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HLA-DP Allele-Specific T Cell Responses to Beryllium Account for DP-Associated Susceptibility to Chronic Beryllium Disease

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Occupational exposure to metals, such as molecules, is frequently associated with hypersensitivity reactions. Chronic beryllium (Be) disease (CBD) is a multisystem granulomatous disease that primarily affects the lung, and occurs in ~3% of individuals exposed to this element. Immunogenetic studies have demonstrated a strong association between CBD and possession of alleles of HLA-DP containing glutamic acid (Glu) at position 69 in the HLA-DPβ-chain. T cell clones were raised from patients with CBD in whom exposure occurred 10 and 30 years previously. Of 25 Be-specific clones that were obtained, all were restricted by HLA-DP alleles with Glu at DPβ69. Furthermore, the proliferative responses of the clones were absolutely dependent upon DPβ Glu69 in that a single amino acid substitution at this position abolished the response. As behs a disease whose pathogenesis involves a delayed type hypersensitivity response, the large majority of Be-specific clones secreted IFN-γ (Th1) and little or no IL-4 (Th2) cytokines. This study provides insights into the molecular basis of DP2-associated susceptibility to CBD. The Journal of Immunology, 2001, 166: 3549–3555.

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The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. This work was in part supported by the European Economic Community Biomed 1 Contract BMH1-CT92-0934, European Economic Community Environment Contract EVSV-CT92-0208, and U.S. Department of Energy Contract DE-FG02-93ER61714. C.G. was supported by a Medical Research Council Program Grant.

1 This work was in part supported by the European Economic Community Biomed 1 Contract BMH1-CT92-0934, European Economic Community Environment Contract EVSV-CT92-0208, and U.S. Department of Energy Contract DE-FG02-93ER61714. C.G. was supported by a Medical Research Council Program Grant.

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3 Abbreviations used in this paper: CBD, chronic beryllium disease; BAL, bronchoalveolar lavage; B-LCL, B lymphoblastoid cell line; Xh B9W, Xh International Histocompatibility Workshop; Pl, propidium iodide; CD, celiac disease.

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**FUNCTIONAL RELEVANCE OF HLA-DP ASSOCIATED SUSCEPTIBILITY IN BE DISEASES**

**Table I. PBMC from CBD patients proliferated to Be**

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>MHC Class II Tissue Typing</th>
<th>− Be</th>
<th>+ Be</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDC</td>
<td>DRB1<em>1501, 1101; DQB1</em>0601, −07; DPB1*0201</td>
<td>457 ± 181</td>
<td>134,283 ± 27,599</td>
</tr>
<tr>
<td>FC</td>
<td>DRB1<em>0301, 0701; DQB1</em>0201; DPB1*0201, 1101</td>
<td>322 ± 67</td>
<td>3,447 ± 617</td>
</tr>
<tr>
<td>NG</td>
<td>DRB1<em>0301, 04−05; DQB1</em>0201, 0302; DPB1*0201, 0001</td>
<td>253 ± 21</td>
<td>34,701 ± 2,632</td>
</tr>
</tbody>
</table>

* PBMC derived from CBD patients typed for HLA class II molecules as indicated were cultured for 5 days with Be. Proliferative responses in the presence and in the absence of Be are shown.

**Be and mitogen**

The beryllium sulfate tetrahydrate (BeSO₄·4H₂O), cobalt chloride hexahydrate (CoCl₂·6H₂O), and nickel (II) sulfite 7-hydrate (NiSO₄·7H₂O) were purchased from Sigma-Aldrich (Dorset, U.K.).

**Monoclonal Abs**

The B7/21 (anti-HLA-DP; American Type Culture Collection (ATCC), Manassas, VA), L2 (anti-HLA-DQ, ATCC), and L243 (anti-HLA-DR, ATCC) mAbs were used after purification on protein A-Sepharose beads by standard methods. Eluted Ab was dialyzed against three changes of PBS.

**Cell lines**

EBV-transformed B lymphoblastoid cell lines (B-LCLs) were obtained from the Xth International Histocompatibility Workshop (Xth IHW). HLA-DP⁺ B-LCLs (9045: DRB1*1104/1201, DPA1*01, DPB1*0201/0402; 9038: DRB1*1201, DPA1*01, DPB1*0201/0402; 9039: DRB1*1102, DPA1*01, DPB1*0201/0402; 9063: DRB1*1101, DPA1*01, DPB1*0201/0402) and HLA-DP² B-LCLs (9037: DRB1*1101, DPA1*01, DPB1*0201/0402; 9043: DRB1*1101, DPA1*0201, DPB1*1001; 9091: DRB1*1001, DPA1*01, DPB1*0402; 9013: DRB1*1501, DPA1*01, DPB1*0201) were used for this study. B-LCLs were cultured in RPMI 1640 tissue culture medium (Life Technologies, Paisley, U.K.) supplemented with 10% FCS, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 µg/ml streptomycin in 25-cm² flasks, and were regularly passaged.

Murine DAP.3 cell transfectants expressing either HLA-DPB1*0201 (workshop number 8301) or HLA-DPB1*0402 (workshop number 8305) were obtained from the Xth IHW. They were maintained in DMEM supplemented with 10% FCS, 0.2% sodium bicarbonate, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 µg/ml streptomycin, and MXH (6 µg/ml mycophenolic acid, 250 µg/ml xanthine, and 15 µg/ml hypoxanthine) to maintain expression of the transfected genes. Cells were grown in 25-cm² flasks and passed, following trypsinization, twice weekly.

**Generation and maintenance of Be-specific T cell clones**

PBMC were isolated from heparinized whole blood from the three patients by density centrifugation on a Lymphoprep gradient (Nycomed, Birming- ham, U.K.). PBMC were cultured with BeSO₄·4H₂O in 24-well plates (Costar, High Wycombe, U.K.). After 6 days, the cultures were enriched for lymphoblasts by centrifugation on Ficoll-Hypaque and cultured for two more weeks in the presence of Be-pulsed autologous irradiated PBMC and 20 U/ml rIL-2 (Boehringer Mannheim, East Sussex, U.K.). The T cells were then cloned by limiting dilution at 0.3–1 cell/well in Terasaki trays (Greiner, Gloucester shine, U.K.) in the presence of PHA (2 µg/ml) and 10³ allogeneic irradiated PBMC and rIL-2. After 10 days, cell growth was detected microscopically, and the contents of wells with growing cells were expanded further in medium with PHA, allogeneic PBMC, and rIL-2. The clones were maintained in culture by weekly stimulation with PHA, allogeneic PBMC, and rIL-2, in RPMI 1640 medium supplemented with 10% human serum, 2 µM L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, and MXH (6 µg/ml mycophenolic acid, 250 µg/ml xanthine, and 15 µg/ml hypoxanthine) to maintain expression of the transfected genes. Cells were grown in 25-cm² flasks and passaged, following trypsinization, twice weekly.

**Proliferation assay**

PBMC (10⁵ cells/well) were cultured with different doses of Be. T cell clones (10⁵ cells/well) were cultured with either B-LCL (3 × 10⁵ cells/well) or DP-expressing murine DAP.3 transfectants (3 × 10⁵ cells/well) pulsed with BeSO₄·4H₂O for 4 h and then treated with 120 Gy X-irradiation or with mitomycin C (50 µg/ml), respectively. The cells were plated out in flat-bottom microtiter plates, in a total volume of 200 µl, in RPMI 1640 medium supplemented with 10% human serum, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 µg/ml streptomycin. Wells were pulsed with 1 µCi of [³H]Tdr (Amersham International, Amersham, U.K.), after either 5 days (PBMC) or 48 h (T cell clones), and the cultures were harvested onto glass fiber filters 18 h later. Proliferation was measured as [³H]Tdr incorporation by liquid scintillation spectrometry. The results are expressed as the mean of triplicate cultures.

**Annexin V staining**

The B-LCL 9036 was pulsed with BeSO₄·4H₂O for 4 h and irradiated. The B-LCL were then incubated either in the absence or in the presence of anti-HLA-DP mAb (B7/21) or anti-HLA-DR mAb (L243) at a concentration of 10 µg/ml. The cells were then stained at various time points (45 min, and 1.5 and 3 h) with annexin V-FITC and the vital dye propidium iodide (PI) to assess apoptosis. The cells were then analyzed by flow cytometry on a Becton Dickinson (Mountain View, CA) FACSCalibur.

**Lymphokine production**

 Supernatants were collected after 24 h of T cell culture. Th1 and Th2 cytokines were measured using a standard ELISA. IFN-γ primary and secondary Abs were purchased from AMS Biotechnology (Oxon, U.K.), and IL-4 Ab were obtained from BioSource (Hertfordshire, U.K.). Briefly, primary Abs were coated overnight onto 96-well plates. The plate was then washed, and 1% BSA (Sigma-Aldrich) was used as the blocking reagent for 2 h. After washing, samples for testing were added to the wells along with IL-4 Abs obtained from BioSource (Hertfordshire, U.K.).

**Table II. Proliferative responses to Be of T cell clones derived from CBD patients**

<table>
<thead>
<tr>
<th>T Cell Clones</th>
<th>Proliferative Responses (Δ cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− Be</td>
</tr>
<tr>
<td>TDC1</td>
<td>0</td>
</tr>
<tr>
<td>TDC3</td>
<td>0</td>
</tr>
<tr>
<td>TDC4</td>
<td>0</td>
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<td>TDC5</td>
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<tr>
<td>TDC6</td>
<td>0</td>
</tr>
<tr>
<td>TDC13</td>
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<tr>
<td>TDC14</td>
<td>136</td>
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<tr>
<td>TDC17</td>
<td>740</td>
</tr>
<tr>
<td>TDC22</td>
<td>817</td>
</tr>
<tr>
<td>TDC30</td>
<td>0</td>
</tr>
<tr>
<td>TDC38</td>
<td>231</td>
</tr>
<tr>
<td>Be7</td>
<td>22</td>
</tr>
<tr>
<td>Be13</td>
<td>464</td>
</tr>
<tr>
<td>Be23</td>
<td>1,370</td>
</tr>
<tr>
<td>Be24</td>
<td>1,223</td>
</tr>
<tr>
<td>Be31</td>
<td>1,666</td>
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<tr>
<td>Be35</td>
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<td>Be41</td>
<td>223</td>
</tr>
<tr>
<td>Be73</td>
<td>143</td>
</tr>
<tr>
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</tr>
<tr>
<td>P1.2</td>
<td>0</td>
</tr>
<tr>
<td>P1.15</td>
<td>67</td>
</tr>
<tr>
<td>P2.2</td>
<td>108</td>
</tr>
<tr>
<td>P2.8</td>
<td>8</td>
</tr>
</tbody>
</table>

* T cell clones derived from patient TDC, FC (Be and P1), and NG (P2) were cultured for 3 days with B-LCL 9036 prepulsed with Be. Proliferative responses in the absence and presence of Be are shown.
with biotinylated secondary Ab. After 2 h, wells were washed and streptavidin peroxidase conjugate (BioSource) was added for 45 min. Wells were washed thoroughly, and tetramethylbenzidine substrate was added. This led to color development, which was stopped using H$_2$SO$_4$. The plates were then read at 540 nm.

**RNA extraction and cDNA synthesis**

Cells (5 × 10$^5$) were placed in a sterile Eppendorf tube for RNA extraction. RNA was extracted using the method of Chomczynski and Sacchi (16). To each tube, 1 ml of cold RNAzol was added and the tube was vortexed. After a 5-min incubation on ice, 100 μl of chloroform was added, and the samples were centrifuged for 15 min at 4°C. The upper phase was then transferred to a clean tube containing an equal volume of isopropanol and 10 μg of transfer RNA as a carrier. After a 15-min incubation on ice, the sample was again centrifuged at 4°C for 15 min, the supernatant was removed, and the RNA pellet was washed with 1 ml of cold ethanol. The air-dried pellet was resuspended in 10 μl of autoclaved distilled water and stored at −70°C. cDNA was synthesized using oligo-dT-primed, Moloney murine leukemia virus-derived reverse transcriptase (Life Technologies) and 5 μl of RNA sample in 20-μl reactions. cDNA reactions were diluted to 100 μl and stored at −70°C; 2 μl of diluted cDNA was used for each PCR.

**PCR amplification and sequencing**

For analysis of TCR V-gene usage, cDNAs were amplified using a panel of primers specific for TCR V$\alpha$ or V$\beta$ families in combination with a C$\beta$B primer. TCR primer sequences were as in Reference (17). Samples were amplified by PCR for 30 cycles (30 s at 94°C, 30 s at 64°C, and 30 s at 72°C) for both TCR V$\beta$ and TCR V$\alpha$ primers. The TCR C$\beta$ reverse primer sequence was: 5′-GGCACAGCAGACCCCT TGCTGTTAGGACAC-3′. The TCR C$\alpha$ reverse primer sequence was: 5′-ACTTTGTGACACATTGTGAG-3′.

The amplified fragments were separated by agarose gel electrophoresis and stained with ethidium bromide. The specific bands were cut, and the amplified products were purified using a gel band purification kit (Pharmacia Biotech, Uppsala, Sweden). Direct sequencing was performed using a primer upstream of the TCR C$\beta$ reverse primer: 5′-TGTCGACCTCCTTCCCATTCA-3′ and a primer upstream of the TCR C$\alpha$ reverse primer: 5′-AGCGACAGACCTT GTCACTG-3′. A PCR-based sequence kit (Perkin-Elmer, Norwalk, CT) was used following the manufacturer’s instructions and using [35S]dATP as isotope; 1 μl of each sequencing reaction was loaded onto a 6% urea-polyacrylamide gel, and the bands were separated by electrophoresis. The gels were dried and analyzed by autoradiography.

**Results**

**T cell clones generated from patients with CB are specific for Be**

Three patients with CBD were selected for this study. They all expressed the susceptibility-conferring Glu at position 69 in one or both of their DP$\beta$-chains. PBMC were separated and cultured with different doses of Be. Dose-dependent T cell proliferation was seen in all three patients. In Table I is shown the proliferation of PBMC to the optimal dose of Be. PBMC from healthy controls did not show any significant response to Be, although three of six control T cell clones generated from patients with CBD are specific for Be. Their reactivity to different doses of Be was then investigated using homogenous BeSO$_4$-treated, MOLT-3 cells. PBMC were separated and cultured with 10$^5$ cells/well, and TDC1 and TDC22, were cultured with B-LCL 9036 pulsed with Be ( ), Co ( ), and Ni ( ). After 48 h [3H]TdR was added and the plates were harvested 18 h later. Proliferation is expressed as Δcpm.

**T cell clones specific for Be are restricted by HLA-DP**

The restriction element used by the Be-specific T cell clones was first examined by using a panel of homozygous B-LCLs from the Xth IHW as APCs. In Fig. 2 is shown the proliferation of three representative Be-specific T cell clones. All the T cell clones tested responded to Be only when it was presented by B-LCLs expressing HLA-DP2-expressing B-LCL 9036 (3 × 10$^5$ cells/well) pulsed with different doses of BeSO$_4$ and Ni. T cell clones, TDC1 and TDC22, were cultured with B-LCL 9036 pulsed with Be ( ), Co ( ), and Ni ( ). After 48 h [3H]TdR was added and the plates were harvested 18 h later. Proliferation is expressed as Δcpm.

**FIGURE 1.** T cell clones generated from patients with CBD respond specifically to Be in a dose-dependent manner. T cell clones, TDC17 (a), TDC38 (b), Be 13 (c), and PI.1 (d), were cultured (10$^5$ cells/well) with HLA-DP2-expressing B-LCL, 9036 (3 × 10$^5$ cells/well) pulsed with different doses of BeSO$_4$ and Ni. T cell clones, TDC17 and TDC22, were cultured with B-LCL 9036 pulsed with Be ( ), Co ( ), and Ni ( ). After 48 h [3H]TdR was added and the plates were harvested 18 h later. Proliferation is expressed as Δcpm.
observed with the anti-DR mAb was due to cell death of the B-LCL presenting Be. This hypothesis is in agreement with the work of Drenou et al. (18). They showed, in a slightly different system, that cross-linking of MHC-class II molecules on a B cell lymphoma led to apoptosis (18). To address this possibility, B-LCL were incubated with the anti-DR mAb (L243) for different lengths of time as described in Materials and Methods, and the percentage of apoptotic and necrotic cells was measured using PI and annexin V staining. After 45 min nearly 10% of the cells were undergoing apoptosis and close to 20% had already died (data not shown). After 3 h the addition of anti-DR (L243) mAb induced >30% cell death and another 14% undergoing apoptosis (Fig. 4, e and f). In contrast, anti-DP mAb (Fig. 4, c and d), which inhibited T cell proliferation, did not cause any cell death compared with untreated cells (Fig. 4, a and b).

Position 69 in the DPβ-chain is critically important in T cell recognition of Be

The genetic analysis of patients with CBD has revealed an association with alleles of HLA-DPB1 encoding a DPβ-chain with glutamic acid at residue 69. To investigate the functional relevance of Glu at residue 69 in the β-chain, we tested the response of Be-specific T cell clones to B-LCLs expressing either HLA-DPB1*0201 or HLA-DPB1*0402 that shared the same DPα-chain and differ only at position 69 in the β-chain. All the Be-specific T cell clones obtained responded only to the B-LCL with Glu at residue 69. The dose-dependent proliferation for four representative T cell clones is shown in Fig. 5, a–d.

The MHC restriction of the Be-specific T cell clones and, in particular, the importance of Glu at residue 69 were further investigated using murine DAP.3 cells transfected with cDNA clones encoding either HLA-DPB1*0201 or HLA-DPB1*0402 (8305). Only 3 of 12 T cell clones tested were capable of responding to the DAP.3-DP2 transfectants. In Fig. 5 are shown the proliferative responses of Be 23 (e) and Be41 (f) to different doses of Be presented by DAP.3-DP2 transfectants. No proliferation was seen to DAP.3 cells expressing comparable levels of DPB1*0402 (Fig. 5). Altogether, these data suggest that HLA-DP is the restriction element for Be-specific T cells and that the presence of Glu at residue 69 is essential for the reactivity of these T cell clones to Be.

The Be-specific T cell clones are predominantly Th1 cells with biased TCR usage

It has been shown previously that delayed type hypersensitivity reactions are mediated by T cells of the Th1 phenotype. In this...
context, we have analyzed the cytokine production profile of Be-specific T cell clones. As shown in Table III, all the Be-specific T cell clones tested produced IFN-γ and little or no IL-4. These results confirm similar data obtained using bronchoalveolar lavage (BAL) cells derived from patients with CBD (19) and suggest that the Be-specific T cell clones raised from PBMC of CBD patients are likely to be derived from pathogenic T cells. Further data supportive of this suggestion were provided by TCR gene analysis on four of the TDC clones (Table IV). Two distinguishable patterns were recognizable. T cell clones TDC1 and 30 shared a “hydrophobic-Ser-negatively charged amino acid” sequence motif at the N-terminal of CDR3α region as well as a Thr at position 94 of the CDR3β region, whereas TDC17 and 38 showed an identical motif “Ser-Gly-Gly-Ser” in the CDR3 region of the α-chain as well as a Ser at position 97 of the CDR3β region. However, the most striking observation is that TDC30 displayed a CDR3 region identical, besides a conservative substitution (Val-Leu) at position 92, to a sequence previously reported as present at high frequency in the BAL of a patient with CBD (20). The associated β-chains also showed a common motif “Gly-Asp”, also overrepresented in the CDR3β region of all patients with CBD reported in the same paper (20). Moreover, the T cell clone TDC38 used the Vα22 chain as reported for the patients analyzed by Fontenot et al. (20).

Discussion
To investigate the molecular basis of HLA-DP-associated susceptibility to CBD (13), T cell clones were generated from three patients with this disease. Be recognition by all the established T cell clones was restricted by HLA-DP with Glu at residue 69, and led to the preferential secretion of IFN-γ. The DP restriction of the Be-specific clones observed here suggests strongly that the DP association in CBD reflects the role of certain alleles of DP in presentation of this metal to T cells in vivo.

There are many examples of HLA-DR and -DQ associations with resistance or susceptibility to a variety of diseases. In particular, celiac disease (CD) has been shown to be primarily associated with a pair of HLA genes: DQA1*0501 and DQB1*0201 (21). More recently, the same group has demonstrated that the CD-associated heterodimer is used as a restriction element in the recognition of gliadin peptides by T cells derived from the gut of CD patients (22). HLA-DP associations have also been described in autoimmune diseases, such as CD (23), insulin-dependent diabetes mellitus (24), pauciarticular juvenile rheumatoid arthritis (25–27), and juvenile ankylosing spondylitis (28). In addition, an HLA-DP association has recently been demonstrated in primary biliary cirrhosis (29), in childhood common acute lymphoblastic leukemia (30), and also in Thai individuals with enhanced vaccine-induced Ab response to a malaria sporozoite Ag (31). However, some of these associations have not been confirmed (32, 33), and in none of

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** Anti-HLA-DR mAb induces cell death of the APC. B-LCL 9036, pulsed with Be and irradiated, were then incubated for 3 h either in the absence (a and b) or in the presence of anti-HLA-DP mAb (B7/21) (c and d) or anti-HLA-DR mAb (L243) (e and f). The percentage of cells undergoing apoptosis (annexin V-FITC-positive and PI-negative) and already dead (annexin V-FITC- and PI-positive) is indicated.

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** The presence of Glu at position 69 in DPβ is necessary for Be recognition by T cells. T cell clones (10^4 cells/well), TDC1 (а), TDC17 (б), Be23 (в), and Be41 (г) were cultured with 3 × 10^4 cells/well of Be-prepulsed B-LCLs expressing either 9036, DPB1*0201 (■) or 9013, DPB1*0402 (●), pre-pulsed with different doses of BeSO_4·4H_2O. a and f, Be 23 and Be 41, respectively, were cultured in the presence of murine DAP3 cell transfectants expressing either 8301, DPB1*0201 (●) or 8305, DPB1*0402 (▲). Proliferation is expressed as Δcpm.
**Table III. Be-specific T cell clones are mostly of Th1 phenotype**

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>IFN-γ Concentration (pg/ml)</th>
<th>IL-4 Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− Be</td>
<td>+ Be</td>
</tr>
<tr>
<td>TDC1</td>
<td>0</td>
<td>&gt;1,250</td>
</tr>
<tr>
<td>TDC14</td>
<td>110</td>
<td>1,098</td>
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<tr>
<td>TDC17</td>
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<tr>
<td>TDC30</td>
<td>98</td>
<td>&gt;1,250</td>
</tr>
<tr>
<td>TDC38</td>
<td>2.8</td>
<td>812</td>
</tr>
<tr>
<td>Be23</td>
<td>4.4</td>
<td>51.2</td>
</tr>
<tr>
<td>Be41</td>
<td>3.1</td>
<td>593.8</td>
</tr>
</tbody>
</table>

* T cell clones were cultured with B-LCL 9036, and supernatants were harvested after 24 h. IFN-γ and IL-4 were measured using a standard ELISA kit.

**Table IV. TCR sequences of Be-specific T cell clones**

| TCRAV1 | TDC1 | AV12 | CA   | L S E A D A G G T S Y G | K L T F G Q G | J14.3 |
|        | TDC30 | AV1  | CA   | V S D N Q G A Q | K L V F G Q G | J14.4 |
|        | TDC38 | AV22 | CA   | L S D N Q G A Q | K L V F G Q | J9.3  |
|        | TCD17 | AV23 | CA   | V Q A S G G S Y | I F T F G R G | J15.3 |

| TCRBV1 | TDC1 | BV5  | CA   | S G T A F L | Y G Y T F G S G | J1.2  |
|        | TDC30 | BV12 | CV   | R Q T G D | Q P Q H F G D G | J1.5  |
|        |       | BV3  | CAS  | Y G D | T Q Y F G D | J2.3  |
|        | TDC17 | BV14 | CA   | S K L G T S | D T Q Y F G P G | J2.7  |
|        | TDC38 | BV20 | CA   | V S V A A S K | Q Y F G P G | J2.7  |

* The sequencing of the TCR was performed using the primers indicated in Materials and Methods.

* The sequences in italics indicate the TCR analysis performed by Fontenot et al. (20).
Finally, the pattern of cytokine production by the Be-specific clones provides further evidence that these in vitro observed responses to Be reflect in vivo events in that the T cell clones appeared mostly to be of the Th1 subset that would be predicted to be pathogenic in a chronic inflammatory lung disease.

These data give a functional basis to the genetic association between CBD and HLA-DP Glu69 previously reported by us (13) and represent a step forward to the understanding of the pathogenesis of CBD.

Acknowledgments

We thank Ilaria Potolocchio and Luca Richeldi for HLA-DP tissue typing of controls and patients.

References