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Identification of Pathogenic T Cells in Patients with Beryllium-Induced Lung Disease

Andrew P. Fontenot,*†§ Michael T. Falta,†¶ Brian M. Freed,*† Lee S. Newman,*†§ and Brian L. Kotzin,*† §

Chronic beryllium disease (CBD) is caused by beryllium exposure and is characterized by granulomatous inflammation with accumulation of CD4+ T cells in the lungs. We analyzed TCR β-chain and α-chain genes expressed by these CD4+ T cells. In the lungs of individual patients, as well as among four of five CBD patients studied, different oligoclonal expansions within the Vβ3 subset were found to express homologous or even identical CDR3 amino acid sequences. These related expansions were specific for CBD patients, were compartmentalized to lung, and persisted at high frequency in patients with active disease. Limiting dilution cloning and analysis of coexpressed TCR α-chain genes confirmed that these TCRs were selectively expanded by a common Ag involving beryllium. Overall, homologous TCR β- and α-chains showed identical V regions and invariant charged residues within the CDR3 but considerable variability in TCRJ usage. Remarkably, CBD patients expressing nearly identical TCRs did not share common HLA-DRB1 or DQ alleles. These results implicate particular CD4+ cells in the pathogenesis of CBD and provide insight into how beryllium is recognized in human disease. The Journal of Immunology, 1999, 163: 1019–1026.

Due to its low density and high stiffness to weight ratio, beryllium continues to be utilized in the aerospace, automotive, ceramics, electronics, and defense industries (1, 2). Exposure to beryllium, which occurs almost always in the workplace, continues to be a public health concern with approximately 800,000 individuals currently at risk for developing chronic beryllium disease (CBD) (1). This disorder is estimated to develop in 1–16% of exposed individuals, depending on the nature of the exposure (3–6). The disease is characterized by granulomatous inflammation, primarily involving the lung (1). Disease severity can vary from histological abnormalities without clinical impairment or symptoms to severe lung destruction requiring transplantation. The diagnosis of CBD requires a history of exposure to beryllium, the presence of granulomatous inflammation in a biopsy specimen, and a proliferative response of blood or lung T cells to beryllium in vitro (7).

Evidence suggests that CD4+ T cells are important in the immunopathogenesis of CBD (7–10). For example, sensitization to beryllium is detected by the ability of CD4+ T cells to proliferate in response to beryllium sulfate (BeSO4) in culture, and the development of granulomatous inflammation in the lung is associated with the accumulation of CD4+ T cells in the bronchoalveolar lavage (BAL) (3, 8, 9, 11, 12). The in vitro T cell response to BeSO4 requires presenting cells expressing class II MHC molecules and has been shown to be MHC restricted (9). How the TCR interacts with beryllium and the MHC remains unknown. Recent studies of BAL CD4+ T cells from CBD patients showed alterations of TCR β-chain V region (Vβ) expression compared with peripheral blood (10). In particular, 11 of 28 CBD patients demonstrated 16 different T cell subset expansions in the BAL, and approximately one-third of these expansions expressed Vβ3. We hypothesized that these increased percentages of Vβ3+ T cells in CBD patients represent clonal or oligoclonal expansions, consistent with conventional Ag stimulation. In the present study, we analyzed the expressed TCR β-chain (TCRB) and α-chain (TCRA) genes and specificity of expanded CD4+ T cells in the lungs of CBD patients. We identified oligoclonal expansions within the Vβ3 subset that persisted in BAL over long periods of time but were rarely present in blood of the same patients. In addition, expressed TCRBV3 genes with identical or homologous CDR3 amino acid sequences were apparent within individual patients as well as among four of five CBD patients studied. Limiting dilution cloning and analysis of BAL CD4+ T cells expressing homologous TCR genes confirmed the coexpansion of highly homologous TCR genes and strongly suggest that these clones were selected in the lung by a common Ag involving beryllium. Together, the results implicate these CD4+ T cells in the pathogenesis of CBD and provide new insight into T cell recognition of beryllium and possibly other metals in human disease.

Materials and Methods

Study population

The diagnosis of CBD was established using previously defined criteria (8, 13), including the presence of granulomatous inflammation on lung biopsy, a history of exposure to beryllium, and a positive proliferative response of BAL T cells to BeSO4 in vitro. Certain relevant characteristics of the patients studied are shown in Table I. A group of three healthy individuals and three sarcoidosis patients served as control subjects for the BAL study. Informed consent was obtained from each CBD patient and control subject, and the protocol was approved by the Human Subject Institutional Review Board at the National Jewish Medical and Research Center.

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3 Abbreviations used in this paper: CBD, chronic beryllium disease; BeSO4, beryllium sulfate; BAL, bronchoalveolar lavage; TCR, TCR α-chain gene; TCRB, TCR β-chain gene; Vβ, TCR β-chain V region.

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HLA typing was performed by standard molecular techniques at the University of Colorado Health Sciences Center Clinical Immunology and Histocompatibility Laboratory (Denver, CO).

Analysis of TCRBV3 gene expression in peripheral blood and BAL CD4+ T cells

Mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque density gradient separation and from BAL as previously described (14). Peripheral blood cells from CBD patients and BAL cells from healthy control subjects were stained with FITC-labeled CD4 (Becton Dickinson, San Jose, CA), and CD4+ T cells were sorted using an Epics 751 sorter (Coulter, Hialeah, FL). Sorting of BAL cells from CBD and sarcoidosis control subjects for CD4+ T cells was not performed due to the predominance of CD4+ T cells in this compartment (>80% of CD4+ cells expressed CD4). However, as indicated below, all repeated and homologous TCR sequences in the CD4 sample were subsequently shown to be derived from the CD4+ population because subsequent studies were performed on cells sorted for expression of both CD4 and Vβ.

Total RNA was isolated using an acid guanidinium-phenol chloroform method, and cDNA was synthesized using 2 µg RNA/20 µl reaction. cDNA synthesis reagents included the following: reverse transcriptase (SuperScript RT; Life Technologies, Grand Island, NY), ribonuclease inhibitor (Promega, Madison, WI), and dNTPs and random hexamers (both from Pharmacia Biotech, Piscataway, NJ). One microliter of the cDNA reaction was added per 50-µl PCR reaction mixture. PCR amplification (AmpliTaq; Perkin-Elmer, Branchburg, NJ) was performed for 35 cycles, and both 5′ and 3′ oligonucleotide primers were present at a concentration of 0.3 µM. Sequences of the 5′ TCRBV3 primer and the 3′ primer are 5′-CTCTCTAGAGAGAAGAAGGAGCGC-3′ and 5′-TTCTGATGCTCAAAACGAC-3′, respectively. The PCR products were ligated into the PCR II TA cloning vector (Invitrogen, San Diego, CA), and the ligated products were transformed into Epicurian Coli XL-1 Blue supercompetent Escherichia coli cells (Strategene, La Jolla, CA). Colonies containing inserts were randomly selected for nucleotide sequencing. Cycle sequencing was performed using M13 reverse (5′-CAGGAAACAGCTATGAC-3′) and/or M13 forward (5′-CCCCAGTCACGACGTTGAAACG-3′) sequencing primers and an automated ABI 377 sequencer (Applied Biosystems, Perkin-Elmer, Foster City, CA).

Generation of T cell clones from the BAL of CBD patients

To further analyze the TCRBV3 gene repertoire and to identify the TCRA genes coexpressed by particular T cell clones, BAL mononuclear cells were stained for CD4 and Vβ3 (clone 8F10; T Cell Sciences, Cambridge, MA) (15, 16), and double-positive cells were sorted and cloned by limiting dilution. Variable numbers (1, 3, or 10 cells per well) of sorted T cells and 1 × 10^6 irradiated (9000 rad) TK6 feeder cells per well were cultured in 96-well microtiter plates (Falcon, Becton Dickinson Labware, Bedford, MA) in RPMI 1640 media (BioWhittaker, Walkersville, MD) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), 20% HL-1 culture media (BioWhittaker), 20 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine (all from Life Technologies), 0.25 µg/ml PHA (Murine Diagnostics, Dartford, England), and 10% T cell growth factor supernatant (a gift from R. J. Albertini, University of Vermont, Burlington, VT) (17). After 12–14 days of culture, T cell colonies were transferred to 1-ml cultures. Cells from confluent cultures were harvested and total cellular RNA was isolated using a commercially available kit (RNAid PLUS, BIO 101, La Jolla, CA). cDNA was prepared, and the TCRBV3 primer and TCRBC primer as described above. Each PCR product was purified using a DNA binding membrane spin column (QIAquick PCR Purification Kit, Qiagen, Chatsworth, CA) and sequenced using a TCRBV3 sequencing primer (5′-CGACCTCGGGTGGAACACTAC-3′).

T cell clones were also derived by limiting dilution from peripheral blood and BAL CD4+ T cells of a normal individual after PHA stimulation using methods similar to those described above. After 14 days of culture, Vβ3 clones were selected and transferred to 1-ml cultures in the absence of PHA. One week later, proliferation assays were performed using T cell clones (1 × 10^5 cells) cultured in 96-well flat-bottom microtiter plates with 1 × 10^5 autologous, irradiated (3000 rad) PBMCs in the presence of 1 × 10^{-5} M BeSO_{4} or 1 µg/ml PHA for 3 days. The wells were then pulsed with 1 µCi of [3H]thymidine for 16 h, and incorporation of radioactivity was determined by β-emission spectroscopy. Proliferation assays were performed in triplicate.

Beryllium-specific clones were also derived by limiting dilution of BAL cells in 24-well plates (Costar, Cambridge, MA) in 2 ml of culture media in the presence of 1 × 10^{-5} M BeSO_{4} for 5 days. Subsequently, Ag was removed, and the lymphoblasts were expanded further in culture media containing 10% T cell growth factor as described above. After 5 days, the lymphoblasts were stained with mAbs to Vβ3 and CD4 as described above, and double-positive cells were sorted and cloned by limiting dilution with 1 × 10^{-5} M BeSO_{4} in the presence of autologous, irradiated (3000 rad) PBMCs for 12 days. Subsequently, T cell clones were further expanded in culture media containing 10% T cell growth factor supernant (described above). T cell clones were maintained in culture by cycles of restimulation (every 2–3 wk) with 1 × 10^{-5} M BeSO_{4} in the presence of autologous, irradiated (3000 rad) PBMCs and further expansion in culture with growth factors. Proliferation assays were performed as described above in the presence or absence of BeSO_{4} (10^{-5} M or 10^{-4} M). Proliferation assays were performed in triplicate.

Analysis of TCRBV and TCRA gene segments expressed by the T cell clones

T cells were harvested, and total cellular RNA was extracted as described above. cDNA was synthesized, and TCRBV3 gene segments were amplified via PCR as described above. Each PCR product was purified using a DNA binding spin column (QIAquick PCR Purification Kit; Qiagen), and cycle sequencing was performed using a TCRBC sequencing primer (5′-CGACCTCGGGTGGAACACTAC-3′) and a TCRAC sequencing primer (5′-TTCAGTCAAACGAGGTGAC-3′). RNA from T cell clones expressing the TCRBV of interest was used for anchored PCR amplification of TCRA cDNA, utilizing the 5′ RACE system (Life Technologies). In brief, TCRA first strand cDNA was synthesized using a TCRA-specific primer, designated GSP1 (5′-TTCAGTCAAACGAGGTGAC-3′) and SuperScript II reverse transcriptase (Life Technologies). RNA was digested with an RNase mixture, and the cDNA was purified using a silica-based membrane column. A homopolymeric dC tail was added to the cDNA using dCTP and TdT. The dC-tailed cDNA was amplified for 35 cycles of PCR using the Abridged Universal Amplification Anchor Primer and a nested TCRAC primer (designated GSP2, 5′-TGTTGACACGCGGAGGTC-3′) and SuperScript II reverse transcriptase (Life Technologies). The amplified PCR product was sequenced using a TCRAC-specific primer, designated GSP3 (5′-GCACCTCGGGTGGAACACTAC-3′) and a TCRAC sequencing primer (5′-TTCAGTCAAACGAGGTGAC-3′). TCRBV3 and TCRA gene segments were also amplified using a DNA binding spin column (QIAquick PCR Purification Kit; Qiagen), and cycle sequencing was performed using a TCRBC sequencing primer (5′-CGACCTCGGGTGGAACACTAC-3′) and SuperScript II reverse transcriptase (Life Technologies). The amplified PCR product was sequenced using a TCRAC-specific primer, designated GSP3 (5′-GCACCTCGGGTGGAACACTAC-3′) and a TCRAC sequencing primer (5′-TTCAGTCAAACGAGGTGAC-3′).
PCR utilizing GSP2 and a TCRAV-specific primer (TCRAV22S1, 5'-CCTCCTGAAAGCCACGAAGGCTGA-3').

Results

Analysis of expressed TCRB genes in patients with CBD

We previously identified a subset of CBD patients with increased percentages of Vβ3+ T cells in BAL compared with blood (10). We focused the current analysis on five of these patients who were available for additional studies. Certain characteristics of these patients are shown in Table I. Four of the CBD patients were exposed to beryllium oxide in the ceramics industry, whereas patient 5 was exposed to beryllium metal in the nuclear defense industry. The duration of beryllium exposure varied from 9 to 282 mo. The diagnosis of CBD was established subsequent to the development of symptoms in patients 1, 2, and 3 while patients 4 and 5 were diagnosed via a screening program. The presence of symptoms correlated with more severe functional and radiographic abnormalities (data not shown). Although three of the five patients were being treated with corticosteroids at the time of study, all of the patients had active disease based on worsening symptoms and progressive physiologic and radiographic abnormalities. We have previously shown (10) that alterations of TCR V region expression and T cell subset expansions persist in CBD patients with active disease, despite treatment with corticosteroids. In that study, we noted that longitudinal changes in TCR V region expression did not appear to be different in patients treated with corticosteroids, compared with untreated patients.

Table I also shows the percentages of Vβ3+ CD4+ cells in the BAL and blood of these patients, as well as their HLA-DRB1, -DQB1, and -DPB1 alleles, determined by molecular typing. The low percentage of Vβ3+ cells in the peripheral blood of patient 4 is likely to be genetically determined and related to a polymorphism in the spacer region of the recombination signal sequence of the BV3.1 gene (16, 18, 19). The relatively small Vβ3+ CD4+ cells in the lung of this individual may therefore be secondary to this baseline inefficient generation of Vβ3+ T cells. All of the CBD patients in this study possessed DPB1*0201, which has a glutamic acid (E) at position 69 and has been associated with an increased risk of developing CBD (20–22). In contrast, no common HLA-DR or -DQ alleles were shared among all individuals. Of note, CBD patient 5 shared DRB1*0701 with patient 1, and DQB1*0303 with patient 1.

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We sorted CD4+ T cells from the blood and BAL of individual patients from which RNA and cDNA were prepared. TCRBV3 fragments were amplified, cloned in bacteria, and sequenced. Fig. 1A shows CDR3 sequences that were found at least three times or that appeared to have an identical or homologous CDR3 amino acid sequence, compared with other cDNA clones in the same sample. In Fig. 1A, identical amino acid sequences are shown more than once if they were encoded by a different nucleotide sequence. Oligoclonal expansions were apparent in the BAL CD4+ TCRBV3 subset in all five individuals. Each individual had several clones that occupied over 10% of the Vβ3+ subset, and a few clonal expansions approached 20–30% of this subset. The probability for one set of three repeated sequences to be found by chance alone (assuming >5000 cells within a particular TCRBV subset from >100,000 CD4+ T cells sorted) was calculated to be $p = 1.6 \times 10^{-7}$. Clonal expansions were much less frequent and smaller in blood. For example, no repeated sequences were found in the blood Vβ3+ CD4+ subset of patient 2, and the others demonstrated only a few repeated sequences. Furthermore, although BAL samples from patients 1–4 were enriched for sets of clones with homologous TCRs, only patient 1 had matched isolates in blood (see below). Conversely, of five clonal expansions (≥3 repeated sequences) found in the peripheral blood of these individuals, none were found in the BAL. These results clearly demonstrate oligoclonality that predominates in the BAL of these patients and shows the independent repertoires of BAL vs blood T cells in CBD patients.

The Vβ3 CDR3 expansions in individual patients as well as among four different individuals (patients 1–4) showed marked similarities (Fig. 1A). In particular, an aspartic acid (D) was present at position 96 of the β-chain chain (at position 90 (25). These sequence data are available from GenBank under accession numbers AF078961 to AF079024.

FIGURE 1. Analysis of deduced Vβ3 CDR3 amino acid sequences expressed in peripheral blood and BAL CD4+ lymphocytes of five CBD patients (A) and from BAL CD4+ lymphocytes of three normal controls and three sarcoidosis patients (B). TCRBV3 sequences found ≥3 times or with CDR3 homology to other isolates are shown. The same CDR3 amino acid sequence is shown more than once when encoded by different TCRBV3 nucleotide sequences. The number of identical sequences (defined at the nucleotide level) is shown over the total number of sequences analyzed for a given anatomic site. Sequences that possess related CDR3 length and amino acid sequence with an aspartic acid (D) at position 96 of the β-chain chain are shown in bold type. The conserved cysteine (C) of the β-chain is designated as position 90 (25). These sequence data are available from GenBank under accession numbers AF078961 to AF079024.
and usually had a glycine (G) or lysine (K) at position 95 and an arginine (R) or glutamine (Q) at position 97. The relatedness of some of these sequences is also shown in Fig. 2. For example, sets of TCRs had the sequence CASSFGD, CASSLGD, or CASSSGD, usually with an arginine (R) or glutamine (Q) at the next position. Although the Vb and NDβN regions of these clones were similar, the Jβ segments varied considerably. Fig. 2 also shows examples of identical β-chain amino acid sequences in the same patient and in different patients that were encoded by different nucleotide sequences, i.e., expressed by different Vb3 T cell clones. One Vb3-CASSFGD-Jβ2.7 sequence was expressed in three different CBD patients. Nucleotide sequence differences among these clones precluded the possibility of a PCR contamination or artifact.

To determine whether these homologous Vβ3 CDR3 sequences were associated with beryllium-induced disease, we sequenced the same TCRBV subset in the BAL of three normal individuals and three patients with sarcoidosis. Sarcoidosis served as a particularly important control disease, since the granulomatous inflammation is essentially identical to that in CBD, and sarcoidosis is also associated with the accumulation of CD4+ T cells in BAL (23, 24). Although clonal populations were identified in the BAL of both normal subjects and sarcoidosis patients, none showed the CDR3 motif found in CBD patients (Fig. 1B). Furthermore, none of the 108 and 137 sequences from normal subjects or sarcoidosis patients, respectively, showed an aspartic acid (D) at position 96 of the TCRB sequence (p < 10^-10, compared with the frequency in CBD patients).

**Persistence of related Vβ3+ clones in BAL**

If CD4+ T cells expressing this CDR3 motif are important in the disease process, we predicted that they should persist at sites of organ involvement in patients with continued disease activity. We therefore studied BAL samples, which were available from prior time points in two patients. Table II shows serial sequence analyses in two patients studied over 3 to 5 years. All clones were detected in at least two different serial samples, and the majority of clones were detected at every time point. Some clones varied considerably in frequency. For example, in patient 1, one clonal sequence (Vb3-CASSLKD-Jβ2.7) decreased from over 50% of the total sequences to 1 in 44 sequences 3 yr later. An explanation for the dramatic decrease in the frequency of this one T cell clone is unclear since patient 1 continued to require corticosteroid therapy for progressive lung dysfunction. However, over this same time period, other clones with the shared CDR3 motif increased in frequency such that the total frequency of clones with homologous TCRs at the last time point was about 50%. In patient 2, the frequency of clones with the shared CDR3 motif persisted at 40–50% of the Vβ3+ subset over the 4-yr study period.

**TCRA gene sequences coexpressed by T cell clones with the related TCRB motif**

Since recognition of Ag by the TCR involves both TCR β- and α-chains, TCRs having the same Ag specificity and related β-chain sequences should express homologous α-chain sequences. To test this prediction, Vβ3+ CD4+ T cells from the BAL of two CBD

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**Figure 2.** Similarity of CDR3 sequences and length in CBD patients 1–4. Three groups of related CDR3s are shown, revealing different nucleotide sequences that encode identical or homologous amino acid sequences. Bold letters indicate nucleotides that differ in each patient. Boxes surround the NDβN regions contained within CDR3s of identical length. The cysteine (C) of the β-chain is designated as position 90 with the conserved aspartic acid (D) at position 96.
patients were cloned by limiting dilution, and TCR genes expressed by the different clones were sequenced. T cells expressing the $\beta$-chain CDR3 motif described above were found among the cloned cells at about the frequency predicted (data not shown). The TCRAs expressed by these clones was determined by anchored PCR. As shown in Fig. 3, TCRAs genes expressed by these T cell clones showed remarkable similarity. Thus, all utilized TCRAV22S1, out of a possible 75–100 TCRAV gene segments to choose from (25). Most used AJ49, and nearly all had similar junctional sequences and length. In addition, all of the coexpressed TCRAs encoded an asparagine (N) at amino acid position 95 and all but one had a leucine (L) at position 92. In addition, two subsets of TCRAs were apparent: one encoding a serine (S) at position 93 and utilization of AJ8 or AJ11 and another encoding an arginine (R) at position 93 and utilization of AJ49.

**Analysis of TCR expression after in vitro stimulation with beryllium**

It was important to determine whether T cells with the related CDR3 motif could be stimulated by BeSO$_4$ in culture. BAL and blood T cells from CBD patients were cultured for 5 days with optimal concentrations of BeSO$_4$ (3, 12, 26). Consistent with previous studies (3, 12), cultures of cells from CBD patients showed vigorous proliferation, whereas cultures containing cells from control subjects showed no response and were similar to cultures without added BeSO$_4$ (data not shown). TCR-$\beta$-chain sequences expressed by cells before and after stimulation are shown in Fig. 4. At day 0, the frequency of TCRs with the shared CDR3 motif was 53% and 30% in patients 2 and 3, respectively. After 5 days of stimulation with BeSO$_4$, Mopping CD4$^+$ T cells predominated in the cultures and expressed the shared motif at about the same frequency. The continued presence of these cells indicates that they did proliferate in culture. Otherwise, their expressed TCRs would have been diluted out by the surrounding proliferating cells. The results also suggest that these cells with the particular $\beta$-chain CDR3 sequence are not the only V$\beta$$^+$ beryllium-responsive T cells in the lungs of these patients and that perhaps most of the BAL V$\beta$$^+$ population, as well as selected cells in other BAL V$\beta$ subsets, are capable of responding to BeSO$_4$ in culture.

Although peripheral blood cells from patient 2 also proliferated in culture after addition of BeSO$_4$, none of the sequences before or after stimulation showed the shared V$\beta$3 CDR3 motif. Thus, the beryllium-responsive cells in BAL vs blood appear to be derived from separate populations.

**V$\beta$$^+$ T cell clones with the shared $\beta$-chain CDR3 motif respond to beryllium in vitro**

BAL cells from patient 2 were stimulated in culture in the presence of BeSO$_4$ and then cloned at limiting dilution in the presence of autologous, irradiated PBMCs in culture media containing BeSO$_4$ and T cell growth factors. After several rounds of repeated stimulation, 24 clones were expanded. Eight clones were randomly chosen for sequence analysis, and four (50%) expressed the homologous CDR3 motif as defined above. Two isolates were identical with the $\beta$-chain junctional sequence V$\beta$-CASSLG-DQPQHFG-J$\beta$1.5 and two others were identical with the sequence V$\beta$3-CASSWADREAFF-J$\beta$1.1. In addition, all four expressed TCRAV22S1 and had the invariant asparagine (N) at position 95 and leucine (L) at position 92 of the $\beta$-chain, as shown above for other T cell clones with the conserved CDR3 motif. Four clones were analyzed for proliferative response to BeSO$_4$ using autologous, irradiated PBMCs as APCs, and all four clones proliferated in an Ag-specific manner (Fig. 5). One of the four clones studied, RP1, expressed the shared CDR3 motif. The frequency of this TCR $\beta$-chain sequence ranged from ~9 to 17% of the V$\beta$$^+$ T cells in this patient’s BAL over 4 yr. The same $\beta$-chain sequence

![Image](http://www.jimmunol.org/Downloadedfrom)
was found in two other T cell clones, including one from patient 3. Irrelevant Vβ3+ CD4+ T cell clones were also derived from peripheral blood CD4+ T cells of a normal individual after stimulation with PHA and selection of clones expressing Vβ3. As shown in Fig. 5, these clones proliferated vigorously to PHA but all failed to respond to BeSO4 in vitro. In fact, exposure to BeSO4 appeared to inhibit proliferation in six of eight T cell clones analyzed.

Discussion

CBD is characterized by the accumulation of CD4+ T cells within the lung, and these cells appear to play a critical role in the development of granulomatous inflammation (7–9). However, little is known about how sensitization to beryllium occurs and why only a minority of individuals exposed to beryllium subsequently develop chronic granulomatous lung disease, usually years after leaving the workplace in which exposure occurred (4–6, 27). Identification and characterization of the pathogenic lung T cells is likely to provide important insight into these questions. In the present work, we focused on the expanded Vβ3+ CD4+ subset previously found in the BAL of some patients with CBD (10).

Sequence analysis of the TCRs expressed in the lung showed evidence of clonally expanded CD4+ populations that expressed TCRs related to each other. These CD4+ clones appeared to be specific for CBD patients and expressed TCRs that were shared among different patients. Cells expressing the shared CDR3 motif were present at high frequency at the site of pathology and appeared to be compartmentalized to the lungs of individual patients. The results strongly support the contention that these expanded clones, which persist in patients with active disease, represent one subset of pathogenic beryllium-responsive CD4+ T cells.
of healthy individuals, including clones that occupied 25–30% of this subset. These clones appear to be larger than those previously identified in normal individuals (31). The clonal expansions in normal controls may represent resident lung T cell populations or result from recent exposures to foreign Ags and indicate that the presence of oligoclonal populations in lung is not necessarily evidence of pathology. In regard to the current study, the repertoire of BAL Vβ3+ T cells in CBD was found to be distinct from that in sarcoidosis patients and normal controls and, therefore, does not appear to represent the stimulation of a resident lung population. Thus, the conserved TCR β-chain CDR3 motif found in four of the five CBD patients was not seen once in the BAL of sarcoidosis patients or normal controls. Furthermore, despite a data bank of hundreds of TCRBV3 sequences in our laboratory derived from the synovial fluid and/or peripheral blood CD4+ T cells from rheumatoid arthritis patients, other patient groups, and healthy individuals, we have not previously found this related CDR3 motif.

Considering the enormous diversity of the TCR repertoire, related TCRs are predicted to occur very rarely by chance alone and almost certainly represent selection by the same or similar Ag. In four of the five CBD patients investigated, related CDR3 sequences appeared to be present in the BAL Vβ3+ subset. This CDR3 motif was not stringent in a number of positions but appeared to require an aspartic acid (D) at the 96th position of the β-chain, frequently followed by a glutamine (Q) or arginine (R) at the next position. These CDR3s also had a similar length of 7 or 8 aa. Interestingly, nearly any TCRBJ usage was allowed, which raised questions about whether these TCRs were truly related. The ultimate form of related TCRs is that which expresses different TCRB genes (i.e., different CDR3 nucleotide sequences) and therefore is derived from different T cell clones, but expresses identical TCR β-chain amino acid sequences. Examples of such clones were found in each CBD patient as well as between different patients. Selection by the same conventional Ag should also be reflected in both the TCRα and TCRβ gene sequences coexpressed in related clones. Therefore, we cloned BAL CD4+ T cells by limiting dilution, identified those clones that expressed TCRB sequences predicted to be related, and sequenced the coexpressed TCRα genes. Despite variation in TCRBJ usage among these T cell clones, the coexpressed TCRα sequences were remarkably related to each other, with identical TCRBV usage and invariant amino acids at certain nongermline-encoded positions of the α-chain CDR3. The similarity of the TCRαs provides unequivocal evidence for the relatedness of these TCRs and their selection by a common Ag.

Although conserved usage of homologous TCRs among different animals has been frequently seen after immunization with particular proteins or peptides in rodent studies, their demonstration in different patients with the same disease has been rare. In a recent study of patients with rheumatoid arthritis, despite selection of patients for particular HLA-DR4 alleles, we could not demonstrate similar TCRs in the synovial fluid among different patients (32). We have also not been able to demonstrate related TCRs in patients with sarcoidosis (30).

It is of interest that the only patient who did not share the conserved CDR3 motif was exposed to beryllium in a nuclear weapons plant whereas the other four patients were exposed in the ceramics industry (1). Our present results suggest that qualitative differences in CD4+ T cell recognition may also be involved. Further studies with larger numbers of patients will determine whether different TCR repertoires are related to these different types of beryllium exposures.

We also noted a remarkable separation of lung and peripheral blood CD4+ T cell repertoires in these patients. The compartmentalization in lung of a subset of beryllium-reactive cells occurred despite the presence of beryllium-responsive cells in peripheral blood in most patients with CBD (12), including the two patients tested in our study. It is well-known that beryllium-exposed individuals may demonstrate evidence of sensitization and yet display no evidence of lung pathology or physiologic abnormalities (27). Thus, in a subset of exposed individuals, peripheral blood T cells will proliferate in culture after the addition of BeSO4, but these patients may demonstrate no clinical, physiologic, or lung histopathologic abnormalities even after long-term follow-up (27). Therefore, only a subset of beryllium-reactive CD4+ T cells appears to have pathogenic potential. The present results support the contention that these clones are compartmentalized because of their different beryllium-related specificity.

We also simulated BAL and blood cells with BeSO4 and analyzed TCR expression after T cell stimulation. Numerous studies have indicated that BeSO4 is not a polyclonal T cell activator (8, 9, 13, 26). In contrast to patients with CBD and those individuals sensitized by a previous exposure to beryllium, cells from normal individuals or patients with other diseases do not proliferate upon BeSO4 exposure. This forms the basis for the diagnostic test used in the evaluation of beryllium-exposed individuals (3, 7, 8, 12, 13). In the present work, T cells expressing the shared CDR3 motif were prevalent in the blast population after in vitro stimulation of BAL cells, indicating their ability to respond to BeSO4 in vitro. This capability to respond to beryllium in vitro was also shown for T cell clones derived from the BAL of a patient but not for irrelevant Vβ3+ T cell clones.

Previous studies have shown that T cells from patients with CBD respond to BeSO4 in culture in a class II MHC-restricted manner (9). Considering the expression of related TCRs among four different patients, it is surprising that these CBD patients demonstrated such diverse HLA-DR and -DQ types. Thus, a shared HLA-DRB1 or -DQB1 allele was not found in this group of CBD patients. Patient 5 did share HLA-DR and -DQ alleles with other CBD patients studied and yet did not demonstrate BAL Vβ3+ cells utilizing the related CDR3 motif. In contrast to HLA-DR and -DQ, all of the patients expressed DPB1*0201, which has been associated with an increased risk of developing CBD after exposure to beryllium (20–22). In preliminary studies, however, the responses to BeSO4 of lung-derived CD4+ T cell clones, including those with the shared CDR3 motif, were blocked by anti-DR mAbs and therefore appear to be restricted to HLA-DR (A. P. Fontenot, L. S. Newman, and B. L. Kotzin, unpublished observations).

The nature of the Ag that interacts with the TCR and MHC is not known. It has been hypothesized that beryllium binds to different self proteins (or peptides), which are then presented as “foreign” to T cells by surface class II MHC molecules. There is no direct evidence to suggest that metals (e.g., beryllium, nickel, and gold) can directly interact with the MHC molecule (33). Studies of nickel-induced contact dermatitis have demonstrated recognition of nickel in association with HLA-DRw11 (34) and suggested that nickel binds directly to the peptide component of the MHC-peptide complex via a histidine residue. The interaction of other transitional metals (Co2+, Cu2+, and Zn2+) with histidine residues has also been demonstrated (35). Whether beryllium might interact with a particular exposed amino acid position of the bound peptide remains unknown. However, our finding of a CDR3 motif with
invariant charged residues at particular positions, such as the β-chain aspartic acid, is consistent with a direct interaction of the TCR with a positively charged beryllium molecule.

In summary, we believe that these studies have identified pathogenic T cells in patients with CBD and have provided insight into an unusual form of T cell recognition. Based on the repertoire of beryllium-reactive peripheral blood cells, the TCRs expressed appear to be diverse. However, only a subset of these clones, including a subset expressing homologous TCRs, appears to have pathogenic potential. Why these particular cells seem to be enriched in the lungs of patients with CBD is of critical importance in our understanding the immunopathogenesis of this disease.

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References