences at junctional VDJ regions. CFTS4:2.22 and CFTS4:2.24 proved to be identical to CFTS4:2.20 and CFTS4:2.15, respectively, whereas CFTS4:3.1 [13] expressed a unique TCR sequence.

Randomly Selected APC Expressing HLA-DPw4 Induce Antigen-specific Proliferation in HLA-DPB1*0401-restricted TCC

The phenotypic frequency of HLA-DPB1*0401 in caucasians, as reported by al Daccak *et al.* [9], predicts compatibility between the three DP-restricted TCC with APC from 64% of the population. However, antigen-specific T-cell stimulation of CFTS4:3.1 was induced with 9 out of 10 selected B cell, 10 out of 14 EBV B cell,

TABLE 2 TCR N-D β -N nucleotide sequences with random nucleotide insertions and V β , J β , and C β subfamily usage in the TCC which antigen-specifically reacted with HLA-DPB1*0401-positive EBV B cell lines

Clone	V β	3' ^a	N-D β -N ^b	5' ^c	J β	C β
CFTS4:2.20	2	TAGAGA	ATGACGACAGGGGAGG	GCAATC	1.5	1
CFTS4:2.22	2	TAGAGA	ATGACGACAGGGGAGG	GCAATC	1.5	1
CFTS4:2.15	13	GTGCCA	TCAACCTTGGGACTAGCGGGGGCT	ATGAGC	2.1	2
CFTS4:2.24	13	GTGCCA	TCAACCTTGGGACTAGCGGGGGCT	ATGAGC	2.1	2
CFTS4:3.1	6	AGCAGC	CTAGTTGTACC	AGCCAA	2.4	2

^a 3' terminal nucleotides from V β subfamily.^b D β gene with randomly inserted nucleotides.^c 5' terminal nucleotides from J β subfamily.

From five isolated clones, three different sequences were obtained. Sequences are available from EMBL/Genbank/DDBJ under accession numbers X86361 (TCC 4:2.20), X86360 (TCC 4:2.15) and X86363 (TCC 4:3.1).

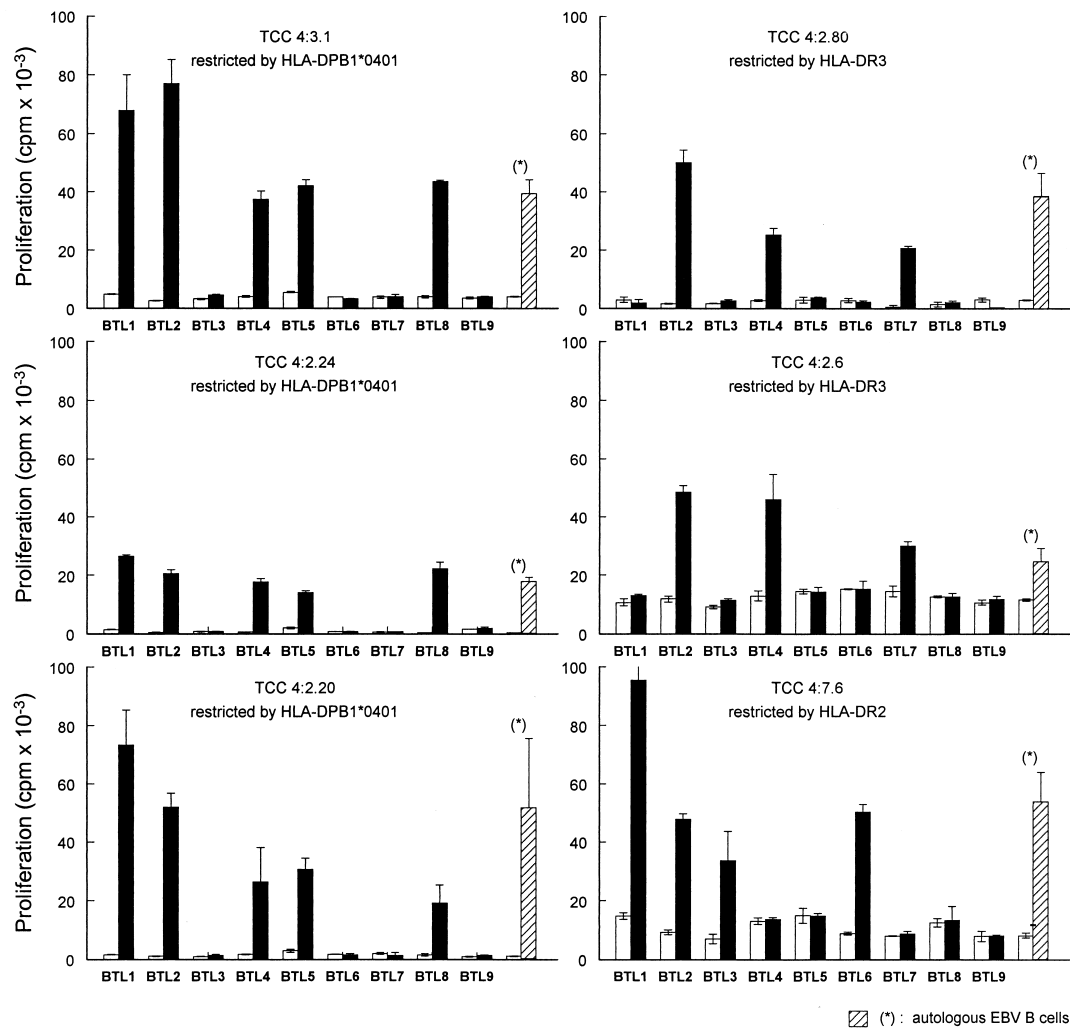


FIGURE 1 TCR restriction of Der-P1-specific TCC was determined in an antigen-specific LST with nine EBV B cell lines expressing overlapping HLA molecules, present in the autologous donor (see Table 1). APC were preincubated without (open bars) or with antigen (solid bars) and cultured with the TCC for 4 d in the presence of 50 U/ml exogenous IL-2. T-cell proliferation was subsequently measured by [³H]-thymidine incorporation, 16 h after addition. The autologous B-cell line was always taken as control to compare proliferation efficiency of APC, in and among experiments. Results are depicted as mean CPM $\pm$ SD from triplicate values of a representative experiment from six individual TCC.

and 20 out of 24 monocyte batches which equates to approximately 81% of randomly selected donors (Figure 2). Differences in stimulation indices between the various APC were not related to APC type, but represent variation between experiments, probably as a result of the relative resting state of T cells. When monocytes, tonsillar B cells, and EBV B cells were used in one experiment no significant differences were found (data

not shown). To confirm that randomly selected positive donors indeed expressed the right restriction element, the HLA-DPB1 allelic expression in 5 EBV B cell lines, 3 tonsillar B cell fractions, and 2 monocyte fractions, was analyzed in retrospect (Table 3). Three donors were homozygous for HLA-DPB1*0401 and two donors expressed HLA-DPB1*0401 on one allele. Three donors were negative for HLA-DPB1*0401 but expressed HLA-DPB1*0402, an isoform of HLA-DPw4 that differs from HLA-DPB1*0401 by three amino acids in the first domain [21].

Using an EBV B cell line that expressed HLA-DPB1*0402, we subsequently tested whether the clones CFTS4:2.15 and CFTS4:2.20, like CFTS4:3.1 were unable to discriminate between both DPw4 variants. Both the autologous HLA-DPB1*0401 and the HLA-DPB1*0402 expressing B cell lines induced comparable T-cell proliferation in CFTS4:3.1 and CFTS4:2.15 as depicted in Figure 3. However, CFTS4:2.20 was not

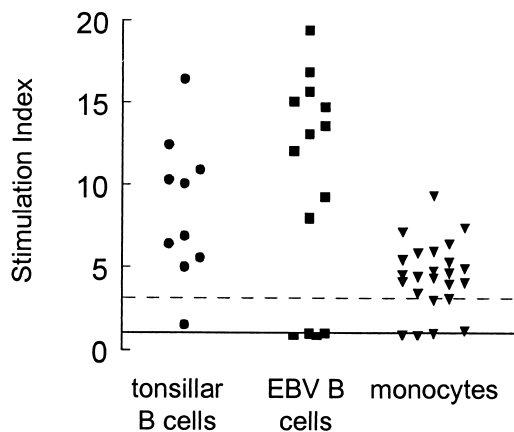


FIGURE 2 The HLA-DPB1*0401-restricted CFTS4:3.1 reacts in an antigen-specific cognate interaction with 81% of randomly selected APC fractions that were derived from donors in the caucasoid population. Tonsillar B cells (●), EBV B cells (■), or monocytes (▼) were preincubated $\pm$ antigen and added to the T cells in the presence of 50 U/ml exogenous IL-2. After 5 d, [^{3} H]-thymidine incorporation was measured and results are presented by stimulation index. Donors were considered compatible when antigen-pulsed APC could induce a 3 times higher [^{3} H]-thymidine incorporation compared to unpulsed APC (SI ≥ 3). Restriction compatibility was observed with 9 out of 10 tonsillar B cell, 10 out of 14 EBV B cell, and 20 out of 24 monocyte donors.

compatible with HLA-DPB1*0402 molecules, suggesting that the TCR of this clone recognizes antigen in the context of one of the three amino acid substitutions that have been reported [21] in the first domain of HLA-DPB1*0401 and HLA-DPB1*0402.

TCC Induce IgE Production in Normal Human Tonsillar B Cells Upon Antigen-specific Cognate Activation

The high incidence of DPw4 in the caucasoid population can be functionally put to use to study, for example, immunoglobulin production in antigen-presenting B cells. Because cognate interactions are required for class-switch induction in primary B cells, it was tested whether IL-4 secretion upon antigen-specific activation of CFTS4:3.1 and CFTS4:2.15 was able to induce IgE synthesis in tonsillar B cells. When DPw4-compatible B cells, preselected by their capacity to induce antigen-specific T-cell proliferation as described (section above), were loaded with antigen and added to the T cells, specific increases of IgE, IgG1, IgM, and IgA titers could be detected compared with antigen negative controls (Figure 4A). CFTS4:3.1 was not able to induce significant immunoglobulin production over background levels in antigen pulsed tonsillar B cells that did not express HLA-DPw4, or when matched B cells presented irrelevant antigen (data not shown). On the other hand, ad-

TABLE 3 Spot-check restriction determination for HLA-DPB1* with INNO-LiPA kit in five randomly chosen EVB B cell lines (EBVB*), three tonsillar B cell (TB*) fractions, and two monocyte fractions (EM*) that showed compatibility for CFTS4:3.1 in an antigen specific LST

Donor	HLA-DPB1 alleles	Associated HLA-DP specificity ^a
EBVBCE	0401	DPw4
	0401	DPw4
EBVBSZ	0401	DPw4
	0401	DPw4
EBVBRST	0401	DPw4
	0501 or 3801	DPw5 or ?
EBVBSIS	0401	DPw4
	0401	DPw4
EM110297	0401 or 2301	DPw4
	0402 or 5101	DPw4 or ?
EBVBEL	0402	DPw4
	4501	?
TB#3	0402	DPw4
	1701	?
TB#1	0402	DPw4
	2301	DPw4
TB200996	0401 or 2301	DPw4
	0402 or 5101	DPw4 or ?
EM210197	0301	DPw3
	0401	DPw4

^a Associated HLA-DP specificities are based on DPB1 determination.

? Indicated that for the DPB1 sequences typed "3801", "5101" and "1701", no matching serological HLA-DP specification is known [38].

dition of exogenous IL-4 (50 U/ml) significantly enhanced both antigen-specific and nonspecific IgE production, without affecting the other isotypes (Figure 4B). This indicates that the induction of IgE correlated to the IL-4 production that is induced in the TCC. Anti-IL-4 Fab₂ fragments could reverse antigen-specific induced IgE production (Figure 4C).

DISCUSSION

In this article, a novel model to study human cognate interactions involving well characterized antigen-specific human TCC and APC derived from randomly selected donors is presented. The model is based on the dominant frequency of the HLA-DPB1*0401 allele in the caucasoid population.

First, three Der-P1-specific TCC were identified, which responded to peptide presented by the HLA-DPB1*0401 molecules. Further analysis indicated that two of these clones also recognized antigen presented by HLA-DPB1*0402 molecules, which are not expressed by the autologous APC. Both HLA-DPB1*0401 and HLA-DPB1*0402 belong to the serologically defined HLA-

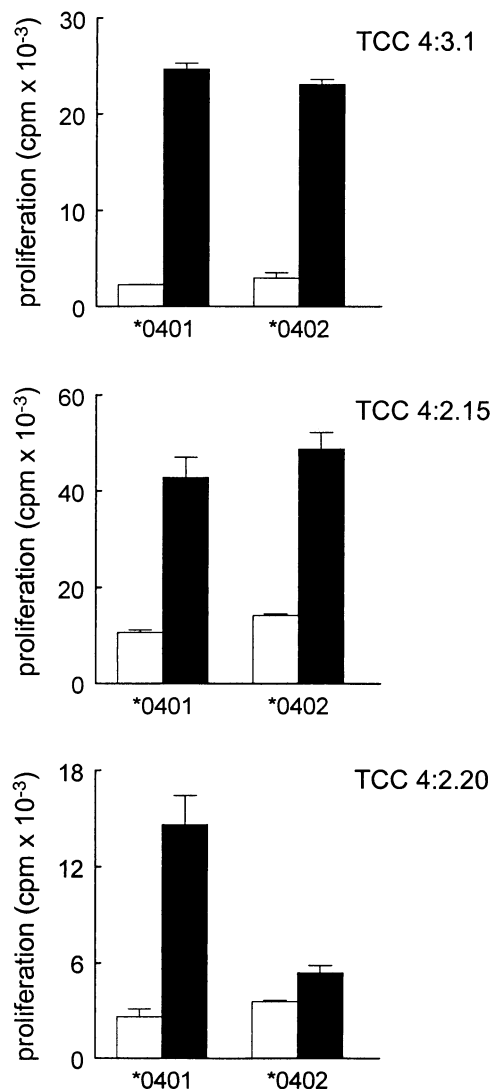


FIGURE 3 CFTS4:3.1 and CFTS4:2.15 are compatible with APC expressing either HLA-DPB1*0401 or HLA-DPB1*0402 molecules. Either autologous HLA-DPB1*0401-expressing or HLA-DPB1*0402-expressing EBV B cells were preincubated without (open bars) or with antigen (solid bars) and cultured with a TCC for 5 d in the presence of 50 U/ml of IL-2. [³H]-thymidine incorporation revealed that CFTS4:2.20 does not recognize antigen presented by HLA-DPB1*0402 molecules. Data are presented as mean CPM $\pm$ SD from triplicate values and represent one out of three experiments.

DPw4 phenotype, which is expressed by 78% of the caucasoid population. The high frequency of HLA-DPw4 allows random use of, for example, patient material, because expensive and laborious HLA-typing, which are necessary for this type of experiments will produce a positive result in 78% of the cases. The *in vitro* model based on human TCC with HLA-DPw4 restriction, for example, can be used to study the following:

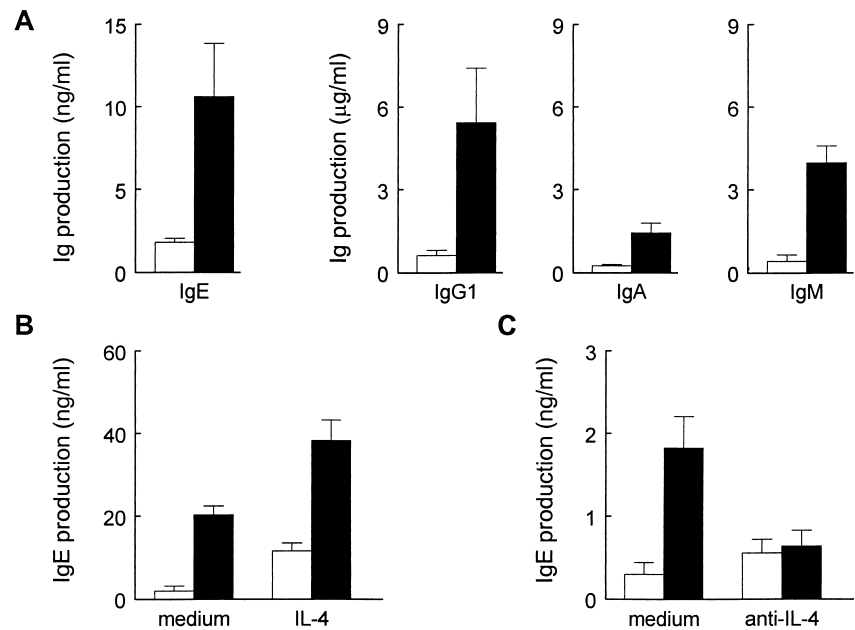
1. Pathways and efficiency of antigen-uptake by different APC within one donor and/or between donors.
2. The contribution of different costimulatory molecules and soluble factors from different APC in antigen presentation within one donor and/or between donors.
3. The functional capacity of APC from healthy donors versus diseased donors.
4. Isotype-specific immunoglobulin formation in antigen stimulated cultures by B cells from different organs and from different donors.

The advantage of the model is that the unpredictable variables, related to the responding antigen-specific T cells, become a constant and predictable factor in each experiment. The potential of this human model has been demonstrated, by a simple antigen-specific *in vitro* assay for induction of IgE synthesis in normal human tonsillar B cells.

Determination of the restriction element for the Der-P1-specific TCC was accomplished with a functional antigen-presentation assay. T-cell proliferation in response to antigen identified five from eight of the TCC tested, with HLA-DPB1*0401 restriction. However, further analyses of the hypervariable regions of the various TCR β -chains [13], indicated that only three genetically different TCC were identified with the required restriction.

Subsequent random testing of various APC donors with the HLA-DPB1*0401-restricted clone CFTS4:3.1 resulted in a ~81% compatibility percentage, which is 17% higher than the predicted phenotypic frequency for HLA-DPB1*0401 of 64% [9]. A higher restriction compatibility percentage indicated promiscuity of selected TCC, as has been described by others, for TCC that have the ability to recognize antigen in association with different class II molecules [22, 23]. Considering that serological tests with patient anti-sera cannot discriminate between the different DPw4-subtypes, it was hypothesised that the TCR of CFTS4:3.1 also did not distinguish between HLA-DPB1*0401 and HLA-DPB1*0402, which shows only a three amino acid difference in the first domain [21]. To calculate the phenotypic frequency for HLA-DPw4 in the caucasoid population, the following formula was used: $\{ \text{allele frequency} / 100 \times (2 - \text{allele frequency} / 100) \} \times 100 = \text{phenotypic frequency}$, where the allele frequency is composed of the relative frequencies of HLA-DPB1*0401 and HLA-DPB1*0402 (40% and 13.3%, respectively). This would imply that $0.533 \times (2 - 0.533) \times 100 = 78.2\%$ of randomly selected donors would match with CFTS4:3.1, which was indeed what we found. HLA-DPB1* determination in 10 compatible APC donors confirmed that five donors expressed HLA-DPB1*0401 and three donors expressed HLA-DPB1*0402. In addition, the remaining two do-

FIGURE 4 (A) Antigen-specific IgE, IgG1, IgA, and IgM induction in DPw4-compatible tonsillar B cells by CFTS4:2.15. B cells were preincubated without (open bars) and with antigen (solid bars) followed by an 8-d culture in the presence of T cells and 50 U/ml of IL-2. Data are presented as mean immunoglobulin amount $\pm$ SD from triplicate ELISA values of ten pooled replicates (3, 3, and 4 wells) and are representative for experiments with six different B-cell donors. (B) Exogenous IL-4 (50 U/ml) enhanced both bystander and antigen-specifically induced IgE production in cultures of tonsillar B cells and CFTS4:3.1. (C) F(ab')₂ fragments against IL-4 (1 μ g/ml) inhibited antigen-specifically induced IgE production in cultures of tonsillar B cells and CFTS4:3.1.



nors could be classified as HLA-DPw4, because they either expressed HLA-DPB1*0401 or HLA-DPB1*2301, both of which are serologically determined as HLA-DPw4 [21, 24, 25]. The relative frequency of both antigens makes it likely that the two donors expressed HLA-DPB1*0401. On the other hand, it may also be possible that the TCC did not differentiate between HLA-DPB1*0401 and HLA-DPB1*2301. However, the data do not support such a statement.

Human *in vitro* models of predominantly non-cognate T-cell-dependent B-cell activation clearly show that CD40L-CD40 interactions play a crucial role in class switch induction [3, 26–29]. Other stimulatory signals involved in B cell help include TNF α , CD58 (LFA-3), CD2, CD21, and CD28 [26–31]. Little is known about the relative role of the BCR, the TCR and the cognate interaction with the HLA-class II molecules and other co-receptors on the two cell types in this process. So far, only superantigens, such as staphylococcal enterotoxins, have been used to mimic *in vitro* cognate physiological T-to B-cell interactions [29, 32–36]. These models still bypass the role and need of antigen uptake and processing by the antigen-presenting B cell, processes that are included in the model described in this article. We have shown that, in the presence of antigen and in the presence of the right TCC, increased immunoglobulin production of IgM, IgG, IgA, and IgE class can be induced in normal tonsillar B cells. Only IgE induction was dependent on the production of IL-4 by the responding TCC, which in addition needed to proliferate, as indicated by the dependence of the model on exogenous IL-2 [14]. IgE titers, induced by antigen-specific stimulation

of the T cells, were inhibited by addition of anti-IL-4 antibodies, suggesting a class switch to IgE rather than plasma cell differentiation of already switched B cells [37]. The level of IL-4 production by the responding TCC appeared to be a limiting factor for the amount of IgE that was produced in the assay, as addition of exogenous IL-4 enhanced IgE production. However, in the presence of exogenous IL-4, also bystander IgE production, that is in the absence of antigen, was enhanced. This indicates that the TCC expressed a certain basal level of CD40L, which depends on the relative resting state of the cells before they were used in the experiments.

Considering the other immunoglobulin isotypes, addition of IL-2 enhanced immunoglobulin production in all cultures whereas IL-4 had no significant influence on IgM, IgG1, and IgA titers (data not shown). The antigen-specific rise in immunoglobulin production of these isotypes probably reflects the synthesis derived from memory B cells.

In conclusion, we show the unique use of TCC restricted by HLA-DPw4, in antigen-specific *in vitro* assays. In these assays the APC can be derived from randomly selected donors, that present a 78% chance for a correct match with these TCC. In addition we report a simple human *in vitro* model for IgE induction in antigen stimulated cultures with cognate cell-cell interactions.

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